PHARMACEUTICAL MANUFACTURING HANDBOOK

Regulations and Quality

SHAYNE COX GAD, PH.D., D.A.B.T.
Gad Consulting Services
Cary, North Carolina

A JOHN WILEY & SONS, INC., PUBLICATION

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PREFACE

This *Handbook of Manufacturing: Regulations and Quality* focuses on all regulatory aspects and requirements that govern how drugs are produced for evaluation (and, later, sale to and use in) humans. The coverage ranges from what the issues are at the early stages (when the amounts are small and the materials of limited sophistication) up to until the issue is reproducibly and continuously making large volumes of a highly sophisticated manufactured product. These 25 chapters cover the full range from preformulation of a product (the early exploratory work that allows us to understand how to formulate and deliver the drug) to identification of sources of contamination and assessment of stability.

The *Handbook of Manufacturing: Regulations and Quality* seeks to cover the entire range of available approaches to satisfying the wide range of regulatory requirements for making a highly defined product that constitutes a successful new drug and how to do so in as effective and as efficient a manner as possible.

Thanks to the persistent efforts of Michael Leventhal, these 25 chapters, which are written by leading practitioners in each of these areas, provide coverage of the primary approaches to the fundamental regulatory challenges that must be overcome to manufacture successfully a deliverable and stable new drug.

SECTION 1

GOOD MANUFACTURING PRACTICES (GMP) AND OTHER FDA GUIDELINES

1.1

GOOD MANUFACTURING PRACTICES (GMP) AND RELATED FDA GUIDELINES

James R. Harris
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Pharmaceutical Manufacturing Handbook: Regulations and Quality, edited by Shayne Cox Gad
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1.1.1 FDA REGULATIONS: REAL AND IMAGINED

A regulation is a law. In the United States, all federal laws have been arranged or codified in a manner that makes it easier to find a specific law. The *Code of Federal Regulations* (CFR) is a compilation of all federal laws published in the *Federal Register* by the executive departments and agencies of the federal government. This code is divided into 50 titles which represent broad areas of federal regulation. Each title is further divided into chapters. The chapters are then subdivided into parts covering specific regulatory areas. Changes and additions are first published in the *Federal Register*. Both the coded law and the *Federal Register* must be used to determine the latest version of any rule. All food- and drug-related laws are contained in Title 21 of the CFR. Each title of the CFR is updated annually. Title 21 is updated as of April 1 of each year.

Because virtually all of the drug regulations are written to state what should be done but do not tell how to do it, the Food and Drug Administration (FDA) also publishes guidance documents. These documents are intended to provide precisely what the name implies—guidance. In this context, *guidance documents are not law and do not bind the FDA or the public*. Manufacturers are not required to use the techniques or approaches appearing in the guidance document. In fact, FDA representatives have repeatedly stated that the regulations were not written to suggest how something should be done in order to encourage innovation. While following the recommendations contained in the guidance documents will probably assure acceptance (agency philosophy and interpretation may have changed since the guidance document was published), other approaches are encouraged. No matter how they choose to proceed, manufacturers should be prepared to show that their methods achieve the desired results.

A method used by the FDA to "float" new ideas is to discuss them at industry gatherings such as FDA-sponsored seminars or meetings of industry groups such as the Pharmaceutical Manufacturers Association (PMA), the Parenteral Drug Association (PDA), and the International Society of Pharmaceutical Engineering (ISPE). Again, it must be remembered that while these comments reflect current FDA thinking, they are simply thoughts and recommendations. They are not law.

Several industry groups also publish comments, guidelines, and so on, that put forth current thinking of the group writing the document. These publications are interesting and often bring out valuable information. However, it is important to remember that *these publications are not regulations or even official guidance documents.* If a firm chooses to follow the recommendations of such documents, they are probably following good advice. However, since the advice comes from a nonofficial source, firms should still be prepared to defend their actions with good scientific reasoning.

1.1.2 21 CFR 210 AND 211: CURRENT GOOD MANUFACTURING PRACTICE FOR FINISHED PHARMACEUTICALS

Parts 210 and 211 of CFR Title 21 are the laws defining good manufacturing practices for finished pharmaceutical products. All manufacturers must follow these regulations in order to market their products in the United States. When a firm files an application to market a product in the United States through a New Drug Application (NDA), abbreviated NDA, (ANDA), Biological License Application (BLA),

or other product application, one of the last steps in approving the application is a preapproval inspection of the manufacturing facility. A major purpose of this inspection is to assure adherence to the GMP regulations. Preapproval inspections are a part of every application approval. Thus, if a firm has 10 applications pending, it should expect 10 inspections. The fact that the manufacturing facility has already been inspected will not alter the need for another inspection.

The FDA also has the right to visit and inspect any manufacturing facility that produces a product or products sold in the United States. Such inspections are unannounced. A manufacturer must admit an inspector when he or she appears at that facility and must do so without undue delay.

GMP requirements for manufacturers of pharmaceutical dosage forms are discussed below. This information should not be considered to be an exact statement of the law. We have attempted to show intent and, occasionally, add some comments that will clarify how that particular regulation is interpreted. For precise wording of a regulation, refer to the CFR and then check the *Federal Register* to determine if there have been any changes since the last update.

General Provisions

1. This section pertains to the manufacture of drug products for humans or animals.
2. These requirements will not be enforced for over-the-counter (OTC) drug products if the products and all their ingredients are ordinarily marketed and considered as human foods and which products may also fall within the legal definition of drugs by virtue of their intended use.

Organization and Personnel

1. Responsibilities of quality control unit
   (a) A quality control unit *must* be a part of the facility organization.
   (b) This unit must be given responsibility and authority to approve or reject all components, drug product containers, closures, process materials, packaging material, labeling, and drug products, and the authority to review production records.
   (c) Adequate laboratory facilities for testing and approval or rejection of the above listed materials must be available.
   (d) The quality control unit is responsible for approving or rejecting all procedures or specifications that impact on the identity, strength, quality, and purity of the drug product.
   (e) Responsibilities and procedures applicable to the quality control unit must be written and these procedures must be followed.
2. Personnel qualifications
   (a) Every person involved in the manufacture, processing, packing, or holding of a drug product must have education, training, and experience that enable that individual to perform their duties. Employees must be trained in the particular operations that they perform and in Current GMPs (CGMPs). The GMP training must be conducted by qualified individuals and with sufficient frequency to assure that workers remain familiar with the requirements applicable to them.

(b) Persons responsible for supervision must have the education, training, and experience to perform their assigned functions in such a manner as to assure that the drug product has the safety, identity, strength, quality, and potency that it is represented to possess.

(c) There must be an adequate number of qualified personnel to perform the needed tasks.

3. Personnel responsibilities
   (a) Personnel shall wear clean clothing appropriate for the duties they perform. Protective apparel must be worn as necessary.
   (b) Personnel shall practice good sanitation and health habits.
   (c) Only personnel authorized by supervisory personnel shall enter those areas designated as limited-access areas.
   (d) Any worker considered to have an apparent illness or open lesions that may adversely affect safety or quality of drug products shall be excluded from direct contact with product, components, or containers.
4. Consultants that advise on the manufacture, processing, packing, or holding of drug products must have sufficient education, training, and experience to advise on the subject for which they are retained. The manufacturer must maintain records of name, address, and qualifications of any consultants and the type of service they provide.

Buildings and Facilities

1. Design and construction features
   (a) Buildings should be of suitable size, construction location to facilitate cleaning, maintenance, and proper operations.
   (b) Space should be adequate for the orderly placement of equipment and materials to prevent mix-ups between different components, drug product containers and closures, labeling, in-process materials, or drug products and to prevent contamination.
   (c) The movement of components and product through the building must be designed to prevent contamination.
   (d) Operations should be performed within specifically defined areas having adequate control systems to prevent contamination or mix-ups during each of the following procedures:
      (i) Receipt, identification, storage, and withholding from use of components, drug product containers, closures, and labeling, pending the appropriate sampling, testing, and release for manufacturing or packaging.
      (ii) Holding rejected materials listed in (a) above.
      (iii) Storage of released components, drug product containers, closures, and labeling.
      (iv) Storage of in-process materials.
      (v) Manufacturing and processing operations.
      (vi) Packaging and labeling operations.
      (vii) Quarantine storage before release of drug products.
      (viii) Storage of drug products after release.
      (ix) Control and laboratory operations.

(x) Aseptic processing, which includes:
   (1) Floors, walls, and ceilings of smooth, hard surfaces that are easily cleanable.
   (2) Temperature and humidity controls.
   (3) An air supply filtered through High-Efficiency Particulate Air (HEPA) filters under positive pressure regardless of whether flow is laminar or nonlaminar.
   (4) A system for monitoring environmental conditions.
   (5) A system for cleaning and disinfecting the room and equipment to produce aseptic conditions.
   (6) A system for maintaining any equipment used to control the aseptic conditions.

(e) Operations relating to the manufacture, processing, and packing of penicillin must be performed in facilities separate from those used for other drug products for humans. *Note*: For all purposes of these GMP regulations, the FDA considers cephalosporins to be penicillin.

2. Adequate lighting should be provided in all areas.
3. Heating, ventilation, and air conditioning (HVAC)
   (a) Adequate ventilation is required in all areas.
   (b) Equipment for adequate control over air pressure, microorganisms, dust, humidity, and temperature must be provided when appropriate for the manufacture, processing, packing, or holding of a drug product.
   (c) When appropriate, air supplied to production areas should be filtered to avoid any possibility of contamination or cross-contamination.
   (d) Air-handling systems for the manufacture, processing, and packing of penicillin shall be completely separate from those for other drug products for humans.
4. Plumbing
   (a) Potable water should be supplied in a continuous positive-pressure system free from defects that could contribute to contamination of any drug product.
   (b) Potable water must meet the standards prescribed in the Environmental Protection Agency (EPA) Primary Drinking Water Regulations defined in 40 CFR Part 141.
   (c) Drainage must be of adequate size. Where connected directly to a sewer, an air break or other suitable mechanical device must be provided to prevent back-siphonage.
5. Sewage, trash, and other refuse in and from the building and immediate premises must be disposed of in a safe and sanitary manner.
6. Adequate washing facilities should be provided. This is to include hot and cold water, soap or detergent, air driers or single-service towels, and clean toilet facilities easily accessible to all work areas.
7. Sanitation
   (a) Any building used for manufacture, processing, packing, or holding of a drug product should be maintained in a clean and sanitary condition. Such buildings should be free of infestation by rodents, birds, insects, and other vermin.
   (b) Trash and organic waste matter should be held and disposed of in a timely and sanitary manner.

(c) Written procedures assigning responsibility for sanitation and describing in sufficient detail the cleaning schedules, methods, equipment, and materials to be used in cleaning the buildings and facilities are required. Such procedures must be followed.

(d) Written procedures for use of suitable rodenticides, insecticides, fungicides, fumigating agents, and cleaning and sanitizing agents are required and must be followed. These written procedures should be designed to prevent the contamination of equipment, components, product containers, closures, packaging, labeling materials, or drug products. Agent may not be used unless registered and used in accordance with the Federal Insecticide, Fungicide, and Rodenticide Act (7 U.S.C. 135).

(e) All sanitation procedures apply equally to contractors or temporary employees as to regular employees.

8. All buildings used for GMP-related purposes must be maintained in a good state of repair.

Equipment

1. Equipment should be of appropriate design, adequate size, and suitably located to facilitate operations for its intended use and for cleaning and maintenance.
2. Equipment construction
   (a) Equipment should be constructed so that surfaces that contact components, in-process materials, or drug products should not be reactive, additive, or absorptive so as to alter the safety, identity, strength, quality, or purity of the drug product beyond official or other established requirements.
   (b) Any substance required for operation such as lubricants or coolants shall not come into contact with drug products, containers, and so on, so as to alter the safety, identity, strength, quality, or purity of the drug product beyond established requirements.
3. Equipment cleaning and maintenance
   (a) Equipment and utensils should be cleaned, maintained, and sanitized at appropriate intervals to prevent malfunctions or contamination that would alter the drug product beyond the official requirements.
   (b) Written procedures must be established and followed for cleaning and maintenance of equipment and utensils used in the processing of a drug product. These procedures must include but are not limited to the following:
      (i) Assignment of responsibility for cleaning and maintaining equipment.
      (ii) Maintenance and cleaning schedules, including sanitizing schedules if appropriate.
      (iii) A sufficiently detailed description of the methods, equipment, and materials used in cleaning and maintenance operations and the methods of disassembling and reassembling equipment as a part of cleaning and maintenance.
      (iv) Removal or obliteration of previous batch identification.
      (v) Protection of clean equipment from contamination prior to use.
      (vi) Inspection of equipment for cleanliness immediately before use.

(vii) Records should be kept of maintenance, cleaning, sanitizing, and inspection of all processing equipment.

4. Automatic, mechanical, and electronic equipment
   (a) All such equipment, including computers or related systems that will perform a function to be used in any GMP-related activity, must be routinely calibrated, inspected, or checked according to a written program designed to assure proper performance. Written records must be maintained for all such activities.
   (b) Appropriate controls should be exercised to assure that changes in master production and control records or other similar records are made only by authorized personnel. Input to and output from such systems should be checked for accuracy.

   A backup file of data entered into a computer-related system must be maintained except where certain data such as calculations performed in connection with laboratory analysis are eliminated by computerization or other automated processes. In this situation, a written record of the program should be maintained along with validation data.
5. Filters for liquid filtration used as a part of the manufacture, processing, or packing of injectable drug products intended for human use must not release fibers into such products. Fiber-releasing filters may not be used unless it is not possible to manufacture the product without the use of such a filter. In this situation, an additional non-fiber-releasing filter of 0.22 μm maximum must be used after the fiber-releasing filtration. Use of an asbestos-containing filter is permissible only upon submission of proof to the appropriate FDA bureau that use of a non-fiber-releasing filter will compromise the safety or effectiveness of the drug product.

Control of Components and Drug Product Containers and Closures

1. General requirements
   (a) There must be written procedures describing in sufficient detail the receipt, identification, storage, handling, sampling, testing, and approval or rejection of product components, containers, and closures. Of course, all such procedures must be followed. It is quite common and even more embarrassing to be cited for not following your own written procedures. *Note:* For the rest of this discussion, the term *components* will mean product ingredients, containers, closures, and so on.
   (b) All components listed above must be handled and stored in a manner that will prevent contamination.
   (c) Bagged or boxed components should be stored off the floor. Spacing should allow cleaning and inspection.
   (d) Every container of components must be identified with a distinctive code or lot number for each receival of that product. Even if the next receival is the same vendor lot number, it must be a new identifying number by the pharmaceutical manufacturer. Each lot must be appropriately identified as to its status (quarantined, approved, or rejected).

2. Receipt and storage of untested components
   (a) Upon receipt each container of components must be visually examined for appropriate labeling and any damage or contamination to the component container.
   (b) Components must be stored under quarantine until they have been tested as appropriate and released for use.
3. Testing and approval or rejection of components
   (a) Each lot of components shall be withheld from use until it has been sampled, tested, and released by the quality control unit.
   (b) Representative samples must be taken from every receival of every component. The number or amount of component to be sampled should be based on component appearance, statistical confidence levels, the past history of the supplier, and the quantity needed to analyze and reserve samples if required.
   (c) Sampling procedures
      (i) The component containers should be cleaned where necessary.
      (ii) The containers should be opened, sampled, and resealed in a manner designed to prevent contamination of the sample and remaining contents of the container.
      (iii) If appropriate, sterile equipment and aseptic sampling techniques should be used.
      (iv) Where sampling is done from various parts of a container, samples should not be composited for testing.
      (v) Containers from which samples have been taken must be marked to show that samples have been removed.
   (d) Examination and testing of samples
      (i) At least one test should be conducted on each lot of component drug product to verify identity.
      (ii) Each component must be tested for conformity with all appropriate written specifications for purity, strength, and quality if an ingredient or for conformity with written specifications for containers or closures.
      (iii) In lieu of the above testing by the manufacturer, a report of analysis may be accepted from the supplier provided that at least one specific identity test is conducted on the component by the manufacturer and provided that the manufacturer has established the reliability of the supplier's analyses through appropriate validation.
      (iv) When appropriate, components should be examined microscopically.
      (v) Each lot of a component that is liable to contamination with dirt, insect infestation, or other extraneous adulterant should be examined against established specifications for such contamination.
      (vi) Each lot of a component that is subject to microbial contamination that is contrary to its intended use should be subjected to microbiological tests before use.
   (e) If a lot of components meets the written specifications, it may be approved and released for use. Any lot of such material that does not meet such specifications must be rejected.
4. Use of approved components (including drug product containers and closures) must be rotated to assure that the oldest approved stock is used first.

5. Components must be retested and/or reexamined after storage for a long period of time or after exposure to the atmosphere, heat, or other condition that might adversely affect the component.
6. Rejected components should be identified and controlled under a quarantine system designed to prevent their use in manufacturing or processing.
7. Containers and closures
   (a) Containers and closures must not be reactive, additive, or absorbent so as to alter the drug beyond established acceptance criteria.
   (b) Container closure systems must provide adequate protection against foreseeable external factors in storage that can cause deterioration or contamination of the product.
   (c) Containers and closures should be clean and, if necessary, sterile and processed to remove pyrogens.
   (d) Standards or specification, methods of testing, and, if appropriate, sterilization and depyrogenation must be written and followed.

Production and Process Controls

1. Written procedures and procedure deviations
   (a) Written procedures for production and process control must be written and followed. These procedures should be designed to assure that the drug products have the identity, strength, quality, and purity they are represented to possess. These procedures must include all requirements given below and must be drafted, reviewed, and approved by the affected organizational units and reviewed and approved by the quality control unit.
   (b) When following the above identified procedures, all actions must be documented at the time of performance. Any deviations from the written procedure must be recorded and justified.
2. Charge-in of components—Written production and control procedures must include the following, which are designed to assure that the drug products produced meet all specifications and standards.
   (a) The batch must be formulated with the intent to provide not less than 100% of the labeled amount of active ingredient.
   (b) Components used must be weighed, measured, or subdivided appropriately. If a component is removed from its original container and placed in another, the new container should be identified with the following information:
      (i) Component name and/or item code.
      (ii) Receiving or control number.
      (iii) Weight or measure of material in the new container.
      (iv) Batch or lot number for which the component was dispensed, including its product name, strength, and lot number.
   (c) Weighing, measuring, or subdividing operations for all components must be adequately supervised. Each container of component dispensed to manufacturing must be examined by a second person to assure that:
      (i) The component was released by the quality control unit.
      (ii) The weight or measure is correct as stated in the batch production records.

(iii) The containers are properly identified and contain the quantity stated on the label.

(d) Addition of each component must be performed by one person and verified by a second person.

3. Actual yield and percentage of theoretical yield should be determined at the completion of each appropriate phase of manufacturing, processing, packaging, or holding. These calculations should be performed by one person and independently verified by a second individual.
4. Equipment identification
   (a) All compounding and storage containers, processing lines, and major equipment used during the production of a batch of a drug product must be properly identified at all times to indicate their contents and the phase of processing of the batch.
   (b) Major equipment should be identified by a distinctive identification that shall be recorded in the batch production record to indicate the specific equipment used. In cases where only one of a particular type of equipment exists in a given manufacturing facility, the name of the equipment may be used instead of creating a distinctive identification.
5. Sampling and testing of in-process materials and drug products
   (a) To assure batch uniformity and integrity, it is necessary to write and follow procedures that describe the in-process controls and tests or examinations that will be conducted on samples taken according to procedure. Procedures should be written to monitor the output and to validate the performance of those manufacturing processes that may be responsible for causing variability in the product being manufactured. These control procedures should include but are not limited to the following:
      (i) Tablet or capsule weight variation.
      (ii) Disintegraton time.
      (iii) Adequacy of mixing or blending to assure uniformity and homogeneity.
      (iv) Dissolution time and rate.
      (v) Clarity of solutions.
      (vi) pH of solutions.
   (b) In-process specifications for all characteristics must be consistent with the drug product final specifications and must be developed from previous acceptable product average and process variability data.
   (c) In-process materials should be tested for identity, strength, quality, and purity as appropriate. As a part of the production process, they must be approved for continued use or rejected by the quality control unit before production continues.
   (d) Rejected in-process materials must be identified and controlled under a quarantine system designed to prevent their use in manufacturing operations for which they have been found to be unsuitable.
6. When appropriate, time limits should be established for the completion of each phase of production. The purpose of this is to assure the quality of the drug product. Deviation from the established time limits may be acceptable if this deviation does not compromise the quality of the product. Any deviation must be documented, including the justification for such deviation.

7. Control of microbial contamination
   (a) To prevent the growth of objectionable microorganisms in products not required to be sterile, appropriate written procedures designed to prevent such growth should be written and followed.
   (b) If sterilization is a part of any procedure described in (a) above, this procedure must be validated.
8. Reprocessing
   (a) Written procedures describing any system used to reprocess batches that do not conform to the established standards must be written and followed.
   (b) Reprocessing must not be performed without the review and approval of the quality control unit.

Packaging and Labeling Control

1. Materials examination and usage criteria
   (a) Written procedures describing in detail the receipt, identification, storage, handling, sampling, examination, and/or testing of labeling and packaging materials must be developed, approved, and followed. These materials must be representatively sampled, examined, or tested on receipt and accepted by the quality control unit before use.
   (b) Any materials that do not fully meet acceptance criteria must be rejected to prevent their use.
   (c) Records of each receival of each different label and packaging material must be maintained indicating receipt, examination or testing, and whether accepted or rejected.
   (d) Labels and other labeling materials for each different drug product, strength, dosage form, or quantity of contents must be stored separately with suitable identification. Access to the storage area must be limited to authorized personnel.
   (e) Obsolete and outdated labels, labeling, and other packaging materials must be quarantined and destroyed.
   (f) The use of gang-printed labels for different drug products or different strengths or different net contents is prohibited. The only exception to this rule is if labels from gang-printed sheets are adequately differentiated by size, shape, or color that will prevent mixing of labels.
   (g) If cut labeling is used, packaging and labeling operations must include one or more of the following special control procedures:
      (i) Dedication of a labeling and packaging line to each different strength of each different drug product.
      (ii) Use of appropriate electronic or electromechanical equipment to conduct a 100% examination for correct labeling during or after completion of the finishing operation.
      (iii) Use of visual inspection to conduct a 100% examination for correct labeling. If visual inspection is used, the inspection should be performed by one person and independently verified by a second individual.
   (h) Printing devices on or associated with the manufacturing line used to imprint labeling upon the drug product unit label or case must be monitored to assure

that the printing conforms to the print specified in the batch production record.

2. Issuance of labeling
   (a) Strict control should be exercised over the issuance of labeling for use in drug product labeling operations.
   (b) Labeling materials issued for a batch must be carefully examined for identity and conformity to the labeling specified in the batch production record.
   (c) Procedures should be written and followed for reconciliation of the quantities of labeling issued, used, destroyed, and returned. Procedures should require evaluation of discrepancies found between the number of packages finished and the amount of labeling issued if discrepancies outside narrow preset limits occur. Limits should be established on the basis of historical operating data. Labeling reconciliation is waived for either cut or roll labeling if a 100% examination for correct labeling is performed.
   (d) All excess labeling bearing a lot or control number must be destroyed.
   (e) Returned labeling should be maintained and stored in a manner to prevent mix-ups.
   (f) Written procedures should describe the control procedures used for the issuance of labeling.
3. There must be written procedures designed to assure that correct labels, labeling, and packaging materials are used. These procedures should incorporate the following features:
   (a) Prevention of mix-ups and cross-contamination by physical or spatial separation of operations on other drug products.
   (b) Identification and handling of filled drug product containers that are set aside and held in unlabeled condition for future labeling operations. Such procedures should be designed to prevent mislabeling individual containers, lots, or portions of lots. It is not necessary to apply identification to each individual container, but the procedure should be adequate to determine the name, strength, quantity of contents, and lot or control number of each container.
   (c) Identification of the drug product with a lot or control number that permits determination of the history of the manufacture and control of the batch.
   (d) Examination of packaging and labeling materials for suitability and correctness before issuing for use and before packaging operations. These examinations must be documented in the batch production record.
   (e) Inspection of the packaging and labeling facility immediately before use to assure that all drug products and labeling materials from the previous operation have been removed. Inspection results must be documented in the batch production record.
4. Tamper-evident packaging requirements for OTC human drug products
   (a) An OTC product (with the exception of a dermatological, dentifrice, insulin, or lozenge product) intended for retail sale is considered adulterated or misbranded or both if it is not packaged in a tamper-resistant package.
   (b) Requirements for a tamper-evident package
      (i) With the exceptions listed above, all OTC products must be packaged in a tamper-evident package if the product is accessible to the public while being held for sale. A tamper-evident package must have

one or more indicators or barriers to entry which, if breached or missing, can reasonably be expected to provide visible evidence to consumers that tampering has occurred: A tamper-evident package may involve an immediate container and closure system or a secondary container or carton system or a combination of systems intended to provide a visual indication of package integrity. The tamper-evident feature must be designed to and shall remain intact when handled in a reasonable manner during manufacture, distribution, and retail display.

(ii) In addition to the tamper-evident packaging feature described above, any two-piece hard gelatin capsule covered by this regulation must be produced using an acceptable tamper-evident technology.

(c) Labeling
- (i) In order to alert consumers to the specific tamper-evident features used, each retained package of an OTC drug product covered by this regulation is required to bear a statement that:
  - (1) Identifies all tamper-evident features and any capsule-sealing technologies.
  - (2) Is prominently placed on the package.
  - (3) Is so placed that it will be unaffected if the tamper-evident feature of the package is breached or missing.
- (ii) If the tamper-evident feature chosen to meet the requirement uses an identifying characteristic, that characteristic is required to be referred to in the labeling statement. For example, the labeling statement on a bottle with a shrink band could say *For your protection, this bottle has an imprinted seal around the neck*.

(d) A manufacturer or packer may request an exemption from the tamper-evident requirement. A request for exemption is required to be submitted in the form of a petition and should be clearly identified on the envelope as a "Request for Exemption from the Tamper-Evident Packaging Rule." This petition is required to contain the following:
- (i) The name of the drug product or, if the petition seeks an exemption for a drug class, the name of the drug class and a list of products within that class.
- (ii) The reasons that the drug product's compliance with the tamper-evident packaging and labeling requirements is unnecessary or cannot be achieved.
- (iii) A description of alternative steps that are available or that the petitioner has already taken to reduce the likelihood that the product or drug class will be the subject of malicious adulteration.
- (iv) Other information justifying an exemption.

(e) Holders of approved new drug applications for OTC drug products are required to provide the FDA with notification of changes in packaging and labeling to comply with the requirements of this section. Changes in packaging and labeling required by the regulation may be made before FDA approval. Manufacturing changes by which capsules are to be sealed require prior FDA approval.

(f) This section does not affect any requirements for "special packaging" as required under the Poison Prevention Packaging Act of 1970.

5. Drug product inspection
   (a) Packaged and labeled products must be examined during finishing operations to provide assurance that containers and packages in the lot have the correct label.
   (b) A representative sample of units should be collected at the completion of finishing operations and should be visually examined for correct labeling.
   (c) Results of these examinations must be recorded in the batch production records.
6. Expiration dating
   (a) All packaged drug products must carry an expiration date that has been determined from appropriate stability testing.
   (b) Expiration dates must be related to the recommended storage conditions stated on the label as determined by stability studies.
   (c) If the drug product is to be reconstituted at the time of dispensing, its label must carry expiration information for both the reconstituted and unreconstituted forms.
   (d) Expiration dates must appear on labeling in accordance with the requirements stated elsewhere in this regulation.
   (e) Homeopathic drug products are exempt from the requirements of this section.
   (f) Allergenic extracts that are labeled "No U.S. Standard of Potency" are exempt.
   (g) New drug products for investigational use are exempt provided that they meet appropriate standards or specifications as demonstrated by stability studies during their use in clinical investigations. If new drug products for investigational use are to be reconstituted at the time of dispensing, their labeling must bear expiration information for the reconstituted product.
   (h) Pending consideration of a proposed exemption published in the *Federal Register*, September 29, 1978, the requirements in this section will not be enforced for human drug products if their labeling does not bear dosage limitations and they are stable at least three years as supported by stability data.

Holding and Distribution

1. Warehousing procedures
   (a) Written procedures describing the warehousing of drug products must be written and followed. These procedures should include:
      (i) Quarantine of drug products before release by the quality control unit.
      (ii) Storage of drug products under appropriate conditions of temperature, humidity, and light so that the quality of the drug products is not affected.
2. Distribution procedures
   (a) Written procedures concerning the distribution of drug products must be established and followed. These procedures should include:
      (i) A procedure that assures the distribution of the oldest approved stock first. Deviation from this procedure is acceptable if it is temporary and appropriate.

(ii) A system for documenting distribution so that distribution of each lot of drug product can be readily determined to facilitate its recall if required.

Laboratory Controls

1. General requirements
   (a) The establishment of any specifications, standards, sampling plans, test processes, or other laboratory control mechanism required by this part of the regulation, including any changes to the above must be drafted by the appropriate organizational unit and reviewed and approved by the quality control unit. All actions must be documented at the time of performance and any deviation must be recorded and justified.
   (b) Laboratory controls must include the establishment of scientifically sound and appropriate specifications, standards, sampling plans, and test procedures designed to assure that all materials conform to appropriate standards of identity, strength, quality, and purity. Laboratory controls should include:
      (i) Determination of conformance to written specifications for the acceptance of each lot within each shipment of raw materials. The specifications should include a description of the sampling and testing procedures used. Samples must be representative and adequately identified. These procedures must also require appropriate retesting of any material that is subject to deterioration.
      (ii) Determination of conformance to written specifications and a description of sampling and testing procedures for in-process materials.
      (iii) The calibration of instruments, apparatus, gauges, and recording devices at specified intervals in accordance with an established written program containing specific directions, schedules, limits for accuracy and precision, and provisions for remedial action in the event that the limits are not met. Any such devices that do not meet the established specifications must not be used.
2. Testing and release for distribution
   (a) Laboratory testing of each lot of drug product must be conducted to establish conformance to final specifications for the product. Testing must include identity and strength of each active ingredient. Where sterility and/or pyrogen testing are required on short-lived radiopharmaceuticals, batches may be released prior to completion of this testing provided that such testing is completed as soon as possible.
   (b) Each batch of product required to be free of objectionable microorganisms must be tested appropriately.
   (c) All sampling and testing plans must be described in written procedures that include the method of sampling and the number of units to be tested.
   (d) Acceptance criteria for the sampling and testing conducted by the quality control unit must be adequate to assure that the batch being tested meets all specifications. Appropriate statistical quality control criteria should be used. The statistical quality control criteria must include acceptance levels and/or rejection levels.

(e) The accuracy, sensitivity, specificity, and reproducibility of test methods used must be established and documented. Validation and documentation must be accomplished in accordance with this regulation.
(f) Drug products failing to meet established standards or specifications and any relevant quality control criteria must be rejected. Reprocessing may be performed, however, prior to acceptance and use, and reprocessed material must meet all standards, specifications, and other relevant criteria.

3. Stability testing
   (a) There must be a written testing program designed to assess the stability characteristics of every drug product. The results of such testing must be used to determine appropriate storage conditions and expiration dates. The written program must include:
      (i) Sample size and test intervals based on statistical criteria for each attribute examined.
      (ii) Storage conditions for sampled retained for testing.
      (iii) Reliable, meaningful and specific test methods.
      (iv) Testing of the product in the same container-closure system as the one in which the product is to be marketed.
      (v) Testing of drug products for reconstitution at the time of dispensing as well as after they are reconstituted.
   (b) An adequate number of batches of each drug product must be tested to determine appropriate expiration date. A record of such data must be maintained. Accelerated studies, combined with basic stability information on the components and drug product in its container-closure system may be used to project a tentative expiration date that is beyond the date supported by shelf life studies. However, there must be stability studies conducted including drug product testing at appropriate intervals until the tentative expiration date is verified.
   (c) The requirements for homeopathic drug products are as follows:
      (i) There must be a written assessment of stability based on testing or examination of the drug product for compatibility of the ingredients, and based on marketing experience with the drug product to indicate that there is no degradation of the product for the normal or expected period of use.
      (ii) Evaluation of stability must be based on the same container-closure system as the one in which the drug product is to be marketed.
   (d) Allergenic extracts that are labeled "No U.S. Standard of Potency" are exempt from the requirements of this section.
4. Special testing requirements
   (a) For each batch of drug product claimed to be sterile and/or pyrogen free, there must be appropriate laboratory testing to establish conformance to this claim. The test procedures must be in writing and must be followed.
   (b) For each batch of ophthalmic ointment, there must be appropriate testing to determine conformance to specifications regarding the presence of foreign particles and harsh or abrasive substances. The test procedures must be in writing and must be followed.
   (c) For each batch of controlled-release dosage form, there must be appropriate laboratory testing to determine conformance to the specifications for the rate

of release of each active ingredient. The test procedures must be in writing and must be followed.

5. Reserve samples
   (a) An identified reserve sample that is representative of each lot or of each shipment of each active ingredient must be retained. This reserve sample should contain at least twice the quantity needed for all tests required to determine whether the active ingredient meets its established specifications with the exception of sterility and pyrogen testing. The required retention time is as follows:
      (i) For an active ingredient in a drug product other than those described in paragraphs (b) and (c) below, the reserve sample must be retained for one year after the expiration date of the last lot of drug product containing that lot of active ingredient.
   (b) For an active ingredient in a radioactive drug product except for nonradioactive reagent kits, the reserve sample must be retained for:
      (i) Three months after the expiration date of the last lot of the drug product containing that lot of active ingredient if the expiration dating period of the drug product is 30 days or less.
      (ii) Six months after the expiration date of the last lot of the drug product containing that lot of active ingredient if the expiration dating period of the drug product is more than 30 days.
   (c) For an active ingredient in an OTC drug product that is exempt from bearing an expiration date, the reserve sample must be retained for three years after distribution of the last lot of drug product containing that lot of active ingredient.
   (d) A properly identified reserve sample that is representative of each batch of drug product must be retained and stored under conditions consistent with the product labeling. The reserve sample must be stored in the same immediate container closure system in which the drug product is marketed or in one that has essentially the same characteristics. The reserve sample consists of at least twice the quantity needed to perform all the required tests except those for sterility and pyrogens. Reserve samples from representative sample lots or batches selected by acceptable statistical procedures must be examined visually at least once a year for evidence of deterioration unless visual examination would affect the integrity of the reserve sample. Any evidence of reserve sample deterioration must be investigated. The results of the examination must be recorded and maintained with stability data concerning that drug product. Retention times are as follows:
      (i) For a drug product other than the exceptions noted above, the reserve sample must be retained for one year after the expiration date of the drug product.
      (ii) For a radioactive drug product, except for nonradioactive reagent kits, the retention sample must be retained for:
         (1) three months after the expiration date of the drug product if the expiration date is 30 days or less or
         (2) six months after the expiration date of the drug product if the expiration date is more than 30 days.

(iii) For an OTC drug product that is exempt from bearing an expiration date, the reserve sample must be retained for three years after the batch of drug product is fully distributed.

6. Animals used in testing components, in-process materials, or drug products for compliance with established specifications must be maintained and controlled in a manner that assures their suitability for their intended use. They must be identified and adequate records must be maintained showing the history of their use.
7. If a reasonable possibility exists that a nonpenicillin drug product has been exposed to cross-contamination with penicillin, the nonpenicillin drug product must be tested for the presence of penicillin. The drug product may not be marketed if a detectable level of penicillin is found when tested according to procedures specified in "Procedures for Detecting and Measuring Penicillin contamination in Drugs" which is incorporated in the regulation by reference.

Records and Reports

1. General Requirements
   (a) Any production, control, or distribution record that is associated with a batch of a drug must be retained for at least one year after the expiration date of the batch OR, for OTC drug products that do not have expiration dates, three years after complete distribution of the batch.
   (b) Records must be retained for all components, containers, closures, and labeling for the same time periods shown in (a) above.
   (c) All retained records or copies of these records must be readily available for authorized inspection at any time in the required retention period. Records must be available for inspection where the activities described therein occurred. Photocopying or similar reproduction by investigators must be permitted.
   (d) Retained records may be original records or true copies such as photocopies, microfilm, microfiche, or other accurate reproduction of the original.
   (e) Written records that must be retained must be maintained so that data contained therein can be used for evaluating the quality standards of each drug product to determine the need for changes in drug product specifications or manufacturing or control procedures. Such reviews should be conducted at least annually. Written procedures must be established and followed for these evaluations and must include provisions for:
      (i) A review of a representative number of batches, whether approved or rejected, and records associated with the batch.
      (ii) A review of complaints, recalls, returned or salvaged drug products, and investigations conducted under Section 211.192 of the GMP regulations for each drug product.
   (f) Procedures must be established to assure that the responsible officials of the firm are notified in writing of any investigations conducted under Sections 211.198, 211.204, or 211.208 of any recalls, reports of inspectional observations issued by the FDA, or any regulatory actions relating to GMP brought by the FDA.
2. A written record of major equipment cleaning, maintenance (except routine maintenance), and use must be included in individual equipment logs that show

the date, time, product, and lot number of each batch processed. The persons performing and double checking the cleaning and maintenance should date and sign or initial the log indicating that the work was performed. Entries in the log must be in chronological order.

3. Component, drug product container, closure, and labeling records must include the following:
   (a) The identity and quantity of each shipment of each lot of components, drug product containers, closures, and labeling. Also required are the identity of the supplier, the supplier's lot number(s), the receiving code, the date of receipt, and name and location of the prime manufacturer if different from the supplier.
   (b) The results of any test or examination performed and any conclusions derived from these results.
   (c) An individual inventory record of each component and a reconciliation of the use of each lot of such component. The inventory record must contain sufficient information to allow determination of any batch or lot of drug product associated with the use of each component.
   (d) Documentation of the examination and review of labels and labeling for conformance with established specifications.
   (e) The disposition of rejected materials.
4. Master production and control records

   Batch production and control records should be prepared for each batch of drug product produced and must include complete information about the production and control of that batch. These records must include:
   (a) A full and complete reproduction of the appropriate master production or control record. The copy must be checked for accuracy, dated, and signed.
   (b) Documentation that each significant step in the manufacture, processing, packaging, and holding of the batch was accomplished as prescribed, including:
      (i) Dates.
      (ii) Identity of individual major equipment used. This includes packaging lines.
      (iii) Complete and specific identification of each batch of component or in-process material used.
      (iv) Weight and measures of components used in the course of processing.
      (v) In-process and laboratory control results.
      (vi) Inspection of the packaging and labeling area before and after use.
      (vii) Documentation of the actual yield and the percentage of theoretical yield that this represents at critical stages of processing.
      (viii) Complete labeling control records, including specimens or copies of all labeling used.
      (ix) A description of drug product containers and closures.
      (x) Any sampling performed.
      (xi) Identification of the persons performing and directly supervising or checking significant steps in the operation.
      (xii) Any investigations conducted.
      (xiii) Results of examinations made.

5. All drug product production and control records, including those for packaging and labeling, must be reviewed and approved by the quality control unit to determine compliance with all established written procedures before a batch is released or distributed. Any unexplained discrepancy or the failure of a batch or any of its components to meet any of the established specifications must be thoroughly investigated. The investigation must be extended to other batches of the same drug product and other drug products that may have been associated with the specific fault or discrepancy. A written record of the investigation must be made and include the conclusions and any required follow-up.
6. Laboratory records
   (a) Laboratory records must include complete data derived from all tests needed to assure compliance with established specifications and standards. This includes examinations and assays as follows:
      (i) A description of the sample received for testing with identification of source. For example, location where the sample was obtained, quantity, lot number or other distinctive code, date the sample was taken, and the date that it was received for testing.
      (ii) A statement of each method used in the testing of the sample. The statement must indicate the location of data that establish that the methods used in the testing of the sample meet proper standards of accuracy and reliability as applied to the product tested. (If the method used is in the current revision of the U.S. Pharmacopeia (USP), National Formulary (NF), or other recognized standard reference or if it is detailed in an approved NDA, this statement will not be required.)
      (iii) A statement of the weight or measure of sample used for each test.
      (iv) A complete record of all data secured in the course of each test, including all graphs, charts, and spectra from laboratory instrumentation properly identified to the specific component and lot tested.
      (v) A record of all calculations performed in connection with the test, including units of measure, conversion factors, and equivalency factors.
      (vi) A statement of the results of tests and how the results compare with established standards of identity, strength, quality, and purity for the component tested.
      (vii) The initials or signature of the person who performed each test and the date the tests were performed.
      (viii) The initials or signature of a second person showing that the original records have been reviewed for accuracy, completeness, and compliance with established standards.
   (b) Complete records must be maintained of any modification of an established method employed in testing. These records must include the reason for the modification and verify that the modification produced results that are at least as accurate and reliable for the material being tested as the established method.
   (c) Complete records must be maintained of any testing and standardization of laboratory reference standards, reagents, and standard solutions.
   (d) Complete records must be maintained of the periodic calibration of laboratory instruments, apparatus, gauges, and recording devices.

(e) Complete records must be maintained of all stability testing performed in accordance with Section 211.166 of the regulation.

7. Distribution records must contain the name and strength of the product and description of the dosage form, name and address of the consignee, date and quantity shipped, and lot or control number of drug product. For compressed medical gas products, distribution records are not required to contain lot or control numbers.
8. Complaint files
   (a) Written procedures describing the handling of all written and oral complaints regarding a drug product must be established and followed. These procedures must include provisions for review by the quality control unit of any complaint involving the possible failure of a drug product to meet any of its specifications and a determination as to the need for an investigation. These procedures must include provisions for review to determine whether the complaint represents a serious and unexpected adverse drug experience which is required to be reported to the FDA.
   (b) A written record of each complaint must be maintained in a file designated for product complaints. The file may be maintained at another facility if the written records of such files are readily available for inspection at that other facility. Written reports involving a drug product must be maintained until at least one year after the expiration date of the drug product or one year after the date that the complaint was received, whichever is longer. In the case of certain OTC drug products lacking expiration dating because they meet the criteria for exemption, such written records must be maintained for three years after distribution of the drug product.
      (i) The written record must include the following information where known: the name and strength of the drug product, lot number, name of complainant, nature or complaint, and reply to the complainant.
      (ii) Where an investigation is conducted, the written record must include the findings of the investigation and follow-up. The record or a copy of the record of investigation must be maintained at the location where the investigation occurred.
      (iii) Where an investigation is not conducted, the written record must include the reason that an investigation was not considered to be necessary and the name of the responsible person making the determination.

Returned and Salvaged Drug Products

1. Returned drug products—Returned drug products must be identified as such and held. If the conditions under which returned drug products have been held, stored, or shipped before or during the return or the condition of the drug product, its container, carton, or labeling is a result of storage or shipping casts doubt on the safety, identity, strength, quality, or purity of the drug product, the returned drug product must be destroyed unless examination testing or other investigation proves the drug product meets appropriate standards. Records of returned drug products must be maintained and must include the name and label potency of the drug

product dosage lot number, reason for the return, quantity returned, date of disposition, and ultimate disposition of the returned product. If the reason for a drug product being returned implicates associated batches, an investigation must be conducted. Procedures for the holding, testing, and reprocessing of returned drug products must be in writing and must be followed.

2. Drug product salvaging—Drug products that have been subjected to improper storage conditions, including extremes in temperature, humidity, smoke, fumes, pressure, age, or radiation due to natural disasters, fires, accidents, or equipment failures, must not be salvaged and returned to the marketplace. Whenever there is a question whether drug products have been subjected to such conditions, salvaging operations may be conducted only if there is (a) evidence from laboratory tests and assays that the drug products meet all applicable standards of identity, strength, quality, and purity and (b) evidence from inspection of the premises that the drug products and associated packaging were not subjected to improper storage conditions as a result of the disaster or accident. Organoleptic examinations are acceptable only as supplemental evidence that the drug products meet appropriate standards of identity, strength, quality, and purity. Records including name, lot number, and disposition must be maintained for drug products subject to this section.

1.1.3 GUIDANCE FOR INDUSTRY: QUALITY SYSTEMS APPROACH TO PHARMACEUTICAL CURRENT GOOD MANUFACTURING PRACTICE REGULATIONS

This guidance document was written by the FDA to help manufacturers implement what they consider to be modern quality systems and risk management approaches that will meet the requirements of the FDA's GMP regulations. The guidance describes what the FDA considers a comprehensive quality systems (QS) model. It also explains how manufacturers can be in full compliance with the GMP regulations by implementing such quality systems. The FDA does not intend this guidance to place new expectations on manufacturers nor does this replace the GMPs.

As is true with all guidance documents, this document does not establish legally enforceable responsibilities, but rather it describes the FDA's current thinking. Thus, this guidance should be viewed as a set of recommendations unless a regulation is cited.

The objective of this guidance is to describe a quality systems model and demonstrate how and where the elements of this model can fit within the requirements of the CGMP regulations. The philosophy being put forward is that *quality should be build into the product, and testing alone cannot be relied on to ensure product quality*.

1.1.3.1 CGMPS and the Concepts of Modern Quality Systems

The FDA believes that several key concepts are critical for any discussion of modern quality systems. The following concepts are used throughout this guidance as they relate to the manufacture of pharmaceutical dosage forms:

Quality For the purposes of this guidance, the phrase *achieving quality* means achieving the identity, strength, purity, and other quality characteristics designed to ensure safety and effectiveness.

Quality by Design and Product Development This means designing and developing a product and its associated manufacturing processes that will be used to ensure that the product consistently attains a predefined quality at the end of the manufacturing process.

Quality Risk Management This component of a quality systems framework can help guide the setting of specifications and process parameters for dosage form manufacturing, assess and mitigate the risk of changing a process or specification, and determine the extent of discrepancy investigations and corrective actions.

Corrective and Preventative Action (CAPA) This is a regulatory concept that focuses on investigating, understanding, and correcting discrepancies while attempting to prevent their recurrence. This model separates CAPA into three separate concepts:

- Remedial corrections of an identified problem
- Root cause analysis with corrective action to help understand the cause of the deviation and prevent recurrence of a similar problem
- Preventative action to prevent recurrence of similar problems

Change Control This process focuses on managing change to prevent unintended consequences.

Quality Unit While the GMPs refer to a quality unit, current industry practice is to divide the responsibilities of this unit between two groups:

- Quality control (QC) usually involves (a) assessing the suitability of incoming components and the finished products, (b) evaluating the performance of the manufacturing process, and (c) determining the acceptability of each batch for release and distribution
- Quality assurance (QA) involves (a) review and approval of all procedures related to manufacturing and maintenance, (b) review of records, and (c) auditing and performing/evaluating trend analyses.

Six-System Inspection Model The FDA's instruction manual for its investigators is a systems-based approach to inspection consistent with this guidance. The FDA defines six interlocked systems: (1) the quality system which encompasses all the other systems, (2) a materials system, (3) a production system, (4) a packaging and labeling system, (5) a facilities and equipment system, and (6) a laboratory controls system. The agency believes that use of this overall system approach will help firms achieve better control.

1.1.3.2 Quality Systems Model

This section was written to describe a model for use in pharmaceutical manufacturing that can supply the controls to consistently produce a product of acceptable quality. The model is described by four major factors:

- Management responsibilities
- Resources
- Manufacturing operations
- Evaluation

Management Responsibilities The FDA feels that a robust quality system model calls for management to play a key role in the design, implementation, and management of the quality system.

Resources Sufficient resources should be provided to create a robust quality system that complies with the GMP regulations. Senior management or a designee should be responsible for providing adequate resources.

Facilities and Equipment The technical experts who have an understanding of pharmaceutical science, risk factors, and manufacturing processes related to the product are responsible for defining specific facility and equipment requirements. The equipment must be qualified, calibrated, cleaned, and maintained to prevent contamination and product mix-ups. It is important to remember that the GMPs place as much emphasis on process equipment as on testing equipment while most quality systems focus only on testing equipment.

Control Outsourced Operations Quality systems call for contracts with outside suppliers that clearly describe the materials or service, quality specification responsibilities, and communication mechanisms.

Manufacturing There is an overlap between the elements of a quality system and the GMP regulation requirements for manufacturing operations. One should always remember that the FDA's enforcement programs and inspectional coverage are based on the GMPs. The FDA feels that the following factors are essential in a manufacturing quality system:

1. Design, develop, and document product and processes
2. Examine inputs
3. Perform and monitor operations
4. Address nonconformities

Evaluation Activities This includes the following activities:

1. Analyze data for trends
2. Conduct internal audits
3. Quality risk management
4. Corrective action
5. Preventative action
6. Promote improvements

1.1.4 GUIDANCE FOR INDUSTRY: PAT—FRAMEWORK FOR INNOVATIVE PHARMACEUTICAL DEVELOPMENT, MANUFACTURING, AND QUALITY ASSURANCE

This guidance is intended to describe a regulatory framework that the FDA chooses to call *process analytical technology*, or PAT. It is the FDA's hope that this will

encourage the voluntary development and implementation of innovative pharmaceutical development, manufacturing, and quality assurance. The FDA intended this guidance for a broad audience in different organizational units. To a large extent, the guidance discusses principles with the goal of highlighting opportunities and developing regulatory processes that encourage innovation.

Conventional pharmaceutical manufacturing is usually accomplished using batch processing with laboratory testing of samples at various stages of manufacturing to evaluate quality. The FDA believes that opportunities exist for improving the development, manufacturing, and quality assurance steps through innovation in product and process development, process control, and analysis.

Typically, the pharmaceutical industry has been reluctant to try something new due to the fear that the new approach will not find favor with the FDA. An FDA rejection would result in costly delays and processing revisions that industry is unwilling to risk. The FDA now says that this hesitancy is undesirable from a public health perspective and it would like to see more innovation introduced. According to the FDA, pharmaceutical manufacturing should be based on:

- The design of effective and efficient manufacturing manufacturing processes
- Product and process specifications based on an understanding of how formulation and process factors affect product performance
- Continuous real-time quality assurance
- Relevant regulatory policies and procedures tailored to accommodate the most current level of scientific knowledge
- Risk-based regulatory approaches that recognize:
  - The level of scientific understanding of how formulation and manufacturing process factors affect product quality and performance
  - The capability of process control strategies to prevent or mitigate the risk of producing a poor-quality product

It is the intent of this guidance to facilitate progress to this state. So far, the FDA's stated goal is not being met. FDA representatives have stated the agency's concern about the failure of industry to rush to implement change. However, the economies of change continue to favor the status quo.

1.1.4.1 PAT Framework

Quality should be built into pharmaceutical products through a comprehensive understanding of:

- Intended therapeutic objectives, patient population, route of administration, and pharmacokinetic characteristics of a drug
- Chemical, physical, and biopharmaceutic characteristics of a drug
- Design of a product and selection of product components and packaging based on drug attributes
- Design of manufacturing processes using principles of engineering, material science, and quality assurance to ensure acceptable and reproducible product quality and performance throughout a product's shelf life

Process Understanding A process is considered to be well understood when all critical sources of variability are identified and explained, variability is managed by the process, and product quality attributes can be accurately and reliably predicted.

Principles and Tools Pharmaceutical manufacturing often consists of a series of unit operations, each of which is intended to change certain properties of the materials being processed. To assure these changes are acceptable and reproducible, consideration should be given to the quality attributes of incoming materials and their acceptability for the given unit operation. Most current pharmaceutical processes are based on time-defined endpoints such as "blend for ten minutes." In some cases, these time-defined endpoints do not consider the effects of physical differences in raw materials. Processing difficulties can arise that result in the failure of a product to meet specifications even if the raw materials conform to established specifications. Use of PAT tools and principles can provide relevant information relating to physical, chemical, and biological attributes. The process understanding gained from this information will enable process control and optimization, address the limitation of the time-defined endpoints, and improve efficiency.

PAT Tools There are many tools available that enable process understanding. These tools, when used within a system, can provide effective and efficient means for acquiring information to facilitate process understanding, continuous improvement, and development of risk mitigation strategies. Such tools are categorized as follows:

- Multivariate tools for design, data acquisition, and analysis
- Process analyzers
- Process control tools
- Continuous improvement and knowledge management tools

Strategy for Implementation To enable successful implementation of PAT, flexibility, coordination, and communication with manufacturers are critical. The FDA believes that current regulations are sufficiently broad to accommodate these strategies. In the course of implementing the PAT framework, manufacturers may want to evaluate the suitability of a tool on experimental and/or production equipment and processes. It is recommended that risk analysis of the impact on product quality be conducted before installation. This can be accomplished within the facility's quality system without prior notification to the agency. Data collected using an experimental tool should be considered research data. If conducted in a production facility, it should be done under the facility's quality system. The FDA does not intend to inspect research data collected on an existing product for the purpose of evaluating the suitability of an experimental PAT tool. Its routine inspection of a firm's manufacturing process that incorporates a PAT tool for research purposes will be based on current regulatory standards.

The FDA has posted much of the information that firms will need in order to implement a PAT program on the Web at http://www.fda.gov/cder/ops/pat.htm.

All marketing applications, amendments, or supplements to an application should be submitted to the appropriate Center for Drug Evaluation and Research (CDER) or Center for Veterinary Medicine (CVM) division in the usual manner. In general,

PAT implementation plans should be risk based. The FDA has suggested the following possible implementation plans, where appropriate:

- PAT can be implemented under the facility's own quality system. CGMP inspections by the PAT team or PAT-certified investigator can precede or follow PAT implementation.
- A supplement [Changes Being Expected (CBE), Changes Being Expected in 30 Days (CBE-30), or Prior Approval Supplement (PAS)] can be submitted to the agency prior to implementation, and, if necessary, an inspection can be performed by a PAT team or PAT certified investigator before implementation.
- A *comparability protocol* can be submitted to the agency outlining PAT research, validation and implementation strategies, and time lines. Following approval of this *comparability protocol* by the agency, one or a combination of the above regulatory pathways can be adopted for implementation.

To facilitate adoption or approval of a PAT process, manufacturers may request a preoperational review of a PAT manufacturing facility and process by the PAT team by contacting the FDA Process Analytical Technology Team at PAT@cder.fda.gov. It should be noted that when certain PAT implementation plans neither affect the current process nor require a change in specifications, several options can be considered. Manufacturers should evaluate and discuss with the agency the most appropriate option for their situation.

1.1.5 GUIDANCE FOR INDUSTRY: PART 11. ELECTRONIC RECORDS; ELECTRONIC SIGNATURES—SCOPE AND APPLICATION

Of the many regulations written by the FDA, the least understood is undoubtedly 21 CFR Part 11. Rather than review the regulation itself, which is under review and possible revision, we will review the guidance for industry that FDA published in August 2003 to "aid" industry in their puzzlement. Depending on the source, it appears to be questionable as to whether this guidance document aids or confuses. It exists, however, and like it or not, understand it or not, the regulation must be followed.

The guidance indicates that the FDA's approach is based on three main components:

- The regulation will be interpreted narrowly. Fewer records will be considered subject to Part 11.
- Those records that are considered subject to Part 11 will be subject to enforcement discretion with regard to the requirements for validation, audit trails, record retention, and record copying in the manner described and with regard to all Part 11 requirements for systems that were operational before the effective date of this regulation.
- All predicate rule requirements will be enforced. This includes record and record-keeping requirements.

The FDA does intend to enforce all other provisions of Part 11, including certain controls for closed systems. The following controls and requirements will be enforced:

- Limiting system access to authorized individuals
- Use of operational system checks
- Use of authority checks
- Use of device checks
- Determination that persons who develop, maintain, or use electronic systems have the education, training, and experience to perform their assigned tasks
- Establishment of and adherence to written policies that hold individuals accountable for actions initiated under their electronic signatures
- Appropriate controls over systems documentation
- Controls for open systems corresponding to controls for closed systems
- Requirements related to electronic signatures

Part 11 Records Under the narrow interpretation, the FDA considers Part 11 to be applicable to the following records or signatures in electronic format:

1. Records that are required to be maintained under predicate rule requirements and that are maintained in electronic format in place of paper format.
2. Records that are required to be maintainer under predicate rules, that are maintained in electronic format in addition to paper format, and that are relied on to perform regulated activities.
3. Records submitted to the FDA under predicate rules in electronic format. However, a record that is not itself submitted but is used in generating a submission is not a Part 11 record.
4. Electronic signatures that are intended to be the equivalent of handwritten signatures, initials, and other general signings required.

FDA's Approach to Specific Part 11 Requirements

1. *Validation* With respect to validation, the agency intends to exercise enforcement discretion regarding specific Part 11 requirements. However, compliance with all applicable predicate rules for validation is still expected. The FDA suggests an approach to validation be based on a justified and documented risk assessment and a determination of the potential of the system to affect product quality, safety, and record integrity.
2. *Audit Trail* The agency also intends to exercise enforcement discretion regarding specific requirements related to computer-generated, time-stamped audit trails and any corresponding requirements in Part 11. Compliance with all applicable predicate rule requirements related to documentation of date, time, or sequencing of events is still expected. It is also required to comply with rules for ensuring that changes to records do not obscure previous entries.
3. *Legacy Systems* The FDA intends to exercise enforcement discretion with respect to all Part 11 requirements for systems that otherwise were operational prior to August 20, 1997. Thus they do not intend to take enforcement action to enforce compliance with any Part 11 requirements if all of the following criteria are met for a specific system:

- The system was operational before the effective date.
- The system met all applicable predicate rule requirements before the effective date.
- The system currently meets all applicable predicate rule requirements.
- There is documented evidence and justification that the system is fit for its intended use.

4. *Copies of Records* Enforcement discretion will be applied with respect to specific Part 11 requirements for generating copies of records and any corresponding requirements in this part. An investigator should be provided with reasonable and useful access to records during an inspection. All records held by a manufacturer are subject to inspection.
5. *Record Retention* The FDA intends to exercise enforcement discretion with regard to the Part 11 requirements for the protection of records to enable their accurate and ready retrieval at any time throughout the records retention period.

1.1.6 GUIDANCE FOR INDUSTRY AND FDA: CURRENT GOOD MANUFACTURING PRACTICE FOR COMBINATION PRODUCTS

This document discusses the applicability of GMPs to combination products as defined under 21 CFR 3.2(e). Manufacturers must ensure that the product is not adulterated; the product possesses adequate strength, quality, identity, and purity; and the product complies with performance standards as appropriate. This guidance does not address technical manufacturing methods or make recommendations for manufacturers' selection of facilities used in manufacturing.

A combination product is a product composed of a drug and a device, a biological product and a device, a drug and a biological product, or a drug, a device, and a biological product. For the purposes of this document, a constituent part of a combination product is an article in a combination product that can be distinguished by its regulatory identity as a drug, device, or biological product.

For regulatory purposes, a combination product is assigned to an agency center or alternative organizational component that will have primary jurisdiction for its premarket review and regulation. Manufacturers will be required to use the applicable GMP for their products. Regulations that may apply are:

- GMP regulations for finished pharmaceuticals (21 CFR Parts 210 and 211).
- Quality system regulations for devices (21 CFR Part 820).
- The biological product regulations (21 CFR Parts 600–680) may also apply to the manufacture of drugs that are also biological products along with the drug provisions.

There are no GMP regulations specifically for combination products. Until such regulations are promulgated, the manufacture of each constituent part is governed by the regulations for that component.

The Office of Combination Products is available as a resource to sponsors throughout the lifecycle of a combination product. This office can be reached at

(301) 427-1934 or by E-mail at combination@fda.cov. Updated guidance documents are available at the office's Internet website, http://www.fda/gov/oc/combination.

1.1.7 GUIDANCE FOR INDUSTRY: POWDER BLENDS AND FINISHED DOSAGE UNITS—STRATIFIED IN-PROCESS DOSAGE UNIT SAMPLING AND ASSESSMENT

This guidance is intended to assist manufacturers in meeting the GMP requirements for demonstrating the adequacy of mixing to ensure uniformity of in-process powder blends and finished dosage units.

Stratified Sampling In this process dosage units are sampled at predefined intervals and representative samples collected from specifically targeted locations in the compression/filling operations that have the greatest potential to yield extremes of drug concentration.

This guidance describes methods of sampling that might be used to demonstrate active ingredient homogeneity. These methods are put forward as suggestions and are not intended to be the only methods for meeting FDA requirements for demonstration of the adequacy of a powder mix.

Assessment of Powder Mix Uniformity The following procedures are recommended:

1. Conduct blend analysis on batches by extensively sampling the mix in the blender and/or intermediate bulk containers.
2. Identify appropriate blending time and speed ranges, dead spots in blenders, and locations of segregation in intermediate bulk containers (IBCs).
3. Define the effects of sample size (1–10 times the dosage unit range) while developing a technique capable of measuring the true uniformity of the blend. Sample quantities larger than 3 times the dosage size can be used with adequate scientific justification.
4. Design blend-sampling plans and evaluate them using appropriate statistical analyses.
5. Quantitatively measure any variability that is present among the samples. Attribute the sample variability to either lack of uniformity of the blend or sampling error. Significant variances in the blend data within a given location can be an indication of one factor or a combination of factors such as inadequacy of blend mix, sampling error, or agglomeration. Significant between-location variance can indicate that the blending operation is inadequate.

Correlation of Powder Mix Uniformity with Stratified In-Process Dosage Unit Data The following steps are recommended for correlation:

1. Conduct periodic sampling and testing of the in-process dosage units by sampling them at defined intervals and locations throughout the compression or filling process. Use a minimum of 20 appropriately spaced in-process dosage

unit sampling points. There should be at least 7 samples taken from each of these locations for a total minimum of at least 140 samples.

2. Take 7 samples from each additional location to further assess each significant event, such as filling or emptying of hoppers and IBCs, start and end of the compression or filling process, and equipment shutdown. This may be accomplished by using process development batches, validation batches, or routine manufacturing batches for approved products.
3. Significant events may also include observations or changes from one batch to another (e.g., batch scale-up and observations of undesirable trends in previous batch data).
4. Prepare a summary of the data and analysis used to correlate the stratified sampling locations with significant events in the blending process.
5. Compare the powder mix uniformity with the in-process dosage unit data described above.
6. Investigate any discrepancies observed between powder mix and dosage unit data and establish root causes. At least one troubleshooting guide is available that may be helpful with this task. Possible corrections may range from going back to formulation development to improve powder characteristics to process optimization. Sampling problems may also be negated by use of alternate state-of-the-art methods of in situ real-time sampling and analysis.

Correlation of Stratified In-Process Samples with Finished Product The following steps are recommended:

1. Conduct testing for uniform content of the finished product using an appropriate procedure or as specified in the ANDA or the NDA for approved products.
2. Compare the results of stratified in-process dosage unit analysis with uniform content of the finished dosage units from the previous step. This analysis should be done without weight correction.
3. Prepare a summary of the data and analysis used to conclude that the stratified in-process sampling provides assurance of uniform content of the finished product.

1.1.7.1 Validation of Batch Powder Mix Homogeneity

This section describes sampling and testing the powder mix of demonstration and process validation batches used to support implementing the stratified sampling method described in this guidance.

The guidance document recommends that during the manufacture of demonstration and process validation batches, the following uniformity characteristics be assessed: (1) the powder blend, (2) the in-process dosage units, and (3) the finished product. Each attribute should be determined independently. It is further recommended that the following steps be used to identify sampling locations and acceptance criteria prior to the manufacture of the exhibit and/or validation batches:

1. Carefully identify at least 10 sampling locations in the blender to represent potential areas of poor blending. For example, in tumbling blenders (such as V-blenders, double cones, or drum mixers), samples should be selected from at least two depths along the axis of the blender. For convective blenders (such as a ribbon blender), a special effort should be made to implement uniform volumetric sampling to include the corners and discharge area (at least 20 locations are recommended to adequately validate convective blenders).
2. Collect at least three replicate samples from each location. Samples should meet the following criteria:
   - Assay one sample per location (number of samples $n = 10$, or $n = 20$ for ribbon blender).
   - RSD *(relative standard deviation)* of all individual results is 5.0%.
   - All individual results are within 10.0% (absolute) of the mean of the results.

It is also recommended that you not proceed any further with implementation of the methods described in this guidance until the criteria are met.

Sampling errors may occur in some powder blends, sampling devices, and techniques that make it impractical to evaluate adequacy of mix using only the blend data. In such cases, it is recommended that in-process dosage unit data be used in conjunction with blend sample data to evaluate blend uniformity.

Some powder blends may present an unacceptable safety risk when directly sampled. The safety risk, once described, may justify an alternate procedure. In such cases, process knowledge and data from indirect sampling combined with additional in-process dosage unit data may be adequate to demonstrate the adequacy of the powder mix. Data analysis used to justify using these alternate procedures should be described in a summary report that is maintained at the manufacturing facility.

1.1.7.2 Verification of Manufacturing Criteria

The assessment of powder mix uniformity and correlation of stratified in-process dosage unit sampling development procedures should be completed before establishing the criteria and controls for routine manufacturing. It is also recommend that the normality be assessed and that the RSD be determined from the results of stratified in-process dosage unit sampling and testing that were developed. The RSD value should be used to classify the testing results as either *readily pass* (RSD 4.0%), *marginally pass* (RSD 6.0%), or *inappropriate* for demonstration of batch homogeneity when RSD > 6.0%.

The FDA recommends that routine manufacturing batches be evaluated against the following criteria after completing the procedures described above to assess the adequacy of the powder mix and uniform content in the finished dosage form:

1. Standard criteria method (SCM)—This method is recommended when either of the following conditions is met:
   1.1. Results of establishing initial criteria are classified as *readily pass*.

1.2. Results of testing to the marginal criteria method (MCM) pass the criteria for switching to the SCM.
   1.2.1. *Stage 1 Test* To perform the stage 1 test, collect at least three dosage units from each sampling location, assay one dosage unit from each location, weight correct the results, and compare the results with the following criteria:
      1.2.1.1. RSD of all individual results is less than 5%.
      1.2.1.2. Mean of all results is 90–110% of target assay.

      If the results pass these criteria and the adequacy of mix and uniformity of dosage unit content for the batch are adequate, the SCM can be used for the next batch. If test results fail stage 1 criteria, extended testing to stage 2 is required.
   1.2.2. *Stage 2 Test* To perform the stage 2 test, assay the remaining two dosage units from stage 1 for each sampling location and compute the mean and RSD of data combined from both stage 1 and stage 2. Compare the results with the following criteria:
      1.2.2.1. For all individual results, the RSD should be less than 5.0%.
      1.2.2.2. Mean of all results is 90–110% of target assay.

      If results pass the above criteria, the adequacy of mix and uniformity of content for the batch are adequate and stage 1 can be used for the next batch. If test results fail the criteria, use the MCM described in the section below.
2. Marginal criteria method—The MCM can be used when either of the following conditions is met:
   2.1. Results of initial criteria establishment qualified as *marginally pass*.
   2.2. Results of initial criteria establishment qualified as *readily pass* or a batch was tested according to SCM and the test results failed both stage 1 and stage 2 criteria.
   2.3. If either of the above two criteria apply, use the weight corrected results from the stage 2 SCM analysis and compare this with the MCM criteria:
      2.3.1. For al individual results, the RSD is less than 6.0%.
      2.3.2. The mean of all results is 90.0–110.0% of target assay.
   2.4. It is acceptable to switch to the SCM when five consecutive batches pass the MCM criteria and result in RSD of less than 5.0%.

1.1.8 GUIDANCE FOR INDUSTRY: IMMEDIATE-RELEASE SOLID ORAL DOSAGE FORMS SCALE-UP AND POSTAPPROVAL CHANGES (SUPAC)—CHEMISTRY, MANUFACTURING AND CONTROLS, IN VITRO DISSOLUTION TESTING, AND IN VIVO BIOEQUIVALENCE DOCUMENTATION

This guidance provides recommendations to NDA and ANDA sponsors who intend to make changes to the product during the postapproval period. Changes include any change in components or composition of the product, the site of manufacture, the scale-up/scale-down of batch size, and/or the manufacturing process and/or equipment of an immediate-release oral formulation.

Changes in Components (Excipients) and Composition Changes in the amount or source of drug substance are not addressed by this guidance. Changes in components or composition that have the effect of adding a new excipient or deleting an excipient are defined at level 3 except as described below:

1. Level 1 changes
   1.1. Level 1 changes are those that are unlikely to have any detectable impact on formulation quality and performance.
   1.2. Allowed changes (changes that can be made without prior FDA approval) are shown below. This is based on the assumption that the drug substance in the product is formulated to 100% of label potency. To be considered a level 1 change, the total additive effect of all excipient changes should not be more than 5% relative to the target dosage form weight.

Excipient	Percentage of Excipient (W/W) Out of Total Target Dosage Form Weight
Filler	±5
Disintegrant	
Starch	±3
Other	±1
Binder	±0.5
Lubricant	
Calcium or magnesium Stearate	±0.25
Other	±1
Glidant	
Talc	±1
Other	±0.1
Film coat	±1

   1.3. Test documentation
      1.3.1. Chemistry—Application/compendial release requirements and stability testing. For stability testing, one batch should be on long-term stability testing with data being reported in the annual report.
      1.3.2. Filing documentation—All information must be included in the annual report (including long-term stability data).
2. Level 2 changes
   2.1. Level 2 changes are those that could have a significant impact on formulation quality and performance. Tests and filing documentation for a level 2 change depend on three factors: (1) therapeutic range, (2) solubility, and (3) permeability. Therapeutic range is defined as either narrow or nonnarrow. Drug solubility and drug permeability are defined as either low or high. Changes in excipients, expressed as percent (w/w) of total formulation, greater than those listed for a level 1 change but less than or equal to the following percent ranges are acceptable level 2 changes:

Excipient	Percentage of Excipient (w/w) of Total Target Dosage Form Weight
Filler	±10
Disintegrant	
Starch	±6
Other	±2
Binder	±1
Lubricant	
Ca or Mg stearate	±0.5
Other	±2
Glidant	
Talc	±2
Other	±0.2
Film coat	±2

These percentages are based on the assumption that the drug substance in the finished product is formulated to 100% of labeled potency. The total additive effect of all excipient changes should not change by more than 10%.

All components in the formulation should have numerical targets that represent the nominal composition of the product on which any future changes in the composition of the product are based. Allowable changes in the composition should be based on the approved target composition and not on the composition based on previous level 1 or level 2 changes.

2.2. Test documentation
- 2.2.1. Chemistry
  - 2.2.1.1. Application/compendial release requirements and batch records.
  - 2.2.1.2. Stability testing—Test one batch with three months of accelerated stability data in supplement and on batch on long-term stability.
- 2.2.2. dissolution
  - 2.2.2.1. High-permeability, high-solubility drugs—Dissolution of 85% in 15 min in 900 mL of 0/1 *N* HCl. If a drug product fails to meet this criterion, tests in 2.2.2.2 or 2.2.2.3 below should be performed.
  - 2.2.2.2. Low-permeability, high-solubility drugs—Multipoint dissolution profile should be performed in the application/compendial medium at 15, 30, 45, 60, and 120 min or until an asymptote is reached. The dissolution profile of the proposed and currently used product formulations should be similar.
  - 2.2.2.3. High-permeability, low-solubility drugs—Multipoint dissolution profiles should be performed in water, 0.1 *N* HCl, and USP buffer media at pH 4.5, 6.5, and 7.5 (five different profiles) for the proposed and currently accepted formulations. Adequate sampling should be performed at 15, 30, 45, 60, and 120 min until either 90% of drug from the drug product is

dissolved or an asymptote is reached. A surfactant may be used, but only with appropriate justification. The dissolution profile of the proposed and currently used product formulations should be similar.

2.2.3. In vivo bioequivalence documentation is not required for level 2. If the product does not meet any of the level 1 cases above, refer to level 3 changes.

2.2.4. Filing documentation—A prior approval supplement with all data including the accelerated stability data is required. This change should also be documented in the annual report along with the long-term stability data.

2.3. Level 3 changes

2.3.1. Level 3 changes are those that are likely to have a significant impact on formulation quality and performance. Tests and filing documentation vary depending on the following three factors: therapeutic range, solubility, and permeability. For example:

2.3.1.1. Any qualitative and quantitative excipient changes to a narrow therapeutic drug beyond the ranges specified in the level 1 table.

2.3.1.2. All other drugs not meeting the dissolution criteria under level 2.

2.3.1.3. Changes in the excipient ranges of low-solubility, low-permeability drugs beyond those listed in level 1.

2.3.1.4. Changes in the excipient ranges of all drugs beyond those listed in the level 2 table.

2.3.2. Test documentation

2.3.2.1. Chemical

(a) Application/compendial release requirements and batch records:
- Information available—One batch with three months accelerated stability data reported in a supplement and one batch on long-term stability reported in the annual report.
- Information NOT available—Up to three batches with three months accelerated stability data reported in the supplement and one batch on long-term stability data reported in annual report.

(b) Dissolution documentation—Case B dissolution profile as described in the table for level 2.

(c) In vivo bioequivalence documentation—Full bioequivalence study. This requirement may be waived with a verified acceptable in vivo/in vitro correlation.

2.3.2.2. Filing documentation—Prior approval supplement including accelerated stability data plus an annual report showing long-term stability data.

Site Changes Site changes are changes in the location of manufacture for both company-owned and contract manufacturing facilities. A site change does not

include, for example, scale-up changes, changes in manufacturing equipment or a manufacturing process, and changes in Standard Operating Procedures (SOPs) or environmental changes. Each change must be considered separately.

1. Level 1 changes—A level 1 change consists of a site change within a single facility where the same equipment, SOPs, environmental conditions, and personnel are used and where no changes are made to the manufacturing batch records other than location of the facility and administrative changes.
   1.1. Required documentation—No documentation is required beyond the usual application/compendial requirements. No in vivo bioequivalence documentation is required.
   1.2. Filing requirements—Annual report.
2. Level 2 changes—A level 2 change is a site change within a contiguous campus or between facilities in adjacent city blocks where the same equipment, SOPs, environmental conditions and controls, and personnel common to both manufacturing sites are used. There must be no changes to the manufacturing batch records except for administrative information and the location of the facility.
   2.1. Required documentation
      2.1.1. Chemistry—Identify location of new site and updated batch records. No other documentation is required beyond application/compendial release requirements, although one batch produced at the new site should be placed on long-term stability and the data should be reported in the annual report. Dissolution data other than normal release requirements are not required nor is in vivo bioequivalence testing required.
      2.1.2. Filing documentation—A supplement should be filed showing the changes being effected. Long-term stability test data should be included in the annual report.
3. Level 3 changes—A level 3 change is a change in manufacturing site to a different campus. However, the same equipment, SOPs, environmental conditions, and controls should be used in the manufacturing process at the new site. No changes may be made to the manufacturing batch records except for administrative information, location, and language translation if needed.
   3.1. Documentation
      3.1.1. Chemistry—Location of new site and updated batch records.
      3.1.2. Stability
         3.1.2.1. If a significant body of data is available, one batch with three months accelerated stability data must be reported in a supplement. One batch should be on long-term stability with the stability data reported in the annual report.
         3.1.2.2. If a significant body of data is not available, up to three batches with three months accelerated stability data should be reported in the supplement. Up to three batches should be on long-term stability with these data being reported in the annual report.
      3.1.3. Dissolution—A multipoint dissolution profile should be performed in the application/compendial medium at 15, 30, 45, 60, and 120 min or until an asymptote is reached. The dissolution profile of the drug product at the current and proposed site should be similar.
      3.1.4. In vivo bioequivalence—None required.

3.2. Filing documentation required—Changes being effected should be identified in a supplement. Long-term stability data are reported in the annual report.

Changes in Batch Size Postapproval changes in the size of a batch from the pilot scale used to manufacture product for clinical trials to larger or smaller commercial batch sizes require submission of additional information in the application. Scale-down below 100,000 dosage units is not covered by this guidance. All scale-up changes should be properly validated and, where needed, inspected by appropriate FDA personnel.

1. Level 1 changes—A change in batch size, up to and including a factor of 10 times the size of the pilot batch, is considered a level 1 change. However, (1) the equipment used must be of the same design and operating principles, (2) the product is manufactured in full compliance with the prevailing GMPs, and (3) the same formulation and manufacturing procedures are used as well as the same SOPs and controls.
   - 1.1. Chemistry documentation—(1) Application/compendial release requirements, (2) notification of change to the FDA and submission of updated batch records in the annual report, and (3) one batch should be on long-term stability with results being provided in the annual report.
   - 1.2. Dissolution documentation—None beyond application/compendial release requirements.
   - 1.3. In vivo bioequivalence—None.
   - 1.4. Filing documentation—Annual report with long-term stability data.
2. Level 2 changes—Level 2 consists of changes in batch size beyond a factor of 10 times the size of the pilot batch where (1) the equipment used to produce the pilot batches is of the same design and operating principles, (2) the product is manufactured in full compliance with the prevailing GMPs, and (3) the same formulation and manufacturing procedures are used as well as the same SOPs and controls.
   - 2.1. Chemistry—Application/compendial release requirements. Notification of change in batch size and submission of updated batch records to the FDA. One batch must be placed on accelerated stability testing and one on long-term stability.
   - 2.2. Dissolution—None beyond application/compendial release requirements.
   - 2.3. In vivo bioequivalence—None.
   - 2.4. Filing requirements—Must submit changes being effected in the supplement. Long-term stability data are reported in the annual report.

Manufacturing Manufacturing changes may be either the equipment used in the manufacturing process or the process itself:

1. Equipment
   - 1.1. Level 1 equipment changes—This category includes change from the use of nonautomated or nonmechanical equipment to automated or mechanical equipment to move ingredients and a change to alternative equipment of the same design and operating principles of the same or different capacity.

- 1.1.1. Chemistry documentation—Application/compendial release requirements, notification of change, and submission of updated batch records. One batch should be placed on long-term stability.
- 1.1.2. Dissolution documentation—None other than application/compendial release requirements.
- 1.1.3. In vivo bioequivalence documentation—None.
- 1.1.4. Filing documentation—Annual report with long-term stability data.

1.2. Level 2 equipment changes—This type of change involves a change in equipment to a different design and different operating principles.

- 1.2.1. Chemistry documentation—Application/compendial release requirements, notification of change, and submission of updated batch records.
  - 1.2.1.1. If a significant body of data are available, one batch with three months of accelerated stability data reported in the supplement and one batch on long-term stability with data reported in the annual report.
  - 1.2.1.2. If a significant body of data are not available, submit up to three batches with three months accelerated stability data in the supplement and up to three batches on long-term stability with data reported in the annual report.
- 1.2.2. Dissolution documentation—A multipoint dissolution profile should be performed in the application/compendial medium at 15, 30, 45, 60, and 120 min or until an asymptote is reached. The dissolution profile of the drug product at the current and proposed site should be similar.
- 1.2.3. In vivo bioequivalence documentation—None.
- 1.2.4. Filing documentation—Prior approval supplement with justification for change; long-term stability data must be reported in the annual report.

2. Process changes

2.1. Level 1 process changes—This includes process changes such as changes in mixing times and operating speeds within application/validation ranges.

- 2.1.1. Chemistry documentation—None beyond application/compendial release requirements.
- 2.1.2. Dissolution documentation—None beyond application/compendial release requirements.
- 2.1.3. In vivo bioequivalence documentation—None.
- 2.1.4. Filing documentation—Annual report.

2.2. Level 2 process changes—Level 2 changes include process changes such as mixing times and operating speeds outside of application/validation ranges.

- 2.2.1. Chemistry documentation—Application/compendial release requirements; notification of change and submission of updated batch records. One batch on long-term stability.
- 2.2.2. Dissolution documentation—A multipoint dissolution profile should be performed in the application/compendial medium at 15, 30, 45, 60, and 120 min or until an asymptote is reached. The dissolution profile of the drug product at the current and proposed site should be similar.

2.2.3. In vivo bioequivalence documentation—None.
2.2.4. Filing documentation—A supplement with changes being effected. Long-term stability data should be reported in the annual report.

2.3. Level 3 process changes—Level 3 includes change in the type of process used in the manufacture of the product, such as a change from wet granulation to direct compression.

2.3.1. Chemistry documentation—Application/compendial release requirements. Notification of change and submission of updated batch records. Stability testing varies depending on the amount of data available:

2.3.1.1. Significant body of data available—One batch with three months accelerated stability data should be reported in the supplement; one batch should also be put on long-term stability with data being reported in the annual report.

2.3.1.2. No significant body of data available—Up to three batches with three months accelerated stability data should be reported in the supplement. Up to three batches should be on long-term stability with data being reported in the annual report.

2.3.2. Dissolution documentation—A multipoint dissolution profile should be performed in the application/compendial medium at 15, 30, 45, 60, and 120 min or until an asymptote is reached. The dissolution profile of the drug product at the current and proposed site should be similar.

2.3.3. In vivo bioequivalence documentation—An in vivo bioequivalence study should be performed. This may be waived if a suitable in vivo/in vitro correlation has been verified.

2.3.4. Filing documentation—A prior approval supplement must be filed with justification for the change. Long-term stability data should be submitted in the annual report.

1.1.9 OTHER GMP-RELATED GUIDANCE DOCUMENTS

This chapter has discussed the CGMP regulations and some of the more important guidances. There have been a number of additional guidance documents related to GMPs published by the FDA. These documents are all posted on the FDA website. They are listed below along with their URL:

- Current good manufacturing practice for combination products: http://www.fda.gov/cder/guidance/OCLove1dft.pdf
- Formal dispute resolution: Scientific and technical issues related to pharmaceutical CGMP: http://www.fda.gov/cder/guidance/5880fnl.pdf
- Questions and answers on current good manufacturing practices (cGMP) for drugs: http://www.fda.gov/cder/guidance/cGMPs/default.htm
- Powder blends and finished dosage units—Stratified in-process dosage unit sampling and assessment: http://www.fda.gov/cder/guidance/5831dft.pdf
- Sterile drug products produced by aseptic processing—Current good manufacturing practice: http://www.fda.gov/cder/guidance/5882fnl.pdf

- Current good manufacturing practice for medical gases: http://www.fda.gov/cder/guidance/3823dft.pdf
- General principles of process validation: http://www.fda.gov/cder/guidance/pv.htm
- SUPAC-IR: Immediate-release solid oral dosage forms: Scale-up and post-approval changes: Chemistry, manufacturing and controls, in vitro dissolution testing, and in vivo bioequivalence documentation: http://www.fda.gov/cder/guidance/cmc5.pdf
- SUPAC-IR/MR: Immediate release and modified release solid oral dosage forms manufacturing equipment addendum: http://www.fda.gov/cder/guidance/1721fnl.pdf
- SUPAC-MR: Modified release solid oral dosage forms scale-up and postapproval changes: Chemistry, manufacturing, and controls; in vitro dissolution testing and in vivo bioequivalence documentation: http://www.fda.gov/cder/guidance/1214fnl.pdf
- SUPAC-SS: Nonsterile semisolid dosage forms; scale-up and post-approval changes: Chemistry, manufacturing and controls; in vitro release testing and in vivo bioequivalence documentation: http://www.fda.gov/cder/guidance/1447fnl.pdf
- SUPAC-SS: Nonsterile semisolid dosage forms manufacturing equipment addendum: http://www.fda.gov/cder/guidance/1722dft.pdf

1.2

ENFORCEMENT OF CURRENT GOOD MANUFACTURING PRACTICES

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1.2.1 INTRODUCTION AND BACKGROUND

The legal authority for the Food and Drug Administration (FDA) to impose minimum manufacturing standards is set forth in the federal Food and Drug and Cosmetic Act (FDCA), 21 U.S.C. sec. 301 et seq. Section 351(a)(2)(B) of 21 U.S.C. requires manufacturers of drugs to operate in conformance with manufacturing regulations established by the FDA. The regulations are primarily contained in Title 21 of the U.S. *Code of Federal Regulations* (CFR), Parts 210 and 211, and are called the current good manufacturing practice (cGMP) regulations.

The cGMP regulations stem from congressional concern that impure and otherwise adulterated drugs might escape detection under a system predicated only on seizure of drugs shown to be *in fact* adulterated. That is, the U.S. Congress desired

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to require manufacturers to utilize manufacturing practices designed to prevent pharmaceuticals from such defects as contamination, nonconforming bioavailability, or potency defects.

Congress stated the rationale for imposing cGMP on the pharmaceutical industry this way[1]:

> The manufacturing of drugs is a business that requires highly qualified and trained personnel, and special laboratory and other facilities and most careful internal manufacturing, packaging, and labeling controls. These requirements are necessary to the assurance that the drugs will be safe for the user and will have, and so far as possible retain, the identity, strength, quality, purity, and effectiveness that they purport to have.

The purpose of the cGMP requirement is to prevent injury and death "by building quality into the design and production of pharmaceuticals," [2] so that substandard prescription drugs do not jeopardize the health and safety of the patients.

The cGMPs require manufacturers to have adequately equipped manufacturing facilities, adequately trained personnel, precisely controlled manufacturing processes, appropriate laboratory controls, complete and accurate records and reports, appropriate finished product examination, and so on. Current GMPs are not "best practices"; rather, they establish threshold or *minimum* standards which must be satisfied in order for a pharmaceutical manufacturing operation to be compliant.

The cGMPs were modified only once between 1963 and 2002 with changes made in 1978 to update them in light of the current technology and also to describe the requirements more explicitly and with more specificity. Meanwhile, the intervening decades saw myriad advances in manufacturing science, engineering, and technology, including the development of better quality systems. These advances, combined with the desire to harmonize manufacturing standards in an increasingly globalized production environment, created the impetus to revamp the cGMPs again.

In August 2002, the FDA announced a comprehensive review of the pharmaceutical cGMPs. The agency identified its cGMP initiative "Pharmaceutical cGMPs for the 21st Century: A Risk-Based Approach." The FDA's articulated goals for the initiative, relevant to enforcement, were:

- The submission review program and the inspection program operate in a coordinated and synergistic manner.
- Regulation and manufacturing standards are applied consistently.
- FDA resources are used most effectively and efficiently to address the most significant health risks.

One of the major products of the cGMP initiative was issued by the FDA in September 2006 in a document entitled "Guidance for Industry—Quality Systems Approach to Pharmaceutical cGMP Regulations."[3] The FDA described the guid-

[1]H. R. Rep. No. 2464, 87th Cong., 2d Sess. 2 (1962). See also 1962 U.S. Cong. and *Admin. News*, p. 2884.
[2]FDA, Pharmaceutical cGMPs for the 21st century: A risk-based approach, Rockville, MD, August 21, 2002.
[3]The FDA's guidance documents advise the reader that they "do not establish legally enforceable responsibilities. Instead, guidances describe the [the FDA's] current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited."

ance as a comprehensive quality systems model which, if followed, would improve quality control and satisfy the requirements of the cGMP regulations. Quality systems and quality assurance are important parts of the cGMP modernization process because quality assurance problems have been the cGMP issues most frequently cited by FDA investigators in recent years.

Drugs which are manufactured not in accordance with any cGMP requirement, including the quality control and quality process mandates, are "adulterated" under the FDCA. Section 351 of 21 U.S.C. defines a drug as adulterated

> [if] the methods used in, or the facilities or controls used for, its manufacture, processing, packing, or holding do not conform to or are not operated or administered in conformity with current good manufacturing practice to assure that such drug meets the requirement of the act as to safety and has the identity and strength, and meets the quality and purity characteristics, which it purports or is represented to possess.

1.2.2 ENFORCEMENT PLAYERS

The FDA is obviously one of the most important regulatory agencies in the United States. It may also be characterized as the most important consumer protection agency in the world. Its decisions involving approval of drugs have a direct effect on testing, approval, access, and distribution of prescription drugs worldwide. As a regulatory agency in a largely scientific role, it is involved in shaping pharmaceutical science and drug access throughout the world. As a scientific agency, the FDA employs physicians, pharmacists, biologists, biochemists, engineers, biostatisticians, and other highly educated and specialized professionals.

But the FDA also has very important law enforcement responsibilities. The agency employs civil and criminal investigators, auditors, attorneys, and other enforcement professionals. One of the FDA's many enforcement functions is investigation, remediation, and prosecution of cGMP violations.

The FDA district offices operate under the auspices of the agency's Office of Regulatory Affairs (ORA). The ORA field organization is divided into five regional offices (northeast, central, southeast, southwest, Pacific). Each region includes district offices, of which there are 20 nationwide. Most district offices have three or four branches, including either a compliance branch or an enforcement branch. The branch offices are the primary regulatory contacts within the districts and act as the "eyes and ears" for FDA headquarters.

The FDA's Office of Criminal Investigations (OCI) is responsible for reviewing allegations which if proven would violate the U.S. criminal code, including potential violations of the cGMPs. The OCI investigators conduct such investigations as is deemed appropriate, sometimes in connection with other federal investigative agencies, including the FBI and the Office of Inspector General of the Department of Health and Human Services. If the OCI chooses not to recommend to the Department of Justice (DOJ)[4] that criminal indictment be pursued, then the district office is at liberty to pursue the matter through administrative or civil proceedings.

[4]The DOJ is under the direction of the attorney general of the United States. Its mission, relevant to this chapter, is to enforce federal statutes and uphold the rule of the law. It pursues violations brought to its attention by the FDA as well as other federal agencies.

Although the FDA's Office of General Counsel is involved with enforcement of both civil and criminal matters, cases involving court enforcement are handled by assistant U.S. attorneys (AUSAs), who are located in U.S. attorneys' offices located across the United States. U.S. attorneys are the local representatives of the DOJ; they are appointed by and serve at the discretion of the president, with advice and consent of the Senate. There are 93 U.S. attorneys, and they are located (by district) across the United States and its territories. Each U.S. attorney is the chief federal law enforcement officer of the United States within his or her particular district.

The AUSAs are the principal trial attorneys for the U.S. government. Each U.S. attorney exercises wide discretion in the use of his or her resources to further the priorities of the local jurisdiction. Discretion and expertise are big factors in case decisions. There may be significant disparity in the experience, interest, and capability of U.S. attorneys' offices with respect to their pursuit of cGMP violations.

The impact of this disparity is mitigated or eliminated by the expertise of the DOJ's Office of Consumer Litigation (OCL), which is charged with coordinating and supporting FDCA prosecutions nationwide. The OCR's attorneys exercise considerable influence over and discretion in deciding what to and what not to prosecute, thus fostering consistent prosecutive decision making. Many civil actions, particularly those seeking injunctive relief, cannot be brought by a U.S. attorney without OCL approval, minimizing the risk that an inconsistent policy position is taken by a U.S. attorney's office.

1.2.3 FDA ENFORCEMENT TECHNIQUES

1.2.3.1 Inspections

The FDA has the right to conduct surveillance inspections of manufacturing facilities for the purpose of enforcement. The goal of inspections is "to minimize consumers exposure to adulterated products."[5]

The FDCA, 21 U.S.C. 374, provides that the FDA is authorized to enter and "to inspect, at reasonable times and within reasonable limits and in a reasonable manner … all pertinent equipment, finished and unfinished materials, containers, and labeling" in the manufacturing or related facility. This statute further authorizes the inspection to "extend to all things therein (including records, files, papers, processes, controls, and facilities)" as long as the records, for example, are relevant to any potential adulteration or misbranding[6] or other FDCA violations. The statute denies the agency the right to review "financial data, sales data, pricing data, personnel data (other than data as to qualifications of technical and professional personnel performing functions)" and certain other types of documents.

Inspectors are required to notify the company that the inspection is occurring but need not provide their reasons. They may take samples and photographs related

[5]*Compliance Program Guidance Manual for FDA Staff: Drug Manufacturing Inspection Program*, 7356.002, available: www.fda.gov.

[6]Misbranding involves labeling a pharmaceutical product in a misleading way. See 21 U.S.C. 331(k).

to the subject of the inspection. It is a criminal offense to deny entry to FDA inspectors or other officials who have appropriately made attempts to conduct an inspection. [21 U.S.C. 331(f)]

In addition to the for-cause inspections, the FDCA mandates that the FDA routinely inspect a manufacturer's facilities for cGMP compliance every two years. This applies to domestic and foreign facilities which manufacture drugs for sale within the United States.[7] Unfortunately, this two-year mandate is rarely satisfied because the FDA's district offices, which are charged with the responsibility for the inspections, lack sufficient resources to conduct regular cGMP compliance inspections.

The FDA conducts two categories of facility inspections—surveillance inspections and compliance inspections. Surveillance inspections are periodic. Whether and when to inspect a particular manufacturing facility is decided in part by application of an analytical model to determine high risk sites. In late 2004, the FDA issued a report entitled "Risk-Based Method for Prioritizing cGMP Inspections of Pharmaceutical Manufacturing Sites—A Pilot Risk Ranking Model," which allows the agency to rank manufacturing plants' risk of noncompliance by using an analytical process to (1) pose a risk question, (2) identify potential hazards and risks, (3) characterize factors that can be used as variables for quantifying risk, and (4) mathematically combine the variables to yield an overall risk score. Since the publication of the report, the FDA has added adverse events reports data to the model. Surveillance inspections are supposed to involve audit coverage of two or more systems,[8] with mandatory coverage of the quality system.[9]

Compliance inspections are for the purpose of evaluating or verifying compliance corrective actions after a problem has been identified and regulatory action has been taken. Compliance inspections cover the areas found deficient and subjected to corrective actions. One type of compliance inspection is a "for-cause" inspection, which is conducted to investigate a specific problem that has come to the attention of the FDA. The sources that trigger a compliance inspection include field alert reports, industry complaints, and recalls.

In fiscal year 2005, the FDA field office conducted 1437 cGMP inspections, resulting in 15 warning letters, six injunctions, and one seizure. These enforcement actions are discussed later in this chapter. Data for the years 2000–2005 are set forth in Figures 1 and 2.

1.2.3.2 After the Inspection: Form 483

If the inspector determines that there are deviations from cGMP, he will complete a form FDA-483 (Inspectional Observations) detailing the violations. The findings are presented to the manufacturer, which is given an opportunity to respond. The FDA-483 advises:

[7]The other cGMP basic enforcement strategy is collection and analysis of drug samples during factory inspections as well as collecting and analyzing drug products in distribution.

[8]The FDA has separated the cGMP regulation into six systems: quality, facilities and equipment, production, materials, packaging and labeling, and laboratory controls.

[9]*Compliance Program Guidance Manual*, 7356.002, February 1, 2002.

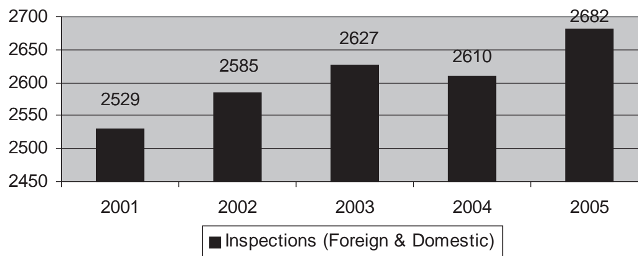

FIGURE 1 CDER five-year Inspection data. (*Source*: FDA.)

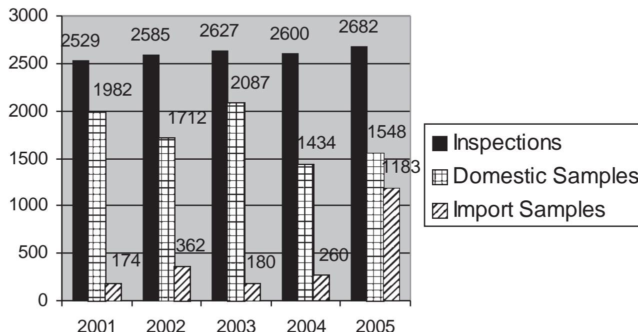

FIGURE 2 Surveillance activity. (*Source*: FDA.)

> This document lists observations made by the FDA representative(s) during the inspection of your facility. They are inspectional observations, and do not represent a final Agency determination regarding your compliance. If you have an objection regarding an observation, or have implemented, or plan to implement, corrective action in response to an observation, you may discuss the objection or action with the FDA representative(s) during the inspection or submit this information to FDA at the address above. If you have any questions, please contact FDA at the phone number and address above.

Most manufacturers provide a written response to the FDA-483, either disputing the findings or addressing how they will correct the issues and how problems are to be corrected. Negotiations typically proceed for months or years until the inspectional problems and issues are resolved or the FDA elects to pursue elevated enforcement. The agency retains discretion to pursue elevated enforcement if it concludes that there is a significant risk of harm to patients, with such action being more likely where patient harm is more likely or more serious.

In addition to providing a form FDA-483, FDA investigators prepare an establishment inspection report (EIR), which is sent to FDA headquarters, which then evaluates the report and determines the corrective action, if any. The FDA then classifies the inspection as "no action indicated," "voluntary action indicated," or "official action indicated." The EIR contains much greater detail than contained in the 483 and is not provided to the manufacturer until after the inspection is deemed closed.

When the FDA conducted an analysis of past FDA-483 reports,[10] the two most reported violations were:

1. Violations of 21 CFR 211.100(b) (failure to follow and/or document production and process control procedures), occurring in over half of all of the 483's
2. Violations of 21 CFR 122d (failure to create adequate, written responsibilities and procedures for the quality control unit or failure to follow them), occurring in 42% of the 483's

The next eight violations, in order of prevalence, were as follows:

- Failure to have written procedures for production and process controls.
- Failure to have testing and release of drug product for distribution for determination of satisfactory conformance to the final specifications/identity and strength of each active ingredient prior to release.
- Batch production and control records were not prepared or are incomplete.
- Control procedures are not established to monitor the output/validate the performance of manufacturing processes that may be responsible for causing variability in the characteristics of the drug product.
- Employees were not given appropriate training.
- Laboratory controls do not include the establishment of scientifically sound and appropriate specifications/standards/sampling plans/test procedures.
- Drug product production and control records are not certified by the quality control unit to assure compliance with all established, approved written procedures before a batch is released or distributed.
- Procedures describing the handling of all written and oral complaints regarding a drug product either not established or not followed.

1.2.3.3 Recalls

Chapter 7 of the *Regulatory Procedures Manual* (March 2007, available at www.fda.gov) provides detailed instructions to FDA personnel regarding recalls. The FDCA does not authorize the FDA to "order" a manufacturer to recall a drug product.[11] In practice, however, the manufacturers or distributors of the drug products are encouraged to implement and carry out recalls voluntarily to fulfill their responsibility to protect the public. It is not uncommon for a company to discover that one of its products is defective and recall it entirely on its own; or the FDA informs a company of its findings that one of its products is defective and suggests or requests

[10]The data for the analysis were compiled by the FDA and derived from 614 Turbo EIR reports completed from 2001 to 2003. (FDA investigators enter their inspections observations on the FDA's Turbo EIR system. The Turbo's electronic format prompts investigators to select the specific cGMP violation in question and then to explain their findings uncovered during the inspection.)

[11]The FDCA gives authority to the FDA to order a recall in some cases involving infant formulas, biological products, and devices that present a "serious hazard to health," but not involving pharmaceuticals.

Class I:	18
Class II:	314
Class III	170
TOTAL:	502

FIGURE 3 2005 recall by class. [*Source*: Centre for Drug Evaluation and Research (CDER) 2005 Report to the Nation.]

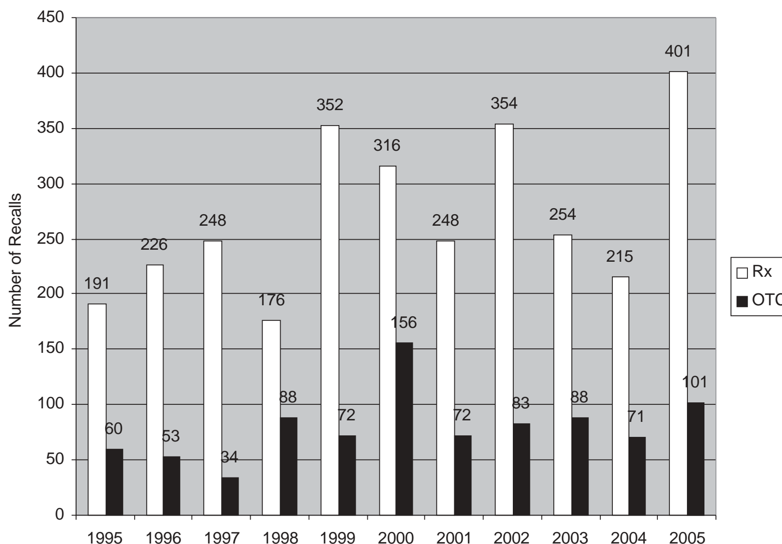

FIGURE 4 Drug recalls. One firm had over 100 recalls in 2005, which caused a spike in the 2005 recall figures. (*Source*: CDER 2005 Report to the Nation.)

a recall.[12] Once a voluntary recall is initiated, the FDA generally follows the following protocol (Figures 3–5):

1. *Classify the Recall* The FDA reviews relevant information and then assigns a recall classification according to the level of health risk involved:

   Class I recalls involve drug products in which the reason for recall predictably could cause serious health problems or death.

   Class II recalls involve drug products which defect might cause a temporary health problem or pose only a slight threat of a serious nature.

   Class III recalls involve products that are unlikely to cause any adverse health reaction but that violate FDA labeling or manufacturing regulations.

2. *Monitor and Audit the Recall* The FDA oversees a recall depending upon the health risk involved. For a class I recall, the FDA checks to make sure that the defective product has been recalled in full. In contrast, for a class III recall, FDA oversight may be to simply spot-check.

[12]If the company does not comply, then FDA can seek judicial enforcement under the FDCA.

- Miscellaneous cGMP deviations (other than below)
- Failed USP dissolution test requirements
- Microbial contamination of non-sterile products
- Lack of efficacy
- Impurities/degradation products
- Lack of assurance of sterility
- Lack of product stability
- Labeling: Label error on declared strength
- Misbranded: Promotional literature with unapproved therapeutic claims
- Labeling: Correctly labeled product in incorrect carton or package

FIGURE 5 Top 10 reasons for drug recalls in fiscal year 2005. (*Source*: FDA.)

3. *Notification and Public Warning* Class I recalls almost always warrant a press release to the media. Classes II and III are not necessarily announced in the media, but all of them are included in the FDA's weekly enforcement report, posted at www.fda.gov/opacom/Enforce.html on the FDA's website.
4. *Termination* The FDA provides written notice to the recalling manufacturer on when the recall should be terminated.
5. *Noncompliance* If applicable, the FDA will take appropriate legal action if a manufacturer fails or refuses to timely complete a recall.

1.2.3.4 Warning Letter

A warning letter is intended to notify manufacturers about violations that the FDA has documented during its inspections or investigations. A warning letter will notify a responsible individual and/or firm that the FDA considers one or more products, practices, processes, or other activities to be in violation of the cGMPs. Warning letters should only be issued for violations of regulatory significance, that is, those that may actually lead to an enforcement action if the documented violations are not promptly and adequately corrected. A warning letter is one of the FDA's principal means of achieving prompt voluntary compliance.

Examples of situations in which the FDA may be expected to issue a warning letter include:

- An active pharmaceutical ingredient (API) batch fails to conform to established specifications and yet the manufacturer distributed it anyway.
- Deliberately blending API batches to dilute or hide noxious contaminant or filth or failing to determine actual yield and percentages of expected yields.
- Contamination of drugs with toxic chemicals, drug residues, airborne contaminants, or filth.
- Failing to comply with commitments in drug applications.
- Combining a batch that does not conform with critical attributes with a batch that does.
- Failing to demonstrate water used in the manufacturing process is suitable.
- Failing to validate water systems.

- Lacking a formal written program to validate an API validation process.
- Failing to demonstrate homogeneity of final blending operations.
- Failing to keep adequate batch records.
- Failing to have a formal process change control system in place.
- Using inadequate or unvalidated laboratory test methods.
- Packaging and labeling processes that could introduce a significant risk of mislabeling.
- Failing to test for residues of organic or inorganic solvents that may carry over to the API.
- Using incomplete stability studies to establish API stability for the intended period of use.

Warning letters detailing cGMP violations typically conclude with the following: "The article(s), (DRUG NAME), is (are) adulterated within the meaning of Section 501(a)(2)(B) of the Act, 21 U.S.C. 351(a)(2)(B), in that the methods used in, or the facilities or controls used for, its manufacture, processing, packing, or holding fails to conform to, or is not operated or administered in conformity with, cGMP regulations [21 CFR 210, 211]." The number of warning letters issued by the FDA concerning prescription and over-the-counter drugs has ranged from 130 letters in 2000 to 79 letters in 2005.

A warning letter is distinguishable from a notice of violation, also called an untitled letter. An untitled letter cites violations that do not meet the threshold of regulatory significance for a warning letter, but the FDA has a need nevertheless to communicate. Unlike a warning letter, an untitled letter does not include a warning statement that failure to take prompt correction may result in enforcement action and does not evoke a mandated FDA follow-up. Further, the untitled letter requests (rather than requires) a written response (from the manufacturer) within a reasonable amount of time (e.g., "Please respond within 45 days").

1.2.4 JUDICIAL ENFORCEMENT: BEYOND THE WARNING LETTER

1.2.4.1 Introduction

The FDA is likely to bypass sending a Warning Letter in certain circumstances. According to Chapter 4 of the FDA *Regulatory Procedures Manual*, the following violations are likely to result in an enforcement action without necessarily issuing a warning letter:

1. The violation reflects a history of repeated or continual conduct of a similar or substantially similar nature during which time the individual and/or firm has been notified of a similar or substantially similar violation.
2. The violation is intentional or flagrant.
3. The violation presents a reasonable possibility of injury or death.
4. Adequate notice has been given by other means and the violations have not been corrected or are continuing.

Written records are required to be kept as set forth in 211.180 to 211.208. Highlights are as follows:

§ 211.182 Equipment cleaning and use log.

A written record of major equipment cleaning, maintenance (except routine maintenance such as lubrication and adjustments), and use shall be included in individual equipment logs that show the date, time, product, and lot number of each batch processed.

§ 211.184 Component, drug product container, closure, and labeling records.

These records shall include the following:

(a) The identity and quantity of each shipment of each lot of components, drug product containers, closures, and labeling; the name of the supplier; the supplier's lot number(s) if known; the receiving code as specified in § 211.80; and the date of receipt. The name and location of the prime manufacturer, if different from the supplier, shall be listed if known.

(b) The results of any test or examination performed (including those performed as required by § 211.82(a), § 211.84(d), or §211.122(a)) and the conclusions derived therefrom.

§ 211.186 Master production and control records.

To assure uniformity from batch to batch, master production and control records for each drug product, including each batch size thereof, shall be prepared, dated, and signed (full signature, handwritten) by one person and independently checked, dated, and signed by a second person. The preparation of master production and control records shall be described in a written procedure and such written procedure shall be followed.

§ 211.188 Batch production and control records.

Batch production and control records shall be prepared for each batch of drug product produced and shall include complete information relating to the production and control of each batch.

§ 211.194 Laboratory records.

Laboratory records shall include complete data derived from all tests necessary to assure compliance with established specifications and standards, including examinations and assays ...

§ 211.198 Complaint files.

A written record of each complaint shall be maintained in a file designated for drug product complaints

FIGURE 6 Written record highlights.

5. The violations, under Title 18 U.S.C. 1001, are intentional and willful acts that once having occurred cannot be retracted. Also, such a felony violation does not require prior notice. Therefore, Title 18 U.S.C. 1001 violations are not suitable for inclusion in warning letters.

In addition, actively deceiving the FDA is almost guaranteed to bring judicial enforcement actions. This includes false representations in the written record-keeping requirements or in written communication with the FDA. Manufacturing record-keeping requirements which give exposure to fraud liability are summarized in Figure 6. Potential violations include the following:

(a) Accepting and validating drug products that failed to meet established standards or specifications and any other relevant quality control criteria (i.e., dissolution rates, content uniformity, purity, potency) and then falsely recording the untruthful data as if the drug products did not fail
(b) Accepting and validating the stability characteristics of drug products and then falsely recording the untruthful data as if the drug products did not fail
(c) Documenting the examination and review of labels, when in truth and fact no review occurred (which results in inaccurate labels distributed with drugs)
(d) Falsely documenting any components of master production and central records
(e) Falsely documenting any component of the batch production and control records
(f) Falsely describing testing methods when no (or inadequate) testing methods were performed
(g) Failing to accurately make a written record of all written and oral complaints regarding a drug product and/or certifying that investigations were performed when they were not, falsely certifying that the findings were negative when they were not, and so on
(h) Falsifying records which would indicate manufacturing changes which require approval by the FDA
(i) False representations that contain statements of fact in correspondence sent to the FDA addressing violations in an inspector's form 483

The FDA typically initiates progressive enforcement, as described in Figure 7. Once the FDA and DOJ decide to bring enforcement action, the U.S. courts have held that the FDA's interpretation of its cGMPs is entitled to substantial deference. As long as the FDA's interpretation of its regulations are "reasonable" and "sensibly conforms to the purpose and wording of the regulations," courts are required to follow the FDA's interpretations.

1.2.4.2 Civil Proceedings

Seizures If during an inspection of a facility the FDA inspector or employee making the inspection has reason to believe that a drug found in such facility is adulterated, such inspector or employee may order the drug detained for a reasonable period which may not exceed 20 days (unless the FDA institutes an action under Subsection 334(a) or an injunction, in which case a longer detention period may be authorized).

The FDCA expressly permits administrative seizure on the basis of an ex parte showing of reasonable belief [21 U.S.C. 334 (g)]. Seizure of a company's inventory deprives the company of both capital investment and potential profit.

If the FDA pursues relief beyond detainment, the United States can file a complaint for forfeiture directing the U.S. marshall to "seize" the pharmaceuticals (or take possession or place in constructive custody of the court). The theory in a complaint for forfeiture is that there is a violation of the law by the pharmaceutical

FDA enforcement mechanisms are often utilized progressively. A good example is the enforcement action against Glaxo SmithKline ("GSK"), which began in July 2002, identifying numerous significant cGMP violations found during a February/April 2002 inspection. A Warning Letter requested that the violations be corrected and stated that failure to correct the violations may result in regulatory action, including seizure and/or injunction. Although a limited follow-up FDA inspection in October 2002, found that some specific corrections were acceptable, the subsequent FDA inspections in November/December 2003 and September/November 2004, revealed continuing significant cGMP violations. FDA concluded that the firm's data and corrective plans were not adequate to correct the cGMP violations. GSK also initiated recall of some, but not all, lots of the two products. On March 4, 2005, in response to ongoing concerns about manufacturing quality, FDA and the DOJ initiated seizures of two GSK pharmaceuticals. The Agency initiated these seizures actions based on concerns that GSK's violation of manufacturing standards may have resulted in the production of poor quality drug products that could potentially pose risks to consumers. On April 28, 2005, FDA announced that GSK had signed a Consent Decree with FDA to correct manufacturing deficiencies at its Cidra, Puerto Rico, facility. The Consent Decree was initiated based on FDA's continued concerns that GSK's violation of manufacturing standards may have resulted in the production of drug products that could potentially pose risks to consumers.

FIGURE 7 Progressive enforcement.

product itself. Accordingly, the government asks the court to condemn the article and declare forfeiture. Upon filing of the complaint, the clerk automatically issues a warrant. Thus, the FDA is able to obtain a warrant without review by a judicial officer or even a finding of probable cause.

There are three types of seizures: mass, open ended, and lot specific. A mass seizure is the seizure of all FDA-regulated products at an establishment/facility. Mass seizures might be conducted when all of the products are produced under the same conditions (e.g., nonconformance with cGMPs). An open-ended seizure is the seizure of all units of a specific product or products, regardless of lot or batch number, when the violation is expected to be continuous. An open-ended seizure may be conducted when a specific product extends to all lots or batches of a product but not to all of the products in the facility.

Following seizure of its drugs a manufacturer has three courses of action. First, it may do nothing, in which case the drug will be disposed of. Second, it can enter into a consent decree, admitting the violation, agreeing to pay costs, and seeking to destroy or rehabilitate the article. The consent decree will typically provide for (1) condemnation of the article as being in violation of the law; (2) a penal bond in approximately twice the retail value of the article under seizure; (3) provisions for payment of costs for storage and handling by the U.S. marshall and for supervision by the FDA before release of the product; and (4) a provision that the manufacturer will attempt to bring the article into compliance under the supervision of and to the satisfaction of the FDA.[13]

Third, it can contest the action. If the manufacturer contests the action, the case is then treated like any other civil case under the federal rules of civil procedure, and the government must prove its case by a preponderance of the evidence. The government must produce evidence, in support of its allegations, including proof of interstate shipment of the drug or its components. FDA employees may testify, but

[13]*Regulatory Procedures Manual*, Chapter 6-1-11, March 2007. This contemplates that seizure of a specific product(s) is the sole issue. More complex consent decrees are described hereinafter.

Major recent consent decrees are United States v. Abbott Labs., Consent Decree of Permanent Injunction filed Nov. 2, 1999; United States v. Various Articles of Drug Identified in Attachment A & Wyeth-Ayerst Labs., Consent Decree of Condemnation and Permanent Injunction filed Oct. 4, 2000; and United States v. Schering-Plough Corp., Consent Decree of Permanent Injunction filed May 20, 2002. To avoid giving manufacturers the wrong message by allowing them to keep on the market what FDA had determined to be produced in violation of the cGMPs, the FDA included three separate types of "disgorgement" payments in the Abbott, Wyeth and Schering consent decrees: (1) a lump sum payment (Abbott, $100 million; Wyeth, $30 million; and Schering, $500 million) (2) if the remedial work was not achieved by the deadline established in the decree, (i) a percentage of sales (Abbott, 16%; Wyeth, 18.5%; and Schering, 24.6%) and (ii) daily payments of a certain flat amount. Both were to be paid until compliance was achieved.

FIGURE 8 Disgorgement.

also outside experts testify such as to the significance of failure to comply with cGMP requirements. If a decree of condemnation is entered (either after trial or by consent), the court may direct disposition of the article by destruction.

Injunctions The FDCA expressly authorizes the courts to restrain and enjoin acts that are in violation of 21 U.S.C. 331, which includes prohibition of adulterated products. FDA policy provides that an injunction action is appropriate where:

(a) there is a current and definite health hazard or a gross consumer deception requiring immediate action to stop the violative practice;
(b) there are significant amounts of violative products owned by the same person in many locations, voluntary recall by the firm was refused or is significantly inadequate to protect the public, and seizures are impractical or uneconomical; or
(c) there are long-standing (chronic) violative practices that have not produced a health hazard or gross consumer fraud, but which have not been corrected through use of voluntary or other regulatory approaches.[14]

A complaint for injunction is typically accompanied by a motion for preliminary injunction.[15] The court schedules a court hearing to determine whether to grant a preliminary injunction, often very quickly and on short notice. The government's main focus at this preliminary stage will be to prove that there is a "substantial likelihood" that the defendant has been producing adulterated drugs in violation of 21 U.S.C. 331, by substantial noncompliance with the cGMPs. The government will also typically present evidence, if applicable, that the defendant has had a history of prior noncompliance with the FDCA and implementing regulations. No specific finding of irreparable harm is necessary as is required in the typical injunction, because the passage of the statute proscribing adulterated products has

[14]*Regulatory Procedures Manual*, March 2007.

[15]The government may also apply for a temporary restraining order (TRO) seeking immediate, temporary relief (for a period of 10 days, which may be extended for 10 additional days) prior to the hearing for preliminary injunction. The FDA will typically recommend a TRO when it believes that the violation is so serious that it must be controlled *immediately*.

been held itself to be an implied finding by Congress that violations will harm the public.

United States courts are imbued with authority to enjoin present and future violations of Section 331 based upon proof by the FDA that such violations have occurred and could recur. Factors that courts consider when determining whether there is a reasonable chance of future infractions include (1) the degree of scienter involved on the part of the defendant; (2) the isolated or recurrent nature of the infraction; (3) the defendant's recognition of the wrongful nature of his or her conduct; (4) the sincerity of the defendant's assurances against future violations; and (5) the nature of the defendant's violation. The court also considers whether the defendant voluntary ceased the challenged conduct, the genuineness of the defendant's efforts to conform to the law, the defendant's progress toward improvement, and the defendant's compliance with any recommendations made by the government.

Good faith is not a defense to the issuance of an injunction. Nor may a defendant successfully defend against the issuance of an injunction by asserting that the injunction would drive it out of business.

Consent Decrees and Disgorgement A consent decree is a judgment (legal order) issued by the court that has been agreed to by the parties whereby the defendant agrees to stop illegal or improper activity as alleged by the government. Once court approval is obtained, the seizure or injunctive lawsuit, for instance, is dropped, and the government's remedy is then based upon any breach of the consent decree, itself which is enforceable by the court.

Consent decrees typically involve a defendant agreeing to address the areas of noncompliance in a manner satisfactory to the FDA within a certain amount of time. It can also provide for the hiring of an expert consultant to certify in detailed reports that the manufacturing facility, at periodic dates, is in full compliance with the cGMPs, and has adequate adverse-event controls, adequate training, and adequate recall procedures. It may also require the payment of money to the U.S. Treasury such as under the equitable remedy of "disgorgement," as described in Figure 8.

As part of a court action, the FDA will sometimes pursue "disgorgement." The purpose of disgorgement is to deprive the wrongdoer of ill-gotten gains as well as provide deterrence. The amount of disgorgement is not necessarily directly tied to restitution. In practice, the amount the FDA exacts is supposed to be enough to send a message but certainly does not provide for full disgorgement of profits of the drug product(s) at issue.

False Claims Act The U.S. Civil False Claims Act, 31 U.S.C. 3729 et seq., is the government's principal means of redressing fraud by government contractors. The act has implications for cGMP violations because the United States (funding as it does the Medicare program, the state Medicaid programs, the Veterans Administration, the TRICARE program, and others) is the world's largest purchaser of prescription medications.

Nonetheless, the government has yet to bring a False Claims Act case which seeks damages for cGMP. One reason may be that the government has multiple other remedies within which to recover damages from noncompliant manufacturers, such as criminal fines and penalties and disgorgement.

Qui tam whistleblowers,[16] however, have already begun bringing such cases. Because the False Claims Act imposes liability on any government contractor which knowingly submits false claims to the United States or which uses false documents to get a false claim paid, a pharmaceutical manufacturer which knew or was recklessly indifferent to the fact that the manufacturing process was compromised by cGMP violations is in the same position as any other contractor which is required to conform to contractual or regulatory standards. The basis of liability under the False Claims Act is that false records have been generated which caused (false) claims for drugs to be paid by the United States.[17] The monetary damages result because the payor (in this case, the United States) is potentially paying for substandard drugs due to the cGMP violations—later covered up by false statements in documents required to be completed under the cGMP.

It makes sense, too: The cGMPs are a set of regulations which, by their very nature, are designed to ensure that drugs are manufactured in such a way that they meet the requirements of the federal Food, Drug and Cosmetic Act as to safety and have the identity and strength and meet the purity characteristics that they purport or are represented to possess. The major federally funded government health care programs, Medicare and Medicaid, operate under the express provisions that they will only pay for medical services and products that are "reasonable and necessary." Unsafe or ineffective drug products are neither reasonable nor necessary. Accordingly, as the theory goes, the United States suffers monetary damages if Medicare and Medicaid programs pay for unsafe or less effective products. These and other federally funded health care programs spend billions of dollars every year on pharmaceuticals.

False representations concerning minor or technical violations will not be the basis for FCA liability. Distribution of products that are not totally cGMP compliant (but have been falsely documented to be) does not necessarily result in unsafe (or subpotent) products. Substantial violations of the cGMP, later covered up in writing, however, could very well be the basis for FCA liability. The common thread through each violation is that the violation is severe enough so that the drug product that

[16]*Qui tam* is shorthand for the Latin phrase, *qui tam pro domino rege quam pro seipso*, meaning "He who is as much for the king as for himself." *Qui tam* statutes date back to thirteenth-century England. The actions were a means of enabling private parties to allege the king's interest and therefore gain access to the royal courts.

The *qui tam* provisions of the federal False Claims Act allow any citizen who has knowledge of fraud that has taken place against the government to bring a civil action in federal court in the name of the United States. In return for his or her efforts, the citizen is entitled to share in the proceeds of the recovery. The *qui tam* provisions raise the incentive for insiders to put the spotlight on the criminals, thereby providing the government with tangible and detailed evidence upon which to base an investigation and prosecution.

In 1986, Congress enacted amendments to the False Claims Act which strengthened the law and increased monetary awards. When hearings were held in 1985 and 1986, the climate was favorable for strengthened antifraud legislation, and Congress expected that most *qui tam* cases would involve defense contractor fraud. In the last decade, the majority of cases have instead been against the health care industry.

[17]Even so, factual questions will be raised, including: (1) Even with the false representations, was a false claim "caused" to be submitted? (2) Had the FDA known about the falsities, would it have enjoined the manufacturer from any further production, etc? (3) What about the false record or statement made the claims for such drugs false?

finally reaches the public is foreseeably and substantially less safe or less effective than if the cGMPs were not violated.

1.2.4.3 Criminal Proceedings

Introduction Criminal prosecutions of violations of the FDCA are intended to further the goal of protecting the health and safety of the public. The FDA historically has not pursued criminal charges unless the defendant shows a continuous or repetitive course of violative conduct, with the exception of intentional violations, fraud, or danger to health.[18]

While the FDCA contains various prohibitions and restrictions which a drug company could violate, the most common FDCA violation arising out of cGMPs is charged by using 21 U.S.C. 331(a), which specifically prohibits introducing an adulterated[19] drug into interstate commerce. In addition to introducing an adulterated drug into interstate commerce, some other acts prohibited by Section 331(a) which could be involved in manufacturing violations include 331(e), which prohibits the refusal to allow access to records mandated elsewhere in the act and 331(f), which prohibits the refusal to allow inspection of production facilities (21 U.S.C. 374).

Commission of any act prohibited by Section 331 is a federal misdemeanor (21 U.S.C. 333). However, violations of Section 331(a) may be charged as felonies where there is intent to defraud or mislead or where the defendant previously has been convicted of a misdemeanor under the FDCA [21 U.S.C. 333(b)]. Federal misdemeanor charges are typically resolved in proceedings before U.S. magistrate judges, and federal felonies are resolved by U.S. district judges.

Individual versus Corporate Liability Introducing an adulterated product into interstate commerce is a strict liability crime that can be enforced against individuals in positions of sufficient authority and responsibility as well as their company. Persons at risk are those who, at minimum,[20] fail to take adequate measures to *prevent* the cGMP violations. As such, warning letters and other communications are often directed at presidents and CEOs as well as their companies. As stated by the U.S. Supreme Court[21] in 1964, just two years after the FDCA as we know it was passed:

> Food and drug legislation, concerned as it is with protecting the lives and health of human beings, under circumstances in which they might be unable to protect themselves, often "dispenses with the conventional requirement for criminal conduct—awareness of some wrongdoing. In the interest of the larger good it puts the burden of acting at hazard upon a person otherwise innocent but standing in responsible relation to a public danger. . . ."

[18]A government review of recent FDA enforcement has suggested that adequate FDA enforcement activity is lacking. See "Prescription for Harm: The Decline in FDA Enforcement Activity," House Committee on Government Reform, June 2006.

[19]Failure to follow cGMP is the most common form of violating the prohibition against introducing an adulterated drug into interstate commerce.

[20]They may also be directly implicated in fraud and cover-ups.

[21]*United States v. Wiesenfeld Warehouse Co.*, 376 U.S. 86, 91 (1964).

In 1975, the Supreme Court made clear that individual responsibility is very important[22]:

> The [FDCA] imposes not only a positive duty to seek out and remedy violations when they occur but also, and primarily, a duty to implement measures that will insure that violations will not occur. The requirements of foresight and vigilance imposed on responsible corporate agents are beyond question demanding, and perhaps onerous, but they are no more stringent than the public has a right to expect of those who voluntarily assume positions of authority in business enterprises whose services and products affect the health and well-being of the public that supports them.

Manufacturing executives therefore carry a great liability burden. The government only need establish that the individual defendant failed to act on his or her own authority and that such an action could have prevented or corrected the violation. The individual need not have formed any intent to break any laws in order to be found guilty. What is relevant is, did the executive have the power to prevent the acts or omissions complained of? This includes a consideration of whether the executive could have prevented the acts or omission by the systems and processes alone. The job is not made easier to the extent that the cGMP regulations are open to varying interpretations or that the technology is constantly changing.

Section 305 Proceedings Due to the nature of the inspection process, a company that the FDA deems is in violation of the cGMPs should not be surprised when a warning letter or more elevated enforcement techniques are implemented. Even so, the FDA sometimes issues a formal form of notice that criminal charges will be brought by what is called a Section 305 notice.

Section 305 of 21 U.S.C., the statutory basis for a 305 notice, seemingly requires that before any violation of the FDCA is reported to the DOJ for institution of a criminal proceeding, the target defendant must "be given appropriate notice and an opportunity to present his views, either orally or in writing, with regard to such contemplated proceeding." The U.S. Supreme Court has watered down this provision, holding that a notice under Section 305 is not a legal prerequisite to government prosecution.

In practice, then, the FDA only sometimes issues a 305 notice and conducts a 305 hearing when it is considering a misdemeanor prosecution. A very informal process, the manufacturer can approach it with as much or as little of a defense as counsel deems appropriate, as there are pros and cons to providing the government with the company's full defense at that juncture.

A prototype Section 305 notice appears in Figure 9.

Grand Jury Proceedings If the government will be pursuing felony criminal charges against a manufacturing facility or persons associated with such facility, it will proceed by grand jury. The Fifth Amendment to the U.S. constitution requires that charges for all capital or "infamous" crimes be brought by an indictment

[22] *United States v. Park*, 421 U.S. 658 (1975).

In reply refer to:
Sample No.
Product

Firm Name and Individual Date
Street Address
City, State, Zip

Investigation by this Administration indicates your responsibility for violations of the Federal Food, Drug, and Cosmetic Act, and other Federal Laws, as described in the attached Charge Sheet, with respect to the following:

[describes specifics of cGMP violations]

A meeting has been scheduled for (day, date, time) at (location), to give you an opportunity to present your views on this matter. The enclosed **INFORMATION SHEET** explains the purpose and nature of the meeting, and how you may reply. If no response is received on or before the date set, our decision on whether to refer the matter to the Department of Justice for prosecution will be based on the evidence in hand.
By direction of the Secretary of the Department of Health and Human Services:

Compliance Officer

Enclosures:
Legal Status Sheet (3)
Charge Sheet
Information Sheet
Regulations

FIGURE 9 Section 305 notice.

returned by a grand jury. This has been interpreted by the U.S. courts to require that an indictment be used to charge federal felonies.

The activities, deliberations, or matters occurring before a grand jury are secret.[23] Strict adherence to grand jury secrecy is important to the integrity of the investigative process and ensures that the grand jury will be able to deliberate without outside pressure, to encourage people with information about a crime to come forward without fear of disclosure, and to protect the rights of the accused, specifically the innocent accused, from disclosure of the fact that he or she or it was investigated. Other than attorneys for the government, only the witness, interpreters when needed, and a court reporter are authorized persons permitted to be present while a grand jury is in session.

A grand jury's function is to determine whether there is probable cause to believe that a certain person(s) or company(ies) have committed a federal offense.[24] Prosecutors are permitted to appear before the grand jury and, in practice, conduct the grand jury proceedings. In general, the prosecutor is the one who makes the decision

[23]See Rule 6(e) Fed. R. Crim. Pro.

[24]The grand jury system is not presently used by countries outside the United States. The United Kingdom, New Zealand, Canada, and Australia, for instance, all have abolished the use of grand juries. See http://enwikipedia.org/wiki/Grand_jury.

as to which witnesses to call and what evidence should come before the grand jury. The prosecutor asks the witness questions and subsequently members of the grand jury may also question witnesses directly or through the prosecutor.

During the course of a grand jury investigation regarding cGMP violations/adulterated product allegations, for instance, the grand jury may hear testimony from not only federal investigators, federal agents, and federal inspectors but also former employees of the company (or current if a custodian of records) and/or experts in the pharmaceutical manufacturing field. These persons are considered witnesses. Witnesses are typically subpoenaed and may not refuse to appear before the grand jury or be subject to contempt charges.

In the federal grand jury system, a witness is not permitted to bring an attorney into the grand jury room. However, a witness is permitted to consult with his attorney outside the grand jury room even interrupting his own testimony.[25] It is typical for corporations such as pharmaceutical manufacturing companies to provide an attorney for any and all employees subpoenaed by a grand jury of which the manufacturer is a target.

A target is the person who is the focus of the grand jury investigation and is likely to be indicted. This company or person may receive a "target letter" from the grand jury which officially advises them of their jeopardy and serves as a formal warning of their status.

In practice, if a manufacturer is the target, the government will likely attempt to develop evidence by subpoena of persons and materials which will help prove culpability. It is likely the subpoenas will ask for correspondence, notes, and memos during a particular time period and involving a particular subject matter.

The grand jury may also issue a subpoena to the manufacturer's designated "custodian of records" for specific document production. The description of subpoenaed documents can include statements or charts of an organization, announcements, statements of policy and procedure, diaries, records of email, manufacturing logs, emails, travel vouchers, financial records and statements, correspondence, notes of conversations, and any other documents that relate to the manufacturing of certain drugs. A document subpoena may also request every writing or record of whatever type and description in the possession, custody, or control of the company that relates to a particular element of the criminal violation the grand jury is investigating. The request typically includes all handwritten, typed, printed, recorded, or transcribed records, including computer records tapes or disks.

The burden is on the government to prove that the crime was committed in the district in which the prosecution is brought. The grand jury should not consider a case unless venue lies in the district where the grand jury is sitting.[26] In the case of adulterated drugs, courts have generally held that it is proper to have venue in a district from which the defendant caused the unlawful introduction of goods into commerce, even though the physical shipment commenced from a different district.

[25]See Rule 6(d) Fed. R. Crim. Pro.; 28 U.S.C. secs. 515, 542, 547.
[26]See Rule 18 Fed. R. Crim. Pro.

Form of Charges and Penalties A grand jury investigation may culminate in the return of an indictment. This means that the grand jury found probable cause to believe that a violation of law occurred. While the focus of the initial inquiry can surround adulterated drugs by virtue of failure to abide by the cGMPs, additional criminal violations may be charged, as, for instance, where there are actions to evade or mislead a grand jury. The end result could, for instance, include accusations of making false statements to the FDA and obstructing the FDA's or DOJ's investigation, in addition to the "adulterated" drug charges.

An indictment consists of a statement describing the time, place, and manner through which the defendant violated the law. Each violation of the law is set out in a separate count. A defendant charged by an indictment is entitled to a trial by jury, although this right can be waived. A defendant has the right to a trial by jury for any criminal offense punishable by imprisonment for more than six months.[27]

If the matter only involves a misdemeanor violation, the prosecutor charges "by information." The information is often referred to as a complaint. An information, like an indictment, is simply a pleading that accuses the defendant of committing crimes. The distinction between an information and an indictment is that a prosecutor can issue and file an information without the grand jury's participation or finding of probable cause but a grand jury must approve and return an indictment.[28]

The penalty for a violation of 21 U.S.C. 331(b) by violating the cGMPs resulting in the adulteration of drug products in interstate commerce is set forth in 21 U.S.C. sec. 333(a). Each separate count for violating the cGMPs (where a misdemeanor is charged) carries with it a possible imprisonment of not more than one year or a fine not to exceed $1000 or both. If the government charges a felony for violation of the cGMPs, then the penalties are imprisonment of not more than three years or a fine not to exceed $10,000 or both. Of course, there may be other charges with greater or lesser penalties which are not related to the adulterated drug charges.

The criminal fine amounts (but not the imprisonment durations), however, are superceded by the criminal fine amounts contained in a different federal statute enacted later. Section 3571 of 18 U.S.C. provides for much greater fines than those provided for within the FDCA itself. For a manufacturer convicted of a felony, the fine can be as much as $500,000; for a misdemeanor (not resulting in death), it could be $200,000. Fines for individuals include a maximum up to $150,000 for a felony and an amount up to $100,000 for a conviction of a misdemeanor not resulting in death. The statute also provides for a multiplier of 2 based upon a finding that the defendant derived a pecuniary gain from the offense. The FDA and DOJ are able to elevate the monetary recoveries against the manufacturers for violations of the cGMP under a civil disgorgement theory explained infra. Recoveries have been in the hundreds of millions in recent years, typically agreed to in a negotiated consent decree.

[27]See Sixth Amendment, U.S. Constitution.

[28]Sometimes, prosecutors are in communication with defendants and their counsel during the investigatory stage. If there are negotiations concerning a plea to a felony and they are successful, a defendant can waive his or her right to be indicted by a grand jury, and the prosecutor can charge them by information.

1.2.5 CONCLUSION

The diligent enforcement of good manufacturing practices is a cornerstone of the safety net for drugs in the United States. Congress, the courts and, manufacturers, most importantly, expect a degree of consistency and responsibility in enforcement policy over a statute as powerful and central to public health and safety as the FDCA. To the extent there is consistency and effective and evenhanded enforcement, it not only protects the public, but it provides a level playing field for those manufacturers who operate in accordance with the cGMPs.

1.3

SCALE-UP AND POSTAPPROVAL CHANGES (SUPAC) REGULATIONS

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1.3.1 INTRODUCTION

Product development aims at formulating active drug ingredient in a palatable form. Technology transfer of a pharmaceutical product from research to the production floor (referred to as "shop floor") with simultaneous increase in production outputs is commonly known as scale-up. In simple terms, the process of increasing batch size is termed as scale-up. Conversely, scale-down refers to decrease in batch size in response to reduced market requirements.

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Often, changing of scale from the research lab to the shop floor is fraught with problems. The basic reason for such problems is the usage of different processing equipment in research and on the shop floor. Moreover, insufficient information about the equipment, various requirements of process control, complexity of a particular pharmaceutical process which may have a several different unit operations, limited information about the behavior of ingredients at different scales, and adoption of trial-and-error methodology also add significantly to scale-up issues. Every product coming from research should be manufacturable and the process should be capable to demonstrate its ruggedness at the shop floor level. This statement points toward the criticality and significance of scale-up and technology transfer in a pharmaceutical development process. After successful accomplishment of technology transfer and validation activity, a product usually has a smooth run on large-scale production machines. Changes are being made in the manufacturing process and chemistry of a drug product following approval and continue throughout its life. Depending upon foreseen (or unforeseen) requirements, there can be changes in the raw materials, process, equipment or manufacturing site, and batch size which ultimately affect quality attributes of a drug or finished product. Therefore, there is a need to anticipate and fully evaluate the impact of any kind of change on the quality of a drug or finished product. There can be several reasons for these changes, such as changed market requirement affecting batch size, new source of raw material, change in manufacturing process, upgrades of packaging material, or shifting to a new analytical methodology.

The intensity of the adverse effect produced by a particular change depends on the type of dosage form. For example, a change in the inactive ingredient beyond a certain range will have more effect on a modified-release (MR) dosage form than it would on an immediate-release (IR) dosage form, where bioavailability is not rate limiting. Likewise, a change in the primary packaging of liquid parenteral may have more pronounced effect on its effectiveness than it would have on a solid dosage form. Hence, depending upon the intensity of change or the adverse effect it may have on the critical parameters of a dosage form, reporting requirements to regulatory authorities also vary.

A drug or drug product may experience many changes during its life cycle. These changes may have an adverse effect on the overall safety and effectiveness of the drug or drug product. After a number of changes over a long time period, the product coming to market may be completely different from the one that was approved. Hence, data submitted to regulatory authorities in support of a change must have a comparison record of the drug or drug product to the one that was approved initially. Documentation generated in support of any change to the approved drug or drug product is submitted to regulatory authority for review, and based on the benefit-to-risk ratio, the drug or drug product is approved. Depending upon the intensity of change, supporting documents are provided to the regulatory agency.

Regulatory authorities such as the U.S. Food and Drug Administration (FDA), the European Commission, the Agencia Nacional de Vigilancia Sanitaria (ANVISA) (in english the National Health Surveillance Agency—Brazil, and others require the pharmaceutical industries in respective countries to follow guidelines on scale-up and postapproval changes (SUPAC) to maintain the quality of the pharmaceutical produced. From time to time these guidelines are assessed so as to keep pace with

the technological advances and new guidelines are developed to reduce the burden on the pharmaceutical industry and regulatory authorities. Apart from these guidelines, there are other checkpoints within an industry to assure production of quality products, such as change control and validation exercises, which will be discussed in detail in this chapter. These operations are controlled through the principles of good manufacturing practices issued by regulatory authorities.

This chapter describes the regulations imposed by different regulatory authorities and measures taken by a pharmaceutical industry to assure quality and performance of pharmaceuticals. The FDA guidelines, being most descriptive, have been discussed at length. Other guidelines have been described in general terms and the interested reader is referred to the references or the regulatory websites for more specific details.

1.3.2 SCIENTIFIC AND REGULATORY RATIONALE FOR SUPAC

Guidelines pertaining to postapproval changes classify these changes in various categories depending upon the effect a particular change may have on the quality and performance of a drug or drug product. Irrespective of the terminologies used by regulatory agencies, in general terms, changes can be described as *mild, moderate*, and *major* and the extent of supporting document varies with the nature of the change. For example, U.S. FDA guideline “Changes to an Approved NDA or ANDA” describe these changes as mild changes that can be implemented immediately and filed in the next periodic report, moderate changes that can be implemented immediately, moderate changes that require 30 days notice before implementation, and major changes that require FDA approval before implementation [1]. Similarly, any changes in an approved drug or drug product under European Union (EU) domain type I (type IA and type IB) and II variation are filed prior to marketing products [2]. The therapeutics Good Administration—Australia (TGA) describes postapproval changes in three categories: nonassessable, self-assessable, and changes requiring prior approval [3].

1.3.2.1 Supporting Documents and Extent of Change

As per FDA guidelines, changes in excipients (%w/w) of total formulation not greater than 5% are considered minor and all information is provided in the annual report. However, changes likely to have significant effect on the quality and performance of a drug product calls for submission of a prior approval supplement on all information (in vitro dissolution and in vivo dissolution), including accelerated stability and long-term stability testing in the annual report [4]. Similarly, in EU guidelines, a change in the batch size of the finished product up to10-fold compared to the original batch size approved at the grant of the marketing authorization (or downscaling to 10-fold) has been defined as type IA and requires batch analysis data (in a comparative tabulated format) on a minimum of one production batch manufactured to both the currently approved and the proposed sizes. Batch data on the next two full production batches should be made available upon request and reported by the marketing authorization holder if outside specifications (with proposed action). However, for type IB (more than 10-fold), in addition to the above

data, a copy of an approved release and end-of-shelf-life specifications as well as the batch numbers (≥3) used in the validation study should be indicated or a validation protocol (scheme) be submitted and the number of batches used in the stability studies should be indicated.

1.3.2.2 Supporting Documents for Change in Specifications

Changes in any type of specification also need to be supported by documentation. In all the guidelines, relaxing an acceptance criterion or deleting any part of the specification is classified as a major change and hence extensive documentation is required, for example, submission of a prior approval supplement to the FDA or comparative table of current and proposed specifications and details of any new analytical method and validation data and batch analysis data on two production batches of the finished product for all tests in the new specification to EU. The specifications are benchmarks for comparison of performance of any product. For example, content uniformity specification of 90–110% assay limit of a 20-mg (average weight) tablet of a potent drug signifies the challenge in maintaining the uniformity of such a low-dose drug during the blending operation. Any relaxation in specification of this potent drug should be justified with extensive documentation to assure the performance. However, tightening of an acceptance criterion is considered as a minor level change and to have minimal potential for an adverse effect on the identity, quality, purity, or potency of a product.

1.3.2.3 Comparability Protocols

The FDA has introduced the concept of comparability protocols to expedite the process of approval after submission of supporting document for a particular change [5]. The protocol covers anticipated changes a product may experience during it shelf life. Its recently published draft guidance "Comparability Protocols—Chemistry, Manufacturing, and Controls (CMC) Information" describes the general principles and procedures to prepare comparability protocols. The FDA suggests a less stringent reporting category for any future change, where appropriate. Additionally, if a detailed comparability protocol is provided, the FDA is less likely to request additional supporting documents while comparing pre- and postapproval change, and this could also help in implementing a particular CMC change, thereby moving the product in the distribution line sooner. According to the FDA:

> A comparability protocol is a well-defined, detailed, written plan for assessing the effect of specific CMC changes in the identity, strength, quality, purity, and potency of a specific drug product as these factors relate to the safety and effectiveness of the product. A comparability protocol describes the changes that are covered under the protocol and specifies the tests and studies that will be performed, including the analytical procedures that will be used, and acceptance criteria that will be achieved to demonstrate that specified CMC changes do not adversely affect the product. The submission of a comparability protocol is optional.

A comparability protocol may be submitted with a new drug application (NDA), abbreviated new drug application (ANDA), or supplements to these applications.

Comparability protocols can have single or multiple changes provided that each change is discrete and specification of the acceptance criteria for a change is well defined.

1.3.2.4 In Vitro–In Vivo Requirements

Stability of a drug product, in vitro dissolution, and in vivo bioequivalence are prerequisites for performance of a drug product and play a key role in establishing the quality of a drug product after a postapproval change has been implemented. Any type of major change, for example, in the manufacturing process from dry granulation to wet granulation could affect the bioavailability and stability of a drug product. Careful selection of the dissolution condition can obviate the need for a costly bioequivalence study. Guidelines by the FDA [4] and ANVISA [6] take into consideration the solubility and permeability of a drug substance for selection of dissolution criteria for a particular drug product (immediate release or modified release) whereas guidelines by the EU and TGA recommend submitting comparison records between a particular number of manufacturing batches pre- and postapproval.

While categorizing a change or variation for its effect, sufficient consideration should be given to those parameters of a drug product which could affect its bioavailability. Critical parameters like the particle size of active ingredient or excipients, solid-state characteristics, and surface wettability may change during the process variation and could adversely affect product performance resulting in an altered dissolution profile. The effect would be more pronounced in drug products containing poorly soluble potent drugs and could have a deleterious effect on bioavailability. The FDA guideline considers recommendation of the Biopharmaceutic Classification System (BCS) regarding solubility and permeability characteristics to see whether any in vivo bioequivalence study is needed along with an in vitro dissolution study. In the same pattern, ANVISA places drugs in three categories for solid TM dosage form: case A, active substances with high permeability and high solubility; case B, active substances with low permeability and high solubility; and case C, active substances with high permeability and low solubility. As per the guideline, for alteration of registration due to excipient change, for level 2 alterations (that could cause significant impact on quality and performance) the following requirements should be met:

Case A “The required documentation must include the undertaking of the technical report and assessment of the results of the dissolution test, carried out as described in the Brazilian Pharmacopoeia and, in its absence, other codes authorized by the legislation in force. There must be dissolution of at least 85% of the active substance in up to 15 minutes, using 900 ml of HCl 0.1 *M*. In case this criterion is not complied with, the tests described for Cases B or C must be carried out.”

Case B “The required documentation must include the undertaking of the technical report and assessment of the results of the dissolution profile employing Pharmacopeial conditions and removing samples from the medium at appropriate time points until the plateau is reached. The dissolution profile obtained must be similar to the profile of the unaltered formulation.”

Case C "The required documentation must include the undertaking of the technical report and assessment of the results of the dissolution profile in five different conditions: distilled water, HCl 0.1 *M* and phosphate buffer pH 4.5, 6.5 and 7.5 for the proposed formulation and the previous formulation, without change. Samples of the dissolution medium must be removed at appropriate time points until 90% of the active substance is dissolved or the plateau is reached. A tensoactive may be used only when appropriately justified. The profile obtained must be similar to the profile of the unaltered formulation."

In addition, for level 2 change, no additional bioequivalence study is required if the proposed alteration matches with the situation for cases A, B, and C. However, if there is any deviation, then documentation containing the results and assessment of a new bioequivalence and/or bioavailability study [if proper in vitro/in vivo correlation (*ivivc*) has not been established] should be submitted as per the conditions mentioned in the level 3 alteration.

1.3.3 REGULATORY AGENCIES AND GUIDELINES

1.3.3.1 FDA SUPAC Regulations

The Food and Drug Administration Modernization Act (FDAMA) of 1997 (the Modernization Act) was passed on November 21. With FDAMA in effect, another Section 506A was added to the federal Food, Drug, and Cosmetic Act (the act) and Section 314.70 (21 CFR 314.70) and the section included recommendations for reporting categories (in terms of defined words) for any type of manufacturing changes to an approved application (NDA or ANDA). In accordance with the act, the FDA issued "Guidance for Industry: Changes to an Approved NDA or ANDA" (finalized in 2004). This guidance is a current standard for pharmaceutical manufacturers for making and reporting manufacturing changes to an approved application and for distributing a drug product made with such changes.

"SUPAC-IR: Immediate-Release Solid Oral Dosage Forms: Scale-Up and Post-Approval Changes: Chemistry, Manufacturing and Controls, In vitro Dissolution Testing, and In vivo Bioequivalence Documentation" (issued 1995) was the first attempt to provide the pharmaceutical industry with a clear-cut guideline covering the requirements for notification and submission of documentation to regulatory authorities pertaining to postapproval changes. This guideline was an outcome of (a) a workshop on the scale-up of IR products conducted by the American Association of Pharmaceutical Scientists with the U.S. Pharmacopoeia (USP) Convention and the FDA; (b) research conducted by the University of Maryland at Baltimore on the CMC of IR products; (c) drug categorization research on the permeability of drug substances at the University of Michigan and the University of Uppsala; and (d) SUPAC task force set up by the Centre for Drug Evaluation and Research (CDER) CMC coordination committee. Following the issuance, it became a benchmark for the industry. Two more guidances have been published on the same format (level of changes as defined by SUPAC IR) as of SUPAC for MR drug products (issued in 1997) [7] and nonsterile semisolid drug products (issued in 1997) [8]. "Guideline for Changes to Approved NDA or ANDA" supersedes any previous

guidelines which have information on reporting categories that is inconsistent with this guideline.

Guideline to Industry: Changes to Approved NDA or ANDA "Guideline for Industry: Changes to Approved NDA or ANDA" provided reporting categories for various postapproval changes and relaxed certain requirements that were considered to have minimal or no impact on the drug product [1]. Moreover, it lessened the burden on regulatory authorities and companies as well. Four reporting categories provided in this guideline are as follows:

1. *Prior Approval Supplement* For a major change (substantial potential to have effect on quality and performance), a supplement has to be submitted to the FDA for approval before a product made with the change is distributed. There is also a provision for "Prior Approval Supplement: Expedite Review Requested" for public health reasons and if the delay in approval may cause any substantial concerns for the applicant.
2. *Supplement: Changes Being Effected (CBE) in 30 Days* For a moderate change (moderate potential to have effect on quality and performance), a supplement has to be submitted to the FDA for approval 30 days before a product made with the change is distributed.
3. *Supplement: Changes Being Effected in 0 Days* For some changes a supplement has to be submitted to the FDA and simultaneously the product made with the change can be distributed.
4. *Annual Report* For a minor change (minimal potential to have effect on quality and performance), all information has to be submitted to the FDA in the next annual review and the product made with the change can be distributed.

All three types of changes under this guidance have been categorized as follows:

(a) *Changes in Components and Composition* Any qualitative or quantitative changes in the components and composition of a drug product is considered as major changes. The current *Guideline to Industry: Changes to Approved NDA or ANDA* does not mention these in detail because of the complexity involved in the recommendations and therefore the SUPAC guideline has to be followed for any such type of changes and regarding documentation requirements for regulatory submission.

(b) *Changes in Manufacturing Sites* A change in a manufacturing site (for manufacturing, packaging, labeling of drug products, testing components, drug product containers, closures, packaging materials), either owned or contract site, of drug products from the one that is approved requires prior approval from the CDER. A prior approval supplement has to be submitted for a change to a site that does not have *a satisfactory CGMP inspection* for the *type of operation* to be performed. Further, changes in sites related to operations like labeling, secondary packaging, and testing are considered to have effect independent of drug product dosage form and therefore the

reporting categories for any of type of manufacturing site changes will be the same. However, changes in sites related to operations like manufacturing and primary packaging are considered to have effect that is dependent on dosage form and hence reporting categories may be different.

(c) *Changes in Manufacturing Process* Changes in the manufacturing process can have substantial effect on the identity, strength, quality, purity, or potency of a drug product and there may be a change in the efficacy of the drug product regardless of the testing of drug product for conformance for the approved specification.

(d) *Changes in Specifications* Specification, acceptance criteria, and regulatory analytical procedure are a part of every dossier submitted to regulatory agencies. Specifications are the standards, acceptance criteria are the limits for specifications, and the regulatory analytical procedure is used for testing a specification's acceptance criteria for the test substance that is approved by the regulatory authority. Alternative analytical procedure may be included in the application simultaneously with the main analytical procedure.

(e) *Changes in the Container Closure System* Effects related to changes in the container closure system are largely dependent on route on administration, the operation in which the container closure system is involved, and contact with the drug product. In some cases, there may be an effect in spite of the conformance of drug product with the approved specification.

(f) *Changes in Labeling* Changes in the package insert and package container label are included in the labeling changes and applicant must immediately revise all promotional labeling and drug advertisement in accordance with the change in the approved labeling.

(g) *Miscellaneous Changes* Apart from categories mentioned above, changes like stability protocol, expiration period, and addition of stability protocol or comparability protocol have been included in the miscellaneous category.

(h) *Multiple Related Changes* One change may lead to advertent or inadvertent incorporation of another change, for example, a change in the manufacturing site may lead to a change in the manufacturing equipment and manufacturing process or changes in packaging material may cause changes in stability protocol. For such combination changes, the CDER recommends submitting documents in accordance with the most stringent reporting category for the individual change.

Scale-up and Postapproval Changes: Immediate-Release and Modified-Release Dosage Forms SUPAC guidelines categorized postapproval changes in terms of "levels" [4]. Three levels were defined depending upon the intensity of the adverse effect on the formulation. Level 1 signifies that the resulting effect on the quality would be minimal and less extensive documentation should be presented to the FDA in an annual review. Changes in accordance with level 2 could have significant effect on the quality and performance of the dosage form. Level 3 changes are most likely to affect the quality and performance of the dosage form and hence extensive

TABLE 1 Different Cases and Respective Dissolution Conditions for Immediate-Release Solid Dosage Form

Case A[a]	Case B[b]	Case B[c]
Dissolution of 85% in 15 min in 900 mL of 0.1 *N* HCl. If a drug product fails to meet this criterion, the applicant should perform the tests described for case B or C.	Multipoint dissolution profile should be performed in the application/compendial medium at 15, 30, 45, 60, and 120 min or until an asymptote is reached.	Multipoint dissolution profiles should be performed in water, 0.1 *N* HCl, and USP buffer media at pH 4.5, 6.5, and 7.5 (five separate profiles) for the proposed and currently accepted formulations. Adequate sampling should be performed at 15, 30, 45, 60, and 120 min until either 90% of drug from the drug product is dissolved or an asymptote is reached. A surfactant may be used, but only with appropriate justification.

[a]High-permeability, high-solubility drugs.
[b]Low-permeability, high-solubility drugs.
[c]High-permeability, low-solubility drugs.

documentation justifying those changes should be submitted to the FDA prior to distribution of the products made with these changes. Apart from describing these levels, recommendations were also made on the extent of CMC documentation, in vitro dissolution, and in vivo bioequivalence tests that need to be submitted. Each section in the guideline [(a) components and compositions, (b) site change, (c) scale-up/scale-down; and (d) manufacturing equipment and process] was categorized in terms of these three levels. Further, SUPAC IR also takes into consideration the therapeutic range, solubility, and permeability of the drug for defining any particular change. As per the guideline, three cases have been defined for the dissolution testing (as mentioned in Table 1). Moreover, changes in excipient limits for a narrow therapeutic range drug beyond that mentioned in level 1 have been recommended as level 3 changes and extensive documentation is required for justification. In the SUPAC guideline for MR dosage forms, changes have been described at the same three levels [7].

However, dissolution conditions have been distinguished quite reasonably between extended- and delayed-release dosage form (Table 2). For reporting any level 3 change, three-month accelerated stability data of three batches (significant body of information not available) or three-month accelerated stability data for one batch (significant body of information available) have to be submitted in a supplement along with long-term stability data for one batch in an annual review. A significant body of information has been defined in the guideline as availability of sufficient stability information of the product (stability data of five commercial batches). To provide a comparative outline, the guidelines for MR and IR dosage forms are described in Tables 3–8:

TABLE 2 Dissolution Conditions for Modified-Release Dosage Form

Extended Release	Delayed Release
In addition to application/compendial release requirements, multipoint dissolution profiles should be obtained in three other media, for example, in water, 0.1 *N* HCl, and USP buffer media at pH 4.5 and 6.8 for the changed drug product and the biobatch or marketed batch (unchanged drug product). Adequate sampling should be performed, for example, at 1, 2, and 4 h and every 2 hours thereafter until either 80% of the drug from the drug product is released or an asymptote is reached. A surfactant may be used with appropriate justification.	In addition to application/compendial release requirements, dissolution tests should be performed in 0.1 *N* HCl for 2 h (acid stage) followed by testing in USP buffer media, in the range of pH 4.5–7.5 (buffer stage) under standard (application/compendial) test conditions and two additional agitation speeds using the application/compendial test apparatus (three additional test conditions). Multipoint dissolution profiles should be obtained during the buffer stage of testing. Adequate sampling should be performed, for example, at 15, 30, 45, 60, and 120 min (following the time from which the dosage form is placed in the buffer) until either 80% of the drug from the drug product is released or an asymptote is reached. The above dissolution testing should be performed using the changed drug product and the biobatch or marketed batch (unchanged drug product).

(a) *Changes in Components and Compositions (Table 3)* The guideline for changes to approved NDA or ANDA does not define these changes in detail, and thus the SUPAC guideline has to be followed for reference and reporting. Changes in excipient levels are submitted as a *prior approval supplement* (with accelerated stability data) whereas any changes in the levels of colors or flavors are submitted in an annual review (long-term stability data). In MR dosage forms these changes have been logically categorized as (a) changes in excipient levels not affecting the release profile and (b) changes in excipient levels affecting the release profile. In level 2 changes for IR product and MR dosage forms for a non-narrow therapeutic drugs, three-month accelerated stability data of one batch (in MR dosage form for narrow therapeutic drugs three-month accelerated stability data of three batches) in a supplement and long-term stability data of one batch in an annual review should be submitted. Additionally, for delayed-release MR dosage forms of a narrow therapeutic range drug, the multipoint dissolution profile in the buffer stage of testing should be generated for changed and commercial product using the medium that is approved or in pharmacopeia. For extended-release MR dosage forms of a narrow therapeutic range drug, the multipoint dissolution profile should be generated for changed and commercial product using the medium that is approved or in pharmacopeia.

(b) *Changes in Manufacturing Site (Table 5)* A change in the manufacturing or packaging site (or a contract manufacturing location) that has been approved by the FDA in the original application has to be evaluated for its effect on the product quality and performance. These changes have been described in detail in current guideline changes to approved NDA or ANDA.

TABLE 3 Changes in Nonrelease Controlling Components and Composition

Level	Classification	Therapeutic Range/Type of Drug	Test Documnetation			Filing Documentation
I	Complete or partial deletion of color/flavor Change in inks, imprints SUPAC-IR level 1 excipient ranges No other changes	All drugs	Stability Application/compendial requirements No biostudy			Annual report
II	Change in technical grade and/or specifications Higher than SUPAC-IR level 1 but less than level 2 excipient ranges No other changes Change in technical grade and/or specifications Higher than SUPAC-IR level 1 No other changes	All drugs for MR Depending upon therapeutic range solubility and permeability (as per BCS) for IR	**MR (ER):** Notification and updated batch record Stability Application/ compendial requirements plus multipoint dissolution profiles in three other media (e.g., water, 0.1 *N* HCl, and USP buffer media at pH 4.5 and 6.8) until ≥80% of drug released or an asymptote is reached Apply some statistical test (f2 test) for comparing dissolution profiles No biostudy	**MR (DR):** Notification and updated batch record Stability Application/compendial requirements plus multipoint dissolution profiles in additional buffer stage testing (e.g., USP buffer media at pH 4.5–7.5) under standard and increased agitation conditions until ≥80% of drug released or an asymptote is reached Apply some statistical test (f2 test) for comparing dissolution profiles No biostudy	**IR:** Notification and updated batch record Stability Dissolution requirements: case A, case B, or case C Apply some statistical test (f2 test) for comparing dissolution profiles No biostudy	Prior approval supplement

TABLE 3 ***Continued***

Level	Classification	Therapeutic Range/Type of Drug	Test Documnetation		Filing Documentation
III	Higher than SUPAC-IR level 2 excipient ranges for MR and IR, change in excipient range for low solubility and low permeability drugs beyond level 1	All drugs for MR and all drugs failing dissolution criteria for level 2 for IR	Updated batch record Application/compendial (profile) requirements and as mentioned for level II Stability Biostudy or ivivc	Updated batch record Dissolution profile as for level II Stability Biostudy or ivivc	Prior approval supplement

Note: MR, Modified-release dosage form; ER, extended-release dosage form; DR, delayed-release dosage form; IR, immediate-release dosage form.

TABLE 4 Changes in Release Controlling Components and Composition

Level	Classification	Therapeutic Range	Test Documentation		Filing Documentation
I	≤5% w/w change based on total release controlling excipient (e.g., controlled-release polymer, plasticizer) content No other changes	All drugs	Stability Application/compendial requirements No biostudy		Annual report
II	Change in technical grade and/or specifications ≤10% w/w change based on total release controlling excipient (e.g., controlled-release polymer, plasticizer) content No other changes	Nonnarrow	**MR (ER):** Notification and updated batch record Stability Application/compendial requirements plus multipoint dissolution profiles in three other media (e.g., water, 0.1 *N* HCl, and USP buffer media at pH 4.5 and 6.8) until ≥80% of drug released or an asymptote is reached Apply some statistical test (f2 test) for comparing dissolution profiles No biostudy	**MR (DR):** Notification and updated batch record Stability Application/compendial requirements plus multipoint dissolution profiles in additional buffer stage testing (e.g., USP buffer media at pH 4.5–7.5) under standard and increased agitation conditions until ≥80% of drug released or an asymptote is reached Apply some statistical test (f2 test) for comparing dissolution profiles No biostudy	Prior approval supplement
		Narrow	Updated batch record Stability Application/compendial (profile) requirements Biostudy or ivivc		Prior approval supplement
III	>10% w/w change based on total release controlling excipient (e.g., controlled-release polymer, plasticizer) content	All drugs	Updated batch record and stability Application/compendial (profile) requirements Biostudy or ivivc		Prior approval supplement

TABLE 5 Site Changes

Level	Classification	Therapeutic Range	Test Documentation			Filing Documentation
I	Single facility Common Personnel No other changes	All drugs	Application/compendial requirements No biostudy			Annual report
II	Same contiguous campus Common personnel No other changes	All drugs	**MR (ER):** Identification and description of site change and updated batch record Notification of site change Stability Application/ compendial requirements plus multipoint dissolution profiles in three other media (e.g., water, 0.1 *N* HCl, and USP buffer media at pH 4.5 and 6.8) until ≥80% of drug released or an asymptote is reached Apply some statistical test (f2 test) for comparing dissolution profiles No biostudy	**MR (DR):** Identification and description of site change, and updated batch record Notification of site change Stability Application/ compendial requirements plus multipoint dissolution profiles in additional buffer stage testing (e.g., USP buffer media at pH 4.5–7.5) under standard and increased agitation conditions until ≥80% of drug released or an asymptote is reached Apply some statistical test (f2 test) for comparing dissolution profiles No biostudy	**IR:** Identification and description of site change and updated batch record Notification of site change Stability Application/ compendial requirements No biostudy	Changes being effected supplement (accelerated stability data for MR and no stability data for IR) Annual report (long-term stability data for MR and IR)
III	Different campus Different personnel	All drugs	Notification of site change Updated batch record Application/compendial (profile) requirements (as for level II) Stability Biostudy or ivivc		Notification of site change Updated batch record Case B dissolution as for excipient change (level II) Stability No biostudy	Prior approval supplement (accelerated stability data) for MR and changes being effected supplement for IR Annual report

TABLE 6 Changes in Batch Size: Scale-Up/Scale-Down

Level	Classification	Change	Test Documentation			Filing Documentation
I	Scale-up of biobatch(s) or pivotal clinical batch(s) No other changes	≤10× (all drugs)	Updated batch record Stability Application/compendial requirements No biostudy			Annual report
II	Scale-up of biobatch(s) or pivotal clinical batch(s) No other changes	>10× (all drugs)	**MR (ER):** Updated batch record Stability Application/ compendial requirements plus multipoint dissolution profiles in three other media (e.g., water, 0.1 *N* HCl, and USP buffer media at pH 4.5 and 6.8) until ≥80% of drug released or an asymptote is reached Apply some statistical test (f2 test) for comparing dissolution profiles No biostudy	**MR (DR):** Updated batch record Stability Application/compendial multipoint dissolution profiles in additional buffer stage testing (e.g., USP buffer media at p*H* 4.5–7.5) under standard and increased agitation conditions until ≥80% of drug released or an asymptote is reached Apply some statistical test (f2 test) for comparing dissolution profiles No biostudy	**IR:** Updated batch record Stability Case B dissolution as for excipient change (level II) No biostudy	Changes being effected supplement (accelerated stability data) Annual report (long-term stability data)

TABLE 7 Changes in Manufacturing: equipment

Level	Classification	Change	Test Documentation			Filing Documentation
I	Equipment changes No other changes (all drugs)	Alternate equipment of same design and principle Automated equipment	Updated batch record Stability Application/compendial requirements No biostudy			Annual report
II	Equipment changes No other changes (all drugs)	Change to equipment of a different design and operating principle	**MR (ER):** Updated batch record Stability Application/ compendial requirements plus multipoint dissolution profiles in three other media (e.g., water, 0.1 *N* HCl, and USP buffer media at pH 4.5 and 6.8) until ≥80% of drug released or an asymptote is reached Apply some statistical test (f2 test) for comparing dissolution profiles No biostudy	**MR (DR):** Updated batch record Stability Application/ compendial requirements plus multipoint dissolution profiles in additional buffer stage testing (e.g., USP buffer media at ph 4.5–7.5) under standard and increased agitation conditions until ≥80% of drug released or an asymptote is reached Apply some statistical test (f2 test) for comparing dissolution profiles No biostudy	**IR:** Updated batch record Stability Case C dissolution as for excipient change (level II) No biostudy	Prior approval supplement (accelerated stability data) Annual report (long-term stability data)

TABLE 8 Changes in Manufacturing: Processes

Level	Classification	Change	Test Documentation			Filing Documentation
I	Processing changes affecting the nonrelease/release controlling excipients for MR Changes within validation ranges (IR) No other changes	Adjustment of equipment operating conditions (mixing times, operating speeds)—within approved application ranges	Updated batch record Application/compendial requirements No biostudy			Annual report
II	Processing changes affecting the nonrelease controlling excipients and/or the release controlling excipients Processing changes outside validation ranges for IR No other changes	Adjustment of equipment operating conditions (e.g. mixing times, operating speeds, etc.) Beyond approved application ranges	**MR (ER):** Updated batch record Stability Application/compendial requirements plus multipoint dissolution profiles in three other media (e.g. water, 0.1 *N* HCl, and USP buffer media at pH 4.5 and 6.8) until ≥80% of drug released or an asymptote is reached Apply some statistical test (f2 test) for comparing dissolution profiles No biostudy	**MR (DR):** Updated batch record Stability Application/compendial requirements plus multipoint dissolution profiles in additional buffer stage testing (e. g., USP buffer media at pH 4.5–7.5) under standard and increased agitation conditions until ≥80% of drug released or an asymptote is reached Apply some statistical test (f2 test) for comparing dissolution profiles No biostudy	**IR:** Notification of change Updated batch record Stability Case B dissolution as for excipient change (level II) No biostudy	Changes being effected supplement (accelerated stability data for MR) Annual report (long-term stability data for MR and IR)
III	Processing changes affecting the nonrelease controlling excipients and/or the release controlling excipients	Change in the type of process used (e.g. from wet granulation to dry)	Updated batch record Stability Application/compendial (profile) requirements Biostudy or ivivc		Updated batch record—stability Case B dissolution as for excipient change (level II) No biostudy	Prior approval supplement (accelerated stability data for MR) Annual report (long-term stability data for MR and IR)

The FDA should be notified of the new location. For any type of moderate changes, accelerated stability data of one batch should be submitted with the CBE supplement for MR dosage forms and long-term stability data of one batch should be submitted in the annual review for IR as well as MR dosage forms. Additionally, the CBE should be submitted to the FDA in case of any moderate change. Stability requirements for any major change are the same as those mentioned in the previous section, that is, three-month accelerated stability data of three batches (significant body of information not available) or three-month accelerated stability data for one batch (significant body of information available) have to be submitted in a prior approval supplement along with long-term stability data for one batch in the annual review.

(c) *Scale-Up/Scale-Down (Table 6)* A change in the batch size of a drug product, either scale-up or scale-down, is likely to induce some changes in the operation parameters. This in turn can adversely affect product quality.

(d) *Changes in Manufacturing Equipment (Table 7) and Process (Table 8)* Any manufacturing changes in equipment and process are included in this section. For example, a change in the blending equipment from octagonal blender to double cone blender or a change in the granulation process from wet to dry granulation calls for submission of proper validation documentation for FDA approval. All these changes along with reporting categories have been described in current guidelines for changes to approved NDA or ANDA.

Biowaivers In vitro and in vivo approaches are commonly used for establishment of bioavailability and bioequivalence. Dissolution studies are used as in vitro approaches and also serve as quality control tools for pharmaceuticals. Under certain circumstances, in vitro dissolution may also act as a surrogate marker for in vivo biostudy and enable the establishment of in vitro and in vivo bioequivalence.

"CDER Guidance for Industry: Waiver of In Vivo Bioavailability and Bioequivalence Studies for IR Solid Dosage Form Based on Biopharmaceutic Classification System (BCS)" recommends waiving an in vivo biostudy under specific circumstances. For example, a waiver of the in vivo biostudy of one or more lower strengths is acceptable based on the correlation data and in vivo bioequivalence of the higher strength, provided all strengths are proportionally equivalent in terms of active and inactive ingredients. A biostudy on a lower strength may also be requested based on safety reasons (as for mitrazapine tablets) and a biowaiver for highest strength is acceptable provided elimination kinetics is linear over a dose range, strengths are proportional, and comparative dissolution data of all strengths are acceptable.

The BCS classifies drugs in four classes:

Class I: high solubility, high permeability
Class II: low solubility, high permeability
Class III: high solubility, low permeability
Class IV: low solubility, low permeability

Dissolution, solubility, and permeability are the three fractors that control the bioavailability of a drug for an IR drug product. Provided the inactive excipient

does not control or modify the release and absorption of the active ingredient, the biostudy may be waived. According to the guideline, the solubility class is determined for the highest dose strength of a drug product for which a biowaiver has been requested. When the highest dose strength of a solid dosage form is soluble in 250 mL of water or less across a pH range of 1–7.5, it is considered as highly soluble. For determination of permeability class various in vivo methods like mass balance, absolute bioavailability, and intestinal perfusion approaches and in vitro methods like permeation studies using excised tissue or monolayer of cultured epithelial cells are used. When extent of absorption is greater than 90% of the administered dose in humans, it is considered as highly permeable. For a dissolution study, drug release should be evaluated in three media that are 0.1 N HCl or USP-simulated gastric fluid without enzymes, pH 4.5 buffer, and pH 6.8 buffer or USP-simulated intestinal fluid without enzymes. Rapidly dissolving drug products are those that dissolve more than 80% in 900 mL of the above-mentioned media in less than 30 min using USP apparatus at 100 rpm (or USP II apparatus of 50 rpm).

A biowaiver can be requested for the postchange products if it falls under class I of the BCS and displays a rapidly dissolving profile and there is a similarity (as determined by f2 test) between the pre- and postchanged drug product in all three media. For BCS class II drugs, a meaningful correlation (level A, B, or C correlation) between in vitro drug release and in vivo absorption also may be used for requesting the biowaiver. Deconvolution techniques are used for prediction of in vivo dissolution and absorption.

1.3.3.2 Regulations Guidance on SUPAC by Pharmaceutical Unit of EU

The pharmaceutical market in European countries is one of the largest in the world. To ensure that the EU promotes pharmaceutical trade and ensures safety, efficacy, and quality of medicinal products within the European member states, the pharmaceutical unit of the EU runs a series of information and communication projects, collectively called EUDRA projects. Out of these projects, the EUDRALEX pharmaceutical unit is responsible for making community pharmaceutical legislation, guidelines, and notices for applicants [9]. Under Volume 2, Section C, of Regulatory Guidelines (Pharmaceutical Legislation: Notice to Applicants) of Eudralex, "Guideline on Dossier Requirements for Type IA and Type IB Notifications" has been provided [2].

Regulations were introduced to lessen the administrative load on the authority and to simplify the procedure for granting a postapproval variation without negotiating any quality attribute of drug product [10]. Under these regulations, type IA and type IB were defined; also, clearcut terms were introduced for extension application, parallel/consequential notification/variation, and urgent safety restriction. For streamline operation of these regulations, four documents have been prepared:

(a) A procedural guidance for the member states (reference or concerned) and the applicant for notifications/variations in the mutual recognition procedure
(b) A procedural guidance for the applicant for notifications/variations in the centralized procedure

(c) A common application form which may be used for type IA and type IB notifications or type II variations in both the centralized and mutual recognition procedures
(d) A guideline on the documentation to be submitted for type IA and type IB notifications

All member states of the EU follow the same regulations for a change or "variation" in an already approved medical product. As per the guidance, three types of variations or changes have been identified—type I variation, which is further classified into types IA and IB and type II variations. The guidelines classify some specific changes in type IA or IB. It also provides specific data analysis required for variation and the types of document that need to be submitted to the regulatory authority. Any change that is not listed in this section is classified as type II variation.

According to Commission Regulation (EC, No. 1084/2003), type I variation has been defined as "A '*minor* variation' of type IA or type IB means a variation listed in Annex I, which fulfils the conditions, set out therein." Annex I of the regulation provides a list of changes and conditions (to be satisfied) to be classified as type IA or type IB variation and Annex II provides changes falling under the extension application category. Type II variations in proposed documentation are not type I or extension application. There is also a provision for "urgent safety restrictions." These are any temporary or provisional changes in the product summary characteristics, such as indications, posology, contraindications, warnings, target species, and withdrawal periods, as result of a new information that may cause significant safety concerns about the medicinal product [11].

Any change arising from the primary change has to be notified separately. Consequential changes form part of the same notification whereas parallel changes do not. A consequential change to type IA can only be another type IA whereas a consequential change to type IB can be type IA or type IB. All other variations should be submitted as Type II variations. "Guideline on Dossier Requirements for Type IA and Type IB Notifications" provides a complete list of all changes, conditions required to be met for the particular change, and documentation required by the regulatory authority [10].

1.3.3.3 Regulatory Guidance on SUPAC by Agencia Nacional de Vigilancia Sanitaria

Agencia Nacional de Vigilancia Sanitara (ANVISA) issues Brazil's generic drug policy. Under legislation for industry, Resolution RE N° 893, of May 29, 2003, is described in "Guide for Making Post-Registration Alterations, Inclusions and Notifications of Drug Products" [6]. This guideline describes postregistration changes as "alterations" and "inclusions" and also tells about the documentation and assays that need to be submitted in support of any type of change. As per the guideline, each type of alteration or inclusion has to be submitted separately and approved by the ANVISA before it can be implemented. Table 9 presents some examples for each category.

Under each category, certain requirements have to be met before its implementation. For example, for inclusion in the batch size, the company should notify, in alteration, if the included batch size is more than 10 times. The documentation that needs to be submitted includes the original proof of payment of fee or of exemption;

TABLE 9 Examples of Different Categories

Postregistration Alterations	Postregistration Inclusions	Postregistration Notifications	Postregistration Cancellation
Labeling alteration	Inclusion of new commercial presentation	Temporary suspension of manufacture	Cancellation upon request of registration of drug presentation
Alteration of corporate name	Inclusion of new packing	Resumption of drug manufacture	Cancellation of drug registration
Alteration of date of expiry	Inclusion of new concentration already approved in country		
Alteration of preservation conditions	Inclusion of new dosage form already approved in country		
Alteration of synthesis path of drug	Inclusion of new therapeutic indication in country		
Alteration of manufacturer of drug	Inclusion of manufacture site		
Alteration of manufacturing site	Inclusion of manufacturer of drug		
Alteration of excipient	Inclusion in the batch size		

a copy of the certificate of good manufacturing and control practices (CBPFC) issued by ANVISA; technical justification; production and quality control records of one batch of each strength of the product; a technical report; and a technical report and assessment of the dissolution profile.

1.3.4 HARMONIZATION

It is essential to evaluate the safety and quality of new or changed medical products before they reach the market. However, the need to set specific guidelines has been recognized at different times in different countries. For example, in the United States a tragic incident with a junior paracetamol formulation was the alarm to initiate guidelines for authorization of medical products. European countries followed this trend in the 1960s after the thalidomide incident. Since then there have been a large number of guidelines that have been put into place to evaluate medical products in terms of their quality, safety, and efficacy [12]. However, with the pharmaceutical industries becoming international and aiming for a worldwide market, there is a move toward internationally accepted guidelines and approval systems. In order for medical products to be marketed internationally, companies have found it necessary to duplicate many tests and studies that are time consuming and broad.

TABLE 10 Changes in Specification of Excipients (Addition of New Test Limit): Comparison between Guidelines

Guidelines	Documentation	Type of Change
TGA	Details of the test method must be provided. Appropriate validation data have been generated for the test method. The limits proposed are based on batch analytical data and are in compliance with official standard and/or relevant accepted guidelines if applicable.	Self-assessable changes
EMEA	Comparative table of current and proposed specifications. Batch analysis data on two production batches for all tests in the new specification. Where appropriate, comparative dissolution profile data for the finished product on at least one pilot batch containing the excipient complying with the current and proposed specification. For herbal medicinal products, comparative disintegration data may be acceptable.	Minor change type IB requires approval

Table 10 shows examples of documentation required by different countries when a postapproval change is made during the manufacturing process of medical products. When there are changes in the specification of an excipient, the documents required by the TGA and European Agency for Evaluation of Medicinal Products (EMEA) are variable. Furthermore this would indicate that the benefit of a patent/medical product might not reach globally. There are also many chances of making an error. For example, the 2003 recall of clotrihexal 100-mg vaginal tablets in New Zealand pharmacies was due to the fact that clotrihexal was packed according to TGA guidelines and thus its sale was prohibited in New Zealand. This resulted in much confusion and problems among patients and medical professionals. Harmonization is the process by which the pharmaceutical industries worldwide adopt the same laws and regulations. Harmonization is intended to assure the safety, quality, and efficacy of a medical product globally. The main goal of harmonization is to recognize and minimize the differences in the scientific requirements for medical product development within different regulatory agencies in different countries.

Harmonization activities focus on reducing and simplifying the types of studies that the pharmaceutical industries need to carry out in order to register a medical product in another country, protocols to be followed when performing these studies, techniques used to validate supporting data, and techniques used to perform risk assessment.

Harmonization reduces replications and unnecessary production and registration of new and changed products. The concept of harmonization was explored by European countries in the 1980s. The success of harmonization in these countries has demonstrated that it is practical and possible. Following harmonization in Europe the International Conference on Harmonization (ICH) was set up in 1990 [13]. Table 11 shows some of the harmonized rules that have been successfully developed by ICH.

TABLE 11 Example of Quality Guidelines Harmonized by ICH

Quality Topic	Example of Guideline
Q1: Stability	Q1B: Photostability testing
Q2: Validation of analytical procedure	Q2A: Methodology
Q3: Impurity testing	Q3A: Impurities in new drug substances
Q4: Pharmacopoeias	Q4: Pharmacopoeial harmonization
Q5: Quality of biotechnological products	Q5A: Viral safety evaluation of biotechnological products
Q6: Specifications for new drug substance and products	Q6A: Acceptance criteria for new drug substances
Q7: GMP for pharmaceutical ingredients	Q7A: GMP for active pharmaceutical ingredients

1.3.5 GMP ISSUES: CHANGE CONTROL AND PROCESS VALIDATION

Changes are unavoidable in a manufacturing setup. Manufacturers make changes at some stage of manufacturing during and after approval of a product. However, consistent quality of a drug product can only be assured through well-defined validation procedures. When a change is made in the manufacturing process of a drug product, sponsors are responsible for evaluating the effect of any change on the safety, efficacy, quality, stability, and potency of a drug product and ensuring that these properties are not influenced by the change. In a manufacturing setup, various disciplines like sales, marketing, medical, regulatory affairs, manufacturing, electrical, and technical services work together. Hence, any kind of change in one discipline will have direct consequences on other disciplines. Each company should have a procedure with regard to handling a change. Quality control and quality assurance departments usually keep track of various changes occurring in a GMP environment. Therefore, it is required that personnel performing the job are trained enough to assess the effect of any kind of change or variation and take appropriate action for its evaluation or control. Supporting data should be generated and once evaluated can confirm whether further clinical or nonclinical studies are required.

1.3.5.1 Change Control

When a change is made in a manufacturing setup, it is important to assess its impact. As a change can have impact on regulatory filing, manufacturing parameters, specifications, and technical services, it is important to consider the concerns and objections of various disciplines involved and only through well-defined standard operating procedures should it be properly validated, evaluated, and finally implemented. A properly defined order of evaluation of a change with strategic input of trained personnel is key to delivering a consistent quality product (Figure 1).

When a change is the processed, the manufacturer should have protocols in place with regard to assessing the change. Therefore, "control of change" is important. Control can be implemented effectively only through well-defined standard operating procedures. The main purpose of "change control" exercise is to have a

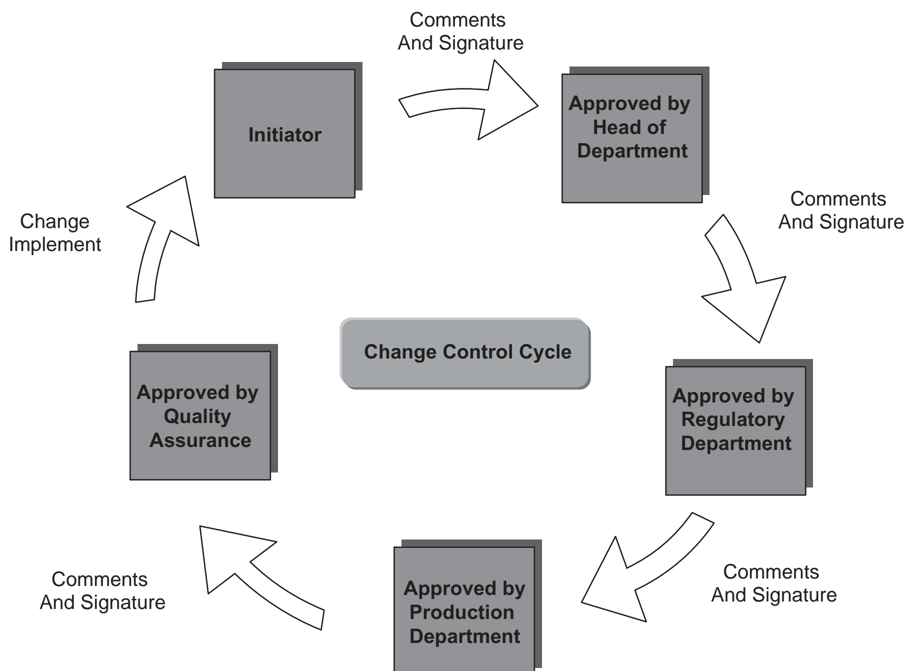

FIGURE 1 Change control cycle for change in manufacturing process.

systematic process in place to accurately evaluate a change using specific tests. Moreover, it aims to measure the effects on quality safety and efficacy before a change is implanted. Change control and its evaluation through proper documentation should include [14]:

(a) Description and purpose of change
(b) Inputs from research and development (R&D) department
(c) Evaluation steps for impact assessment, such as evaluation of stability, validation requirements, and in vivo bioequivalence requirement
(d) Need and extent of regulatory documentation and approval
(e) Implementation schedule
(f) Clear definition of personnel authorized for change approval
(g) Monitoring protocol for change implementation and periodic review of impact

Following the informal proposal of a change, it should be reviewed by the responsible initiator, who will then generate a formal proposal [15]. The proposal should describe accurately what the change is concerned with, how to validate the change, and the time frame within which the change should be implemented. The final proposal should be reviewed and assessed by all functional groups involved. Once the change is approved, it can be implemented and the change cycle is completed. Figure 2 describes responsibilities of different departments of a pharmaceutical company,

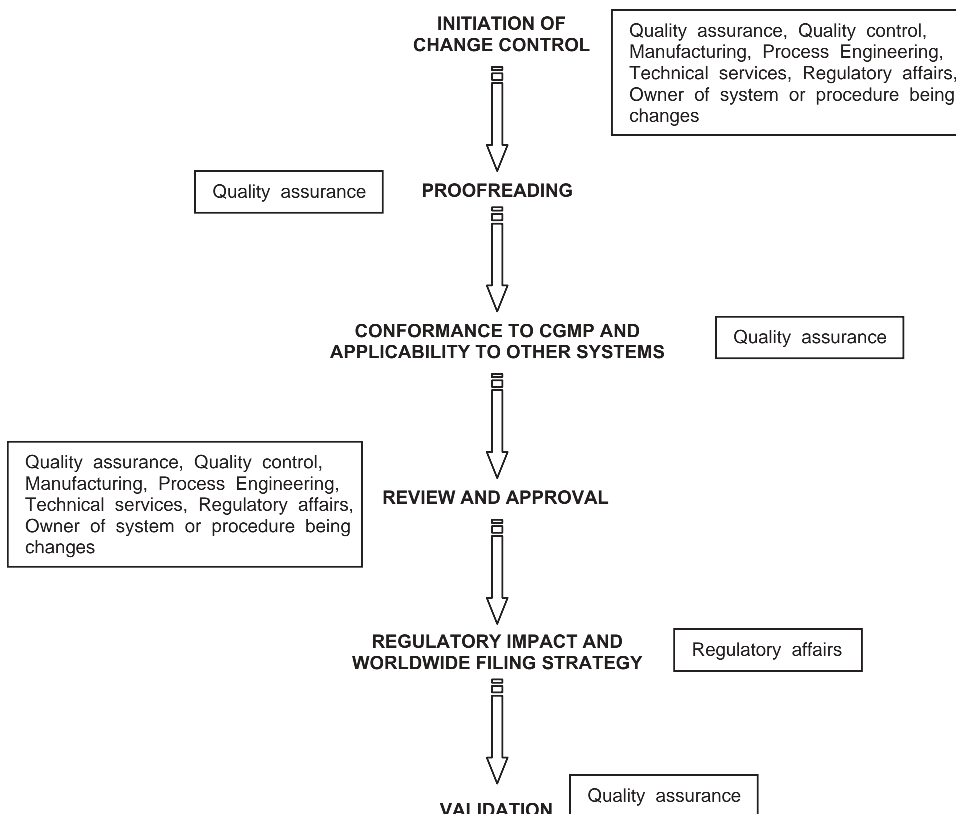

FIGURE 2 Responsibilities of different disciplines of a pharmaceutical company in a change control procedure (modified from ref. 15).

in the change control procedure. Standard operating procedures (SOPs) for change control are an important part of any GMP audit. Hence it is important that it is implemented by trained and qualified personnel from appropriate disciplines.

After a change has been approved by all functional groups within the manufacturing setup and if it has no regulatory concerns, it can be implemented immediately. However, if the impact comes under any regulatory domain, the company may have to wait for regulatory approval.

1.3.5.2 Process Validation

Process validation is an important part in the implementation of a postapproval change. It establishes the documented evidence of conformance of a pharmaceutical operation in accordance with specifications. FDA "Guideline on General Principles of Process Validation" describes in detail the principles and practices of process validation and documentation required by the regulatory authority [13]. In general terms, process validation may be defined as the procedure which generates sufficient assurance and documented evidence that a particular operation is operating and producing drug products in accordance with the specifications and process controls.

Prospective validation, retrospective validation, concurrent validation, and revalidation are the four validation components. Prospective validation is performed before the distribution of drug products in the market or after the manufacturing of a drug product using revised changes that can affect product quality and characteristics. Retrospective validation is conducted for an established drug product whose manufacturing process is stable to ensure that the current pharmaceutical operation is performing as per the protocols and specification and yielding satisfactory product. Concurrent validation is conducted by monitoring in-process critical manufacturing parameters and end-product testing to ensure that the current manufacturing process is per the in-process control specifications. Revalidation is performed after changes to an approved drug product are implemented to ascertain that there is no adverse effect on the quality and performance of a drug product [16].

During a validation process, the products and processes are subjected to testing at extreme conditions of in-process limits and their performance is evaluated against the acceptance criteria. The parameters of different pharmaceutical operations are varied and product properties are recorded and evaluated (Figure 3). When it is found that adjustment is required, necessary actions are taken in consultation with R&D personnel. Generally, validation data of three production scale batches are compared to generate a high level of quality assurance.

Systematic documentation of the effect on the product attributes by varying various process parameters is very important in the validation process. The product development team, engineering and technical services, and production and regulatory departments are also consulted while making any process change or before finalizing any validation protocol or report. Depending on the "level" of change or degree of effect to be produced, the extent of the validation is determined.

Based on the validation requirements, samples are collected at different stages and submitted for analysis per the validation protocol. The data are finally compiled in the form of a validation report. A systematic validation protocol and validation report are the backbone of the validation process. Table 12 gives key components of any validation activity [16]. These protocols and reports should be verified and approved by the relevant functions.

Some changes are often made in the manufacturing process without prior notification, and hence it is advisable to consider revalidation at predetermined frequencies (or whenever an unusual behavior is noted).

When new equipment is purchased or there is a change in the manufacturing site, qualification exercises are performed as part of the validation process. Qualification (installation qualification, operation qualification, and performance qualification) for any equipment or facility is an extreme process which involves testing, verification, and documentation to assure that the particular equipment or facility is per the specification and meets the appropriate standards as defined by vendor and required by manufacturing and engineering personnel [14].

1.3.6 CONCLUSION

The global pharmaceutical industry is continuously growing in a rapidly changing and dynamic environment of the health care sector. New drugs and delivery systems

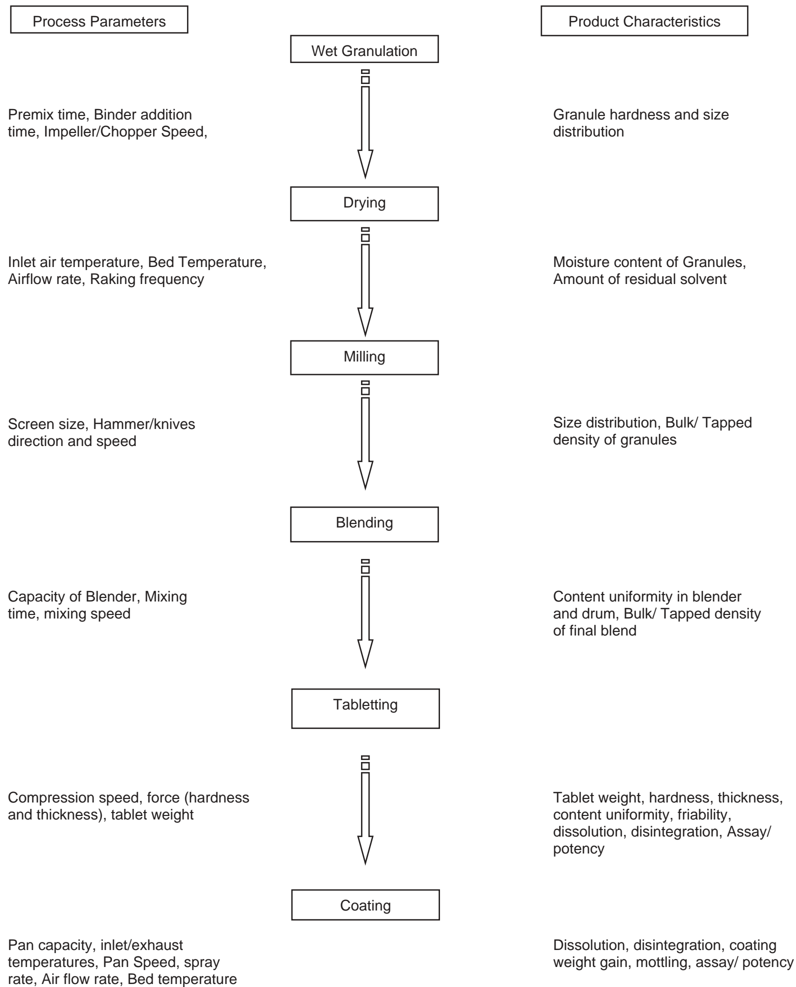

FIGURE 3 Various process parameters and product characteristics associated with validation activity of typical coated tablet.

TABLE 12 Key Components of Validation Activity

Validation Protocol	Validation Report
Purpose of study	Aim of study
Personnel responsibility	List of raw material used in study
Critical process steps	List of manufacturing equipment
Critical process parameters	Critical steps studied
Critical product parameters	Collected data and its analysis
Sampling plan	Acceptance criteria evaluation
Testing plan	Statistical analysis
Acceptance criteria	Recommendations by validation department

surface each year in the market. To maintain the quality of new and existing drugs and delivery technologies, pharmaceutical operations are controlled by regulatory guidelines. The purpose of developing guidelines is to keep the health and safety of a person on the highest priority by delivering quality pharmaceuticals. Implementation of these guidelines and systematic follow-up of the effect of postapproval changes in the form of documentation are essential to safeguard against any possible failure of the whole system. Change control and validation ensure that there is no deleterious impact on the drug product characteristics. Anticipated changes incorporated in comparability protocols reduce significant risk of experiencing unpredictable adverse effects and help to introduce the product in less time. When an impact is anticipated, it should be properly discussed with R&D, process development, and other concerned departments for appropriate regulatory filing by following regulatory guidelines. Provided that these guidelines are followed properly, quality and performance of a drug product can be ensured.

REFERENCES

1. U.S. Department of Health and Human Services, Food and Drug Administration, Centre for Drug Evaluation and Research (CDER), Guidance for industry: Changes to an approved NDA or ANDA, available: http://www.fda.gov/cder/guidance/3516fnl.pdf, accessed Apr. 15, 2006.
2. European Commission, Guideline on dossier requirements for type IA and type IB notifications: Pharmaceuticals: Regulatory framework and market authorizations, available: http://ec.europa.eu/enterprise/pharmaceuticals/eudralex/vol-2/c/gdvartypiab_rev0_200307.pdf, accessed Apr. 20, 2006.
3. Department of Health and Ageing, Therapeutic Goods Administration, Australian regulatory guidelines for prescription medicines. Appendix 12: Changes to the quality information of registered medicines: Notification. Self-assessment and prior approval, available: http://www.tga.gov.au/pmeds/argpmap12.pdf, accessed Apr. 12, 2006.
4. Food and Drug Administration, Centre for Drug Evaluation and Research (CDER), Guidance for industry: SUPAC-IR: Immediate-release solid oral dosage forms: Scale-up and post-approval changes: Chemistry, manufacturing and controls, in vitro dissolution testing, and in vivo bioequivalence documentation, available: http://www.fda.gov/cder/guidance/cmc5.pdf, accessed May 11, 2006.
5. Food and Drug Administration, Centre for Drug Evaluation and Research (CDER), Guidance for industry: Comparability protocols—Chemistry, manufacturing, and controls information (draft), available: http://www.fda.gov/cder/guidance/5427dft.pdf, accessed Apr. 15, 2006.
6. Brazilian Sanitary Surveillance Agency (ANVISA), Resolution: Guide for making post-registration alterations, inclusions and notifications of drug products, Brazil's generic drug policy, industry legislation, available: http://www.anvisa.gov.br/hotsite/genericos/legis/resolucoes/893_03re_e.htm, accessed Apr. 11, 2006.
7. U.S. Department of Health and Human Services, Food and Drug Administration, Centre for Drug Evaluation and Research (CDER), Guidance for industry: SUPAC-MR: Modified release solid oral dosage forms scale-up and postapproval changes: Chemistry, manufacturing, and controls; in vitro dissolution testing and in vivo bioequivalence documentation, available: http://www.fda.gov/cder/guidance/1214fnl.pdf, accessed May 5, 2006.

8. U.S. Department of Health and Human Services, Food and Drug Administration, Centre for Drug Evaluation and Research (CDER), Guidance for industry: SUPAC-SS: Nonsterile semisolid dosage forms; scale-up and post-approval changes: Chemistry, manufacturing and controls; in vitro release testing and in vivo bioequivalence documentation, available: http://www.fda.gov/cder/guidance/1447fnl.pdf, accessed Apr. 24, 2006.
9. Pharmaceutical Unit of European Commission at EUROPA, available: http://ec.europa.eu/enterprise/pharmaceuticals/pharmacos/docs/brochure/pharmaeu.pdf, accessed May 21, 2006.
10. Variations. Pharmaceuticals: Regulatory framework and market authorizations, Chapter 5, in *Procedures for Marketing Authorisation*, Vol. 2A, European Commission, available: http://ec.europa.eu/enterprise/pharmaceuticals/eudralex/vol-2/a/v2a_chap5_r1_2004-02.pdf, accessed Apr. 16, 2006.
11. Commission regulation (EC) No. 1084/2003 of June 3, 2003, concerning the examination of variations to the terms of a marketing authorisation for medicinal products for human use and veterinary medicinal products granted by a competent authority of a member state (Official Journal L 159, 27/6/2003, pp. 1–23), available: http://ec.europa.eu/enterprise/pharmaceuticals/eudralex/homev1.htm, accessed Apr. 10, 2006.
12. The ICH process for harmonisation of guidelines, available: http://www.ich.org/cache/compo/276-254-1.html, accessed May 15, 2006.
13. Food and Drug Administration, Centre for Drug Evaluation and Research (CDER), Guideline on general principles of process validation, available: http://www.fda.gov/cder/guidance/pv.htm, accessed Apr. 26, 2006.
14. Willig, S. H. (2001), Production and process controls, in Swarbrick, J., Ed., *Good Manufacturing Practices for Pharmaceuticals: A Plan for Total Quality Control from Manufacturer to Consumer*, Marcel Dekker, New York, pp. 99–138.
15. Waterland, N. H., and Kowtna, C. C. (2003), Change control and SUPAC, in Nash, R. A., and Wachter, A. H., Eds., *Pharmaceutical Process Validation*, Marcel Dekker, New York, pp. 699–748.
16. Ahmed, S. U., Naini, V., and Wadgaonkar, D. (2005), Scale-up, process validation and technology transfer, in Shargel, L., and Kanfer, I., Eds., *Generic Drug Product Development: Solid Oral Dosage Form*, Marcel Dekker, New York, pp. 95–136.

1.4

GMP-COMPLIANT PROPAGATION OF HUMAN MULTIPOTENT MESENCHYMAL STROMAL CELLS

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1.4.1 INTRODUCTION

Somatic stem cell therapy (SCT) is a rapidly growing field that opens a broad spectrum of therapeutic options. The concept of regenerative SCT is based on the assumption that transplantation of adult human stem cells may support organ regeneration, modulate immunity, and regulate hematopoiesis. Transplantation of bone marrow (BM)–derived hematopoietic stem cells (SCs) for blood and immune system regeneration has been a clinical reality for almost 40 years. The existence of detectable numbers of mesenchymal and endothelial progenitors within blood and BM has promoted the readily harvestable hematopoietic tissue as a source of SCs for nonhematopoietic regenerative SCT (Figure 1).

Multipotent mesenchymal stromal cells (MSCs) are currently undergoing evaluation in a number of clinical trials (www.clinicaltrials.gov). These nonhematopoietic cells have been first described by Friedenstein et al. in a fibroblast colony-forming unit assay (CFU-F) based on low-density culture of adherent BM-derived cells [1–3]. Alternative sources for MSCs have been identified in a number of studies showing the successful isolation of fibroblast precursors from umbilical cord blood, placenta, umbilical cord, amniotic fluid, and adipose tissue [4–13]. To date, most experimental and clinical experience has been accumulated with BM-MSC [14–21]. Ex vivo expansion of these rare BM constituents (representing less than 1% of aspirated BM nucleated cells) is a prerequisite to achieve a reasonable MSC application dose of at least 2×10^6 MSCs/kg of the recipients' body weight. The majority of expansion procedures are currently based on the use of fetal bovine serum (FBS), which carries the risk of xenoimmunization and transmission of known (e.g., prions transmitting bovine spongiforme encephalopathia, BSE) and unknown pathogens. These risks could be avoided by developing MSC expansion protocols that use human alternatives which replace FBS.

The preclinical development of medicinal products in general bears high complexity due to the lack of fixed routines. Long term manipulations of cell-based medicinal products (CBMPs) may enhance the risks for undesirable effects in the course of ex vivo cell expansions. Safety concerns regarding the clinical application of ex vivo generated MSCs require a logistic environment providing an established good manufacturing practice (GMP) background embedded in a highly effective quality system. Demonstration of manufacturing and product consistency is achieved by applying rational in-process controls. Release criteria should ideally emerge from successful product development and optionally include the sterility, safety, purity, identity, and potency.[1] They must to be rapid, sensitive, and reliable and should retain some flexibility in type and timing of testing. The complexity and function of different CBMPs require an array of analytical procedures to adequately characterize the particular product (potency assays). Personalized (patient-specific) CBMPs differ from large drug batches in the pharmaceutical industry in terms of practicability in final product release in that they may require process-oriented rather than single-product potency testing. U.S. regulations demand that "tests for potency shall consist of either in vitro or in vivo tests, or both, which have been specifically

[1] U.S. legislation: 21 CFR 610, General biological products standards, CFR 610.10 Potency, CFR 600.3(s).

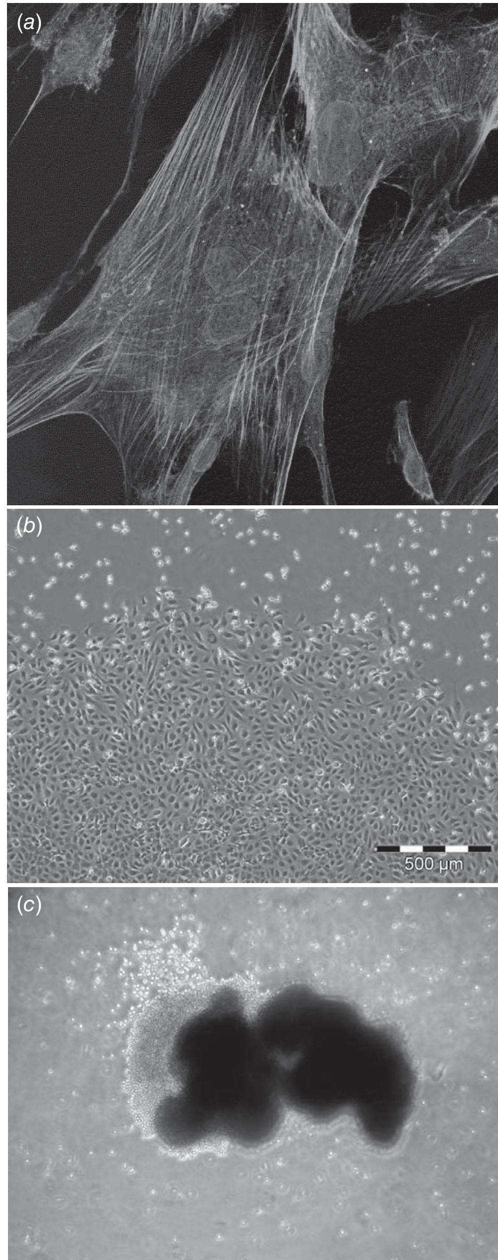

FIGURE 1 Hematopoietic tissue-derived SC and progenitors. Hematopoietic tissue contains (*a*) mesenchymal and (*b*) endothelial in addition to (*c*) hematopoietic progenitor cells. (*a*) Adult human BM-derived MSCs were stained to visualize the actin cytoskeleton, mitochondria, and nuclei. (*b*) The periphery of an umbilical cord blood–derived endothelial progenitor cell (EPC) colony is depicted demonstrating typical cobble stone–like morphology. The entire colony was derived from a single UCB-EPC indicating impressive proliferation potential (more than 70,000 cells were obtained by harvesting single EPC-derived colonies indicating the completion of at least 16 population doublings). Less than 10 mL of adult BM [(*a*) and (*c*)] but at least 40 mL of UCB (*b*) were sufficient to generate appropriate numbers of cells for therapeutic purposes.

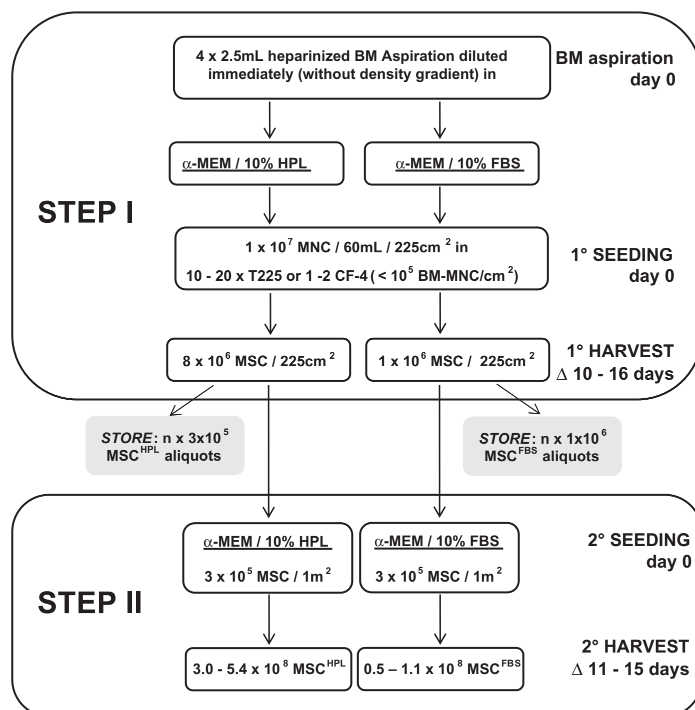

FIGURE 2 GMP-compliant propagation of human MSCs. The summary of a two-step MSC production procedure shows seeding and harvest numbers of BM-MNC and resulting numbers of MSC^{HPL} as compared to MSC^{FBS}. (Reproduced with permission from ref. 23.)

designed for each product so as to indicate its potency in a manner adequate to satisfy the interpretation of potency given by the definition in 21 CFR 600.3(s)." Functional analyses accompanying the expansion process development leading to a full product characterization and optimization of manufacturing steps are prerequisites that allow for the creation of a safe and effective CBMP.

This chapter demonstrates that rapid and standardized expansion of human MSCs to achieve a reasonable cell dose (i.e., $\geq 2 \times 10^6$/kg body weight of a 75-kg person corresponding to $\geq 1.5 \times 10^8$ MSCs) is feasible within less than four weeks. Replacing FBS with human platelet lysate (HPL) provides one strategy toward a safer CBMP (Figure 2). Appropriate preclinical development adhering to GMP principles will enhance safety in the course of a consecutive clinical evaluation of MSCs as a therapeutic agent.

1.4.2 ACRONYMS AND DEFINITIONS

1.4.2.1 Mesenchymal Stromal Cells

Adhesion of mononuclear cells from human bone marrow aspirates (BM-MNC) to tissue culture plastic and removal of nonadherent cells during the first days of culture selects for a population of proliferating spindle-shaped fibroblast-like non-

hematopoietic multipotent MSCs. Mesenchymal stromal cells can also be obtained from umbilical cord blood, umbilical cord, placenta, adipose tissue, and several fetal tissues. The minimum criteria for MSCs are defined in an ISCT (International Society for Cellular Therapy) position paper published in 2006 [22]. The MSCs have a high self-renewal potential and the capacity to be differentiated in vitro into progeny displaying an osteo-, chondro-, or adipogenic phenotype.

1.4.2.2 Somatic Stem Cell Therapy

The concept of regenerative SCT is based on experimental and early clinical observations indicating that the application of adult stem cells can improve organ regeneration after ischemic, toxic, or metabolic injury. Bone marrow harbors hematopoietic and mesenchymal stem cells and endothelial progenitor cells and is an easily accessible but not the sole, source of candidate cells to promote organ repair after systemic or local application. Regulation of hematopoiesis and immune modulation are the two established applications in the broad field of SCT with autologous and allogeneic stem and progenitor cells.

1.4.2.3 Good Manufacturing Practice

Good manufacturing practice is that part of the quality management system (QMS) that is concerned with the production and quality control of medicinal products (drugs) for human and veterinary use. It includes documentation, personnel training, facility, equipment, and process controls for the manufacture of pharmaceuticals.

1.4.2.4 Cell-Based Medicinal Products

Medicinal products containing viable cells are summarized under the umbrella term *cell-based medicinal products*. The term CBMP does not cover products containing nonviable cells or cellular fragments. The CBMPs may have much potential in the treatment of various diseases that to date have no cure. They are heterogeneous in terms of origin and type of cells and with regard to the complexity of the product. Cells may be self-renewing stem cells, more committed progenitors, or terminally differentiated cells exerting a specific regenerative function. Cells may be of autologous or allogeneic origin. Cells may be used alone or in combination with biomolecules, chemical substances, or structural materials that possibly potentiate their desired effects.

1.4.2.5 Human Platelet Lysate

Human platelet lysate can be obtained from buffy coat–derived platelet rich plasma. The platelet fraction is separated from the plasma and the white and red blood cell fraction by centrifugation steps and concentrated to a density of at least 1×10^9 platelets/mL. Platelets can either be activated with thrombin or lysed by repeated freeze–thaw cycles. Both mechanisms result in the release of growth factors and mitogens that are stored in intact platelets. Mediators released from platelets include, among others, epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), platelet-derived growth factors (PDGFs), transforming growth factor (TGF-ß1), and insulinlike growth factor (IGF) [23, 24]. Perhaps HPL may replace FBS in many

cell culture systems that have previously been thought to strictly depend on the presence of FBS.

1.4.3 APPROACHES

1.4.3.1 Adherence to Principles of GMP in a Preclinical Developmental Process

The standardized MSC propagation should be conducted as a well-planned, consistently documented, and optimized procedure that also minimizes risks of microbiological, particulate and pyrogen contamination by reducing manipulation steps and manipulation time. According to current European legislation,[2] the principles of GMP should be applied to CBMP when they are manufactured for use in human subjects in phase 1 studies. These requirements do not apply to cellular or tissue-based medicinal products used in phase 1 studies according to U.S. legislation[3] or to products in the preclinical developmental phase. If it is expected that preclinical findings are to be translated into clinical use rather rapidly, it may be recommended to establish GMP-compliant technology during the preclinical developmental phase of any cell product. As a result, this ensures that products are consistently produced and controlled to meet the quality standards appropriate for their intended use or product specification. The GMP requirements are well described in "PIC/S Guide to Good Manufacturing Practice for Medicinal Products" and include the implementation of an efficiently running quality management system, dedicated areas for manufacture of sterile medicinal products complying with GMP, appropriately qualified and trained personnel, suitable equipment, correct materials, containers and labels, approved procedures and instructions, suitable storage and transport facilities, and a record-keeping system that allows the complete history of a medicinal product to be traced (See http://www.picscheme.org). It is a challenge to conduct preclinical research and development complying to GMP as procedures routinely turn out to be much more time and cost intensive than common laboratory-scale research. These circumstances can advance either the developmental progress at the expense of quality standards or vice versa. It should therefore be decided on a case-by-case basis how closely to adhere to GMP standards depending on the more or less stringent time schedule for the considered clinical use of a CBMP.

1.4.3.2 Efficient Standardized MSC Propagation Using Low Cell Seeding Density

The future use of MSCs in clinical studies may require very high absolute MSC numbers to gain appropriate cell doses ($>5 \times 10^6$/kg body weight) per patient compared to in vivo experimental models with small animals [20]. It is consequently advantageous to develop large-scale MSC expansion protocols that allow for the

[2]European legislation: Directive 65/65/EEC, Directive 75/318/EEC, Directive 75/318/EEC, Commission Communication on the Community marketing authorisation procedures for medicinal products (98/C229/03); Directive 2001/20/EC, EMEA/CHMP/410869/2006.

[3]U.S. legislation: 21 CFR 210; 21 CFR 211; 21 CFR 312.21; 21 CFR 312.22(a) and 21 CFR 312.23(a)(7)(i).

generation of up to 5×10^8–10×10^8 MSCs from the limited starting volume of primary material.

The cell seeding density is of critical importance for the expansion rate of MSCs and must be defined for the primary seeding and the following passaging steps. Most experimental and clinical expansions described to date were started with a high seeding density of more than 1×10^5 BM-MNC/cm^2 [2, 14, 16]. For further passages pioneering studies showed that a very low seeding density between 0.5 and 10 MSCs/cm^2 selects for the expansion of a rapidly proliferating subpopulation of recycling SCs, termed RS cells [25–28]. This seeding density, referred to as "clonal density," would necessitate a theoretical growth area of from 2,000,000 to 100,000 cm^2 (from 200 to 10 m^2) to obtain a clinical quantity of $>1 \times 10^8$ MSCs from 1×10^6 starting MSCs within one passage. Plating 30–100 MSCs/cm^2 therefore is a reasonable compromise density requiring a more realistic growth area between 10,000 and 25,000 cm^2 (1 and 2.5 m^2). We have recently shown that the primary seeding of only 10 mL bone marrow aspirates on approximately 0.2 m^2 culture area for two weeks (culture step 1; BM diluted immediately after aspiration in culture medium without density gradient separation; removal of nonadherent cells at day 3) followed by an expansion on 2.5 m^2 (step 2) is sufficient to consistently generate at least 1.5×10^8 MSCs in FBS-supplemented medium within less than four weeks (Figure 2) [29]. This study furthermore corroborated earlier data on the inverse correlation of the seeding density to MSC proliferation (Figure 3) [25–28].

1.4.3.3 Superior MSC Proliferation Resulting from HPL-Driven as Compared to FBS-Driven Cultures

The most commonly used basic cell culture medium compositions for MSC propagation are minimum essential medium alpha (α-MEM) and low-glucose (1 g/L) Dulbecco's modified Eagle medium (DMEM-LG) supplemented with L-glutamin, antibiotics, and 5–20% FBS [14, 16, 19, 24, 25, 30]. Our experience with MSC propagation relates to the use of α-MEM supplemented with either FBS or HPL. In contrast to HPL that has been recognized only recently as a potent culture medium supplement [24], FBS is a well-known key medium supplement for cell culture and its role has been unchallenged for more than 50 years [31]. The common use of FBS in MSC cultures as a source of growth factors and mitogens bears the risk of transmission of known and unknown pathogens as well as xenoimmunization against bovine pathogens and should therefore be avoided for clinical use [32, 33].

In a recent study we analyzed the capacity of HPL to replace FBS in large-scale (clinical) MSC expansions and were able to demonstrate a superior propagation of MSC cultured with HPL (MSC^{HPL}) as compared to MSC derived from FBS-driven cultures (MSC^{FBS}) [23]. Figure 4 illustrates superior MSC proliferation at low plating density and higher population doublings (PDs) with HPL after a culture period of less than 14 days.

1.4.3.4 Contamination Risks Can Be Minimized in Rational MSC Propagation Procedures

Cell expansion is mainly performed according to labor-intensive time-consuming protocols using open systems that increase the risks of microbiological or particulate contamination and supplementation with potent antibiotics to control these prob-

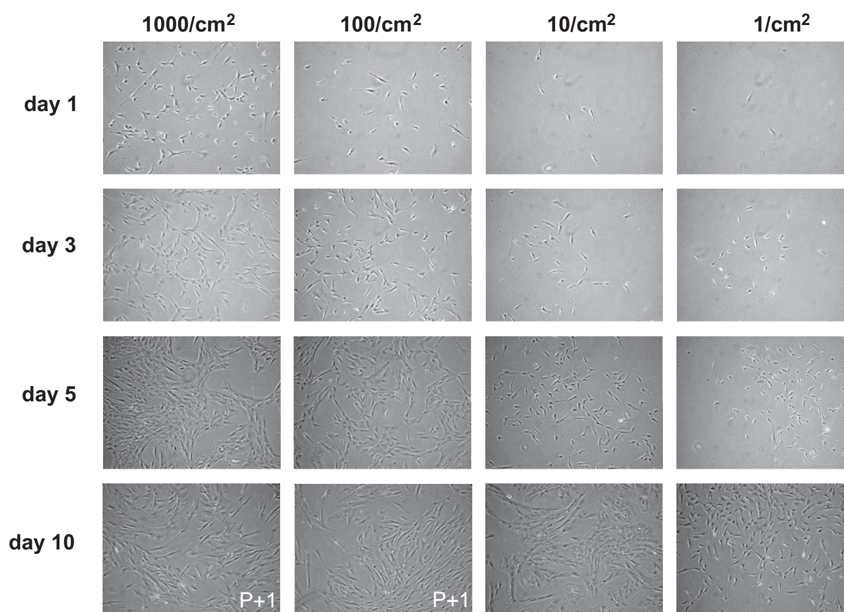

FIGURE 3 Inverse correlation of seeding density to MSC proliferation. BM-derived MSCs derived from passage 2 were seeded at log fold deescalated density of 1000, 100, 10, and 1 cm^{-2}. Photographs were taken after 1, 3, 5, and 10 days of culture in α-MEM/10% FBS (original magnification 40×). In the case of MSC seeded at 100 and 1000 cells/cm^2 confluence necessitated trypsinisation between days 5 and 10 followed by reseeding at 100 and 1000 cells/cm^2, respectively, and is therefore indicated as P + 1.

lems. Avoiding the use of penicillin during clinical-scale cell propagation follows the rationale to reduce the risk of sensitization as well as anaphylactic precipitation. Thus, it may be worthwhile not using other antibiotics for the GMP-compliant MSC propagation. One approach to minimize potential contamination risks is to rigorously reduce handling in the course of MSC propagation to the absolute minimum of necessary steps. In our experience, the commonly used density gradient centrifugation step can be skipped prior to the primary cell seeding of the bone marrow aspirate. Immediate dilution of limited volumes (e.g., 10–20 mL) of heparinized BM aspirate into supplemented α-MEM medium for direct cell seeding does not result in a loss of MSC recovery [23]. Furthermore, the aforementioned low cell seeding density and the employment of an increased growth area in a simplified procedure together with the use of HPL in fact allow for an efficient production of high MSC numbers within one to two harvest-replating cycles. The relatively short ex vivo expansion time of less than three to four weeks may be helpful in reducing the cumulative risk of contamination.

1.4.4 TESTING METHODS

1.4.4.1 Safety and Efficacy of CBMP in Preclinical Stage

The preclinical developmental period should be used for the extensive characterization of the CBMP. Release criteria have to be defined and reasonable time frames

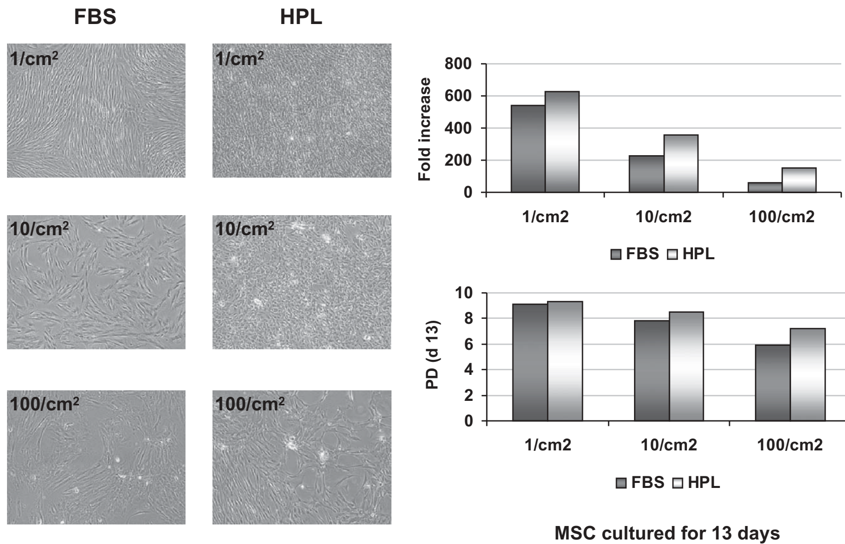

FIGURE 4 MSC proliferation capacity depends on seeding density in xenogeneic FBS and HPL-supplemented cultures. The inverse correlation of MSC proliferation to their seeding density resulted in the formation of a confluent MSC layer in cultures starting with 1 MSC/cm^2 in α-MEM/10% FBS and cultures starting with 1–10 MSCs/cm^2 in α-MEM/10% HPL but not when initiating cultures with the respective higher seeding densities within less than two weeks. The calculated fold increase of the cell number and corresponding population doublings from a representative experiment harvested at day 13 are shown.

must be set to allow for a high safety and quality standard of the final cellular product. On the other hand, the logistic background should allow for a rapid release of the CBMP within a few hours due to the potential short shelf life of many cellular products. Ranges of cell purity, sterility, and absence of pyrogens and endotoxins are factors of utmost importance which must be determined. It is an inherent feature of CBMPs that product specifications must be adapted to the individual application. The challenge in the preclinical developmental phase is to find satisfactory answers to unresolved questions in terms of cell type, source, dose, and mode of application according to the particular target disease. Thus, in-process controls and definitive release criteria must be met by each CBMP. Since many CBMPs are personalized medicine, potency assays must be performed for selected representative products (e.g., before initiating a study and consecutively once per year).

1.4.4.2 Quality Controls During Cell Culture (In-Process Controls) and Final Product Release Criteria

General Safety According the Food and Drug Administration (FDA), cellular therapy products are exempt from general safety testing [21 CFR 610.11(g)(1)].

Cell Dose The preclinical stage can be used to determine the specifications for the minimum effective and maximum tolerable number of viable and functional cells. The optimum dose of cells to be administered still needs to be established [20].

Viability Viability of MSCs can easily be determined immediately after trypsinization via trypan blue or 7-amino-actinomycin D (7-AAD) exclusion. According to the specifications developed from our cell culture studies, viability should be >90%. In selected exceptional cases a lower limit of 70% viability of total harvested cells may be acceptable.

Microbiological Testing Sterility testing that detects fungal, anaerobic, and aerobic bacterial and mycoplasma contamination should be performed after each critical manipulation step during MSC culture that is prone to microbiological contamination [34]. The crucial bacterial sterility check at the end of last harvesting step cannot be evaluated prior to in vivo application if MSCs need to be applied immediately after propagation due to the duration of the cultures. Mycoplasma polymerase chain reaction (PCR) results can be obtained at the day of harvest within less than 6h. MycoAlert® results are available within less than 1h at the day of harvest. Definitive culture results to exclude mycoplasma contamination are available within two to three weeks and therefore are not applicable for CBMPs with a short shelf life that are planned to be administered immediately after production.

Endotoxin and Pyrogenicity Testing Endotoxin measurement using the Limulus amebocyte lysate (LAL) assay is typically done as an alternative to pyrogenicity testing for early phase trials. For any parenteral drugs, except those administered intrathecally, the FDA recommends that the upper limit for endotoxin be 5EU/kg body weight/dose. The LAL assay method can be applied to the safety evaluation of biological preparations according to existing regulations.[4] We use the LAL assay to substitute for the lengthy delay in microbiological data availability to obtain results prior to the clinical application of the final product within less than 2h after harvest.

Phenotypic Identity of MSC In addition to morphological identification by microscopy, the immunophenotypic characterization of MSCs can be done using a broad panel of fluorescence-conjugated antibodies directed against surface molecules. To date there is no specific marker uniquely defining MSCs. Therefore a profile is used to show the expression of certain markers and to exclude the contamination by cells expressing other marker profiles. Flow cytometry is recommended by the ISCT to reveal that MSCs stain positive for CD73, CD90, and CD105 and negative for HLA-DR, CD14, CD31, CD34, and CD45 (Figure 5) [22, 23]. Much more extensive phenotypic analyses have been performed without retrieving additional information about MSC type or function [35]. Gene expression profiling will hopefully result in a better definition of human MSCs [35–42].

1.4.4.3 MSC Functionality and Potency Assays

Clonogenicity The self-renewal capacity of cells and the proportion of proliferating cells within a heterogeneous cell mixture can be evaluated using the CFU assay.

[4]Endotoxin testing, LAL, according to Eur. Pharm. 2.6.14 and Guideline on Validation of the Limulus Amebocyte Lysate Test as an End-Product Endotoxin Test for Human and Animal Parenteral Drug, Biological Products and Medical Devices, 1987, Sections I–IV, http://www.fda.gov/cber/gdlns/lal.pdf.

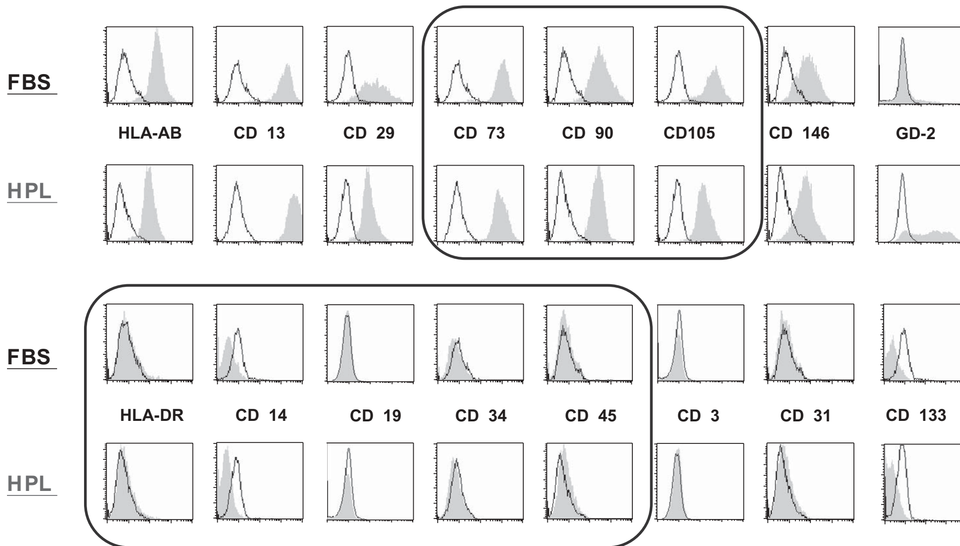

FIGURE 5 Immune phenotype of human MSCs. Flow cytometric analysis of at least 10,000 viable MSCs was used to determine antibody reactivity (gray-filled histograms) compared to appropriately diluted isotype controls (black line). Phenotypic criteria require positivity (≥90%) for CD73, CD90, and CD105 and negativity (≤2%) for HLA-DR, CD14 (or CD11b), CD19 (or CD79α), CD34, and CD45. Absence of CD3+ T cells may be desirable in the case of GvHD treatment. Depending on the culture conditions, MSCs share reactivity with the anti-disialoganglioside antibody GD2 with neuroblastoma cells, melanoma, and small-cell lung cancer cells.

A tissue culture method allowing for the clone counting of cells was first describend in 1956 [43]. The introduction of bone marrow CFU assays led to the discovery of hematopoietic stem cells [44]. Fibroblast precursors existing within the hematopoietic system also have been evaluated with another specific CFU assay method introduced by Friedenstein in 1974 (CFU-F) [2]. We analyzed the clonal expansion capacity of MSC with the CFU-F method. Figure 6 shows differences in CFU-F appearance between MSC^{HPL} and MSC^{FBS}. In the case of primary BM appropriate dilution is necessary to determine the CFU-F frequency (Figure 7). Once MSCs are enriched, the appropriate MSC seeding density recommended for CFU-F enumeration may range from 1 to 5 MSCs/cm^2 [2, 29].

Osteo-, Chondro-, and Adipogenic Differentiation Isolated BM-derived MSCs were shown to differentiate along multiple mesechymal lineages in 1999 [45]. Evidence suggests MSCs can also express phenotypic characteristics of endothelial, neural, smooth muscle, skeletal myoblast, and cardiac myocyte cells [46]. The prototype pathways of MSC differentiation occur along osteogenic, chondrogenic, and adipogenic lineages and have been extensively demonstrated in a large number of publications [47]. This kind of potency assay may be performed regularly if bone or connective tissue repair is intended, although time limits do not enable immediate product release.

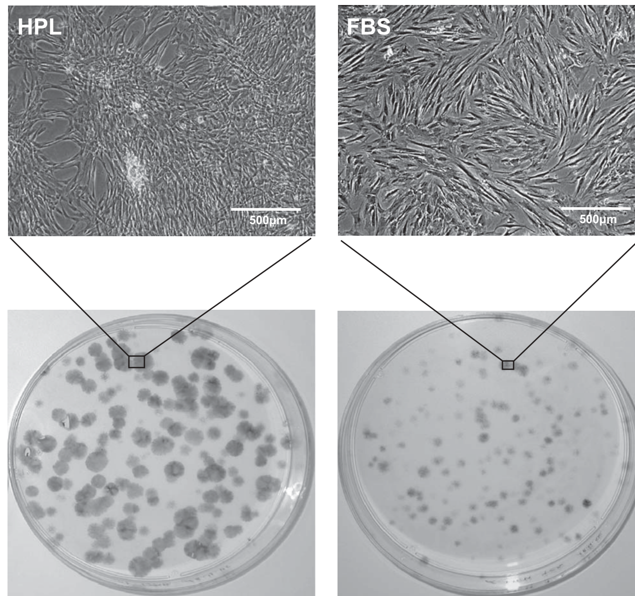

FIGURE 6 Morphological evaluation of MSCs. CFU-F of MSC^{HPL} compared to MSC^{FBS} differ in size, morphology, and density (scale bar identifies magnification in the upper panel; colony photographs taken on day 12, 40× original magnification).

Immune Modulatory Effects Mesenchymal stromal cells inhibit T-cell alloreactivity in mixed lymphocyte cultures (MLCs) or lymphocyte proliferation induced by mitogens, such as phytohemaglutinin (PHA) or concanavalin A [29, 48–51]. It is of note that high concentrations of MSCs (representing 10–40 MSCs per 100 responder lymphocytes) have an inhibitory effect while low MSC concentrations (0.1–1%) may stimulate lymphocyte proliferation in mixed lymphocyte cultures [50]. If MSCs are used for immunosuppressive therapies, these findings may imply that high doses of MSCs are needed to inhibit T-cell proliferation in patients with graft-versus-host disease following allogeneic bone marrow transplantation. The application of low MSC numbers could stimulate lymphocyte proliferation in vivo and hence result in an undesirable boost to graft-versus-host disease as an adverse reaction of the MSC therapy. It is not clear so far whether the precise number of T cells in a given MSC transplant needs to be determined to exclude a potential boost to alloreactivity. Immune modulation can be measured with carboxyfluorescein diacetate *N*-succinimidyl ester (CFSE) labeling of cells to quantify proliferation in response to allogeneic or mitogenic stimuli [52]. We analyzed the loss of CFSE fluorescent intensity indicating cell proliferation by flow cytometry after culturing CFSE-labeled MNCs in the absence or presence of different numbers of MSCs [53]. The immune regulatory capacity of MSC^{HPL} and MSC^{FBS} was studied by measurement of allogenic MNC proliferation after co-culturing pairs of MNC from three different donors with two independent MSC^{HPL} and two other MSC^{FBS} (Figure 8).

Hematopoiesis Regulation Regulation of the behavior of early hematopoietic progenitor cells (HPCs) can be analyzed by MSC-HPC cocultures in vitro [54].

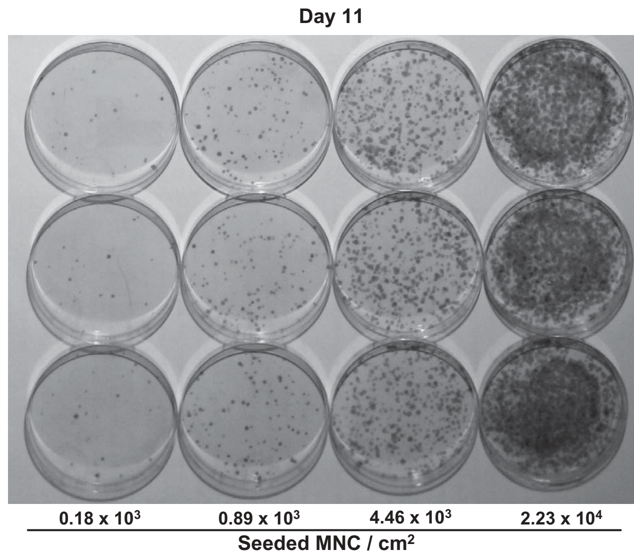

FIGURE 7 CFU-F Evaluation of MSCs depends on BM seeding density. An appropriate dilution of the heparinised BM aspiration is needed for accurate enumeration of the primary CFU-F frequency as indicated in this representative experiment where whole heparinized BM was seeded corresponding to the respective measured BM-MNC number per square centimeters of growth area, cultured for 11 days at 37°C/humidified atmosphere/3% O_2/5% CO_2. Nonadherent cells were removed at day 3. CFU-F are visualized by Harris hematoxylin staining.

Liquid cultures of purified $CD34^+$ (HPC) with a preestablished MSC feeder layer result in the expansion of $CD34^+/38^+$ HPC and $CD34^+/38^-$ hematopoietic SCs and support the growth of mature hematopoietic total nucleated cell (TNC) progeny (Figure 9).

Genetic Stability and Potential Tumorigenicity Genetic analysis of human MSCs is not well established. The significance of standard metaphase chromosome G banding is limited due to the low number of metaphases recovered during standard analyses. Advances in multicolor fluorescence in situ hybridization (FISH) and high-resolution array-based techniques may also soon be translated into practicable diagnostic tools in relation to CBMP safety in regenerative medicine [55].

Genetic instability can occur as a rare event after extended culture of mouse and human MSCs in FBS-supplemented medium [56, 57]. To test for potential in vivo tumor formation, MSCs derived from short-term clinical-scale expansions in FBS- or HPL-supplemented media were injected into immunocompromised athymic nude mice subcutaneously. Putative tumor formation was evaluated by histological analyses three months after injection of 2×10^6 and 2×10^4 MSCs and compared to controls that were injected 48 h prior to euthanasia. A primary cell deposit was visible immediately and 48 h after injection and MSCs could be recovered by conventional microscopic evaluation. However, none of 12 animals tested developed a macroscopic or microscopic detectable tumor over the 90-day observation period [23]. In this situation, genetic testing may be encouraged for prospective data acqui-

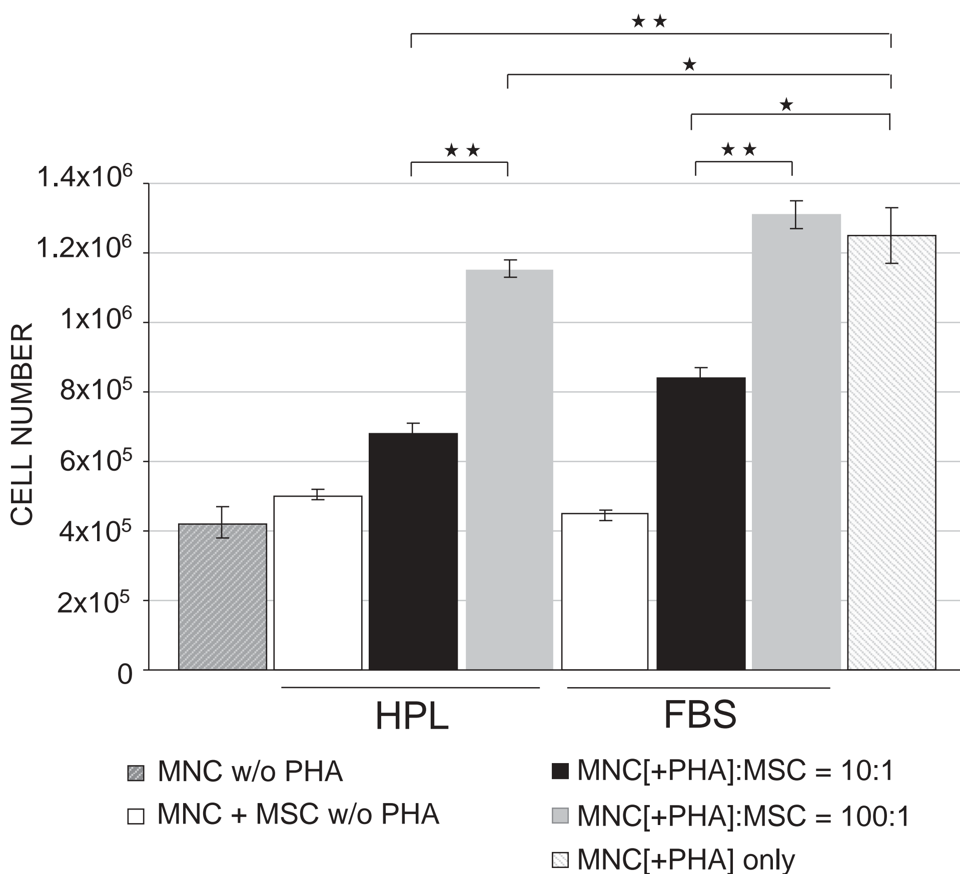

FIGURE 8 MSC-mediated Immune Modulation. Allogeneic MNC proliferation (mean cell number ± SEM) was measured after co-culturing pairs of MNC from three different donors with two independent MSC^{HPL} and two other MSC^{FBS} as shown in the bar chart. MSC were added in a 1:10 (3×10^4 MSC to 3×10^5 MNC/well; MNC[+PHA]:MSC = 10:1) or 1:100 (MNC[+PHA]:MSC = 10:1) ratio to test their influence on PHA-driven proliferation of MNC. MNC numbers were measured by flow cytometric MNC count using BD Truecount™ tubes. As a control numbers of mitogen stimulated MNC without additional MSC (MNC[+PHA] only) and background proliferation without PHA stimulation (MNC w/o PHA) are shown. MSC did not induce MNC proliferation (MNC + MSC w/o PHA). Significant differences are marked by asterisks (*$p < 0.05$ and **$p < 0.01$). (Figure reproduced with permission from reference 53)

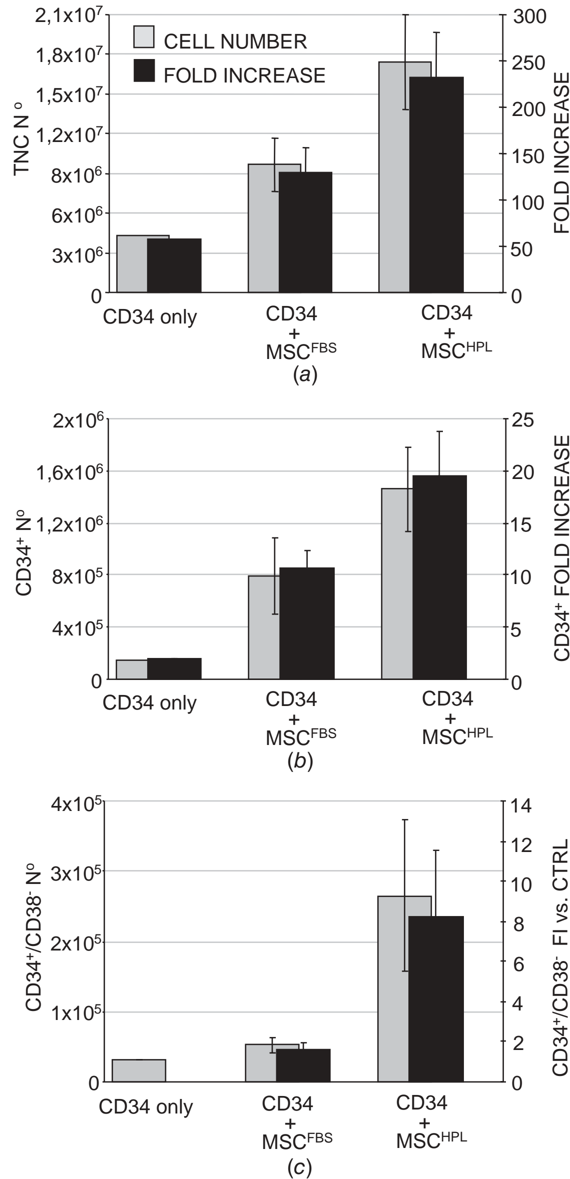

FIGURE 9 MSC-mediated hematopoiesis regulation. (*a*) Umbilical cord blood (UCB)-derived sorted $CD34^+$ cells were expanded in cytokine-supplemented medium [Roswell Park Memorial Institue (RMPI)-1640/10% Fetal Bovine Serum (FBS)/Granulocyte and Macrophage Colony Stimulating Factor (GM-CSF)/Interleukin 3(IL-3)/Stem Cell Factor (SCF)/FMS-like tyrosin kinase 3 ligand (Flt-3L)] in the absence or presence of clinical-scale expanded MSCs. Gray bars show harvested total nucleated cell number (TNC N°.) and black bars show the fold increase (FI) of the TNC N°. compared to the starting $CD34^+$ cell number. (*b*) Harvested number of $CD34^+$ cells (gray bars) and fold increase (black bars) of $CD34^+$ cells after liquid culture with or without MSC support. (*c*) Harvested number (gray bars) and fold increase (black bars) of $CD34^+/CD38^-$ hematopoietic stem cells after liquid culture of $CD34^+$ cells with MSC^{FBS} or MSC^{HPL} support compared to cytokine-supplemented liquid cultures in the absence of MSCs. (Mean ± Standard Error of the Mean (SEM) of two independent expansions.) (Reproduced with permission from ref. 53.)

sition but is not considered as mandatory for product release in current MSC clinical trials.

1.4.5 CONCLUSION

There are considerable limitations of common pharmacological techniques used in determining the safety and efficacy of CBMPs at the preclinical stage. Conventional methods used in the pharmaceutical industry to develop pharmacological profiles and to determine the acute toxicity of drugs in animals as well as toxicity studies may not directly be translated to ex vivo generated cellular products. Nevertheless, it is inevitable that preclinical research and development of cellular products will be conducted under the guidance of either individual or consensus specifications and definitions that will continuously be improved. This approach will be helpful in developing successful therapeutic cellular agents.

ACKNOWLEDGMENTS

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REFERENCES

1. Luria, E. A., Panasyuk, A. F., and Friendenstein, A. Y. (1971), Fibroblast colony formation from monolayer cultures of blood cells, *Transfusion*, 11(6), 345–349.
2. Friedenstein, A. J., Deriglasova, U. F., Kulagina, N. N., et al. (1974), Precursors for fibroblasts in different populations of hematopoietic cells as detected by the in vitro colony assay method, *Exp. Hematol.*, 2(2), 83–92.
3. Kuznetsov, S. A., Friedenstein, A. J., and Robey, P. G. (1997), Factors required for bone marrow stromal fibroblast colony formation in vitro, *Br. J. Haematol.*, 97(3), 561–570.
4. Erices, A., Conget, P., and Minguell, J. J. (2000), Mesenchymal progenitor cells in human umbilical cord blood, *Br. J. Haematol.*, 109(1), 235–242.
5. Kogler, G., Sensken, S., Airey, J. A., et al. (2004), A new human somatic stem cell from placental cord blood with intrinsic pluripotent differentiation potential, *J. Exp. Med.*, 200(2), 123–135.
6. Bieback, K., Kern, S., Kluter, H., and Eichler, H. (2004), Critical parameters for the isolation of mesenchymal stem cells from umbilical cord blood, *Stem Cells*, 22(4), 625–634.
7. Romanov, Y. A., Svintsitskaya, V. A., and Smirnov, V. N. (2003), Searching for alternative sources of postnatal human mesenchymal stem cells: Candidate MSC-like cells from umbilical cord, *Stem Cells*, 21(1), 105–110.
8. Miao, Z., Jin, J., Chen, L., et al. (2006), Isolation of mesenchymal stem cells from human placenta: Comparison with human bone marrow mesenchymal stem cells, *Cell. Biol. Int.*, 30(9), 681–687.
9. In ‘t Anker, P. S., Scherjon, S. A., Kleijburg-van der Keur, C., et al. (2004), Isolation of mesenchymal stem cells of fetal or maternal origin from human placenta, *Stem Cells*, 22(7), 1338–1345.

10. In 't Anker, P. S., Scherjon, S. A., Kleijburg-van der Keur, C., et al. (2003), Amniotic fluid as a novel source of mesenchymal stem cells for therapeutic transplantation, *Blood*, 102(4), 1548–1549.
11. Gronthos, S., Franklin, D. M., Leddy, H. A., Robey, P. G., Storms, R. W., and Gimble, J. M. (2001), Surface protein characterization of human adipose tissue-derived stromal cells, *J. Cell Physiol.*, 189(1), 54–63.
12. Zuk, P. A., Zhu, M., Ashjian, P., et al. (2002), Human adipose tissue is a source of multipotent stem cells, *Mol. Biol. Cell*, 13(12), 4279–4295.
13. Kern, S., Eichler, H., Stoeve, J., Kluter, H., and Bieback, K. (2006), Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue, *Stem Cells*, 24(5), 1294–1301.
14. Lazarus, H. M., Haynesworth, S. E., Gerson, S. L., Rosenthal, N. S., and Caplan, A. I. (1995), Ex vivo expansion and subsequent infusion of human bone marrow-derived stromal progenitor cells (mesenchymal progenitor cells): Implications for therapeutic use, *Bone Marrow Transplant.*, 16(4), 557–564.
15. Koc, O. N., Peters, C., Aubourg, P., et al. (1995), Bone marrow-derived mesenchymal stem cells remain host-derived despite successful hematopoietic engraftment after allogeneic transplantation in patients with lysosomal and peroxisomal storage diseases. *Exp. Hematol.*, 27(11), 1675–1681.
16. Koc, O. N., Gerson, S. L., Cooper, B. W., et al. (2000), Rapid hematopoietic recovery after coinfusion of autologous-blood stem cells and culture-expanded marrow mesenchymal stem cells in advanced breast cancer patients receiving high-dose chemotherapy, *J. Clin. Oncol.*, 18(2), 307–316.
17. Lee, S. T., Jang, J. H., Cheong, J. W., et al. (2002), Treatment of high-risk acute myelogenous leukaemia by myeloablative chemoradiotherapy followed by co-infusion of T cell-depleted haematopoietic stem cells and culture-expanded marrow mesenchymal stem cells from a related donor with one fully mismatched human leucocyte antigen haplotype, *Br. J. Haematol.*, 118(4), 1128–1131.
18. Koc, O. N. Day, J., Nieder, M., Gerson, S. L., Lazarus, H. M., and Krivit, W. (2002), Allogeneic mesenchymal stem cell infusion for treatment of metachromatic leukodystrophy (MLD) and Hurler syndrome (MPS-IH), *Bone Marrow Transplant.*, 30(4), 215–222.
19. Le Blanc, K., Rasmusson, I., Sundberg, B., et al. (2004), Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells, *Lancet*, 1, 363(9419), 1439–1441.
20. Lazarus, H. M., Koc, O. N., Devine, S. M., et al. (2005), Cotransplantation of HLA-identical sibling culture-expanded mesenchymal stem cells and hematopoietic stem cells in hematologic malignancy patients, *Biol. Blood Marrow Transplant.*, 11(5), 389–398.
21. Ringden, O., Uzunel, M., Rasmusson, I., et al. (2006), Mesenchymal stem cells for treatment of therapy-resistant graft-versus-host disease, *Transplantation*, 81(10), 1390–1397.
22. Dominici, M., Le Blanc, K., Mueler, I., et al. (2006), Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement, *Cytotherapy*, 8(4), 315–317.
23. Schallmoser, K., Bartmann, C., Rohde, E., et al. (2007), Human platelet lysate can replace fetal bovine serum for clinical scale expansion of functional MSC, *Transfusion*. 2007 Aug; 47(8), 1436–1446.
24. Doucet, C., Ernou, I., Zhang, Y. Z., et al. (2005), Platelet lysates promote mesenchymal stem cell expansion: A safety substitute for animal serum in cell-based therapy applications. *J. Cell. Physiol.*, 205(2), 228–236.

25. Digirolamo, C. M., Stokes, D., Colter, D., Phinney, D. G., Class, R., and Prockop, D. J. (1999), Propagation and senescence of human marro stromal cells in culture: A simple colony-forming assay identifies samples with the greatest potential to propagate and differentiate, *Br. J. Haematol.*, 107(2), 275–281.
26. Colter, D. C., Class, R., DiGirolamo, C. M., Prockop, D. J. (2000), Rapid expansion of recycling stem cells in cultures of plastic-adherent cells from human bone marrow, *Proc. Nat. Acad. Sci. USA*, 97(7), 3213–3218.
27. Colter, D. C., Sekiya, I., and Prockop, D. J. (2001), Identification of a subpopulation of rapidly self-renewing and multipotential adult stem cells in colonies of human marrow stromal cells, *Proc. Nat. Acad. Sci. USA*, 98(14), 7841–7845.
28. Sekiya, I., Larson, B. L., Smith, J. R., Pochampally, R., Cui, J. G., and Prockop, D. J. (2002), Expansion of human adult stem cells from bone marrow stroma: Conditions that maximie the yields of early progenitors and evaluate their quality, *Stem Cells*, 20(6), 530–541.
29. Bartmann, C., Rohde, E., Schallmoser, K., et al. (2007), Two steps to functional MSC for clinical application, *Transfusion*. 2007 Aug; 47(8), 1426–1435.
30. Horwitz, E. M., Gordon, P. L., Koo, W. K., et al. (2002), Isolated allogeneic bone marrow-derived mesenchymal cells engraft and stimulate growth in children with osteogenesis imperfecta: Implications for cells therapy of bone, *Proc. Natl. Acad. Sci. USA*, 99(13), 8932–8937.
31. Pollard, J. W. (1989), Basic cell culture, in Pollard, J. W., Ed., *Animal Cell Culture*, Human, Clifton, NJ, pp. 1–2.
32. WHO (1997), Medicinal and other products and human and animal transmissible spongiform encephalopathies: Memorandum from a WHO meeting, *Bull. World Health Org.*, 75(6), 505–513.
33. European Union (2004), Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMEA/410/01 Rev. 2—October 2003) adopted by the Committee for Proprietary Medicinal Products (CPMP) and by the Committee for Veterinary Medicinal Products (CVMP), *Off. J. Europ. Union*, C24/26–C24/19.
34. Schallmoser, K., Rosin, C., Vormittag, R., et al. (2006), Specificities of platelet autoantibodies and platelet activation in lupus anticoagulant patients: A relation to their history of thromboembolic disease, *Lupus*, 15(8), 507–514.
35. Wagner, W., Wein, F., Seckinger, A., et al. (2005), Comparative characteristics of mesenchymal stem cells from human bone marrow, adipose tissue, and umbilical cord blood, *Exp. Hematol.*, 33(11), 1402–1416.
36. Ramalho-Santos, M., Yoon, S., Matsuzaki, Y., Mulligan, R. C., and Melton, D. A. (2002), "Stemness": Transcriptional profiling of embryonic and adult stem cells, *Science*, 298(5593), 597–600.
37. Boquest, A. C., Shahdadfar, A., Fronsdal, K., et al. (2005), Isolation and transcription profiling of purified uncultured human stromal stem cells: Alteration of gene expression after in vitro cells culture, *Mol. Biol. Cell*, 16(3), 1131–1141.
38. Shahdadfar, A., Fronsdal, K., Haug, T., Reinholt, F. P., and Brinchmann, J. E. (2005), In vitro expansion of human mesenchymal stem cells: Choice of serum is a determinant of cell proliferation, differentiation, gene expression, and transcriptome stability, *Stem Cells*, 23(9), 1357–1366.
39. Silva, W. A., Jr., Covas, D. T., Panepucci, R. A., et al. (2003), The profile of gene expression of human marrow mesenchymal stem cells, *Stem Cells*, 21(6), 661–669.
40. Panepucci, R. A., Siufi, J. L., Silva, W. A., Jr., et al. (2004), Comparison of gene expression of umbilical cord vein and bone marrow-derived mesenchymal stem cells, *Stem Cells*, 22(7), 1263–1278.

41. Jeong, J. A., Hong, S. H., Gang, E. J., et al. (2005), Differential gene expression profiling of human umbilical cord blood-derived mesenchymal stem cells by DNA microarray, *Stem Cells*, 23(4), 584–593.
42. Monticone, M., Liu, Y., Tonachini, L., et al. (2004), Gene expression profile of human bone marrow stromal cells determined by restriction fragment differential display analysis, *J. Cell. Biochem*, 92(4), 733–744.
43. Puck, T. T., and Marcus, P. I. (1956), Action of x-rays on mammalian cells. *J. Exp. Med.*, 103(5), 653–666.
44. Tepperman, A. D., Curtis, J. E., and McCulloch, E. A. (1974), Erythropietic colonies in cultures of human marrow, *Blood*, 44(5), 659–669.
45. Pittenger, M. F., Mackay, A. M., Beck, S. C., et al. (1999), Multilneage potential of adult human mesenchymal stem cells, *Science*, 284(5411), 143–147.
46. Pittenger, M. F., and Martin, B. J. (2004), Mesenchymal stem cells and their potential as cardiac therapeutics, *Circ. Res.*, 95(1), 9–20.
47. Delorme, B. C. S., and Charbord, P. (2006), The concept of mesenchymal stem cells, *Regenerative Med.*, 1(4), 497–509.
48. Di Nicola, M., Carlo-Stella, C., Magni, M., et al. (2002), Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli, *Blood*, 99(10), 3838–3843.
49. Bartholomew, A., Sturgeon, C., Siatskas, M., et al. (2002), Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin graft survival in vivo, *Exp. Hematol.*, 30(1), 42–48.
50. Le Blanc, K., Tammik, L., Sundberg, B., Haynesworth, S. E., and Ringden, O. (2003), Mesenchymal stem cells inhibit and stimulate mixed lymphocyte cultures and mitogenic responses independently of the major histocompatibility complex, *Scand. J. Immunol.*, 57(1), 11–20.
51. Tse, W. T., Pendleton, J. D., Beyer, W. M., Egalka, M. C., and Guinan, E. C. (2003), Suppression of allogeneic T-cell proliferation by human marrow stromal cells: Implications in transplantation, *Transplantation*, 75(3), 389–397.
52. Muller, I., Kordowich, S., Holzwarth, C., et al. (2006), Animal serum-free culture conditions for isolation and expansion of multipotent mesenchymal stromal cells from human BM, *Cytotherapy*, 8(5), 437–444.
53. Reinisch, A., Bartmann, C., Rohde, E. S. K., Bjelic-Radisic, V., Lanzer, G., Linkesch, W., and Strunk, D. (2007), A humanized system to propagate cord blood-derived mesenchymal stem cells for clinical application, *Regen. Med.* 2007 Jul; 2(4), 371–382.
54. Robinson, S. N., Ng, J., Niu, T., et al. (2006), Superior ex vivo cord blood expansion following co-culture with bone marrow-derived mesenchymal stem cells, *Bone Marrow Transplant.*, 37(4), 359–366.
55. Speicher, M. R., and Carter, N. P. (2005), The new cytogenetics: Blurring the boundaries with molecular biology, *Nat. Rev. Genet.*, 6(10), 782–792.
56. Rubio, D., Garcia-Castro, J., Martin, M. C., et al. (2005), Spontaneous human adult stem cell transformation, *Cancer Res.*, 65(8), 3035–3039.
57. Tolar, J., Nauta, A. J., Osborn, M. J., et al. (2007), Sarcoma derived from cultured mesenchymal stem cells, *Stem Cells*, 25(2), 371–379.

SECTION 2

INTERNATIONAL REGULATIONS OF GOOD MANUFACTURING PRACTICES

2.1

NATIONAL GMP REGULATIONS AND CODES AND INTERNATIONAL GMP GUIDES AND GUIDELINES: CORRESPONDENCES AND DIFFERENCES

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2.1.1 INTRODUCTION

The first predecessors of manufacturing and quality requirements, which later evolved into good manufacturing practices (GMPs), were issued in the 1940s in the United States by the Food and Drug Administration (FDA) [1]. In the general meeting of the World Health Organization (WHO) held in 1969, the World Health Assembly issued a recommendation for the introduction of GMPs [2]. Since then, most industrialized countries have passed laws on control procedures essential for the manufacture of drug products. In some countries GMPs are integrated into national legislation as a part of laws or regulations on production, distribution, marketing, and use of drug products (GMP regulations). In other countries, GMPs are separate guidelines outside the national drug legislation (GMP codes). In addition to national GMPs, also some international organizations and trade blocks have issued their own international GMP guidelines to harmonize the requirements for drug production in different countries. However, regardless of their origin, the main purpose of GMPs is to ensure that manufactured drug products have the safety, identity, potency, purity, and quality that they are presented to have [3]. To fulfill this aim, most GMPs usually cover quality management, personnel, premises, equipment, documentation, materials management, production and in-process controls, packaging and labeling of intermediate and finished products, laboratory controls, validation, and change controls [4].

2.1.2 NATIONAL GMP REGULATIONS AND CODES

2.1.2.1 United States

In the United States the production of drug products is controlled under the federal Food, Drug and Cosmetic Act, which states that a drug product will be deemed to be adulterated unless the methods used in or the facilities or controls used for its manufacture, processing, packaging, or holding conform to or are operated or administered in conformity with current GMP [5]. The actual GMP regulations are issued as a part of the Code of Federal Regulations and as such they are a federal law. The current set of GMP regulations is based on the 1978 revision [6, 7] of the original GMP regulations, which were first promulgated in 1963. The GMP regulations are updated every year in April [8]; however, no major changes have been implemented since 1978. As an addition to GMP regulations, the FDA also publishes other GMP-related guidance documents covering various issues of drug manufacturing [9]. On the other hand, although these documents reflect current views and

TABLE 1 Contents of Part 211 of U.S. GMP Regulations [7]

Section	Subject
Subpart A	General provisions
Subpart B	Organization and personnel
Subpart C	Buildings and facilities
Subpart D	Equipment
Subpart E	Control of components and drug product containers and closures
Subpart F	Production and process controls
Subpart G	Packaging and labeling control
Subpart H	Holding and distribution
Subpart I	Laboratory controls
Subpart J	Records and reports
Subpart K	Returned and salvaged drug products

expectations of the agency, they only provide guidance on principles and practices that are not legal requirements [1]. As a member of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceutical for Human Use (ICH), the United States has adopted the ICH guidance document Q7, *Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients*, and published it as a guidance for industry document [10].

The U.S. GMP regulations are divided into two parts: 210 [6] and 211 [7]. Part 210, "Current Good Manufacturing Practice in Manufacturing, Processing, Packing or Holding of Drugs—General," provides the framework for the regulations [6], and Part 211, "Current Good Manufacturing Practice for Finished Pharmaceuticals," states the actual requirements. Part 211 is further divided into 11 subparts, which cover the requirements for personnel, premises, equipment, control of materials, production and process controls, packaging and labeling control, holding and distribution, laboratory controls, documentation, and returned and salvaged products [7]. The contents of Part 211 are presented in Table 1.

2.1.2.2 Canada

The production of drug products (drugs) in Canada is controlled under the Food and Drugs Act, which states that distributors and importers are not allowed to sell a drug product unless it has been manufactured according to the requirements of GMP. The principles of GMP are laid down by Division 2 in Part C of the Food and Drug Regulations, which is a part of the Food and Drugs Act [11]. The Health Products and Food Branch Inspectorate has also issued a guidance document (GMP code), which has been prepared to assist in the interpretation of GMP regulations. The current set of the Canadian GMP code was issued in 2002 and has not been revised since. It has been written with a view to harmonization with GMP standards of other countries and international organizations [WHO, Pharmaceutical Inspection Cooperation Scheme (PIC/S), ICH]. Canadian Healthcare authorities have also published several annexes to the basic GMP code covering topics such as GMP for medical gases, biological drug products, blood products, and production of investigational new drugs. In addition to the GMP code and its annexes, the Canadian

TABLE 2 Contents of Canadian GMP Code [12]

Introduction
Quality management
Glossary of terms
Regulation
Premises
Equipment
Personnel
Sanitation
Raw material testing
Manufacturing control
Quality control department
Packaging material testing
Finished product testing
Records
Samples
Stability
Sterile products
Medical gases
Annex A: Internationally Harmonized Requirements for Batch Certification
Annex B: Application for Alternate Sample Retention
Annex C: References

authorities have also issued several other specific guidelines dealing with issues related to GMP and manufacturing methods [12].

As shown in Table 2 the Canadian GMP code can be divided into four chapters and three annexes. The first three chapters cover general issues such as scope and applicability of the code, definitions of used terms, and issues concerning quality management and GMP in general. GMP regulations and their application are presented in the fourth chapter ("Regulation"), which is divided into 14 subchapters covering the requirements for premises, equipment, personnel, sanitation, testing of components and packaging materials, testing of finished product, production control, quality control department, documentation, reserve samples, stability testing, and manufacture of sterile drug products and medical gases. Each subchapter contains the corresponding regulation according to regulations in Division 2 [11] issued with a rationale and interpretation to assist in their application. The annexes include requirements for batch certification, application form for alternate sample retention site, and references such as hyperlinks to Canadian laws concerning drug products and other GMP-related national and international guidelines [12].

2.1.2.3 European Union

The production of drug products (medicinal products) in the European Union (EU) is controlled under Directive 2001/83/EC of the European parliament and of the Council, which states that the holder of a manufacturing authorization for medicinal products is obliged to comply with good manufacturing practices as laid down by European Community law [13]. The principles and guidelines of GMP for medicinal products are stated by the Commission directive 2003/94/EC, which provides the

TABLE 3 Contents of Part I of EU GMP Code Covering Basic Requirements for Manufacture of Drug Products [15]

Section	Subject
	Introduction
Chapter 1	Quality management
Chapter 2	Personnel
Chapter 3	Premises and equipment
Chapter 4	Documentation
Chapter 5	Production
Chapter 6	Quality control
Chapter 7	Contract manufacture and analysis
Chapter 8	Complaints and product recall
Chapter 9	Self-inspection
	Glossary

legal basis for GMP in the EU [14]. The actual GMP code with detailed written procedures is published in *The Rules Governing Medicinal Products in the European Union*, volume 4. The current set of the EU GMP code was first introduced in 1989 consisting of nine chapters covering the general requirements of GMP and one annex on the manufacture of sterile drug products. Since then the EU GMP code has been revised many times and several new annexes have been issued [15]. In addition to the GMP code, the EU has also published several other guidelines concerning the quality issues of drug production in *The Rules Governing Medicinal Products in the European Union*, volume 3A [16].

As shown in Tables 3–5, the EU GMP code is presented in two parts of basic requirements and 18 annexes. Part I, "Basic Requirements for Medicinal Products," covers GMP principles for the manufacture of drug products. It consists of nine chapters covering the requirements for quality management and control, personnel, premises, equipment, documentation, production, contract services, complaints, product recall, and self-inspection. Part II, "Basic Requirements for Active Substances Used as Starting Materials," covers GMPs for active substances used as starting materials. It is based on the ICH document Q7, *Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients*, and was originally introduced in 2001 as Annex 18 of the EU GMP code. In the restructured revision of the EU GMP code issued in October 2005, Annex 18 was replaced with Part II. It consists of 19 chapters, which cover basic GMP issues related to quality management, personnel, premises, equipment, documentation, materials, production and process controls, packaging and labeling, storage and distribution, laboratory controls, validation, change control, complaints, recalls, contract services, co-operators, active pharmaceutical ingredients (APIs) manufactured by cell culture/fermentation, and APIs used in clinical trials. The annexes give more detailed specific guidance on the manufacture of sterile drug products, biological drug products, radiopharmaceuticals, veterinary drug products, medical gases, herbal drug products, oral liquids, external preparations (creams, ointments), aerosols, investigational new drugs, and blood and blood products. They also cover sampling of materials, computerized systems, use of ionizing radiation, qualification and validation, batch release, parametric release, reference, and retention samples [15].

TABLE 4 Contents of Part II of EU GMP Code Covering Basic Requirements for Manufacture of Active Substances Used as Starting Materials [15]

Section	Subject
1	Introduction
2	Quality management
3	Personnel
4	Buildings and facilities
5	Process equipment
6	Documentation and records
7	Materials management
8	Production and in-process controls
9	Packaging and identification labeling of APIs and intermediates
10	Storage and distribution
11	Laboratory controls
12	Validation
13	Change control
14	Rejection and reuse of materials
15	Complaints and recalls
16	Contract manufacturers (including laboratories)
17	Agents, brokers, traders, distributors, repackers, and relabelers
18	Specific guidance for APIs manufactured by cell culture/fermentation
19	APIs for use in clinical trials
20	Glossary

TABLE 5 Annexes of EU GMP Code Covering Specific Guidance [15]

Section	Subject
Annex 1	Manufacture of sterile medicinal products
Annex 2	Manufacture of biological medicinal products for human use
Annex 3	Manufacture of radiopharmaceuticals
Annex 4	Manufacture of veterinary medicinal products other than immunological veterinary medicinal products
Annex 5	Manufacture of immunological veterinary medicinal products
Annex 6	Manufacture of medicinal gases
Annex 7	Manufacture of herbal medicinal products
Annex 8	Sampling of starting and packaging materials
Annex 9	Manufacture of liquids, creams, and ointments
Annex 10	Manufacture of pressurised metered-dose aerosol preparations for inhalation
Annex 11	Computerized systems
Annex 12	Use of ionizing radiation in manufacture of medicinal products
Annex 13	Manufacture of investigational medicinal products
Annex 14	Manufacture of products derived from human blood or human plasma
Annex 15	Qualification and validation
Annex 16	Certification by a qualified person and batch release
Annex 17	Parametric release
Annex 19	Reference and retention samples

2.1.2.4 East Asian Countries

Japan In Japan the production of drug products (drugs) is regulated under the Pharmaceuticals Affairs Law (PAL), which states that any drug manufacturer who plans to manufacture a drug product for sale in Japan must have a Japanese drug manufacturing license and comply with Japanese GMP requirements. The first regulations of Japanese GMP were introduced in 1974 as *The Standards for Manufacturing Control and Quality Control*. In 1979 PAL was partially revised and GMPs became legally binding [2].

PAL is managed and enforced via ministerial ordinances and notices, which are detailed regulations prepared by the Japanese government. The requirements for premises for drug manufacture are given in Ministry of Health, Labor and Welfare (MHLW) Ministerial Ordinance No. 73, 2005 *Regulations for Buildings and Facilities for Pharmacies, etc.* [originally Ministry of Health and Welfare (MHW) Ministerial Ordinance No. 2, 1961] [17], and the requirements for manufacturing and quality controls in MHLW Ministerial Ordinance No. 95, 2003 *Regulations for Manufacturing Control and Quality Control of Drugs* (originally MHW Ministerial Ordinance No. 3, 1994). As a member of the ICH Japan has adopted the ICH guidance document Q7, *Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients*, and published it as Pharmaceutical and Food Safety Bureau (PFSB) Director-General Notification No. 1200, 2001 *Guidelines on GMP for Drug Substances*, which states the requirements for the manufacture of APIs. The requirements concerning imported drug products are given in MHLW Ministerial Ordinance No. 97, 2003 *Regulations for Importing/Retail Management and Quality Control of Drugs and Quasi-Drugs* (originally MHW Ministerial Ordinance No. 62, 1999). The requirements specifying manufacture of investigational products are given in PAB Notification No. 480, 1997 *Products and Standards for the Buildings and Facilities of Manufacturing Plants for Investigational Products* (Investigational Product GMP) [2].

South Korea The production of drug products (drugs) in South Korea is regulated under the Pharmaceutical Affairs Law, which was first enacted in 1953 and has since been revised several times [18]. New drug approval and related activities are regulated in much the same way as in the United States and Japan. Korean GMP, which is often called KGMP, was initiated in 1984 and became mandatory in 1995 [19]. A drug manufacturer who intends to manufacture a drug product for sale in Korea must have approval from the Commissioner of the Korea Food and Drug Administration (KFDA). In order to require the license for manufacturing business the manufacturer has to prove the compliance of facility standards with KGMP [20].

China China regulates the production of drug products (drugs) under the Drug Administration Law of the People's Republic of China, which states that a drug manufacturer has to conduct drug manufacture according to the GMP for pharmaceutical products formulated by the Drug Regulatory Department under the State Council on the basis of the Drug Administration Law [21]. In June 2004 GMP became mandatory in China and the State Drug Administration announced that local drug manufacturing establishments lacking approved GMP certification would not be allowed to continue the production of pharmaceuticals [22].

TABLE 6 Contents of Part I of Indian GMP Regulations Covering Good Manufacturing Practices for Premises and Materials [24]

Section	Subject
1	General requirements
2	Warehousing area
3	Production area
4	Ancillary areas
5	Quality control area
6	Personnel
7	Health, clothing, and sanitation of workers
8	Manufacturing operations and control
9	Sanitation in the manufacturing premises
10	Raw materials
11	Equipment
12	Documentation and records
13	Labels and other printed materials
14	Quality assurance
15	Self-inspection and quality audit
16	Quality control system
17	Specification
18	Master formula records
19	Packing records
20	Batch packaging records
21	Batch processing records
22	Standard operating procedures (SOPs) and records
23	Reference samples
24	Reprocessing and recoveries
25	Distribution records
26	Validation and process validation
27	Product recalls
28	Complaints and adverse reactions
29	Site master file

2.1.2.5 India

The production of drug products (drugs) in India is controlled under the Drugs and Cosmetics Rules (1945, last amended in 2005), which states that the holder of the license to manufacture drugs has to comply with the requirements of GMP as laid down in Schedule M [23]. Schedule M is a part of the Drugs and Cosmetics Rules and embodies the Indian GMP regulations [24], which are based on the 1982 version of WHO GMP guidelines [25].

As shown in Tables 6–8 the Indian GMP regulations consists of eight parts: I, IA, IB, IC, ID, IE, IF, and II. Part I covers the general requirements of GMP. It is divided into 29 chapters, which deal with the requirements for personnel, premises, equipment, sanitation, production and process controls, materials, documentation, quality management, validation, reserve samples, recalls, complaints, and self-inspection. Parts IA to IE cover specific requirements for the manufacture of different dosage forms regarding premises, equipment, and methods. Part IA deals with the require-

TABLE 7 Contents of Parts IA, IB, IC, ID, IE, and IF of Indian GMP Regulations Covering Specific Guidance [24]

Section	Subject
Part IA	Specific requirements for manufacture of sterile products, parenteral preparations (small-volume injectables and large-volume parenterals) and sterile ophthalmic preparations
Part IB	Specific requirements for manufacture of oral solid dosage forms (tablets and capsules)
Part IC	Specific requirements for manufacture of oral liquids (syrups, elixirs, emulsions, and suspensions)
Part ID	Specific requirements for manufacture of topical products, i.e., external preparations (creams, ointments, pastes, emulsions, lotions, solutions, dusting powders, and identical products)
Part IE	Specific requirements for manufacture of metered-dose inhalers (MDIs)
Part IF	Specific requirements of premises, plant, and materials for manufacture of active pharmaceutical ingredients (bulk drugs)

TABLE 8 Contents of Part II of Indian GMP Regulations Covering Requirements of Plant and Equipment [24]

Section	Subject
1	External preparations
2	Oral liquid preparations
3	Tablets
4	Powders
5	Capsules
6	Surgical dressing
7	Ophthalmic preparations
8	Pessaries and suppositories
9	Inhalers and vitrallae
10	Repacking of drugs and pharmaceutical chemicals
11	Parenteral preparations

ments for the manufacture of parenteral preparations; Part IB with the requirements for the manufacture of oral solid dosage forms such as tablets and capsules; Part IC with the requirements for the manufacture of oral liquids such as syrups, elixirs, emulsions, and suspensions; Part ID with the requirements for the manufacture of external preparations such as creams, ointments, pastes, emulsions, and lotions; and Part 1E with the requirements for the manufacture of inhalers. Part 1F covers specific requirements for the manufacture of APIs regarding buildings and facilities, utilities, equipment, controls, and containers. Part II of the Indian GMP regulations consist of detailed recommendations for the process equipment to be used in the manufacture of different dosage forms and requirements for the partition of the production area [24].

2.1.2.6 Australia

In Australia the production of drug products (medicinal products) is controlled under the Therapeutics Goods Act, which provides the Minister for Health and Aged Care the right to determine written principles including codes of GMP to be observed in the production of drug products for use in humans [26]. The Therapeutic Goods (Manufacturing Principles) Determination No. 2 of 2002 given by the minister states that drug products must be manufactured in compliance with the *Australian Code of Good Manufacturing Practice for Medicinal Products*, dated August 16, 2002 [27]. The current set of the Australian GMP code is based entirely on the PIC/S GMP guide version PH 1/97 (Rev. 3) published in 2002 with some minor modifications [28].

As shown in Table 9, the Australian GMP code consists of 9 chapters and 13 annexes. The chapters present the general requirements of GMP for the manufacture of drug products, the requirements for quality management and control, personnel, premises, equipment, documentation, production, contract services, complaints, product recall, and self-inspection. The annexes give specific guidance on the manufacture of sterile drug products, biological drug products, radiopharmaceuticals, medical gases, herbal drug products, oral liquids, external preparations (creams, ointments), aerosols, investigational new drugs, blood, and blood products. They also

TABLE 9 Contents of Australian GMP Code [28]

Section	Subject
	Introduction
	Interpretation
Chapter 1	Quality management
Chapter 2	Personnel
Chapter 3	Premises and equipment
Chapter 4	Documentation
Chapter 5	Production
Chapter 6	Quality control
Chapter 7	Contract manufacture and analysis
Chapter 8	Complaints and product recall
Chapter 9	Self-inspection
Annex 1	Manufacture of sterile medicinal products
Annex 2	Manufacture of biological medicinal products for human use
Annex 3	Manufacture of radiopharmaceuticals
Annex 6	Manufacture of medicinal gases
Annex 7	Manufacture of herbal medicinal products
Annex 8	Sampling of starting and packaging materials
Annex 9	Manufacture of liquids, creams, and ointments
Annex 10	Manufacture of pressurised metered-dose aerosol preparations for inhalation
Annex 11	Computerized systems
Annex 12	Use of ionizing radiation in the manufacture of medicinal products
Annex 13	Manufacture of investigational medicinal products
Annex 15	Qualification and validation
Annex 17	Parametric release
	Glossary

cover sampling of materials, computerized systems, use of ionizing radiation, qualification, and validation and parametric release [28].

Australia has not adopted Annexes 4, 5, 14, 16, and 18 of the PIC/S GMP guide. Annexes 4 and 5 cover the manufacture of veterinary drug products. Annex 14 covers the manufacture of products derived from human blood or human plasma, which is excluded from the Australian GMP code. Annex 16 is specific to the EU GMP code and Annex 18 is the ICH GMP guide for the manufacture of APIs, which Australia has adopted separately as a manufacturing principle [28].

2.1.2.7 New Zealand

The production of drug products (medicines) in New Zealand is controlled under the Medicines Act 1981, which states that a drug manufacturer is not allowed to manufacture drug products without a manufacturing license issued by the licensing authority. In order to obtain a manufacturing license the applicant must satisfy the licensing authority with respect to the proposed manufacturing premises and equipment, which must be suitable and adequate for the manufacture of drugs. Moreover, the applicant must show that adequate arrangements have been made or are to be made for the making, maintaining, and safekeeping of adequate records with reference to the drug products that are to be manufactured [29]. The authorities (Medsafe) require that any drug manufacturer who plans to manufacture drug products for sale in New Zealand must deliver evidence of GMP compliance for the manufacturing site. Copies of appropriate certificates, manufacturing licenses, or reports issued by a regulatory authority whose competence is recognized by Medsafe are accepted as proof of GMP compliance [30].

As shown in Table 10 New Zealand's own GMP code consists of five parts. The first part covers the manufacture of drug products and the second part the manufacture of blood products. Part 3 covers compounding and dispensing, including compounding of sterile drug products. Part 4 deals with wholesaling and Part 5 with product recalls. Parts 4 and 5 are combined in one document [31].

2.1.2.8 South Africa

South Africa controls the production of drug products (medicines) under the Medicines and Related Substances Control Act (Act 101 of 1965), which states that the Medicines Control Council may issue to a drug manufacturer a license to manufacture a drug product upon such conditions as to the application of such acceptable

TABLE 10 Contents of New Zealand's GMP Code [31]

Section	Subject
Part 1	Manufacture of pharmaceutical products
Part 2	Manufacture of blood and blood products
Part 3	Compounding and dispensing
Part 4	Wholesaling of medicines and medical devices
Part 5	Uniform recall procedure for medicines and medical devices

TABLE 11 Contents of South African GMP Code [34]

Section	Subject
	Introduction
Chapter 1	Quality management
Chapter 2	Personnel
Chapter 3	Premises and equipment
Chapter 4	Documentation
Chapter 5	Production
Chapter 6	Quality control
Chapter 7	Contract manufacture and analysis
Chapter 8	Complaints and product recall
Chapter 9	Self-inspection
Annex 1	Manufacture of sterile medicinal products
Annex 2	Manufacture of biological medicinal products for human use
Annex 3	Manufacture of radiopharmaceuticals
Annex 4	Manufacture of veterinary medicinal products other than immunologicals
Annex 5	Manufacture of immunological veterinary medical products
Annex 6	Manufacture of medicinal gases
Annex 7	Manufacture of herbal medicinal products
Annex 8	Sampling of starting and packaging materials
Annex 9	Manufacture of liquids, creams, and ointments
Annex 10	Manufacture of pressurized metered-dose aerosol preparations for inhalation
Annex 11	Computerized systems
Annex 12	Use of ionizing radiation in the manufacture of medicinal products
Annex 13	Manufacture of investigational medicinal products
Annex 14	Manufacture of products derived from human blood or human plasma
Annex 15	Qualification and validation
Annex 16	Organisation and personnel
Annex 17	Parametric release
	Glossary

quality assurance principles and GMPs as the council may determine [32]. As a part of the license application the manufacturer must provide acceptable documentary proof of the ability to comply with GMP as determined by the council [33]. The current set of South African GMP code determined by the council is entirely based on the PIC/S GMP guide version PE 009-2, published in 2004 with some minor modifications [34].

As shown in Table 11 the South African GMP code consists of 9 chapters and 17 annexes. The chapters present the general requirements of GMP for the production of drug products covering the requirements for quality management and control, personnel, premises, equipment, documentation, production, contract services, complaints, product recall, and self-inspection. The annexes give specific guidance on the manufacture of sterile drug products, biological drug products, radiopharmaceuticals, veterinary drug products, medical gases, herbal drug products, oral liquids, external preparations (creams, ointments), aerosols, investigational new drugs, and blood and blood products. They also cover sampling of materials, computerized systems, use of ionizing radiation, qualification and validation, organization, and

personnel and parametric release. The original Annex 16, which is specific to the EU GMP code, has been replaced in South African GMP code with an annex covering organization and personnel. Nor has South Africa adopted Annex 18, which covers the ICH GMP guide for the manufacture of APIs, as it has been adopted separately as a manufacturing principle [34].

2.1.3 INTERNATIONAL GMP GUIDES AND HARMONIZATION

2.1.3.1 World Health Organization

The WHO was established in 1948 as a specialized agency of the United Nations (UN). Its purpose is to serve as the directing and coordinating authority for international health matters and public health. One of the main functions of the WHO is to provide objective and reliable information and advice in the field of human health, a task that it partly fulfills through WHO publications [35]. The first WHO draft text on GMP was prepared in 1967 and a revised version was published in 1968 as an annex of the twenty-second report of the WHO expert committee on specifications for pharmaceutical preparations. Over the years the WHO has issued several versions of its GMP guidelines as well as other guidelines related to the GMP and quality issues of the production of therapeutic products. The latest version of the WHO GMP guideline was published in 2003 as an annex of the WHO Technical Report 908 [36].

As shown in Table 12 the WHO GMP guideline is divided into five parts: introduction, general considerations, glossary, quality management in the drug industry, and references. The actual GMP guidelines are presented in the fourth part, which consists of 17 chapters covering the requirements for quality assurance and control, personnel, premises, equipment, sanitation, materials, validation, documentation, production, contract services, complaints, recalls, and self-inspection [36]. In addition to this guideline laying down the main principles of GMP, the WHO has also published several other guidelines covering specific requirements for components, quality of water for pharmaceutical use, APIs, excipients, sterile drug products, biological drug products, investigational drug products, herbal drug products, and radiopharmaceuticals (Table 13).

2.1.3.2 Pharmaceutical Inspection Cooperation Scheme

The Pharmaceutical Inspection Convention (PIC), which is the predecessor of PIC/S, was founded in 1970 by the European Free Trade Area (EFTA). The initial members comprised of the 10 EFTA member countries at that time. From the beginning one of the main goals has been the harmonization of GMP requirements as well as the promotion of mutual recognition of inspections and uniformity of inspection systems by training the inspectors, improving the exchange of information, and mutual confidence [46]. Originally PIC was a formal treaty between member countries and as such it also had a legal status. When countries outside Europe were seeking to join PIC, it became evident that, according to European law, individual EU countries that were members of PIC were not permitted to sign agreements with countries outside Europe. Only the European Commission, which itself was

TABLE 12 Contents of WHO GMP Guideline Covering General Requirements of GMP for Manufacture of Drug Products [36]

Introduction
General considerations
Glossary
Quality management in the drug industry: philosophy and essential elements

Section	Subject
1	Quality assurance
2	Good manufacturing practices for pharmaceutical products (GMP)
3	Sanitation and hygiene
4	Qualification and validation
5	Complaints
6	Product recalls
7	Contract production and analysis
8	Self-inspection and quality audits
9	Personnel
10	Training
11	Personal hygiene
12	Premises
13	Equipment
14	Materials
15	Documentation
16	Good practices in production
17	Good practices in quality control
References	

TABLE 13 GMP-Related WHO Documents Covering Specific Guidance

Document	Subject
TRS 929, Annex 2 [37]	Requirement for the sampling of starting materials
TRS 823, Annex 1 [38]	Active pharmaceutical ingredients (bulk drug substances)
TRS 885, Annex 5 [39]	Pharmaceutical excipients
TRS 902, Annex 6 [40]	Sterile pharmaceutical products
TRS 834, Annex 3 [41]	Biological products
TRS 863, Annex 7 [42]	Investigational pharmaceutical products for clinical trials in humans
TRS 863, Annex 8 [43]	Herbal medicinal products
TRS 908, Annex 3 [44]	Radiopharmaceutical products
TRS 929, Annex 3 [45]	Water for pharmaceutical use

not a member of PIC, was permitted to sign agreements. Consequently, a less formal and more flexible PIC/S was developed to continue the work of PIC. The PIC/S, which became operational in November 1995, is an informal arrangement without legal status between regulatory authorities instead of countries. The PIC and the PIC Scheme, operating together as PIC/S, provide an active and constructive cooperation in the field of GMP [47].

The current members of PIC/S are Australia, Austria, Belgium, Canada, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland,

Italy, Latvia, Liechtenstein, Malaysia, Netherlands, Norway, Poland, Portugal, Romania, Singapore, Slovak Republic, Spain, Sweden, Switzerland, and the United Kingdom. In addition, Estonia, the European Agency for the Evaluation of Medicinal Products (EMEA), UNICEF, and the WHO participate in PIC/S activities as observers [48]. Also many other regulatory authorities have shown interest in joining PIC/S, in particular Argentina, Brazil, Cyprus, Indonesia, Israel, Philippines, Slovenia, Thailand, the United States, Bulgaria, Estonia, Lithuania, Oman, Russia, South Africa, and the Ukraine [49].

To become a PIC/S member, a joining regulatory authority is required to go through a detailed assessment to prove that the authority has the arrangements and competence necessary to apply an inspection system equivalent to inspection systems of existing PIC/S members. To ensure that both new applicants and older members fulfill the same requirements, also existing members are reassessed on a regular basis. One of the main functions of PIC/S is to develop GMP guidance documents, which it carries out in close cooperation with the EU and relevant agencies thereof. Under this cooperation both parties have been able to adopt each others' documents, thus minimizing the duplication of effort in development of GMP-related documents. Among other highly informative guides on various aspects of GMP and quality issues [49], PIC/S has also published its own GMP guide (*Guide to Good Manufacturing Practice for Medicinal Products*), which is harmonized with the EU GMP code [50].

The latest revision of the PIC/S GMP guide (version PE 009-3) was issued in January 2006. As shown in Table 14, it consists of 9 chapters and 16 annexes. Chapters present the general requirements of GMP for the production of drug products covering the requirements for quality management and control, personnel, premises, equipment, documentation, production, contract services, complaints, product recall, and self-inspection. The annexes give specific guidance on the manufacture of sterile drug products, biological drug products, radiopharmaceuticals, veterinary drug products, medical gases, herbal drug products, oral liquids, external preparations (creams, ointments), aerosols, investigational new drugs, and blood and blood products. In addition, there are annexes covering the sampling of materials, computerized systems, use of ionizing radiation, qualification and validation, and parametric release [50].

Although the PIC/S GMP guide is harmonized with the EU GMP code and their contents are similar, there are some minor differences between them. Instead of the term *qualified person*, the PIC/S GMP guide uses the term *authorized person*. Furthermore, all references to EU directives have been deleted from the PIC/S GMP guide. Moreover, PIC/S has not adopted Annexes 16 and 18 of the EU GMP code. Annex 16 is specific to the EU GMP code covering the status of a qualified person in batch release and Annex 18 is the ICH GMP guide for the manufacture of APIs, which the PIC/S Committee has adopted as a stand-alone document (PE 007) [50].

2.1.3.3 International Conference on Harmonization

The ICH was established in 1990. Its main aim is to improve the efficiency of the drug development process and the registration of new drug products in its member countries through harmonization of national guidelines. This is a joint initiative

TABLE 14 Contents of PIC/S GMP Guide [50]

Section	Subject
	Introduction
Chapter 1	Quality management
Chapter 2	Personnel
Chapter 3	Premises and equipment
Chapter 4	Documentation
Chapter 5	Production
Chapter 6	Quality control
Chapter 7	Contract manufacture and analysis
Chapter 8	Complaints and product recall
Chapter 9	Self-inspection
Annex 1	Manufacture of sterile medicinal products
Annex 2	Manufacture of biological medicinal products for human use
Annex 3	Manufacture of radiopharmaceuticals
Annex 4	Manufacture of veterinary medicinal products other than immunologicals
Annex 5	Manufacture of immunological veterinary medical products
Annex 6	Manufacture of medicinal gases
Annex 7	Manufacture of herbal medicinal products
Annex 8	Sampling of starting and packaging materials
Annex 9	Manufacture of liquids, creams, and ointments
Annex 10	Manufacture of pressurised metered-dose aerosol preparations for inhalation
Annex 11	Computerized systems
Annex 12	Use of ionizing radiation in manufacture of medicinal products
Annex 13	Manufacture of investigational medicinal products
Annex 14	Manufacture of products derived from human blood or human plasma
Annex 15	Qualification and validation
Annex 17	Parametric release
	Glossary

involving both regulators and industry as equal partners. The founders and current members of ICH, which represent the regulatory bodies and the research-based industry in the member countries, are the EU, European Federation of Pharmaceutical Industries and Associations (EFPIA), MHLW, Japan Pharmaceutical Manufacturers Association (JPMA), FDA, and Pharmaceutical Research and Manufactures of America (PhRMA). In addition to the actual member countries there are also observers who act as a link between ICH and non-ICH countries and regions. Current observers are the WHO, EFTA, Swissmedic (representing Switzerland), and Health Canada (representing Canada) [51].

Among other guidelines, ICH has also published a guide on GMP for APIs (Q7: *Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients*). It is intended to provide guidance regarding GMP for the manufacture of APIs and to help ensure that APIs meet the quality and purity requirements that they are presented to possess. This covers APIs that are manufactured by chemical synthesis, extraction, cell culture/fermentation, recovery from natural sources, or any combination of these processes. Excluded are vaccines, medical gases, bulk-packaged drug

TABLE 15 Contents of ICH GMP Guideline for API Production [52]

Section	Subject
1	Introduction
2	Quality management
3	Personnel
4	Buildings and facilities
5	Process equipment
6	Documentation and records
7	Materials management
8	Production and in-process controls
9	Packaging and identification labeling of APIs and intermediates
10	Storage and distribution
11	Laboratory controls
12	Validation
13	Change control
14	Rejection and reuse of materials
15	Complaints and recalls
16	Contract manufacturers (including laboratories)
17	Agents, brokers, traders, distributors, repackers, and relabelers
18	Specific guidance for APIs manufactured by cell culture/fermentation
19	APIs for use in clinical trials
20	Glossary

products, radiopharmaceuticals, whole cells, whole blood and plasma, blood and plasma derivatives, and gene therapy APIs. However, APIs that are produced using blood or plasma as raw materials are included [52]. All ICH member countries have adopted this guideline: the EU in November 2000, Japan in November 2001, and the United States in September 2001 [53]. In addition, it has also been adopted by several other non-ICH countries such as Australia [28] and South Africa [34].

The basic structure of the ICH GMP guideline for API production is shown in Table 15. It consists of 19 chapters, which cover the requirements for quality management, personnel, premises, equipment, documentation, materials, production and process controls, packaging and labeling, storage and distribution, laboratory controls, validation, change control, complaints, recalls, contract services, cooperators, APIs manufactured by cell culture/fermentation, and APIs used in clinical trials [52].

2.1.3.4 Association of Southeast Asian Nations (ASEAN)

ASEAN was established in 1967 by Indonesia, Malaysia, Philippines, Singapore, and Thailand. Current members include also Brunei and Darussalam (joined in 1984), Vietnam (joined in 1995), Laos and Myanmar (joined in 1997), and Cambodia (joined in 1999). The aims and purposes of ASEAN involve cooperation in the economic, social, cultural, technical, educational, and other fields [54]. Among other cooperation schemes the ASEAN countries have also developed their own GMP guidelines, which were issued in 1984 [55].

2.1.3.5 Mercado Comun del Sur (MERCOSUR)

MERCOSUR was established in 1991 by Argentina, Brazil, Paraguay, and Uruguay to develop a common market between its member countries. Current members include also Bolivia and Chile (joined in 1996). One of the original aims was to harmonize the pharmaceutical legislation of the member countries. As a part of these harmonization activities MERCOSUR has developed its own GMP guidelines, which are based on WHO recommendations. In addition to the GMP guideline, MERCOSUR has also issued other GMP-related guides covering inspections, requirements for facilities, and quality control [56].

2.1.4 CORRESPONDENCES OF THE U.S. GMP REGULATIONS WITH GMP CODES AND GUIDELINES

The following sections deal with the correspondences and differences between the U.S. GMP regulations and the Canadian and EU GMP codes and the WHO GMP guideline. As the EU GMP code is harmonized with the PIC/S GMP guide, the correspondences between the EU GMP code and the U.S. GMP regulations cover also the correspondences between the U.S. GMP regulations and the PIC/S GMP guide as well as all other national GMPs that are based on the PIC/S GMP guide. Differences between the EU GMP code and the PIC/S GMP guide have been presented in Section 2.1.3.2.

2.1.4.1 General Issues

In the U.S. GMP regulations general issues related to the use and applicability of GMP regulations are presented in Part 210 [6], which consists of regulations 210.1, 210.2, and 210.3 and in Subpart A of Part 211 [7], which consists of regulations 211.1 and 211.3. Contents of Part 210 and Subpart A of Part 211 are presented in Table 16. Regulation 210.1 defines the status, 210.2 deals with the applicability, and 211.1 states the scope of the regulations. Definitions of terms used in the regulations are provided in regulation 210.3 and in regulation 211.3, which states that the definitions provided in regulation 210.3 apply also in Part 211.

Correspondences in Canadian GMP Code In the Canadian legislation general issues related to the use and applicability of the GMP regulations and code are covered in the introduction of the GMP code [12] and in Divisions 1A [57] and 2

TABLE 16 Contents of Part 210 and Subpart A of Part 211 of US GMP Regulations Covering General Issues Related to Use and Applicability of Regulations [6, 7]

Section	Subject
CFR 210.1	Status of current good manufacturing practice regulations
CFR 210.2	Applicability of current good manufacturing practice regulations
CFR 210.3	Definitions
CFR 211.1	Scope
CFR 211.3	Definitions

of the Part C of the Food and Drug Regulations [11]. Definitions for the GMP regulations are covered in regulation C.01A.001 of Division 1A [57] and in regulation C.02.002 of Division 2 [11]. Definitions for the GMP code are covered in the glossary of terms of the code [12].

Correspondences in EU GMP Code In the EU legislation general issues related to the use and applicability of the GMP regulations and the code are covered in the Commission Directive 2003/94/EC [14] and in the introduction of the GMP code [15]. Definitions for the directive are covered in Article 2 of the directive [14] and definitions for the code in the glossary of the GMP code [15].

Correspondences in WHO GMP Guideline In the WHO GMP guideline [36] general issues related to the use and applicability of the GMP guide are covered in section "General Considerations." Definitions for the GMP guide are covered in the glossary of the guideline.

2.1.4.2 Organization and Personnel

For GMP regulations in the United States issues related to organization and personnel are covered in Subpart B [7], which consists of regulations 211.22, 211.25, 211.28, and 211.34. The contents of Subpart B is presented in Table 17. Regulation 211.22 states the responsibilities and authorities of the quality control unit, including requirements for the resources. Regulation 211.25 deals with personnel qualifications covering the requirements for their education and experience and it also states the requirements for the training of the personnel. Regulation 211.28 states the responsibilities of personnel covering the requirements for the clothing and other protective apparel, personal sanitation and health habits, as well as personal health conditions. Furthermore, it states the requirements for the authorization for limited access. Regulation 211.34 deals with consultants and lays down the requirements for their education, training, and experience, including the requirements for documentation.

Correspondences in Canadian GMP Code In the Canadian GMP code [12] issues related to organization and personnel are mainly covered in the interpretation of regulation C.02.006 (Personnel) and partly in the interpretations of regulations C.02.004 (Premises), C.02.008 (Sanitation), C.02.011 (Manufacturing Control), C.02.013 (Quality Control Department), C.02.015 (Quality Control Department), and C.02.024 (Records). Correspondences to regulation 211.22 are covered in

TABLE 17 Contents of Subpart B of Part 211 of U.S. GMP Regulations Covering Organization and Personnel [7]

Section	Subject
CFR 211.22	Responsibilities of quality control unit
CFR 211.25	Personnel qualifications
CFR 211.28	Personnel responsibilities
CFR 211.34	Consultants

Sections 1–5 of the interpretation of regulation C.02.015 and in Section 2 of the interpretation of regulation C.02.013. Sections 1–5 of the interpretation of regulation C.02.015 state the responsibilities of the quality control unit (quality control department), and Section 2 of the interpretation of regulation C.02.013 covers the requirements for resources. Correspondences to regulation 211.25 stating the requirements for the education, training, and experience of the personnel are covered in Sections 1–5 of the interpretation of regulation C.02.006. Correspondences to regulation 211.28 are covered in Section 6.3 of the interpretation of regulation C.02.004, Sections 1–2 of the interpretation of regulation C.02.008, Section 8 of the interpretation of regulation C.02.011, and Section 4 of the interpretation of regulation C.02.013. Section 1 of the interpretation of regulation C.02.008 states the health requirements and Section 2 the requirements for clothing, other protective apparel, and personal hygiene. Section 6.3 of the interpretation of regulation C.02.004, Section 8 of the interpretation of regulation C.02.011, and Section 4 of the interpretation of regulation C.02.013 cover the requirements regarding limited access. Correspondences to regulation 211.34 are covered in Section 6 of the interpretation of regulation C.02.006 and in Subsection 1.3.2 of the interpretation of regulation C.02.024. Section 6 of the interpretation of regulation C.02.006 states the requirements for the education, training, and experience of consultants and contractors and Subsection 1.2.3 of the interpretation of regulation C.02.024 the requirements for documentation.

Correspondences in EU GMP Code In the EU GMP code [15] issues related to organization and personnel are mainly covered in Chapter 2 (Personnel) and partly in Chapters 3 (Premises and Equipment), 5 (Production), and 6 (Quality Control). Correspondences to regulation 211.22 are covered in Subchapters 2.6, 2.7, 6.1, and 6.2. Subchapters 2.6 and 2.7 deal with the responsibilities of the head of the quality control unit (quality control department) and 6.2 with the responsibilities of the quality control unit as a whole. Requirements for resources are covered in Subchapter 6.1. Correspondences to regulation 211.25 are covered in Subchapters 2.1, 2.4, and 2.8–2.12. Subchapters 2.1 and 2.4 deal with the requirements for personnel and Subchapters 2.8–2.12 with the requirements for their training. Correspondences to regulation 211.28 are covered in Subchapters 2.15, 2.16, 3.5, 3.21, 5.16, and 6.4. Subchapter 2.15 deals with requirements for personal health conditions and 2.16 with requirements for clothing and protection. Access limitations are covered in Subchapters 3.5, 3.21, 5.16, and 6.4. In the EU GMP code there is no correspondence to regulation 211.34, which covers the requirements for the use of consultants. However, Chapter 7 of the code deals with the requirements for the contract services in general.

Correspondences in WHO GMP Guideline In the WHO GMP guideline [36] issues related to organization and personnel are mainly covered in Chapter 9 (Personnel) and partly in Chapters 10 (Training), 11 (Personal Hygiene), 16 (Good Practices in Production), and 17 (Good Practices in Quality Control). Correspondences to regulation 211.22 are covered in Subchapters 9.8, 9.10, 17.3, and 17.4. Subchapters 9.8 and 9.10 state the responsibilities of the head of the quality control unit and Subchapter 17.4 the responsibilities of the quality control unit as a whole. Subchapter 17.3 covers the requirements for resources. Correspondences to regula-

tion 211.25 are covered in Subchapters 9.2, 9.4, 9.7, and 10.1–10.4. Subchapters 9.2, 9.4, and 9.7 state the requirements for the personnel covering their education and experience and Subchapters 10.1–10.4 the requirements for the training. Correspondences to regulation 211.28 are covered in Subchapters 11.1–11.8, 9.5, and 16.7. Subchapters 11.1–11.5 state the requirements for the health conditions and personal hygiene and Subchapters 11.6–11.8 the requirements for the clothing and other protective apparel. Subchapters 9.5 and 16.7 cover the requirements for the limited access. Correspondences to regulation 211.34 are covered in Subchapter 10.6, which covers the requirements for the use of consultants.

2.1.4.3 Buildings and Facilities

In the United States GMP regulations on issues related to buildings and facilities are covered in Subpart C [7], which consists of regulations 211.42, 211.44, 211.46, 211.48, 211.50, 211.52, 211.56, and 211.58. Contents of Subpart C are presented in Table 18. Regulation 211.42 deals with design and construction features covering the requirements for the size, construction, and location of buildings used in the production. Furthermore, it states the requirements for the placement of equipment as well as the flow of materials and products and specifies operations, which have to be performed in separate or defined areas to prevent contamination or mix-ups. It also covers the special requirements for the facilities used in aseptic processing and facilities used in the production of penicillin. Regulation 211.44 states the requirements for lighting and 211.46 for ventilation, including the requirements for controls and air-handling systems. Furthermore, it states the special requirements for ventilation in the production of penicillin. Regulation 211.48 deals with plumbing covering requirements for the plumbing system, drains, and the quality of potable water. Regulation 211.50 deals with sewage, trash, and other refuse stating the requirements for their disposal. Regulation 211.52 covers the requirements for washing and toilet facilities. Regulation 211.56 deals with sanitation stating the requirements for the conditions to be maintained in the manufacturing facilities. It also states the requirements for handling of trash and organic waste. Furthermore, it states the requirements for the written procedures for sanitation operations and use of biocides, fumigating, cleaning, and sanitizing agents. It also states the

TABLE 18 Contents of Subpart C of Part 211 of U.S. GMP Regulations Covering Buildings and Facilities [7]

Section	Subject
CFR 211.42	Design and construction features
CFR 211.44	Lighting
CFR 211.46	Ventilation, air filtration, air heating and cooling
CFR 211.48	Plumbing
CFR 211.50	Sewage and refuse
CFR 211.52	Washing and toilet facilities
CFR 211.56	Sanitation
CFR 211.58	Maintenance

requirements for the use of biocides and the scope of sanitation procedures. Regulation 211.58 states the requirements for the maintenance of the buildings used in the production.

Correspondences in Canadian GMP Code In the Canadian GMP code [12] issues related to buildings and facilities are mainly covered in the interpretation of regulation C.02.004 (Premises) and partly in the interpretations of regulations C.02.005 (Equipment), C.02.007 (Sanitation), C.02.009 (Raw Material Testing), C.02.011 (Manufacturing Control), and C.02.029 (Sterile Products). Correspondences to regulation 211.42 are covered in Sections 1, 2, 2.3, 6, 6.2, and 6.4 of the interpretation of regulation C.02.004, in Section 15 of the interpretation of regulation C.02.011, and in section "Premises" of the interpretation of regulation C.02.029. Sections 1 and 2 of the interpretation of regulation C.02.004 cover the requirements for the size, construction, and location of buildings used in the production. Section 6.2 of the interpretation of regulation C.02.004 and Section 15 of the interpretation of regulation C.02.011 state the requirements for the placement of equipment. Requirements for the flow of materials and products are covered in Section 6 of the interpretation of regulation C.02.004 and operations, which have to be performed in separate or defined areas in Sections 2.3 and 6.4 of the interpretation of regulation C.02.004. The requirements for the facilities used in aseptic processing are covered in section "Premises" of the interpretation of regulation C.02.029 and the requirements for the facilities used in the production of penicillin in Section 11.1 of the interpretation of regulation C.02.004. Correspondences to regulation 211.44 stating the requirements for the lighting are covered in Section 6.5 of the interpretation of regulation C.02.004. Correspondences to regulation 211.46 are covered in Sections 3.6 and 4 of the interpretation of regulation C.02.004. Section 3.6 of the interpretation of regulation C.02.004 states the requirements for the air-handling systems and Section 4 the requirements for the control of temperature and humidity. The specific requirements regarding the production of penicillin are covered in Section 11.1. Correspondences to regulation 211.48 are covered in Sections 3.5 and 7 of the interpretation of regulation C.02.004, Section 3.7 of the interpretation of regulation C.02.005, Section 4 of the interpretation of regulation C.02.009, and section "Water Treatment Systems" of the interpretation of regulation C.02.029. Section 7 of the interpretation of regulation C.02.004 states the requirements for the utilities and support systems, including supplies of purified water. Section 3.7 of the interpretation of regulation C.02.005 states the requirements for the operation of water purification, storage, and distribution equipment. Requirements for the quality of water are covered in Section 4 of the interpretation of regulation C.02.009 and in section "Water Treatment Systems" of the interpretation of regulation C.02.029. The requirements for drains are covered in Section 3.5 of the interpretation of regulation C.02.004. Correspondences to regulation 211.50 stating the requirements for the handling of sewage and refuse are covered in Section 2.6 of the interpretation of regulation C.02.007. Correspondences to regulation 211.52 stating the requirements for washing and toilet facilities are covered in Section 5 of the interpretation of regulation C.02.004. Correspondences to regulation 211.56 stating the requirements for sanitation are covered in Sections 1 and 2 of the interpretation of regulation C.02.007. The Canadian GMP code does not state any separate requirements for the handling of organic waste. General requirements for the handling of waste

materials are covered in Section 2.6 of the interpretation of regulation C.02.007. Correspondences to regulation 211.58 stating the requirements for the maintenance of the premises are covered in Section 9 of the interpretation of regulation C.02.004.

Correspondences in EU GMP Code In the EU GMP code [15] issues related to buildings and facilities are mainly covered in Chapter 3 (Premises and Equipment) and partly in Annex 1 (Manufacture of Sterile Medicinal Products). Correspondences to regulation 211.42 are covered in the foreword of Chapter 3 and in Subchapters 3.6–3.8, 3.13, 3.22, 3.23, 3.26, and 3.33. The requirements for the size, construction and location of buildings used in the production are covered in the foreword of Chapter 3. Subchapter 3.8 states the requirements for the placement of equipment and Subchapter 3.7 for the flow of materials and products. Operations, which have to be performed in separate or defined areas, are specified in Subchapters 3.6, 3.13, 3.22, 3.23, 3.26, and 3.33. Annex 1 covers the requirements for facilities used in aseptic processing and Subchapter 3.6 the requirements for the facilities used in the production of penicillin. Correspondences to regulation 211.44 are covered in Subchapters 3.3 and 3.16, which state the requirements for lighting. Correspondences to regulation 211.46 are covered in Subchapters 3.3 and 3.12, which state the requirements for ventilation. The specific requirements for the production of penicillin are covered in Subchapter 3.6. Correspondences to regulation 211.48 are covered in Subchapters 3.10 and 3.11 and in Subsections 35 and 44 of Annex 1. Subchapter 3.10 states the requirements for the plumbing and Subchapter 3.11 the requirements for drains. Section 35 of Annex 1 covers the requirements for water treatment plants and distribution systems and Section 44 the requirements for the monitoring of water sources and water treatment equipment. More guidance on the quality of water is given in the EU guidance document *Note for Guidance on Quality of Water for Pharmaceutical Use* [58]. The EU GMP code does not have correspondence to regulation 211.50, which covers the requirements for the handling of sewage and other refuse. Correspondences to regulation 211.52 are covered in Subchapter 3.31, which covers the requirements for the facilities for washing and toilet purposes. Correspondences to regulation 211.56 are covered in Subchapters 3.2, 3.4, 3.43, and 4.26. Subchapters 3.2 and 3.4 cover the requirements for the conditions to be maintained in the manufacturing facilities. Subchapter 4.26 covers the procedures for cleaning and sanitization and Subchapter 3.43 the requirements for the sanitization of water pipes. The EU GMP code does not cover any separate requirements for the handling of organic waste. Correspondences to regulation 211.58 are covered in Subsection 3.2, which covers the requirements for the maintenance of the buildings used in the production.

Correspondences in WHO GMP Guideline In the WHO GMP guideline [36] issues related to buildings and facilities are mainly covered in Chapter 12 (Premises) and partly in Chapters 3 (Sanitation and Hygiene), 14 (Materials), and 15 (Documentation). Correspondences to regulation 211.42 are covered in Subchapters 12.1, 12.2, 12.4, 12.5, 12.10, 12.14, 12.17, 12.19, 12.22–12.26, and 12.33. The requirements for the size, construction, and location of buildings are stated in Subchapters 12.1, 12.4, and 12.5. Subchapters 12.2 and 12.26 cover the requirements for the placement of equipment and Subchapters 12.10 and 12.25 the requirements for the flow of

materials and products. Operations, which have to be performed in separate or defined areas, are specified in Subchapters 12.14, 12.17, 12.19, 12.22–12.24, and 12.33. The requirements for the facilities used in aseptic processing are covered in Chapter 9 of Annex 6 of the WHO TRS 902 [40] and the requirements for the facilities used in the manufacture of penicillin are in Subchapter 12.24. Correspondences to regulation 211.44 are covered in Subchapters 12.8 and 12.32, which state the requirements for lighting. Correspondences to regulation 211.46 are covered in Subchapters 12.8 and 12.30, which state the requirements for ventilation. The specific requirements for the production of penicillin are covered in Subchapter 12.24. Correspondences to regulation 211.48 are covered in Subchapters 12.28, 12.29, and 14.6 and in Annex 3 of the WHO TRS 929 [45]. Subchapter 12.28 states the requirements for the plumbing and Subchapter 14.6 for the quality of water used in the production of drug products. More guidance on the quality of water is given in Annex 3 of the WHO TRS 929 [45]. The requirements for the drains are stated in Subchapter 12.29. Correspondences to regulation 211.50 are covered in Subchapters 14.44 and 14.45, which state the requirements for the handling of sewage and other refuse. Correspondences to regulation 211.52 are covered in Subchapter 12.12, which states the requirements for the facilities for washing and toilet purposes. Correspondences to regulation 211.56 are covered in Subchapters 3.1, 12.7, 12.9, 14.44–14.46, and 15.48. Subchapters 12.7 and 12.9 state the requirements for the conditions to be maintained in the manufacturing facilities and Subchapter 3.1 the general requirements for sanitation and hygiene. In the WHO GMP guideline there is no separate guidance on the handling of organic waste. General requirements for the handling of waste materials are stated in Subchapters 14.44 and 14.45. Subchapter 15.48 states the requirements for the written procedures for sanitation operations and Subchapter 14.46 for the use of rodenticides, insecticides, fumigating agents, and sanitizing materials. Correspondences to regulation 211.58 are covered in Subchapter 12.6, which states the requirements for the maintenance of the buildings used in drug production.

2.1.4.4 Equipment

For GMP regulations in the United States issues related to equipment are covered in Subpart D [7], which consists of regulations 211.63, 211.65, 211.67, 211.68, and 211.72. Contents of Subpart D are presented in Table 19. Regulation 211.63 states the requirements for the production equipment covering design, size, and location. Regulation 211.65 states the requirements for the construction of equipment cover-

TABLE 19 Contents of Subpart D of Part 211 of U.S. GMP Regulations Covering Equipment [7]

Section	Subject
CFR 211.63	Equipment design, size, and location
CFR 211.65	Equipment construction
CFR 211.67	Equipment cleaning and maintenance
CFR 211.68	Automatic, mechanical, and electronic equipment
CFR 211.72	Filters

ing the characteristics of used materials and special requirements for the structure of the equipment. Regulation 211.67 deals with cleaning, maintenance, and sanitizing of equipment and utensils covering the requirements for the procedures for cleaning and maintenance operations. Regulation 211.68 deals with automatic, mechanical, and electronic equipment covering requirements for their calibration and inspection, including the requirements for the documentation of checks and inspections. Furthermore, it covers the requirements for the controls for computer or related systems, including the requirements for the maintenance of backup data. Regulation 211.72 covers the requirements for the filters for liquid filtration used in the manufacture of injectable products, including the specific requirements for the use of fiber-releasing and asbestos-containing filters.

Correspondences in Canadian GMP Code In the Canadian GMP code [12] issues related to equipment are mainly covered in the interpretation of regulation C.02.005 (Equipment) and partly in the interpretation of regulation C.02.007 (Sanitation) and C.02.024 (Records). Correspondences to regulation 211.63 stating the requirements for the design, construction, and location of equipment used in the manufacture of drug products are covered in Sections 1 and 5 of the interpretation of regulation C.02.005. Correspondences to regulation 211.65 stating the requirements for the construction of equipment are covered in Sections 2.1–2.3 of the interpretation of regulation C.02.005. Correspondences to regulation 211.67 stating the requirements for the sanitation are covered in Sections 1, 2, and 3 of the interpretation of regulation C.02.007. Correspondences to regulation 211.68 are covered in Section 5.4 of the interpretation of regulation C.02.005 and in the foreword of the interpretation of regulation C.02.024. Section 5.4 of the interpretation of regulation C.02.005 states the requirements for the use of automatic, mechanical, and electronic equipment, including computerized systems, and the foreword of the interpretation of regulation C.02.024 the requirements for the maintenance of backup data. The Canadian GMP code does not have correspondence to regulation 211.72, which states the requirements for the filters for liquid filtration used in the manufacture of injectable products. Nor does it cover requirements for the use of fiber-releasing or asbestos-containing filters.

Correspondences in EU GMP Code In the EU GMP code [15] issues related to equipment are mainly covered in Chapter 3 (Premises and Equipment) and partly in Chapter 4 (Documentation) and Annexes 1 (Manufacture of Sterile Medicinal Products) and 11 (Computerised Systems). Correspondences to regulation 211.63 are covered in Subchapter 3.34, which states the requirements for the design and location of equipment used in the manufacture of drug products. Correspondences to regulation 211.65 are covered in Subchapters 3.38 and 3.39, which state the requirements for the construction of equipment. Correspondences to regulation 211.67 are covered in Subchapters 3.36, 3.37, and 3.43, which cover the requirements for cleaning and sanitizing the manufacturing equipment. Correspondences to regulation 211.68 are covered in Subchapters 3.41 and 4.9 and Annex 11. Subchapter 3.41 states the requirements for the maintenance of measuring, weighing, recording, and control equipment and Subchapter 4.9 the requirements for the use of electronic data processing systems and the maintenance of backup data. Additional guidance on the use of computerized systems is given in Annex 11. Correspondences

to regulation 211.72 stating the requirements for filters for liquid filtration used in the sterile filtration are covered in Sections 84–87 of Annex 1. The EU GMP code does not have any separate guidance for the use of fiber-releasing or asbestos-containing filters.

Correspondences in WHO GMP Guideline In the WHO GMP guideline [36] issues related to equipment are mainly covered in Chapter 13 (Equipment) and partly in Chapters 14 (Materials), 15 (Documentation), and 16 (Good Practices in Production). Correspondences to regulation 211.63 are covered in Subchapters 13.1 and 13.2, which state the requirements for the design, location, and installation of equipment used in the manufacture of drug products. Correspondences to regulation 211.65 are covered in Subchapters 13.9 and 14.3, which state the requirements for the construction of equipment. Correspondences to regulation 211.67 are covered in Subchapters 13.6, 13.8, 13.12, 16.17, 16.18, and 16.22, which state the requirements for cleaning and sanitizing the equipment. Correspondences to regulation 211.68 are covered in Subchapters 16.23 and 15.9. The requirements for the maintenance of measuring, weighing, recording, and control equipment and instruments are covered in Subchapter 16.23. Subchapter 15.9 states the requirements for the use of electronic data-processing systems, including the requirements for the maintenance of backup data. Correspondences to regulation 211.72 stating the requirements for the use of filters are covered in Subchapters 7.6–7.9 of Annex 6 of the WHO TRS 902 [40]. Subchapter 7.6 covers the requirements for asbestos-containing filters.

2.1.4.5 Control of Components and Drug Product Containers and Closures

In the United States issues related to control of components and drug product containers and closures are covered in Subpart E [7], which consists of regulations 211.80, 211.82, 211.84, 211.86, 211.87, 211.89, and 211.94. Contents of Subpart E are presented in Table 20. Regulation 211.80 defines the requirements for the procedures for the control of components, containers, and closures. It also states the requirements for their handling, storing, and identification. Regulation 211.82 covers the requirements for receipt and storage of untested components, containers, and

TABLE 20 Contents of Subpart E of Part 211 of U.S. GMP Regulations Covering Control of Components and Drug Product Containers and Closures [7]

Section	Subject
CFR 211.80	General requirements
CFR 211.82	Receipt and storage of untested components, drug product containers, and closures
CFR 211.84	Testing and approval or rejection of components, drug product containers, and closures
CFR 211.86	Use of approved components, drug product containers, and closures
CFR 211.87	Retesting of approved components, drug product containers, and closures
CFR 211.89	Rejected components, drug product containers, and closures
CFR 211.94	Drug product containers and closures

closures. Regulation 211.84 deals with testing and approval or rejection of components, containers, and closures covering the requirements for sampling, testing, and release. Regulation 211.86 deals with the use of approved components, containers, and closures stating the requirements for the rotation of the storage. Regulation 211.87 states the requirements for the retesting of approved components, containers, and closures. Regulation 211.89 covers the requirements for the handling of rejected components, containers, and closures. Regulation 211.94 deals with drug product containers and closures covering the requirements for materials and the cleanliness of containers and closures. Furthermore, it states the requirements for container closure systems, standards and methods.

Correspondences in Canadian GMP Code In the Canadian GMP code [12] issues related to control of components and drug product containers and closures are covered in interpretations of regulations C.02.009 (Raw Material Testing), C.02.010 (Raw Material Testing), C.02.011 (Manufacturing Control), C.02.014 (Quality Control Department), C.02.016 (Packaging Material Testing), and C.02.017 (Packaging Material Testing). Correspondences to regulation 211.80 stating the general requirements for the handling, storing, and identification of components (raw materials) and drug product containers and closures (packaging materials) are covered in Sections 1, 20, and 21 of the interpretation of regulation C.02.011. Correspondences to regulation 211.82 stating the requirements for the receipt, testing, and storage of untested components and drug product containers and closures are covered in Sections 16, 18, and 19 of the interpretation of regulation C.02.011. Correspondences to regulation 211.84 stating the requirements for testing and approval of components and drug product containers and closures are covered in Sections 6 and 7 of the interpretation of regulation C.02.009, Sections 1–8 of the interpretation of regulation C.02.010, Sections 1 and 2 of regulation C.02.016, Section 4 of its interpretation, and Section 1 of the interpretation of regulation C.02.017. Interpretations 6 and 7 of regulation C.02.009 and interpretations 1–8 of regulation C.02.010 cover the requirements for components. Sections 1 and 2 and interpretation 4 of regulation C.02.016 and interpretation 1 of regulation C.02.017 state the requirements for drug product containers and closures. In the Canadian GMP code there is no correspondence to regulation 211.86, which covers the requirements for the rotation of the storage. Correspondences to regulation 211.87 stating the requirements for the retesting of approved components are covered in Sections 8–10 of the interpretation of regulation C.02.009. For the retesting of drug product containers and closures the Canadian GMP code has no guidance. Correspondences to regulation 211.89 stating the requirements for the handling of rejected components and drug product containers and closures are covered in Section 14 of the interpretation of regulation C.02.011 and in Section 5 of the interpretation of regulation C.02.014. The Canadian GMP code does not have correspondence to regulation 211.94, which covers the requirements for containers and closure systems.

Correspondences in EU GMP Code In the EU GMP code [15] issues related to control of components and drug product containers and closures are mainly covered in Chapter 5 (Production) and partly in Chapter 6 (Quality Control). Correspondences to regulation 211.80 are covered in Subchapters 5.2, 5.7, 5.10, 5.29, and 5.40–5.42, which cover the requirements for the handling, storing, and identification

of components (starting materials) and drug product containers and closures (primary packaging materials). Correspondences to regulation 211.82 are covered in Subchapters 5.5, 5.27, and 5.40, which state the requirements for the receipt, testing, and storage of untested components and drug product containers and closures. Correspondences to regulation 211.84 are covered in Subchapters 5.31, 5.40 and 6.11–6.22 and Annex 8. The general requirements for sampling and testing are covered in Subchapters 6.11–6.22. More guidance on sampling is given in Annex 8. The requirements for the approved use of components and drug product containers and closures are stated in Subchapters 5.31 and 5.40. Correspondences to regulation 211.86 are covered in Subchapter 5.7, which states the requirements for the storage conditions and rotation. Correspondences to regulation 211.87 are covered in Subchapters 5.29 and 5.40, which deal with the retesting of components and drug product containers and closures. Correspondences to regulation 211.89 are covered in Subchapter 5.61, which states the requirements for the handling of rejected components and drug product containers and closures. Correspondences to regulation 211.94 are covered in Subchapter 5.48, which states the requirements for drug product containers and closures.

Correspondences in WHO GMP Guideline In the WHO GMP guideline [36] issues related to control of components and drug product containers and closures are covered in Chapters 14 (Materials), 16 (Good Practices in Production), and 17 (Good Practices in Quality Control). Correspondences to regulation 211.80 are covered in Subchapters, 14.5, 14.13, 14.14, 14.19–14.21, and 16.2, which state the requirements for the handling, storing, and identification of components (starting materials) and drug product containers and closures (primary packaging materials). Correspondences to regulation 211.82 are covered in Subchapters 14.4, 14.9–14.11, and 14.19, which state the requirements for receipt, testing, identification, and storage of untested components and drug product containers and closures. Correspondences to regulation 211.84 are covered in Subchapters 14.12, 14.15, and 17.7–17.17. The requirements for sampling and testing are covered in Subchapters 17.7–17.17 and 14.12. Subchapter 14.15 states the requirements for the approved use of components and drug product containers and closures. Correspondences to regulation 211.86 are covered in Subchapter 14.5, which states the requirements for the storage conditions and the rotation of the storage. Correspondences to regulation 211.87 are covered in Subchapter 14.13, which states the requirements for the retesting of approved components. The WHO GMP guideline does not cover the requirements for the retesting of drug product containers and closures. Correspondences to regulation 211.89 are covered in Subchapter 14.28, which states the requirements for the handling of rejected components and drug product containers and closures. Correspondences to regulation 211.94 are covered in Subchapter 16.19, which states the requirements for the drug product containers and closures.

2.1.4.6 Production and Process Controls

In the United States GMP regulations on issues related to production and process controls are covered in Subpart F [7], which consists of regulations 211.100, 211.101, 211.103, 211.105, 211.110, 211.111, 211.113, and 211.115. Contents of Subpart F are presented in Table 21. Regulation 211.100 states the requirements for procedures

TABLE 21 Contents of Subpart F of Part 211 of U.S. GMP Regulations Covering Production and Process Controls [7]

Section	Subject
CFR 211.100	Written procedures, deviations
CFR 211.101	Charge-in of components
CFR 211.103	Calculation of yield
CFR 211.105	Equipment identification
CFR 211.110	Sampling and testing of in-process materials and drug products
CFR 211.111	Time limitations on production
CFR 211.113	Control of microbiological contamination
CFR 211.115	Reprocessing

regarding production and process controls, including the requirements for the documentation and handling of deviations. Regulation 211.101 deals with the requirements for the charge-in of components. Regulation 211.103 states the requirements for the determination of yields. Regulation 211.105 covers requirements for the identification of processing equipment such as containers, processing lines, and major equipment used during manufacture. Regulation 211.110 states the requirements for in-process controls, including the testing and approval of in-process materials and handling of rejected in-process materials. Regulation 211.111 covers the requirements for the time limitations on production, including the handling of deviations from established limits. Regulation 211.113 covers the control of microbiological contaminations. Regulation 211.115 states the requirements for the reprocessing of batches that do not conform to standards or specifications.

Correspondences in Canadian GMP Code In the Canadian GMP code [12] issues related to production and process controls are mainly covered in the interpretation of regulation C.02.011 (Manufacturing Control) and partly in the interpretations of regulations C.02.005 (Equipment), C.02.014 (Quality Control Department), and C.02.029 (Sterile Products). Correspondences to regulation 211.100 are covered in Sections 1–5 of the interpretation of regulation C.02.011. Interpretations 1–4 state the requirements for manufacturing processes and interpretation 5 for the handling of deviations. Correspondences to regulation 211.101 stating the requirements for charge-in of components are covered in Section 22 of the interpretation of regulation C.02.011. Correspondences to regulation 211.103 stating the requirements for the determination of yields including the handling of deviations from the expected yield are covered in Sections 6 and 7 of the interpretation of regulation C.02.011. Correspondences to regulation 211.105 stating the requirements for the identification of piping, containers, equipment, and rooms used in the manufacturing of drug products are covered in Section 3.5 of the interpretation of regulation C.02.005 and in Section 13 of the interpretation of regulation C.02.011. Correspondences to regulation 211.110 are covered in Sections 11 and 14 of the interpretation of regulation C.02.011 and in Section 5 of the interpretation of regulation C.02.014. Section 11 of the interpretation of regulation C.02.011 states the requirements for the in-process controls. The Canadian GMP code does not cover requirements for the testing of in-process materials. The handling of rejected materials is covered in Section 14 of the interpretation of regulation C.02.011 and in Section 5 of the interpretation

of regulation C.02.014. Correspondences to regulation 211.111 dealing with the requirements for the time limitations on production are covered in Section 24.7 of the interpretation of regulation C.02.011. Correspondences to regulation 211.113 are covered in the interpretation of regulation C.02.029, which deals with the manufacture of sterile products. Correspondences to regulation 211.115 stating the requirements for the reprocessing of batches that do not conform to specifications are covered in Sections 7–9 of the interpretation of regulation C.02.014.

Correspondences in EU GMP In the EU GMP code [15] issues related to production and process controls are mainly covered in Chapter 5 (Production) and partly in Chapters 3 (Premises and Equipment), 4 (Documentation), and 6 (Quality Control). Correspondences to regulation 211.100 are covered in Subchapters 5.2, 5.15, and 5.22–5.24. The requirements for manufacturing processes are covered in Subchapters 5.2 and 5.22–5.24. Subchapter 5.15 states the requirements for handling of deviations from instructions or procedures. Correspondences to regulation 211.101 are covered in Subchapters 5.28–5.34, which state the requirements for the charge-in of components. Correspondences to regulation 211.103 are covered in Subchapters 5.8 and 5.39, which state the requirements for determination of yields, including the handling of deviations from the expected yield. Correspondences to regulation 211.105 are covered in Subchapters 3.42 and 5.12, which state the requirements for identification of piping, containers, equipment, and rooms used in the manufacture of drug products. Correspondences to regulation 211.110 are covered in Subchapters 3.17, 4.10, 4.12, 5.38, 5.61, and 6.18. The requirements for in-process controls are covered in Subchapters 3.17, 5.38, and 6.18. Subchapters 4.10 and 4.12 state the requirements for the specifications for in-process materials (intermediate products) and Subchapter 5.61 for handling of rejected materials. The EU GMP code does not cover separate guidance on testing and approval of in-process materials. General guidance on sampling and testing is given in Subchapters 6.11–6.22. Correspondences to regulation 211.111, which deals with the time limitations on production, are covered in Chapter 4.15. Correspondences to regulation 211.113 are covered in Subchapter 5.10 and in Annex 1, which cover the requirements for the control of microbiological contaminations. Correspondences to regulation 211.115 are covered in Subchapters 5.62 and 5.64, which state the requirements for the reprocessing of rejected batches.

Correspondences in WHO GMP Guideline In the WHO GMP guideline [36] issues related to production and process controls are covered in Chapters 13 (Equipment), 14 (Materials), 15 (Documentation), 16 (Good Practices in Production), and 17 (Good Practices in Quality Control). Correspondences to regulation 211.100 are covered in Subchapters 16.1–16.3. Subchapters 16.1 and 16.2 state the requirements for the manufacturing operations and Subchapter 16.3 for the handling of deviations from instructions or procedures. Correspondences to regulation 211.101 are covered in Subchapters 14.12–14.18, which state the requirements for the charge-in of components. Correspondences to regulation 211.103 are covered in Subchapters 16.4 and 16.20, which state the requirements for the determination of yields, including the handling of deviations from the expected yield. Correspondences to regulation 211.105 are covered in Subchapters 13.3, 13.4, and 16.6, which state the requirements for the identification of piping, containers, equipment, and rooms used during pro-

duction. Correspondences to regulation 211.110 are covered in Subchapters 14.28, 15.20, 16.9, 16.16, and 17.8. Subchapters 16.9, 16.16, and 17.8 cover the requirements for the in-process controls and Subchapter 15.20 for the specifications for in-process materials (intermediate products). The requirements for the handling of rejected materials are stated in Subchapter 14.28. Correspondences to regulation 211.111, which deals with the time limitations on production, are covered in Chapter 15.23. Correspondences to regulation 211.113 are covered in Subchapters 16.10–16.14 and in Annex 6 of the WHO TRS 902 [40]. Subchapters 16.10–16.14 cover general requirements for the prevention of cross-contamination and bacterial contamination during production and Annex 6 general requirements for the manufacture of sterile drug products. Correspondences to regulation 211.115 are covered in Subchapters 14.29, 14.31, and 15.40, which state the requirements for the reprocessing of rejected batches.

2.1.4.7 Packaging and Labeling Control

For GMP regulations in the United States issues related to packaging and labeling control are covered in Subpart G [7], which consists of regulations 211.122, 211.125, 211.130, 211.132, 211.134, and 211.137. The contents of Subpart G is presented in Table 22. Regulation 211.122 deals with materials examination and usage criteria covering the requirements for the receipt, identification, storage, handling, sampling, testing, and approval of labeling and packaging materials, including documentation. Furthermore, it covers the requirements for the control of labeling, handling of obsolete and outdated labeling and packaging materials, and special requirements for different labeling methods. Regulation 211.125 states the requirements for the labeling issuance covering the testing of labeling materials, the control of discrepancy between the quantities of labeling issued, used, and returned, and the handling of excess and returned labeling. Regulation 211.130 states the requirements for the packaging and labeling operations covering the written procedures. Regulation 211.132 states the requirements for the tamper-evident packaging. Regulation 211.134 states the requirements for the inspections of packaged and labeled products covering sampling, examination, and documentation. Regulation 211.137 states the requirements for the expiration dates, including exemptions from the requirements.

TABLE 22 Contents of Subpart G of Part 211 of U.S. GMP Regulations Covering Packaging and Labeling Control [7]

Section	Subject
CFR 211.122	Materials examination and usage criteria
CFR 211.125	Labeling issuance
CFR 211.130	Packaging and labeling operations
CFR 211.132	Tamper-evident packaging requirements for over-the-counter (OTC) human drug products
CFR 211.134	Drug product inspection
CFR 211.137	Expiration dating

Correspondences in Canadian GMP Code In the Canadian GMP code [12] issues related to packaging and labeling control are covered in the interpretations of regulations C.02.011 (Manufacturing Control), C.02.017 (Packaging Material Testing), C.02.016 (Packaging Material Testing), C.02.019 (Finished Product Testing), and C.02.027 (Stability). Correspondences to regulation 211.122 are covered in Sections 1, 16, 40, and 43–48 of the interpretation of regulation C.02.011, Sections 1, 8, and 9 of the interpretation of regulation C.02.017, and Sections 1 and 4–7 of the interpretation of regulation C.02.016. Sections 1, 16, 43, and 48 of the interpretation of regulation C.02.011 state the general requirements for the handling of packaging and labeling materials covering receipt and storage. Section 8 of the interpretation of regulation C.02.017 states the requirements for the identification of the packaging and labeling materials. The requirements for the testing of the packaging and labeling materials are covered in Sections 1 and 9 of the interpretation of regulation C.02.017. Sections 1 and 4 of the interpretation of regulation C.02.016 and Sections 6 and 7 of the interpretation of regulation C.02.017 state the requirements for the approval of packaging and labeling materials. Sections 44–47 of the interpretation of regulation C.02.011 cover requirements for the use of roll-fed labels, cut labels, gang printing, and the monitoring of the performance of printing. The requirements for the handling of obsolete and outdated packaging and labeling materials are covered in Section 40 of the interpretation of regulation C.02.011 and in Section 5 of the interpretation of regulation C.02.016. Correspondences to regulation 211.125 are covered in Sections 39 and 42 of the interpretation of regulation C.02.011 and in Section 8 of the interpretation of regulation C.02.017. Section 8 of the interpretation of regulation C.02.017 states the requirements for the examination of packaging and labeling materials. The requirements for the control and handling of discrepancy between the quantities of labeling issued, used, and returned are covered in Section 42 and the requirements for the handling of unused batch-coded packaging and labeling materials in Section 39 of the interpretation of regulation C.02.011. Correspondences to regulation 211.130 stating the requirements for the packaging and labeling operations are covered in Sections 29–38 of the interpretation of regulation C.02.011. In Canadian GMP code there is no correspondence to regulation 211.132 stating the requirements for the tamper-evident packaging. Correspondences to regulation 211.134 stating the requirements for the inspections of packaged and labeled products are covered in Section 1 of the interpretation of regulation C.02.019. Correspondences to regulation 211.137 stating the requirements for the expiration dates are covered in regulation C.02.027 and in Section 1 of its interpretation.

Correspondences in EU GMP Code In the EU GMP code [15] issues related to packaging and labeling control are mainly covered in Chapter 5 (Production) and partly in Chapters 4 (Documentation) and 6 (Quality Control). Correspondences to regulation 211.122 are covered in Subchapters 4.11, 4.19, 4.21–4.23, 5.2, 5.40–5.43, and 5.50–5.52. Subchapters 4.19, 4.21–4.23, 5.2, and 5.40–5.42 cover the requirements for purchase, handling, control, storage, and identification of packaging and labeling materials. Specifications for packaging and labeling materials are stated in Subchapter 4.11 and the requirements for handling of outdated or obsolete packaging and labeling materials in Subchapter 5.43. Subchapter 5.51 covers the requirements for the use of cut-labels, off-line overprinting, and roll-feed labels. The requirements for

the control of the printing and labeling operations are stated in Subchapters 5.50 and 5.52. Correspondences to regulation 211.125 are covered in Subchapters 5.2, 5.56, and 5.57. Subchapter 5.2 states the general requirements for the handling of packaging and labeling materials. The requirements for the control of discrepancy between the quantities of labeling issued, used, and returned are covered in Subchapter 5.56 and the requirements for the handling of unused batch-coded packaging and labeling materials in Subchapter 5.57. Correspondences to regulation 211.130 are covered in Subchapters 5.2 and 5.44–5.49. Subchapter 5.2 states the general requirements for the handling of packaging and labeling materials and Subchapters 5.44–5.49 cover the requirements for the packaging and labeling operations. The European Community GMP code does not have correspondence to regulation 211.132, which covers the requirements for the tamper-evident packaging. Correspondences to regulation 211.134 are covered in Subchapters 5.54 and 6.3, which state the requirements for the control of packaged and labeled products. The EU GMP code does not have correspondence to regulation 211.137, which covers the requirements for expiration dates.

Correspondences in WHO GMP Guideline In the WHO GMP guideline [36] issues related to packaging and labeling control are covered in Chapters 6 (Product Recalls), 12 (Premises), 14 (Materials), 15 (Documentation), 16 (Good Practices in Production), and 17 (Good Practices in Quality Control). Correspondences to regulation 211.122 are covered in Subchapters 12.21, 14.19–14.23, 15.18, 16.2, 17.14, and 17.16. Subchapters 6.2, 12.21, 14.19–14.21, 14.23, and 17.16 state the requirements for the purchase, handling, control, storage, and identification of packaging and labeling materials. The requirements for the approval of packaging and labeling materials are covered in Subchapters 17.14 and 15.18. Subchapter 14.20 states the requirements for the use of roll-feed and cut labels and Subchapter 14.22 the requirements for the handling of outdated and obsolete packaging and labeling materials. Correspondences to regulation 211.125 are covered in Subchapters 16.2, 16.34, and 16.35. Subchapter 16.2 states the general requirements for the handling of packaging and labeling materials and Subchapter 16.34 the requirements for the handling of discrepancy between the quantities of labeling issued, used, and returned. The requirements for the handling of unused batch-coded packaging and labeling materials are covered in Subchapter 16.35. Correspondences to regulation 211.130 are covered in Subchapters 16.25–16.30, which state the requirements for the packaging and labeling operations. The WHO GMP guideline does not cover correspondence to regulation 211.132, which covers the requirements for tamper-evident packaging. Correspondences to regulation 211.134 are covered in Subchapter 16.32, which states the requirements for the control of packaged and labeled products. Correspondences to regulation 211.137 are covered in Subchapter 17.24, which states the requirements for the determination of expiration dates and shelf-life specifications.

2.1.4.8 Holding and Distribution

In the United States GMP regulations on issues related to holding and distribution are covered in Subpart H [7], which consists of regulations 211.142 and 211.150. The contents of Subpart H is presented in Table 23. Regulation 211.142 states the

TABLE 23 Contents of Subpart H of Part 211 of U.S. GMP Regulations Covering Holding and Distribution [7]

Section	Subject
CFR 211.142	Warehousing procedures
CFR 211.150	Distribution procedures

requirements for the warehousing procedures covering quarantine and storage and regulation 211.150 for the distribution procedures covering distribution order and recalls.

Correspondences in Canadian GMP Code In the Canadian GMP code [12] issues related to holding and distribution are covered in the interpretations of regulations C.02.004 (Premises), C.02.011 (Manufacturing Control), C.02.012 (Manufacturing Control), and C.02.019 (Finished Product Testing). Correspondences to regulation 211.142 stating the requirements for the quarantine and storage of products are covered in Sections 1 and 49 of the interpretation of regulation C.02.011, Section 11.4 of the interpretation of regulation C.02.004, and Section 2 of the interpretation of regulation C.02.019. Correspondences to regulation 211.150 stating the requirements for distribution and recalls are covered in Section 1 of the interpretation of regulation C.02.011 and Section 1 of the interpretation of regulation C.02.012.

Correspondences in EU GMP Code In the EU GMP code [15] issues related to holding and distribution are covered in Chapters 4 (Documentation), 5 (Production), and 8 (Complaints and Product Recall). Correspondences to regulation 211.142 are covered in Subchapters 5.2, 5.58, and 5.60, which state the requirements for the storage and quarantine of products. Correspondences to regulation 211.150 are covered in Subchapters 4.25, 5.2, and 8.8–8.15, which state the requirements for distribution and recalls.

Correspondences in WHO GMP Guideline In the WHO GMP guideline [36] issues related to holding and distribution are covered in Chapters 6 (Product Recalls), 14 (Materials), 15 (Documentation), and 16 (Good Practices in Production). Correspondences to regulation 211.142 are covered in Subchapters 14.4, 14.26, and 16.2, which state the requirements for the storage and quarantine of products. Correspondences to regulation 211.150 are covered in Subchapters 6.1–6.8, 15.45, and 16.2, which state the requirements for distribution and recalls.

2.1.4.9 Laboratory Controls

In the United States GMP regulations [7] issues related to laboratory controls are covered in Subpart I, which consists of regulations 211.160, 211.165, 211.166, 211.167, 211.170, 211.173, and 211.176. The contents of Subpart I is presented in Table 24. Regulation 211.160 states the requirements for the establishment of laboratory controls such as specifications, standards, sampling plans, and test procedures. Furthermore, it covers the requirements stated for the calibration of instruments, apparatus, gauges, and recording devices. Regulation 211.165 states the require-

TABLE 24 Contents of Subpart I of Part 211 of U.S. GMP Regulations Covering Laboratory Controls [7]

Section	Subject
CFR 211.160	General requirements
CFR 211.165	Testing and release for distribution
CFR 211.166	Stability testing
CFR 211.167	Special testing requirements
CFR 211.170	Reserve samples
CFR 211.173	Laboratory animals
CFR 211.176	Penicillin contamination

ments for the laboratory testing of batches prior to release covering the requirements for sampling, testing, and approval. Furthermore, it states the requirements for the handling of rejected drug products. Regulation 211.166 states the requirements for stability testing, including the requirements for the determination of expiration dates and the requirements for stability testing of homeopathic drug products. Regulation 211.167 deals with special testing requirements covering sterile products, ophthalmic ointments, and controlled-release dosage forms. Regulation 211.170 states the requirements for reserve samples covering identification, quantity, retention time, and storage. Furthermore it covers the requirements for the deterioration investigations. Regulation 211.173 deals with laboratory animals covering the requirements for their maintenance and control. Regulation 211.176 states the requirements for the testing of penicillin contamination and the handling of penicillin contaminated drug product.

Correspondences in Canadian GMP Code In the Canadian GMP code [12] issues related to laboratory controls are covered in the interpretations of regulations C.02.004 (Premises), C.02.009 (Raw Material Testing), C.02.011 (Manufacturing Control), C.02.014 (Quality Control Department), C.02.015 (Quality Control Department), C.02.016 (Packaging Material Testing), C.02.017 (Packaging Material Testing), C.02.018 (Finished Product Testing), C.02.025 (Samples), C.02.026 (Samples), C.02.027 (Stability), and C.02.028 (Stability). Correspondences to regulation 211.160 stating the general requirements for laboratory controls are covered in regulation C.02.009 and Sections 1–3 and 5–6 of its interpretation, regulation C.02.016 and Sections 1–3 of its interpretation, Section 1 of the interpretation of regulation C.02.017, regulation C.02.018 and Sections 1–5 of its interpretation, and Section 6.4 of the interpretation of regulation C.02.015. Correspondences to regulation 211.165 stating the requirements for the release for distribution including the testing of finished drug products and the handling of rejected drug products are covered in Sections 7 and 14 of the interpretation of regulation C.02.011, Sections 2 and 5 of the interpretation of regulation C.02.014, Section 3 of the interpretation of regulation C.02.015, and Section 2 of regulation C.02.018 and Sections 1 and 4 of its interpretation. Correspondences to regulation 211.166 stating the requirements for stability testing are covered in Section 1 of the interpretation of regulation C.02.027 and Sections 1 and 2 of the interpretation of regulation C.02.028. The Canadian GMP code does not cover separate requirements for the stability testing

of homeopathic drug products. Correspondences to regulation 211.167 stating the requirements for sterility testing are covered in Sections 1–4 of the interpretation of regulation C.02.029 (Sterile Products). The Canadian GMP code does not have any guidance covering the testing of ophthalmic ointments and controlled-release dosage forms. Correspondences to regulation 211.170 stating the requirements for reserve samples are covered in Section 1 of regulation C.02.025 and in regulation C.02.026 and Sections 1 and 3–5 of their interpretation. Correspondences to regulation 211.173 stating the requirements for laboratory animals are covered in Section 2.4 of the interpretation of regulation C.02.004. The Canadian GMP code does not have correspondence to regulation 211.176, which covers the requirements for the testing and handling of penicillin contamination.

Correspondences in EU GMP Code In the EU GMP code [15] issues related to laboratory controls are covered in Chapters 1 (Quality Management), 4 (Documentation), 5 (Production), and 6 (Quality Control) and in Annexes 1 (Manufacture of Sterile Medicinal Products), 9 (Manufacture of Liquids, Creams, and Ointments), and 19 (Reference and Retention Samples). Correspondences to regulation 211.160 are covered in Subchapters 1.4, 4.2, 4.3, 4.10–4.13, 5.15, 6.7, and 6.18, which cover the general requirements for laboratory controls. Correspondences to regulation 211.165 are covered in Subchapters 4.22, 4.23, 5.61, 5.62, 6.3, 6.11, and 6.15, which state the requirements for the release for distribution, the testing of finished drug products, and the handling of rejected drug products. The EU GMP code does not have correspondence to regulation 211.166, which states the requirements for stability testing. However, there is a separate guideline, *Stability Testing on Active Ingredients and Finished Products* [59], which provides guidance on issues related to stability testing. Furthermore, Subchapters 6.23–6.33 cover the requirements for the on-going stability program. Correspondences to regulation 211.167 are covered in Annexes 1 and 9. Section 93 of Annex 1 covers the requirements for sterility testing and Annex 9 the requirements for ointments. In the EU GMP code there is no guidance on the testing of the controlled-release dosage forms. Correspondences to regulation 211.170 are covered in Subchapters 1.4 and 6.12 and Annex 19, which state the requirements for reserve samples. Correspondences to regulation 211.173 are covered in Subchapters 3.33 and 6.22, which state the requirements for the maintenance of animals. The EU GMP code does not have correspondence to regulation 211.176, which covers the requirements for the testing and handling of penicillin contaminations.

Correspondences in WHO GMP Guideline In the WHO GMP guideline [36] issues related to laboratory controls are covered in Chapters 14 (Materials), 15 (Documentation), 16 (Good Practices in Production), and 17 (Good Practices in Quality Control). Correspondences to regulation 211.160 are covered in Subchapters 15.14–15.16, 15.18–15.21, 16.3, and 16.23, which state the general requirements for laboratory controls. Correspondences to regulation 211.165 are covered in Subchapters 14.28, 14.29, 15.13, 15.42, 17.7–17.13, 17.19, and 17.20, which state the requirements for the release for distribution covering the testing of finished drug products and the handling of rejected drug products. Correspondences to regulation 211.166 are covered in Subchapters 17.23–17.26, which state the requirements for stability testing. The WHO GMP guideline does not cover separate requirements

for the stability testing of homeopathic drug products. Correspondences to regulation 211.167 are covered in Annex 6 of the WHO TRS 902 [40], which states the requirements for sterility testing. The WHO GMP guideline does not cover any requirements for the testing of ophthalmic ointments or controlled-release dosage forms. Correspondences to regulation 211.170 are covered in Subchapter 17.22, which states the requirements for reserve samples. The WHO GMP guideline does not have correspondence to regulation 211.173, which covers the requirements for the maintenance of laboratory animals. Nor does it have correspondence to regulation 211.176, which covers the requirements for the testing of penicillin contaminations.

2.1.4.10 Records and Reports

In the United States issues related to records and reports are covered in Subpart J [7], which consists of regulations 211.180, 211.182, 211.184, 211.186, 211.188, 211.192, 211.194, 211.196, and 211.198. The contents of Subpart J is presented in Table 25. Regulation 211.180 states the general requirements for documentation covering maintenance, retention times, and availability of the records. Furthermore, it states the requirements for the annual quality standards evaluation. Regulation 211.182 states the requirements for individual equipment logs. Regulation 211.184 states the requirements for component, drug product container, closure, and labeling records. Regulation 211.186 states the requirements for master production and control records. Regulation 211.188 states the requirements for batch production and control records. Regulation 211.192 states the requirements for the review and approval of production and control records, including the requirements for the investigation of any unexplained discrepancies. Regulation 211.194 states the requirements for laboratory records, including the requirements for the documentation of modifications. Furthermore, it covers the requirements for the documentation of the testing and standardization of reference standards, reagents, and standard solutions; calibration of laboratory instruments and recording devices; and stability tests. Regulation 211.196 states the requirements for the distribution records. Regulation 211.198 states the requirements for the handling of complaints, including the maintenance and retention times of complaint files.

TABLE 25 Contents of Subpart J of Part 211 of U.S. GMP Regulations Covering Records and Reports [7]

Section	Subject
CFR 211.180	General requirements
CFR 211.182	Equipment cleaning and use log
CFR 211.184	Component, drug product container, closure, and labeling records
CFR 211.186	Master production and control records
CFR 211.188	Batch production and control records
CFR 211.192	Production record review
CFR 211.194	Laboratory records
CFR 211.196	Distribution records
CFR 211.198	Complaint files

Correspondences in Canadian GMP Code In the Canadian GMP code [12] issues related to records and reports are mainly covered in regulations C.02.021, C.02.022, C.02.023, and C.02.024 (Records) and in their interpretations and partly in the interpretations of regulations C.02.005 (Equipment), C.02.010 (Raw Material Testing), C.02.011 (Manufacturing Control), C.02.012 (Manufacturing Control), C.02.014 (Quality Control Department), C.02.015 (Quality Control Department), and C.02.017 (Packaging Material Testing). Correspondences to regulation 211.180 stating the general requirements for the maintenance of records, including periodic quality evaluation (self-inspection) and the retention time of the records are covered in regulations C.02.021, C.02.022, C.02.023, and C.02.024 and their interpretations and in Section 2 of the interpretation of regulation C.02.012. Correspondences to regulation 211.182 stating the requirements for individual equipment logs are covered in Section 5.5 of the interpretation of regulation C.02.005. Correspondences to regulation 211.184 stating the requirements for records to be kept on components, drug product containers, closures, and labeling are covered in Sections 4 and 5 of the interpretation of regulations C.02.020–24, Section 5 of the interpretation of regulation C.02.010, and Section 7 of the interpretation of regulation C.02.017. Correspondences to regulation 211.186 stating the requirements for the master production and control records (manufacturing and packaging master formulas) are covered in Sections 23–25 of the interpretation of regulation C.02.011 and Section 1.1 of the interpretation of regulations C.02.020–24. Correspondences to regulation 211.188 stating the requirements for the batch production and control records (manufacturing and packaging batch document) are covered in Sections 26, 27, 29, and 30 of the interpretation of regulation C.02.011 and Section 1.2 of the interpretation of regulations C.02.020–24. Correspondences to regulation 211.192 stating the requirements for review and approval of production and control records including investigation of batch deviations are covered in Section 2 of the interpretation of regulation C.02.014. Correspondences to regulation 211.194 stating the requirements for laboratory records are covered in Sections 6.4, 6.6, and 6.7 of the interpretation of regulation C.02.015. Correspondences to regulation 211.196 stating the requirements for distribution records are covered in Section 1.6 of the interpretation of regulation C.02.012 and Section 2.1 of the interpretation of regulations C.02.020–24. Correspondences to regulation 211.198 stating the requirements for the maintenance of complaint files including retention times are covered in Section 4 of the interpretation of regulation C.02.015, Section 3.1 of the interpretation of regulations C.02.020–24, and regulation C.02.023.

Correspondences in EU GMP Code In the EU GMP code [15] issues related to records and reports are mainly covered in Chapter 4 (Documentation) and partly in Chapters 1 (Quality Management), 5 (Production), 6 (Quality Control), 8 (Complaints and Product Recall), and 9 (Self Inspection). Correspondences to regulation 211.180 are covered in Subchapters 4.1–4.9, 6.8, and 9.1–9.3, which state the general requirements for the maintenance of the records, including periodic quality evaluation (self-inspection) and retention times. Correspondences to regulation 211.182 are covered in Subchapters 4.28 and 4.29, which state the requirements for individual equipment logs. Correspondences to regulation 211.184 are covered in Subchapters 4.19 and 4.20, which state the requirements for the records to be kept on the receipt of components, drug product containers, closures, and labeling. Correspon-

dences to regulation 211.186 are covered in Subchapters 4.14–4.16, which state the requirements for the master production and control records (manufacturing formula, processing, and packaging instructions). Correspondences to regulation 211.188 are covered in Subchapters 4.17 and 4.18, which state the requirements for the batch production and control records (batch processing and packaging record). Correspondences to regulation 211.192 are covered in Subchapters 1.4, 4.3, 4.24, 5.8, and 5.39, which state the requirements for review and approval of production and control records, including the investigation of unexplained discrepancies. Correspondences to regulation 211.194 are covered in Subchapters 3.41, 6.7, 6.17, 6.20, and 6.21, which state the requirements for laboratory records. Correspondences to regulation 211.196 are covered in Subchapter 4.25, which states the requirements for distribution records. Correspondences to regulation 211.198 are covered in Subchapters 4.26 and 8.1–8.8, which state the requirements for the handling of complaints.

Correspondences in WHO GMP Guideline In the WHO GMP guideline [36] issues related to records and reports are covered in Chapters 5 (Complaints), 8 (Self-Inspection and Quality Audits), 13 (Equipment), 14 (Materials), 15 (Documentation), 16 (Good Practices in Production), and 17 (Good Practices in Quality Control). Correspondences to regulation 211.180 are covered in Subchapters 8.1–8.6 and 15.1–15.9, which state the general requirements for the maintenance of the records, including periodic quality evaluation (self-inspection) and retention times. Correspondences to regulation 211.182 are covered in Subchapters 15.46 and 15.47, which state the requirements for individual equipment logs. Correspondences to regulation 211.184 are covered in Subchapters 15.32 and 15.33, which state the requirements for the records to be kept on the receipt of components, drug product containers, closures, and labeling. Correspondences to regulation 211.186 are covered in Subchapters 15.22–15.24, which state the requirements for the master production and control records (master formula and packaging instructions). Correspondences to regulation 211.188 are covered in Subchapters 15.25–15.30, which state the requirements for the batch production and control records (batch processing and packaging records). Correspondences to regulation 211.192 are covered in Subchapters 16.4, 16.20, and 17.21, which state the requirements for review and approval of production and control records covering also the requirements for the investigation of unexplained discrepancies. Correspondences to regulation 211.194 are covered in Subchapters 13.5, 14.34, 14.35, 14.41, 15.12, 15.42, 15.43, and 16.23, which states the requirements for laboratory records. Correspondences to regulation 211.196 are covered in Subchapter 15.45, which states the requirements for the distribution records. Correspondences to regulation 211.198 are covered in Subchapters 5.1–5.10, which state the requirements for the handling of complaints.

2.1.4.11 Returned and Salvaged Drug Products

In the United States GMP regulation [7] issues related to returned and salvaged drug products are covered in Subpart K, which consists of regulations 211.204 and 211.208. The contents of Subpart K is presented in Table 26. Regulation 211.204 states the requirements for the handling of returned drug products, including repro-

TABLE 26 Contents of Subpart K of Part 211 of U.S. GMP Regulations Covering Returned and Salvaged Drug Products [7]

Section	Subject
CFR 211.204	Returned drug products
CFR 211.208	Drug product salvaging

cessing and documentation. Regulation 211.208 states the requirements for drug product salvaging.

Correspondences in Canadian GMP Code In the Canadian GMP code [12] issues related to returned and salvaged drug products are covered in the interpretation of regulation C.02.014 (Quality Control Department). Correspondences to regulation 211.204 stating the requirements for the handling of returned drug products are covered in Section 4 of the interpretation of regulation C.02.014. The Canadian GMP code does not have correspondence to regulation 211.208, which covers the requirements for drug product salvaging.

Correspondences in EU GMP Code In the EU GMP code [15] issues related to returned and salvaged drug products are covered in Chapters 4 (Documentation) and 5 (Production). Correspondences to regulation 211.204 are covered in Subchapters 4.26 and 5.26, which state the requirements for the handling of returned drug products. The EU GMP code does not have correspondence to regulation 211.208, which covers the requirements for drug product salvaging.

Correspondences in WHO GMP Guideline In the WHO GMP guideline [36] issues related to returned and salvaged drug products are covered in Chapter 14 (Materials). Correspondences to regulation 211.204 are covered in Subchapter 14.33, which states the requirements for the handling of returned drug products. The WHO GMP guideline does not have correspondence to regulation **211.208**, which covers the requirements for drug product salvaging.

REFERENCES

1. Immel, B. K. (2001), A brief history of the GMPs for pharmaceuticals, *Pharm. Technol. No. Am.*, 25(7), 44–48.
2. Anonymous *Pharmaceutical Administration and Regulations in Japan*, Japan Pharmaceutical Manufacturers Association, available: http://www.jpma.or.jp/english/library/pdf/2005.pdf.
3. Vesper, J. L. (2003), So what are GMPs, anyway? *BioProcess Int.*, 1(2), 24–29.
4. Rosin, L. J. (2006), Regulatory affairs: If you didn't write it down, it didn't happen, *BioProcess Int.*, 4(3, Suppl), 16–23.
5. Anonymous (2002), Sec. 351: Adulterated drugs and devices, in *United States Code*, Title 21, Chapter 9, Subchapter V, Part A, U.S. Government Printing Office, Washington, DC, available: http://frwebgate.access.gpo.gov/cgi-bin/getdoc.cgi?dbname=browse_usc&docid=Cite:+21USC351.

6. Anonymous (2005), Part 210: Current good manufacturing practice in manufacturing, processing, packing, or holding of drugs: General, in *Code of Federal Regulations*, Title 21, Chapter I, U.S. Government Printing Office, Washington, DC, pp. 118–119, available: http://www.access.gpo.gov/nara/cfr/waisidx_05/21cfr210_05.html.
7. Anonymous (2005), Part 211: Current good manufacturing practice for finished pharmaceuticals, in *Code of Federal Regulations*, Title 21, Chapter I, U.S. Government Printing Office, Washington, DC, pp. 120–141, available: http://www.access.gpo.govnara/cfr/waisidx_05/21cfr211_05.html.
8. Grazal, J. G., and Earl, D. S. (1997), EU and FDA regulations: Overview and comparison, *Qual. Assur. J.*, 2, 55–60.
9. Anonymous (2006), Guidance page, available: http://www.fda.gov/cder/guidance/index.htm.
10. Anonymous (2001), Guidance for industry: Q7A Good manufacturing practice guidance for active pharmaceutical ingredients, Office of Training and Communications, available: http://www.fda.gov/cber/gdlns/ichactive.pdf.
11. Anonymous (2005), Division 2: Good manufacturing practices, in *Consolidated Statutes and Regulations*, Food and Drugs Act, Food and Drug Regulations, Part C, Department of Justice, Canada, available: http://laws.justice.gc.ca/en/f-27/c.r.c.-c.870/230079.html.
12. Anonymous (2002), Good manufacturing practices guidelines, Version 2, Health Products and Food Branch Inspectorate, available: http://www.hc-sc.gc.ca/dhp-mps/alt_formats/hpfb-dgpsa/pdf/compli-conform/2002v2_e.pdf.
13. Anonymous (2001), Directive 2001/83/EC of the European Parliament and of the Council, *Off. J. Eur. Union*, 44(L311), 67–128, available: http://europa.eu.int/eur-lex/pri/en/oj/dat/2001/l_311/l_31120011128en00670128.pdf.
14. Anonymous (2003), Commission directive 2003/94/EC, *Off. J. Eur. Union*, 46(L262), 22–26, available: http://europa.eu.int/eur-lex/pri/en/oj/dat/2003/l_262/l_26220031014en00220026.pdf.
15. Anonymous (2005), EU guidelines to good manufacturing practice, in *The Rules Governing Medicinal Products in the European Union*, Vol. 4, European Commission Enterprise and Industry Directorate-General, available: http://pharmacos.eudra.org/F2/eudralex/vol-4/home.htm.
16. Anonymous (1998), Quality and biotechnology, in *The Rules Governing Medicinal Products in the European Union*, Vol. 3A, European Commission Directorate General III, available: http://pharmacos.eudra.org/F2/eudralex/vol-3/home.htm.
17. Anonymous (2005), *Regulations for Buildings and Facilities of Pharmacies*, etc., MHLW Ministerial Ordinance, Yakuji Nippon Ltd., Tokyo, No.73.
18. Kim, Y.-O., Ha, K.-W., and Choi, K.-S. (2001), Safety evaluation for new drug approval in Korea, *Drug Info. J.*, 35(1), 285–291.
19. Shin, S.-G. (1998), Current status of clinical trials in the Republic of Korea, *Drug Info. J.*, 32(Suppl), 1217S–1222S.
20. Anonymous (2000), *Competition in the Pharmaceutical Industry—Republic of Korea*, Working Party No. 2 on Competition and Regulation, Committee on Competition Law and Policy, OECD Publishing, Paris.
21. Anonymous (2001), *Drug Administration Law of the People's Republic of China*, Order of the President of the People's Republic of China No. 45, available: http://www.sfda.gov.cn/cmsweb/webportal/W45649037/A48335975.html.
22. Deng, R., and Kaitin, K. I. (2004), The regulation and approval of new drugs in China, *Drug Info. J.*, 38(1), 29–39.

23. Anonymous (2005), The Drugs and Cosmetics Act and Rules, Ministry of Health and Family Welfare, Department of Health, available: http://cdsco.nic.in/html/Drugs&CosmeticAct.pdf.
24. Anonymous (2005), Schedule M: Good manufacturing practices and requirements of premises, plant and equipment for pharmaceutical products, in The Drugs and Cosmetics Act and Rules, Ministry of Health and Family Welfare, Department of Health, pp. 386–436, available: http://cdsco.nic.in/html/Drugs&CosmeticAct.pdf.
25. Venkateswarlu, M. (2006), Why do we need revision of schedule M, available: http://www.pharmabiz.com, 2006.
26. Anonymous (2006), Section 36: Manufacturing principles, in *Therapeutic Goods Act 1989*, Office of Legislative Drafting and Publishing, pp. 96–97, available: http://www.tga.gov.au/legis/index.htm#instruments.
27. Slater, T. (2002), Therapeutic goods (manufacturing principles) determination no 2 of 2002, *Commonwealth Austral. Gaz.*, GN 34, 2306–2307.
28. Anonymous (2002), Australian code of good manufacturing practice for medicinal products, Therapeutic Goods Administration, available: http://www.tga.gov.au/docs/pdf/gmpcodau.pdf.
29. Anonymous (2005), Medicines Act 1981, Parliamentary Counsel Office, available: http://www.legislation.govt.nz/browse_vw.asp?content-set=pal_statutes.
30. Anonymous (2001), Guidance notes for applicants for consent to distribute new and changed medicines and related products, in *New Zealand Regulatory Guidelines for Medicines*, Vol. 1, 5th ed., MedSafe, available: http://www.medsafe.govt.nz/downloads/vol1.doc.
31. Anonymous (2005), New Zealand code of good manufacturing practice for manufacture and distribution of therapeutic goods, available: http://www.medsafe.govt.nz/Regulatory/Guideline/code.htm.
32. Anonymous (2002), Medicines and Related Substances Control Act 101 of 1965, Medicines Control Council, available: http://www.mccza.com/showdocument.asp?Cat=27&Desc=Acts%20and%20Regulations.
33. Anonymous (2003), General regulations made in terms of the Medicines and Related Substances Act 1965 (Act no. 101 of 1965) as Amended, Government Notice, Department of Health, available: http://www.mccza.com/showdocument.asp?Cat=27&Desc=Acts%20and%20Regulations.
34. Anonymous (2005), Guide to Good Manufacturing Practice for Medicines in South Africa, Medicines Control Council, available: http://www.mccza.com/showdocument.asp?Cat=21&Desc=Guidelines%20-%20Good%20Manufacturing%20Practices.
35. Anonymous (2002), WHO Expert Committee on Specifications for Pharmaceutical Preparations: 37th Report, WHO Technical Report Series 908, World Health Organization, Singapore, available: http://whqlibdoc.who.int/trs/WHO_TRS_908.pdf.
36. Anonymous (2002), Annex 4: Good manufacturing practices for pharmaceutical products: Main principles, in WHO Expert Committee on Specifications for Pharmaceutical Preparations: 37th Report, WHO Technical Report Series 908, World Health Organization, Singapore, pp. 36–89, available: http://whqlibdoc.who.int/trs/WHO_TRS_908.pdf.
37. Anonymous (2005), Annex 2: Good manufacturing practices: Requirement for the sampling of starting materials (Amendment), in WHO Expert Committee on Specifications for Pharmaceutical Preparations: 39th Report, WHO Technical Report Series 929, World Health Organization, Singapore, pp. 38–39, available: http://whqlibdoc.who.int/trs/WHO_TRS_929_eng.pdf.
38. Anonymous (1992), Annex 1: Good manufacturing practices for pharmaceutical products: Good manufacturing practices for active pharmaceutical ingredients (bulk drug

substances), in WHO Expert Committee on Specifications for Pharmaceutical Preparations: 32th Report, WHO Technical Report Series 823, World Health Organization, Geneva, pp. 72–79, available: http://whqlibdoc.who.int/trs/WHO_TRS_823.pdf.

39. Anonymous (1998), Annex 5: Good manufacturing practices: Supplementary guidelines for the manufacture of pharmaceutical excipients, in WHO Expert Committee on Specifications for Pharmaceutical Preparations: 35th Report, WHO Technical Report Series 885, World Health Organization, Madrid, Spain, pp. 50–71, available: http://whqlibdoc.who.int/trs/WHO_TRS_885.pdf.
40. Anonymous (2002), Annex 6: Good manufacturing practices for sterile pharmaceutical products, in WHO Expert Committee on Specifications for Pharmaceutical Preparations: 36th Report, WHO Technical Report Series 902, World Health Organization, Singapore, pp. 76–93, available: http://whqlibdoc.who.int/trs/WHO_TRS_902.pdf.
41. Anonymous (1993), Annex 3: Good manufacturing practices for biological products, in WHO Expert Committee on Specifications for Pharmaceutical Preparations: 33th Report, WHO Technical Report Series 834, World Health Organization, Geneva, pp. 20–30, available: http://whqlibdoc.who.int/trs/WHO_TRS_834.pdf.
42. Anonymous (1995), Annex 7: Good manufacturing practices: Supplementary guidelines for the manufacture of investigational pharmaceutical products for clinical trials in humans, in WHO Expert Committee on Specifications for Pharmaceutical Preparations: 34th Report, WHO Technical Report Series 863, World Health Organization, Geneva, pp. 97–108, available: http://whqlibdoc.who.int/trs/WHO_TRS_863_(p1-p98).pdf (pp. 97–98); http://whqlibdoc.who.int/trs/WHO_TRS_863_(p99-p194).pdf (pp. 99–108).
43. Anonymous (1995), Annex 8: Good manufacturing practices: Supplementary guidelines for the manufacture of herbal medicinal products, in WHO Expert Committee on Specifications for Pharmaceutical Preparations: 34th Report, WHO Technical Report Series 863, World Health Organization, Geneva, pp. 109–113, available: http://whqlibdoc.who.int/trs/WHO_TRS_863_(p99-p194).pdf.
44. Anonymous (2002), Annex 3: Guidelines on good manufacturing practices for radiopharmaceutical products, in WHO Expert Committee on Specifications for Pharmaceutical Preparations: 37th Report, WHO Technical Report Series 908, World Health Organization, Singapore, pp. 26–35, available: http://whqlibdoc.who.int/trs/WHO_TRS_908.pdf.
45. Anonymous (2005), Annex 3: WHO Good manufacturing practices: Water for pharmaceutical use, in WHO Expert Committee on Specifications for Pharmaceutical Preparations: 39th Report, WHO Technical Report Series 929, World Health Organization, Singapore, pp. 40–58, available: http://whqlibdoc.who.int/trs/WHO_TRS_929_eng.pdf.
46. Anonymous (2006), Background to PIC, available: http://www.picscheme.org/indexnoflash.php?p=backg.
47. Anonymous (2006), Introduction, available: http://www.picscheme.org/indexnoflash.php?p=intro.
48. Anonymous (2006), List of PIC/S participating authorities (& observers), available: http://www.picscheme.org/indexnoflash.php?p=members.
49. Brunner, D. (2004), Pharmaceutical inspection co-operation scheme (PIC/S), *Qual. Assur. J.*, 8, 207–211.
50. Anonymous (2006), Guide to good manufacturing practice for medicinal products, PE 009–3, Pharmaceutical inspection co-operation scheme, available: http://www.picscheme.org/guides.php#.
51. Anonymous (2006), Structure of ICH, available: http://www.ich.org/cache/html/510-272-1.html.

52. Anonymous (2000), Good manufacturing practice guide for active pharmaceutical ingredients Q7, ICH harmonised tripartite guideline, ICH Steering Committee, available: http://www.ich.org/LOB/media/MEDIA433.pdf.
53. Anonymous (2006), Quality guidelines, available: http://www.ich.org/cache/compo/363-272-1.html.
54. Anonymous (2006), The founding of ASEAN, available: http://www.aseansec.org/7069.htm.
55. Anonymous (2006), Pharmaceuticals, available: http://www.aseansec.org/8657.htm.
56. Vernengo, M. J. (1998), Advances in pharmaceutical market integration in MERCOSUR and other Latin American countries, *Drug Info. J.*, 32(3), 831–839.
57. Anonymous (2005), Division 1A establishment licences, in *Consolidated Statutes and Regulations*, Food and Drugs Act, Food and Drug Regulations, Part C, Department of Justice Canada, available: http://laws.justice.gc.ca/en/f-27/c.r.c.-c.870/230049.html.
58. Anonymous (2002), Note for guidance on quality of water for pharmaceutical use, CPMP/QWP/158/01, Committee for Proprietary Medicinal Products, Quality Working Party, London, available: http://www.emea.eu.int/pdfs/human/qwp/015801en.pdf.
59. Anonymous (1998), Stability testing on active ingredients and finished products, in *The Rules Governing Medicinal Products in the European Union*, Vol. 3A, European Commission Directorate General III, pp. 143–151, available: http://pharmacos.eudra.org/F2/eudralex/vol-3/pdfs-en/3aq16aen.pdf.

SECTION 3

QUALITY

3.1

ANALYTICAL AND COMPUTATIONAL METHODS AND EXAMPLES FOR DESIGNING AND CONTROLLING TOTAL QUALITY MANAGEMENT PHARMACEUTICAL MANUFACTURING SYSTEMS

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3.1.1 INTRODUCTION

Total quality management (TQM) and operation control in pharmaceutical manufacturing system design engineering is essential. TQM-focused pharmaceutical manufacturing system engineering involves the continual satisfaction of customer requirements at lowest cost by harnessing the efforts of everybody in the company. Quality assurance means sustaining a system that prevents defects. This includes quality control and quality engineering. Quality control means establishing and maintaining specified quality standards of products; quality engineering is the establishment and execution of tests to measure product quality and adherence to acceptance criteria.

This chapter explains the importance of reducing variation for the purpose of implementing total quality in every process of the pharmaceutical design and manufacturing enterprise. Furthermore, it represents a modular product, process, service design, implementation, and management approach to the introduction of various TQM methods, tools, technologies, and their management issues within a variety of small, medium, and large enterprises for the purpose of designing and controlling pharmaceutical manufacturing systems.

These aspects are very important, clearly illustrated by the fact that the U.S. Food and Drug Administration (FDA) has three classification levels for medical products:

- Class I products are passive devices that do not enter the patient's body or contact only the skin.
- Class II products are active devices or devices that are used to administer fluids to the patient's body.
- Class III products are implanted inside the patient's body.

The FDA is familiar with the complexity of designing pharmaceutical systems. To support this activity, there are several software tools that help product/process and system designers to achieve the above.

It should also be noted that the FDA expects design validation results to accompany some submissions. This is particularly true of class II and III devices. The agency expects such analysis results to match those obtained with established experimental methods. A number of software tools, including finite element analysis (FEA), motion and actuation simulation, computational fluid dynamics (CFD), in conjunction with the computer-aided design (CAD) used for the designs themselves and other solutions are available that help today's pharmaceutical/medical designer/medical manufacturing/assembly system designer to meet the complex requirements of the industry as well as the FDA. (The key, here, is to accept the important

principle that pharmaceutical design and manufacturing/assembly and even packaging must be an integrated approach.)

The main problems when applying a traditional quality management philosophy to any pharmaceutical design/manufacturing/assembly challenge include the following:

- This philosophy focuses on correcting mistakes after they have been made, rather than preventing them in the first place.
- It allows mistakes to be made. It actually builds them into every aspect of the system, typically costing around 20% of the turnover.
- It accepts that quality has to be sacrificed as the volume and the productivity go up.
- As viewed by accountants, it is an expensive add on item of the value chain.

However, modern thinking claims that, because TQM involves every person, aspect, and machine of the organization, it requires a total commitment. It is not a "test-and-fix" approach. It is a preventive system designed into every aspect of the world-class design, manufacturing, and service enterprise, including product design, manufacture, and management (and even in accounting terms costing somewhat less than conventional quality systems, i.e., typically around 10% of the turnover).

The fundamental goal of TQM and TQC (total quality management and control) is to program, measure, and keep process variability under control. Some of these methods discussed in this chapter are as follows:

- Pharmaceutical manufacturing system design methods and tools with examples
- Process modeling for designing and running pharmaceutical manufacturing systems
- Requirements analysis modeling for pharmaceutical manufacturing systems
- Risk analysis modeling for pharmaceutical manufacturing systems
- Dynamic modeling and network simulation for globally distributed pharmaceutical manufacturing systems and other methods and tools

3.1.2 FLEXIBLE PHARMACEUTICAL MANUFACTURING AND ASSEMBLY SYSTEM DESIGN

A flexible pharmaceutical manufacturing/assembly system, (FMS) is a highly automated, distributed feedback-controlled system of data, information, and physical processors, such as computer and manually controlled machines, cells, workstations, and robots, in which decisions have to be made often in real time. This is only possible if all information processors (including the human resources of such systems) are "well informed" and lean/flexible, meaning that they have the exact information at the exact time, format, and mode they need to allow responsible decision making within given time constraints. Note that this is a fundamentally different system design concept than that of the transfer line, operating on a fixed cycle time, and designed for large batch production [1–8].

When designing a flexible manufacturing/assembly system (FMS/FAS), the design team should consider the following steps:

1. Collect all current and possible future user and system requirements.
2. Analyze the system (i.e., the data processing and the FMS/ FAS hardware and software constraints).
3. Design an appropriate data structure and database for describing processors and their resources, such as machines, robots, and tools (and/or robot hands, probes, sensory-based inspection and assembly tools, etc.).
4. Specify and design programs and query routines and dialogues that are capable of accessing this database as well as communicating with the real-time production planning and control system of the FMS/FAS.
5. Design and integrate the system with the rest of the hardware and software, including on-line manuals, education, and training packages, preferably in interactive, engineering multimedia format.
6. Maintain the system and continuously learn for the benefit of the existing as well as future system designs.

Probably the most important questions to be answered before starting to design such a system are: Who is going to use it? For what purposes? With what data? How will it be used?

As an example, consider that tooling data in FMSs will typically be used by several subsystems as well as by human beings are as follows:

- Production planning subsystem
- Process control
- Part programming
- Tool preset and tool maintenance
- Tool assembly (manual or robotized)
- Stock control and material storage

By employing the above subsystems, the production planning system has to be informed in real time about the availability of tools in stock as well as about the current contents of the tool magazines of the machine tools (in the case of FASs the robot hands in the end-of-arm-tool magazines); otherwise it will not be able to generate a proper production schedule.

It must be noted that the real-time aspect is important because tools are changed in the magazines of machines (or cells), not only because they wear, but also because different part programs may need different sets of tools. (The actual tool-changing operation is done in most cases by manipulators or by robots. The tool magazine loading/unloading procedure is performed mostly by human operators, sometimes by robots or special-purpose mechanisms, such as a tool shuttle.)

Both the process control and the production planning systems have to update any changes and act in real time; otherwise the operation of the system can be disrupted.

From the FMS/FAS tooling and tool management points of view one must emphasize the links between the CAD system, in which the parts are designed (using design for manufacturing principles), and the computer-aided manufacturing (CAM) system, where the FMS part programs are written. Typically, an FMS part programmer analyzes the CAD output (i.e., the design drawings of the pharmaceuti-

cal products to be manufactured/assembled on the FMS), the fixturing, the different setup (i.e., work-mounting) tasks, as well as the necessary operations, their alternatives, the required tools, and finally a precedence list of the resources (i.e., the possible candidates of processing stations, or cells, or machines).

Real-time databases and software systems are also important, since they provide the reports and status information that are needed for the smooth operation of the FMS (in particular, its dynamic scheduler and other subsystems such as maintenance should be emphasized here) [4, 9–14].

3.1.3 A FLEXIBLE MANUFACTURING MODEL INTEGRATED WITH DESIGN

The output of the CAM system is a production rule base. This is the knowledge the FMS needs to produce each pharmaceutical product. In this production rule base, among others, tools are assigned to each operation. The tool codes are selected by the FMS process planner or automatically assigned by a process planning system and are obtained from the tool database.

On the basis of the requested tools a list is sent via the network to the tool preparation facility, or station, where the actual tools are prepared (i.e., assembled and preset) and stored in an appropriate way such that the material-handling system of the FMS can pick them up [12–21].

The tool preparation station also deals with other activities, among which the most important are as follows:

- Tool service and maintenance
- Tool assembly to orders (as it is necessary to replace worn tools)
- Tool preset, tool inspection and adjustment
- Real-time tool pickup and tool transportation organized to serve the needs of the real-time FMS

The tool preparation station receives its orders, initially originated by the CAD data processing system, via the FMS network and technically specified by the CAM system in the form of a production rule base. Order data arriving at the tool preparation station include the following:

- Part orders (consisting of part codes and quantities). Note that this is a very important data set for the real-time FMS dynamic scheduler too.
- Notification of when the parts are physically available for FMS processing, representing a due date for tool preparation.
- A priority order (note that this can change because of some real-time changes in the system, and thus this station must be able to cope with this task too).
- The portion of the production rule base describing the requirements regarding tool preparation.

The tool preparation station keeps in touch with the real-time FMS system, as well as with the rest of the system, by feeding back important tooling system-related data:

- Stock reviews (regarding tools)
- FMS status report (regarding tools)
- Part priority status reports (in case dynamic changes must be performed in the FMS which have an effect on tooling needs and tool preparation due dates)

3.1.4 REAL-TIME OPERATION CONTROL

The real-time part of the FMS operation control and management system must deal with the following tasks:

- It must handle the application of tools for a variety of processes as defined in the production rule base and assigned in real time to the FMS/FAS resources by the dynamic scheduler.
- It must provide data to control the transportation of tools and tool magazines within the FMS.
- It must provide information to perform and supervise tool changes and tool magazine changes at all levels.
- It must be notified of tool inspection results (e.g., if it finds a worn-out tool as a result of an inspection procedure, it must generate a command that instructs the tool magazine update system to change the tool in question in the appropriate tool magazine).
- It must provide information in the case of emergency.
- It must provide the necessary interfaces and data to perform diagnostic/recovery operations, preferably using diagnostic expert systems.

Finally, let us underline an important feedback loop starting at the real-time system and ending at the tool preparation station, which contains the real-time tool status, wear, and part priority information. These data are often useful to those people and/or system software systems that deal with the generation of the production rule base. It is also a very useful data set for FMS designers, since a lot of data which would previously have been lost will be saved in this way.

The most important operation control activities in FMS/FAS identify three levels at which simulation and optimization are required prior to or during FMS/FAS part manufacturing:

1. The factory level or business level handled by the business system of the computer integrated manufacturing (CIM) or, even broader, the enterprise resource management system
2. The FMS off-line level representing scheduling, simulation, and optimization activities prior to loading a batch or a single component on the FMS (handled sometimes by the CAM system, sometimes by the FMS part programming computer)
3. The real-time controlled level handled by the FMS/FAS operation control system, a dynamic scheduler with integrated tool management and multimedia support, representing a situation where the parts are already physically as well as logically in the real-time controlled environment

Due to its complexity, a truly integrated approach is required in designing a production rule base to provide the job description for the FMS dynamic scheduler. This is because the dynamic system relies heavily on the knowledge base as represented by the rule base, and an overly restrictive rule base will lead to inefficient, at times even wrong, decisions. In other words, such a structure should represent all the multilevel interactions and their possible precedence rules that relate to the manufacturing process planning and processing decisions in an FMS. This turns out to be a difficult task.

It should be underlined that the application of multimedia at this level is extremely beneficial in terms of part program preparation, teaching/training operators on setting up parts, fixtures, tools, machines, for troubleshooting, for regular maintenance, at the computer numerical control (CNC) level programming, robot programming, placement machine programming, programmable logic controller (PLC) programming, quality control, maintenance, and other tasks.

Most FMSs have some part-buffering capability. This may be not for scheduling reasons, but for technological, that is, process-planning, reasons (e.g., the part must cool before an accurate inspection procedure is performed). Some level of buffering is useful and necessary because of reliability reasons. (The actual number of buffer store locations should be established on the basis of simulation and experience.)

Cells often have some buffers too. The reason for this is that, by providing a part in the input queue of the cell just before the currently processed part is finished at the particular cell, the cell is kept running at its highest efficiency level, since time is only "wasted" for part changing. The other important point to note is that well-designed part buffers offer a direct access pickup/load facility, making the rescheduling process in the queues short, simple, and dynamic [18, 19, 21–27].

3.1.5 INNOVATIVE DESIGN

The key objective of this chapter is to describe a generic and systematic pharmaceutical manufacturing/assembly system design method that includes product, process, service systems, and even innovation project management architecture aspects of such systems.

This architecture must be simultaneously novel as well as compliant with set guidelines by the product/process design industry and the PMI (Project Management Institute), following International Organization for Standardization (ISO) 9000:2000 quality standards. Our tested pharmaceutical manufacturing system design solution integrates object-oriented process modeling, requirements and risk analysis, statistical methods, design of experiments, and three-dimensional (3D) interactive multimedia methods and tools which are 100% Web compatible. Furthermore, our methods and software tools are generic in that they can be applied not only to systems such as the pharmaceutical industries or automobile manufacturing but also to processes such as the oil business or services such as education.

A pharmaceutical manufacturing system design requires significant level of innovation. The broadest definition of innovation is the act of introducing something new to a society or community, whether a product or process. This is often confused with invention, which focuses more on specific objects. Within pharmaceuticals innovation can therefore include new business structures within the company,

manufacturing processes and quality control for the medications, and product materials. Process and service improvements can also qualify as innovation, but note that in this case services are usually counted as processes [4, 13, 21, 23, 28–32].

Discoveries such as the charting of new planets, land masses, or forms of life are not classified as innovations as they had existed before being observed by humans. When new species are introduced into a society and find a specific use, it can be classified as innovation. A pharmaceutical example of this is the antibiotic penicillin. Although it had existed as a fungal secretion, it was only within the past century that it was used to actively eliminate infectious bacteria. In initial analysis, however, it was not thought to survive long enough within the human body to be effective.

This brings a vital aspect of innovation, namely the ability to recognize alternative uses for existing processes or tools. This is difficult as unexpected changes within a system are usually labeled as mistakes or anomalies. The development of the Post-It note is an example of this. The original goal was to create a high-strength adhesive, and an extremely weak one was created by accident. Nonetheless, instead of simply disposing of it, the possibilities of this new substance were examined by technicians and managers alike, allowing the use of easily placed reminders for everyday usage. Possessing an ultraweak adhesive allows Post-It notes to be removed without damaging the surfaces that they are placed on, and they are available in a variety of colors and sizes.

The former example is a radical innovation, not only because it allowed significant changes in message reminders and adhesives, but also because it was completely unexpected. The diversification of Post-It notes into different sizes and colors is an example of incremental innovation, which involves step-by-step changes and improvements to existing products or processes. Radical innovations are far less common, though their effects are farther reaching over both society and history.

The general trend through human history has been one of learning to consciously recognize and direct innovation, particularly through combining science and technology. Human societies have often worked with certain processes even without fully understanding their effects or underlying ideas. Metallurgy shows this clearly, as iron, bronze, and gold have been used for millennia before the molecular structure could be seen and analyzed. Note also that, although our ancestors could not describe their chemical composition, these metals served a great many successful purposes. In these cases, the goals of innovation are highly pragmatic, as successful solutions are passed down and taught to future generations.

Those who innovate can therefore learn from working, viable solutions to begin their own practices. Those who continuously work with a fixed set of designs must be willing to experiment, test, and diversify their practices to avoid stratification, as innovation not only allows survival but also encourages prosperity.

The ability to innovate also involves learning from past mistakes, not just one's own. Mistakes and errors in practices can be both costly and dangerous but can be prevented from occurring successively if their causes are determined. This can be difficult because a near-miss scenario can be seen either as an infrequent event or as an averted disaster. The first reaction to this is usually to continue without changing current practices, allowing for similar mistakes to occur. Learning requires all levels of an organization to participate and create channels of communication to innovate effectively, as the inability to share experience denies new opportunities [13, 29–40].

3.1.6 OPEN INNOVATION ARCHITECTURE

Innovation as a process and the related research-and-development (R&D) project management are considered to be two of the most complex information systems and engineering architectures due to the large number of attributes, processes, and dynamic changes projects go through during their life cycle.

Following our integrated and simultaneously open architectural approach, we look at every innovation process and project as a system built of objects and classes of objects.

Then we look at the way the components of these systems interact with each other. Once we understand these behaviors, we follow our integrated system approach in terms of looking at the project management system as processes, trying to satisfy customer requirements and also representing risks.

We then embed this system model into a statistical analysis and 3D interactive multimedia framework (Figure 1). We use statistical methods to capture processes before they go out of control as well as to perform trend analysis, a great opportunity for innovation, and use 3D interactive multimedia and 3D visualization methods over the Web for communication purposes with global innovation team members. The emphasis on collaboration in today's competitive medical drug field requires these virtual environments to streamline team interaction. (Note that the active

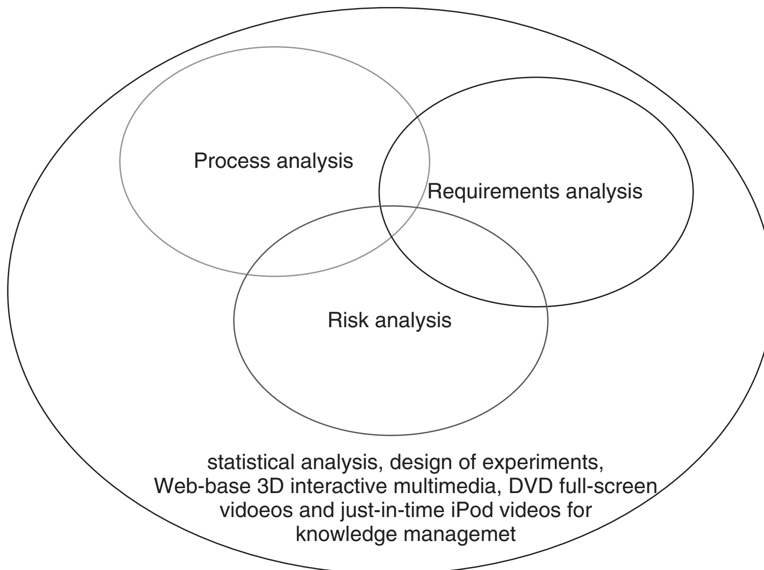


FIGURE 1 When designing lean and flexible pharmaceutical manufacturing/assembly/packaging systems, one needs to analyze the required processes, customer, user, maintenance, quality, reliability, flexibility, lean, design requirements, and risks involved with any of the listed processes, all in a statistical framework. (Note that our 3D interactive multimedia and simulation framework supports integrated digital design and digital manufacturing system design principles, meaning that one should test all designs and systems first on the screen, and only if everything looks fine, in the real world.)

code spreadsheets and 3D objects referred to in this presentation are all part of Ranky's eLibrary and are available at http://www.cimwareukandusa.com.)

To illustrate the importance of the "openness" of our architecture, consider modern simulation/analysis tools by Parametric Technology Corporation (PTC) (Figure 2), and PLM (product life-cycle management) tools, such as the IBM/Dassault Systemes Delmia tools for pharmaceutical manufacturing system modeling and design (Figure 3) with sensory feedback processing (Figure 4).

Since these models can be designed, edited, run, and driven even over the limits, they can be extremely valuable sources for modeling in the digital domain, process analysis, requirements modeling, risk analysis, and even collecting statistical data and modeling breakdowns of complex systems.

Observe the FEA in Figure 2*a*. This is a torsional test of a pharmaceutical manufacturing machine element on the assembly line of a new medication packaging line. The line is still being tested and improved in the virtual environment, which greatly streamlines the refinement process. As can be seen by the von Mises stress distribution, the sharp edges of the shaft will need to be rounded with a fillet. These would also increase the distribution of the same stress and thus reduce the majority of the red zones (high stress) to blue or even green (low stress). Without using the virtual assembly line to test ideas before for the physical, the unexpected failure of this part could create delays or contamination of product or even harm human operators.

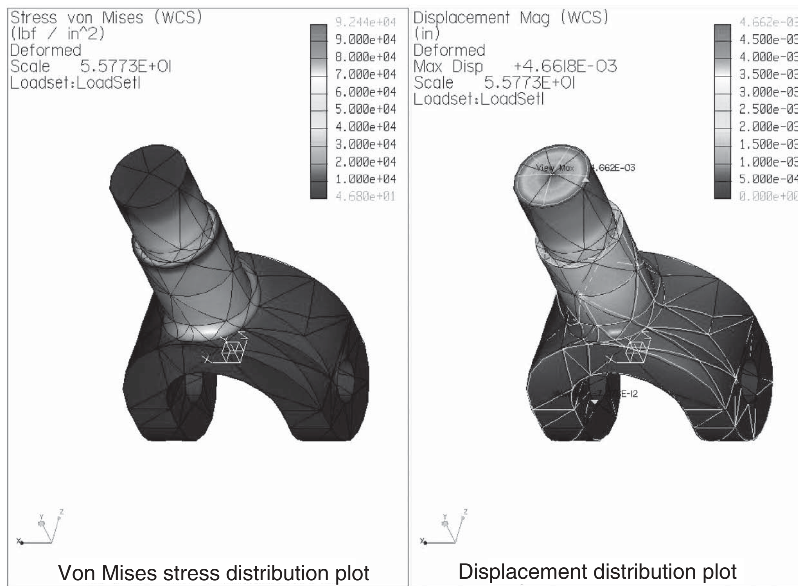
As can be seen, digital pharmaceutical manufacturing/assembly/packaging and factory design tools include not only machines but also advanced sensors, actuators, controls, material-handling systems, labeling machines, and even ergonomically realistic human models and operators performing real-world tasks in extremely realistic model factories. Simulations like these are not just pretty models; they actually save huge investments because the factories are not built until the models are satisfactory. Keep in mind that making changes in a physical factory costs time, money, and possibly production efficiency, even to just check a possible improvement. Virtual models can be simultaneously run thousands of times over a period of days, with hundreds of variables being optimized until the appropriate combination is chosen [30–36, 38, 40–44].

3.1.7 GENERIC, OBJECT-ORIENTED INNOVATION PROCESS MODELING METHOD AND SAMPLE MODEL

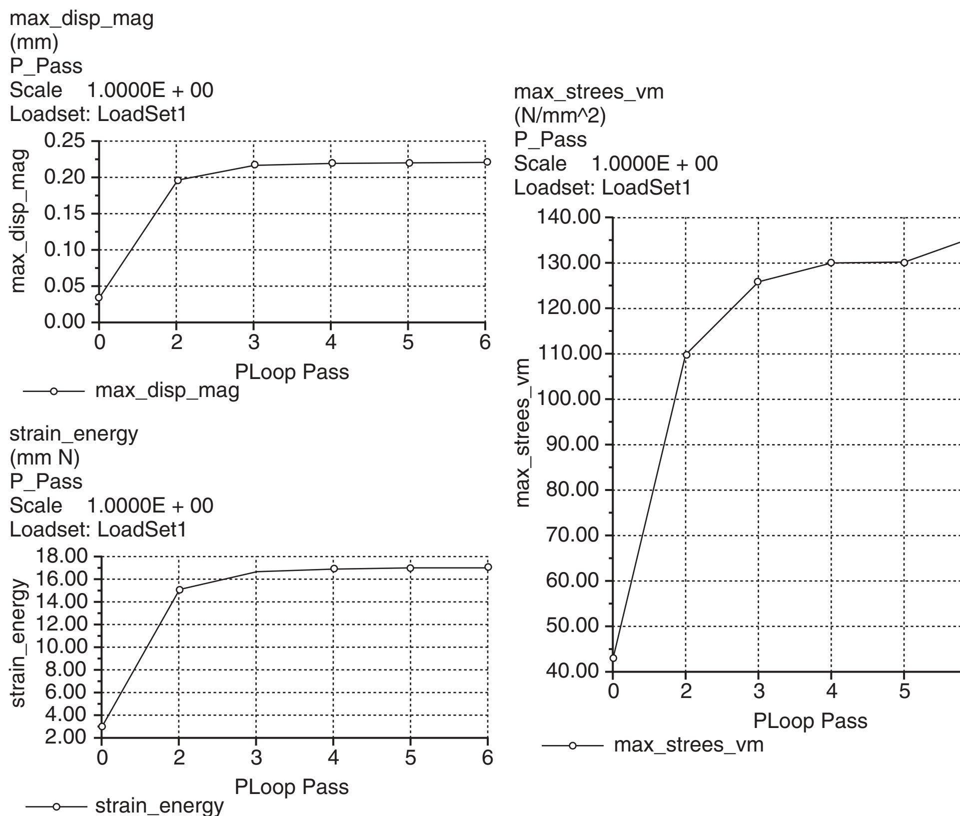
Understanding, modeling, and then following processes, procedures, and best practice reusable processes are essential for every business to stay at the top. The pharmaceutical manufacturing system "innovation business" is not exception.

Major international product/process design standards written and reviewed by thousands of leading researchers and companies around the world always help to create a model for complex problem-solving challenges such as innovation. Therefore this section discusses two of the eight quality management principles of the ISO 9000:2000 international quality standard and the way these rules should be applied to pharmaceutical manufacturing system designs.

We do this for the purpose of developing systematic innovation (with related project modeling skills) and reusable, tested pharmaceutical system design



(*a*)



(*b*)

FIGURE 2 Finite element torsional test of pharmaceutical manufacturing machine element on assembly line of new medication packaging line. The line is still being tested and improved in the virtual environment, which greatly streamlines the refinement process. As can be seen by the von mises stress distribution, the sharp edges of the shaft will need to be rounded with a fillet. These would also increase the distribution of the same stress and thus reduce the majority of the red zones (high stress) to blue or even green (low stress).

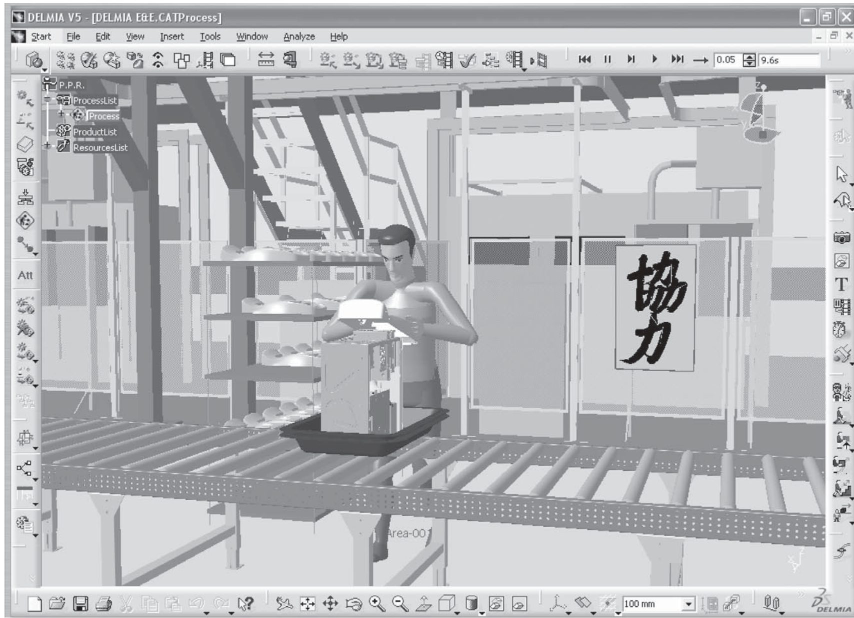


FIGURE 3 Modern simulation/analysis and PLM tool: IBM/Dassault Systemes Delmia for pharmaceutical manufacturing system modeling and design. The benefits are huge, since the system can be built and tested in the digital domain. (Courtesy of IBM/Dassault Systemes Delmia, Inc.)

processes. We use our own object-oriented process modeling method, called CIMpgr. The nature of this method tansforms into UML models [the Unified Modeling Language of information technology (IT)] and also complies with international process modeling standards used in complex system modeling environments.

First, we will discuss a few important definitions that closely relate to ISO 9000:2000 (quality process modeling) standard principle 4:

- A process, or activity, can be defined as a transfer function with one or more inputs, outputs, controls, and resources that together all enable the variables to gain data and then fire.
- Transfer functions, when fired, create a transformation process. A transformation process in a project is made up of methods, steps, tasks, and various algorithms and processes that acquire and manipulate data and then turn it into system output(s). Note that the input data can describe material, human knowledge, technological standing, fiscal information, and others.
- The output of the process is a product that consists of specific technical and/or social products and services that conform to the sponsor's requirements.
- Processes, in terms of quality project management, have visibility, documentation, and traceability.
- In this context visibility, relates to whether we know and transparently (or graphically) see what methods and techniques, system process steps, and technologies are involved when creating the desired output. Do we know the

FIGURE 4 Advanced sensors working in pharmaceutical assembly systems help real-time operation control and quality assurance system to test every product. (This is often referred to as the zero-defect policy designed into a system.) The luminescence sensor illustrated will detect a wide variety of invisible targets. This STEALTH-UV sensor was designed to sense the presence of invisible fluorescent materials contained in or added to many products. Users can detect the most difficult targets, including clear tamper-proof seals, clear labels, and invisible registration marks. This unique sensor is also ideal for solving many of today's toughest problems in product orientation, inspection, and verification. (Courtesy of TRI-TRONICS Co., Inc., www.ttco.com.)

sequence of these steps and the possible parallel process relationships? How does one process affect the other?

- Documentation means that the methods, steps, processes, and technologies are well specified and recorded according to agreed-upon standard specifications.
- Traceability means that the process steps as well as the output(s) can be traced back to actual customer requirements.
- Process capability can be defined as the ability of the production process to meet certain specifications and tolerances.
- Process discrepancy is the deviation of process settings from specifications.
- Process variability is the variation in dimensional or other measurable characteristics of output from a production process. (Note that in any project the ultimate goal is to stay within the predefined limits of process variability and, if possible and feasible, to reduce process variability, because this typically reduces risk too.)
- Variability can be expressed in terms of average range of standard deviation.
- A process variable is a process parameter that fluctuates in the manner of a random variable and hence requires surveillance.
- Process management means getting the activities and procedures that highly skilled and experienced managers carry in their heads into the open by means of a well-documented model, often referred to as the process model [40–46].

3.1.8 SYSTEMS APPROACH TO PHARMACEUTICAL MANUFACTURING SYSTEMS MANAGEMENT

Identifying, understanding, and managing interrelated processes as a system contribute to the organization's effectiveness and efficiency in achieving its objectives. (Note that every one of the key drivers listed below embed one or more innovation opportunities!)

Key drivers and achievable gains include the following:

- Processes that will achieve the desired results will become better integrated and aligned.
- Management and process owners will have the ability to focus their efforts on the key processes.
- Since the consistency, effectiveness, and efficiency of the organization will grow, the confidence of interested parties and collaborators in the organization will grow too.
- System structuring and fine-tuning will become possible to achieve the organization's objectives in the most effective and efficient way.
- Understanding the interdependencies between the processes of the system will yield good results.
- Structured (and object/component-oriented) modeling approaches that harmonize and integrate processes will become reality. Employees will understand them, follow them, and therefore reduce waste and increase quality in every process.
- The resistance created by cross-functional barriers will be reduced, providing a better understanding of the roles and responsibilities necessary for achieving common objectives.
- Organizational capabilities and the establishment of resource constraints prior to action will be better understood by all involved (and mostly by all those who have created the models).
- Targeting and defining how specific activities within a system should operate will become reality.
- Continually improving the system through measurement and feedback-controlled evaluation becomes possible due to the analytical and quantifiable approach of the process models. (Note that at its ultimate level this will lead to a real-time, feedback-controlled enterprise capable of reacting to dynamically changing market needs.)

After this introduction, let us show our object-oriented system components, following the above described ISO 9000:2000 principles, and how we can model complex innovation and related project management processes using them [40–54].

As a simple example, consider, that you are packaging a pharmaceutical product using a line that performs various process steps. Figure 5*a* illustrates one of these steps. It has input(s), output(s), control(s), and resource(s). These data

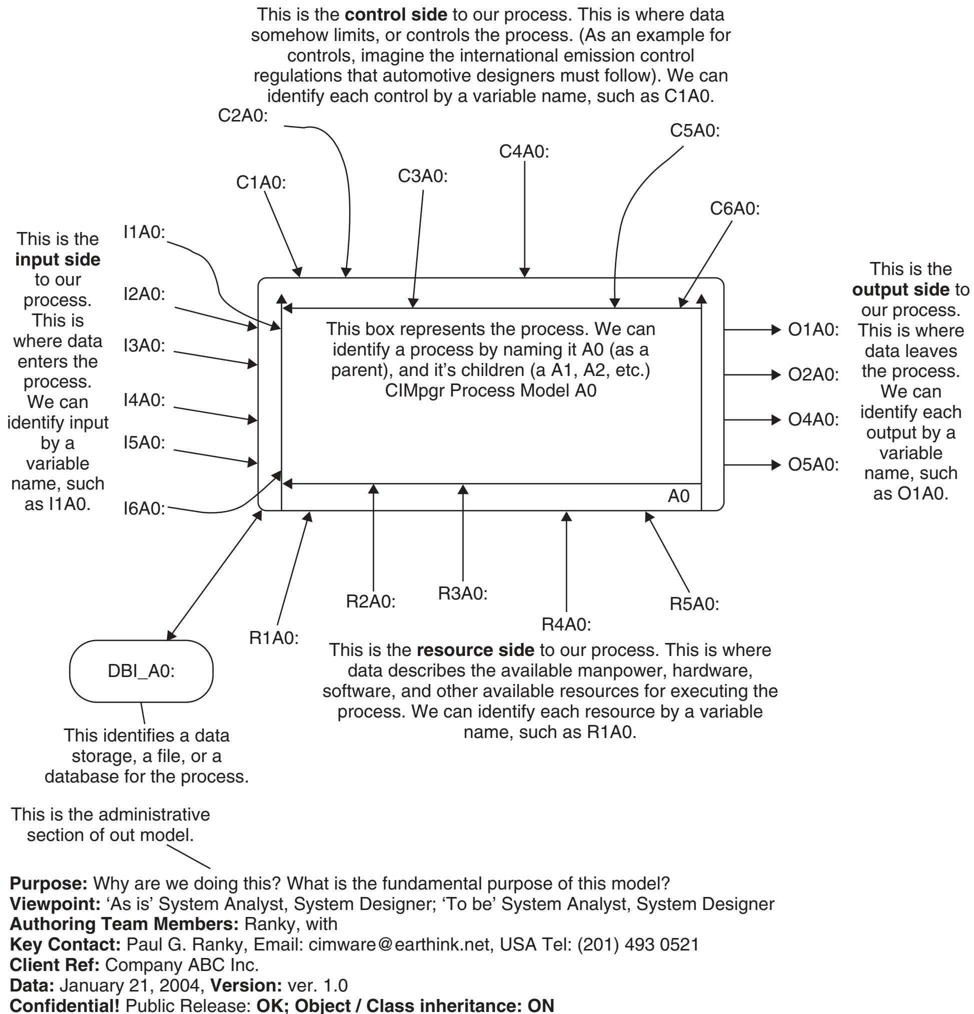
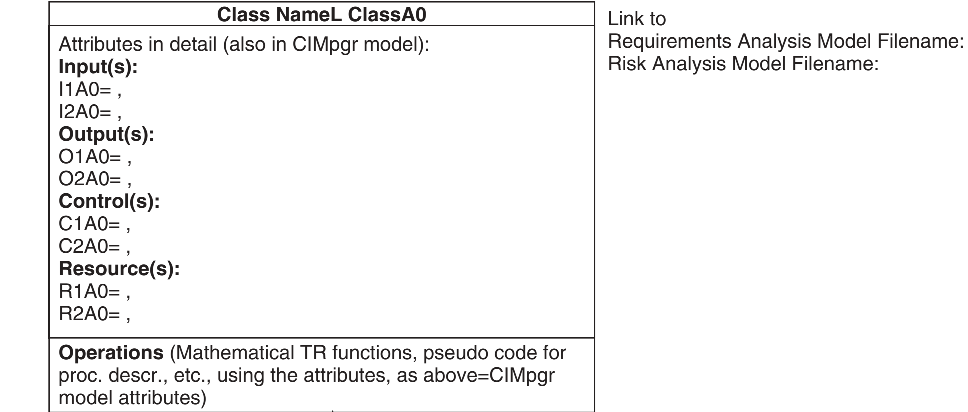
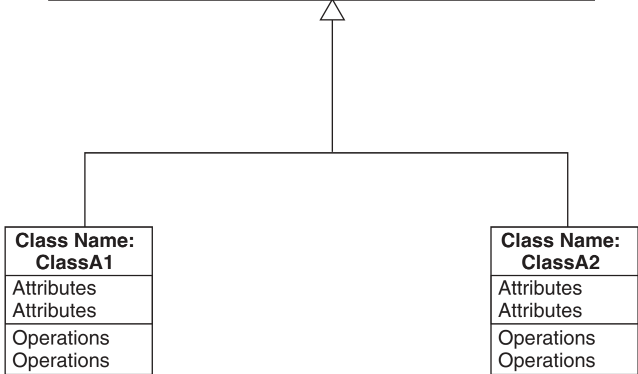


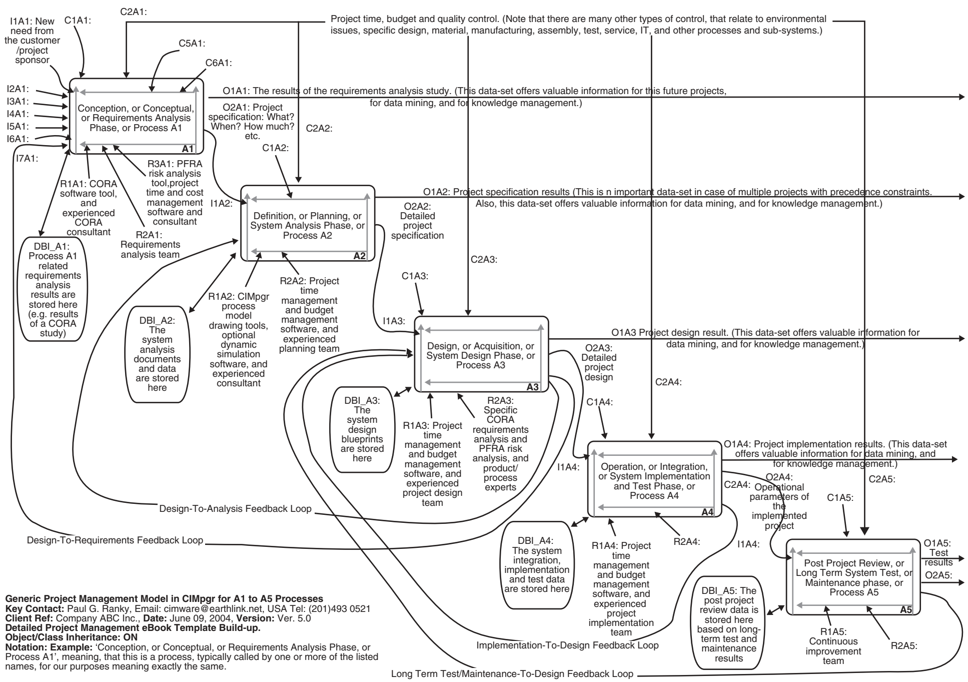
FIGURE 5 Object-oriented process modeling method (CIMpgr) as applicable to pharmaceutical manufacturing/assembly/packaging system design.

types help to identify under what conditions the process should be executed by the pharmaceutical manufacturing system. (Definitions are offered in the diagrams.) We can also see the way the CIMpgr process maps into a UML diagram. This is important, since UML is the modeling language of the IT professionals who will program the PLCs and control systems for the lines. Figure 5*b* shows how the CIMpgr process maps into a UML diagram. We can see in Figure 5*a* how multiple processes have to interact as we design a pharmaceutical assembly system.





(*b*)



(*c*)

FIGURE 5 *Continued*

3.1.9 REQUIREMENTS ANALYSIS FOR SYSTEM PRODUCT, PROCESS, AND SERVICE DESIGN INNOVATION

Processes in a successful innovation project must satisfy requirements set by the market, the sponsors, and/or the inventor's own dreams. Requirements analysis is considered to be one of the most important features of any innovative pharmaceutical manufacturing system project, because if done professionally, it helps to specify, research, and develop appropriate features and processes that customers need.

In our innovation project examples we have focused on generic needs and requirements, and our associated "customers" are the pharmaceutical R&D team members, managers, and operators in various industries.

In terms of our research approach, we have followed a proven method: Analyze the needs and the requirements, the demonstrated processes, and the methods and systems they try to or have to satisfy, and if you find a "gap", you have found an innovation opportunity. Note that when we search for this gap, it will simultaneously appear as a missing process in our CIMpgr model or as an existing process but missing attributes as well as a requirement in our CORA model (component-oriented requirements analysis) model:

- Analyze the actual methods presented. Find the core methodologies, the mathematical models, and the underlying engineering and/or other science foundation.
- Analyze the technologies involved. (How is science turned into a practical solution/engineering and/or computing technology?) Is there a need for a new, novel technology that has not been invented yet or applied in this field?
- Analyze and review the actual processes and the way the process flow is integrated. (Follow an object-oriented process analysis method, i.e., from concept to product.) Focus on the attributes of the processes. Note that by adding a new attribute you create new data types, with new information, and if your process can reason over this in a new way, new knowledge; therefore your combined CIMpgr and UML model becomes a new knowledge representation model too. This is important because innovation is formalized this way and can be communicated among global teams.
- Analyze potential alternative solutions. (A pharmaceutical manufacturing/assembly/packaging system must be very flexible these days, due to dynamically changing customer requirements and even operating conditions.)
- Analyze the benefits and the disadvantages of each process/solution.
- Design alternative methods, processes based on what you have experienced/seen and learned.
- Design an integrated system, based on what you have analyzed in this case.
- Work in a multidisciplinary team and exchange ideas.
- Understand the boundaries as well as the tremendous potential of new ideas and developments by working on this case (realize that in order to survive and win, you must add value) [54–57].

After this short introduction, we demonstrate our CORA spreadsheet solution with a real-world example (Figure 6).

Object / Component Oriented Requirements Analysis Program for Networked Lean Manufacturing by Paul G. Ranky © 1992–2006

© Paul G. Ranky. 2000–2002

Lean Manufacturing Manager: Customer Requirements (Reflecting component object behavior related to customer needs) \ Engineering / Software Solutions Responding to Customer Requirements		Importance Rating (1–5)	Fieldbus Network	Profibus Network	DeviceNet Network	Ethernet	Graphical CNC Progr.	On-site maint. Support	Off-site maint. Support	On-site Redundant Server	Off-site Redundant Server	Link to Factory prod. contr.	Link to Factory TQM/TQC	2D videos/3D multimedia	Cell-based web Camera	PC-based CNC Controller	PC based workkstation contr.	PC based Cell controller
S.No	Describe the Requirement		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	Reliability of data transfer for real-time access should be high	5	9	9	9	9				9	3					9	9	9
2	Reliability of reporting process failure to the line manager's computer	4	9	9	9	9										9	9	9
3	Ease of integration into a system (plug and play networking): important!	4	9	9	9	9										9	9	9
4	Ease of machine programming (CNC machining / inspection)	3				9	9	9								9	3	3
5	Ease of changng CNC part programs (locally, and via the network)	4	9	9	9	9	9	3	9					9	9	9	9	9
6	Ease of adding new sensors to a workstation, CNC, or cell: high	3	9	9	9	9										9	9	9
7	safety of operation: critical!	5	9	9	9	9		9	3	9	3					9	9	9
8	Cost of change/extenaion/system expansion should be low	4	3	3	3	3		9	3	3	3					9	3	3
9	Operator training needs and costs should be low	3					9	9	3							9	3	3
10	Network installation complexity and cost should be low	3	9	9	9	9			3	3						9	3	3

(*a*)

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20			
20 The system history database should be on the network	5	3	3	3	3			3	3	9	9	9	3	3	9		9					4	0	0
Target Values (List here the parameters that specify engineering solutions accurately. If you don't know the range of the acceptable values, use our Taguchi Calculator Program for Designing an Experiment)		Within 27 m sec	Within 27 m sec	Within 27 m sec	Within 27 m sec	GUI, iconized, multimedia	Less than 3 minutes	Less than 24 hrsr	0 sec switch	Less than 30 sec switch	Retresh every 2 minutes	Refresh every 2 minutes	320 240 pxels or better	320 240 pxels or better	Win, Linux, Solans, or OSX	Response within 12 m sec	Response within 24 m sec							
Absolute Importance Rating		416	416	416	489	207	147	111	186	107	144	135	87	87	567	381	588	0	0	0	0			
Relative Importance Rating		9.3	9.3	9.3	11	4.6	3.3	2.5	4.1	2.4	3.2	3	1.9	1.9	13	8.5	13	0	0	0	0			
Enter Our Product ratings (1 = low, 5 = High)		4	5	5	4	5	3	4	3	4	5	5	5	4	5	4	5							
Enter Competitor A's ratings (1-5); Graph		3	4	4	5	4	3	2	2	3	3	4	4	3	4	4	4							
Enter Competitor B's ratings (1-5); Graph		4	4	4	4	4	5	4	4	3	2	3	2	3	4	3	4							
Enter Competitor C's ratings (1-5); Graph		3	2	3	3	2	2	1	1	2	3	4	3	4	3	2	3							

(*b*)

FIGURE 6 CORA method. This is a spreadsheet-based tool, designed to analyze customer requirements. (A "customer" here can mean a pharmaceutical manufacturing line vendor, user, operators, maintenance engineers, and many others.) The key approach is that we create a correlation matrix and then evaluate the results using a quantitative, computational approach. This is much more accurate than just a simple structured list. Our method offers a list of all key requirements as well as the priorities for the pharmaceutical manufacturing system design team they should follow during the design process. (For more about this software tool, see http://www.cimwareukandusa.com.

3.1.10 INNOVATION RISK ANALYSIS AND OPPORTUNITY METHOD AND TOOL WITH PHARMACEUTICAL MANUFACTURING SYSTEM APPLICATIONS

Our failure risk analysis and opportunity method and iterative software tool, as part of our New Product & Process Innovation (NPPI) Tool Library, promotes systematic collaboration and team-oriented engineering thinking when a new pharmaceutical manufacturing system process and/or product are developed. (We call it "opportunity method" too, since most risks, if not all, offer new opportunities for innovation.) It is based on our generic process failure risk analysis method that could be applied to literally any process that involves risk—and innovation is a very risky process.

We follow a rule-based method when we analyze risk objects and components and their attributes. These plug-and-play rules can be different for different subjects, research areas, and industries. They can be designed and standardized for different industry sectors, enabling an analytical approach, systematic standardization, and accurate and predictable results.

Our risk analysis method and tools help the engineering management team to understand some of the following concerns:

- What could go wrong with the processes involved during the innovation project?
- How badly might it go wrong and what could the financial loss be?
- Which are the highest risk processes/operations when working on the product/process/service-related innovative design and project?
- What needs to be done to prevent failures?
- Which processes must be changed to reduce the risk of failure?
- What tools and fixtures are required to prevent failures and reduce the risk?
- What education is needed for participants, innovators, engineers, and process owners, such as line management and operators, to reduce or prevent failures?

After this introduction, we show the risk analysis system components, following the already described ISO 9000:2000 principles, and how we can model complex project management risks using them (Figure 7) (note that the active code spreadsheets and 3D objects are part of Ranky's eLibrary) [50–55].

3.1.11 OPEN-SOURCE COMPUTATIONAL STATISTICAL AND THREE-DIMENSIONAL MULTIMEDIA FOR PHARMACEUTICAL MANUFACTURING SYSTEM INNOVATION AND PROJECT COMMUNICATION

Since we follow an analytical, quantitative, and open-source computational approach, our pharmaceutical product/process and project management method and software toolset are implemented as (Internet browser readable) MS-Excel spreadsheets, integrated with several hyperlinks to the rule base and to optional 2D video and 3D virtual-reality objects for visualization.

Ranky 111601/DFRA_Ver.5	**Disassembly Process Code**	Ranky PC DisassyCode: 05/07/97
11/16/01	**Engineering Rerease Date or Process Methodology**	5/7/97
9/19/01	**Type of Product Disassembled**	Electro-mechanical
Rev.2.1.3. by Ranky	**Product Group Classifier**	Desktop PC
	Engineering Release Date of the Product	Estimated: 1993

List/Identify the Parts/Components Retrieved in Each Disassembly Process Step	Process Time	Process Cost	Accumulated Process Cost	The DFRA Team Describes/Illustrates the Potential Disassembly Failure Mode and the Effect; the Risk of Failure	
		16.40		Proc. ID	Failure Mode(s) and Effect(s)
	[sec]	[USD]	[USD]		
Painted metal PC cover (File: 3DMetalCover. mov)	45	0.21	0.21	ID 1.1	Metal Cover scratched by slipped screwdriver
				ID 1.2	As PC Metal Cover is removed, internal parts are cratched
				ID 1.3	
Floppy drive, hard drive, mounted in a solid sheet metal bracket inside the PC (File: 3DFloppyHDassy. mov)	137	0.62	0.83	ID 2.1	Floppy drive assy. screw removal can damage nother board
				ID 2.2	
				ID 2.3	Movie, illustrating floppy/HD assy. removal risks
Floppy drive (File: 3DFloppyDrive. mov)	35	0.16	0.99	ID 3.1	Can damage Floppy Drive if assy. Is dropped
				ID 3.2	Can damage Hard Drive if assembly is dropped
				ID 3.3	
Hard drive (File: 3DHDobj. mov)	65	0.30	0.28	ID 4.1	Can damage Hard Drive if assembly is dropped
				ID 4.2	
				ID 4.3	
Metal bracket holding the floppy and the hard drive (File: 3DFloppyHDBracket. mov)	12	0.05	1.34	ID 5.1	
				ID 5.2	
				ID 5.3	

(*a*)

	Ranky PC DisassyCode: 05/07/97	This DFRA Study Prepared By	Paul
ss	5/7/97	DFRA Team	NJIT
	Electro-mechanical	Responsible Organization/Department	NJIT
	Desktop PC	Comments	
oduct	Estimated: 1993		

ed ost	The DFRA Team Describes/Illustrates the Potential Disassembly Failure Mode and the Effect; the Risk of Failure		Severity Rating	Detection Rating	Occurrence Rating	RPN Pr Nu
	Proc.ID	Failure Mode(s) and Effect(s)				
			(1-10)	(1-10)	(1-10)	
	ID 1.1	Metal Cover scratched by slipped screwdriver	3	2	3	
	ID 1.2	As PC Metal Cover is removed, internal parts are cratched	5	4	1	
	ID 1.3					
	ID 2.1	Floppy drive assy, screw removal can damage mother board	5	9	2	
	ID 2.2					
	ID 2.3	Movie, illustrating floppy/HD assy. removal risks				
	ID 3.1	Can damage Floppy Drive if assy. Is dropped	8	2	1	
	ID 3.2	Can damage Hard Drive if assembly is dropped	8	2	3	
	ID 3.3					
	ID 4.1	Can damage Hard Drive if assembly is dropped	9	2	3	
	ID 4.2					
	ID 4.3					
	ID 5.1					
	ID 5.2					
	ID 5.3					

(*b*)

FIGURE 7 The process failure risk analysis (PFRA) tool is an analytical and computational tool using rule bases for evaluating process risks. It is an ideal method and tool for reducing costly failures. (For more about this software tool, see http://www.cimwareukandusa.com.

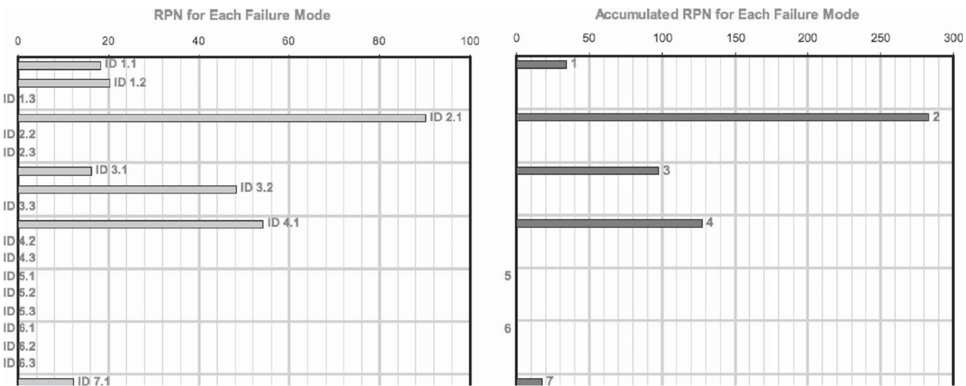
The reason for this is because we would like to offer our users the opportunity not just to understand the method and the coded logic, but also to be able to enjoy the 3D interactive graphics, the digital videos, the color images, and most importantly the active code spreadsheets. Along with any other imaginable visualization, this can be executed and experimented with using their own data.

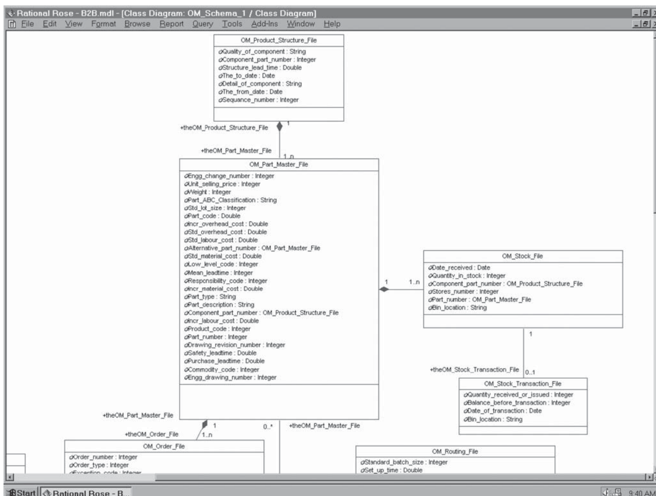
In terms of statistical methods, our NPPI Tool Library has several statistical analysis tools to capture innovation opportunities at processes that are likely to drift and become out of control or processes that execute with random failure.

This DFRA Study Prepared By	Paul G Ranky NJIT/MERC
OFRA Team	NJIT/MERC CFRA Team
Responsible Organization/Department	NJIT/MERC
Comments	

Severity Rating	Detection Rating	Occurrence Rating	RPN (Risk Priority Number)	Max. RPN	Tooling Factor	Clamping/ Fixturing Factor	Skill Factor	Any Other Factor You Define	Accumulated RPN	Risk Associated	
(1-10)	(1-10)	(1-10)			0.1-2.1=100%	0.1-2.1=100%	0.1-2.1=100%	0.1-2.1=100%			
3	2	3	18								
5	4	1	20	20	1.40	1.00	1.20	1.00	33.60	Low	
			0								
5	9	2	90								Mc
			0	90	1.60	1.40	1.40	1.00	282.24	HIGH	
			0								
8	2	1	16								Ha
8	2	3	48	48	1.20	1.20	1.40	1.00	96.77	Low	
			0								
9	2	3	54								
			0	54	1.40	1.20	1.40	1.00	127.01	Low	
			0								
			0								
			0	0	1.00	1.00	1.00	1.00	0.00	Low	
			0								

(*c*)





(*d*)

FIGURE 7 *Continued*

For capturing such critical opportunities for innovation and process improvement, we use a range of control charts for drifting data analysis, Taguchi DOE (design of experiments) methods for developing the desired list of parameters for our engineering solutions in our requirements analysis method, and Weibull methods for process reliability analysis. As we progress, we plan to introduce further statistical and other tools to our NPPI Tool Library [56–70].

3.1.12 RFID APPLICATIONS

Radio-frequency identification (RFID) technologies are being adopted in the United States at a fast pace in pharmaceutical/assembly and packaging, in general manufacturing, warehousing, distribution, and global supply chain management. The market size for this technology is expected to rise from around $500 million in 2005 to about $4 billion in 2010. In this section we outline some of the main application areas with a focus on the pharmaceutical applications.

We also deal with the R&D opportunities and some digital pharmaceutical manufacturing systems with RFID information system modeling results. Furthermore we offer a generic factory assembly and tracking digital model for RFID integration, the most complicated task manufacturing systems engineers, industrial engineers, and IT experts have faced due to the mixed real-time as well as global traceability and messaging challenges one faces with RFID-tagged parts and shipments. RFID opportunities are great since with the appropriate IT infrastructure they help both major distributors and manufacturers as well as other logistics operations, such as in the health care system, defense industries, and others, dealing with complex, global supply chains in which products and product shipments must be traced and identified in a noncontact, wireless fashion using a computer network, because of cost, security, or safety or because parts are subject to corrosion or medicine is subject to quality degradation.

All of these requirements point to an automated, wireless-readable sensory-based identification method and network that offers more functionalities and is significantly “smarter” than the well-known bar code or the unified product code (UPC).

RFIDs are available as passive and/or active radio read/write sensor packages with active read (and often write) capabilities in relatively large areas (e.g., a large distribution center warehouse or a containership), all performed automatically, supervised by computers, and communicated in a wireless fashion over secure intranets. The attraction to a pharmaceutical assembly factory or a supply chain manager is that when the RFID network is integrated with the factories’ material resource IT management systems, accurate information can be obtained on all tagged parts in close to real time throughout the entire supply chain. This can include the globally distributed factories as well as information about parts and assemblies during shipment, including in transit. This is why RFID represents great research and technology as well as huge business opportunities.

We introduce here some of the most important engineering and information systems management principles and challenges that RFID researchers, implementers, and users should keep in mind when developing such systems and/or planning

for such applications as well as offer an RFID digital factory integration model in UML [60–64, 70, 73].

3.1.13 RFID EXAMPLES

To set the scene, consider a large storage house for a variety of medications or their distribution center with thousands of boxes, parts, and assemblies that range from low cost to high value, on occasion even highly sensitive technology or perishable drugs that must be kept in certain environmental conditions, such as temperature, humidity, or pressure, for the entire period of the shipment and/or production/packaging operations.

Just-in-time delivery in an environment like this means that in order to build an order with variety or a new combination of treatments in a medical drug, every component must be in place on time and in good condition, which is a very difficult criterion to satisfy.

Obviously supply chains are global these days, and shipments are typically made by a variety of means, including cargo ships, air, rail, and trucks; all of these can be late or can get in trouble because of the weather, traffic, industrial disputes, or other reasons. Supply chain systems like this are very complex, because of the uncertainties in deliveries, parts and shipments are lost and/or stolen, goods get damaged during shipment, or the number of international ports and customs often take unpredictable time to check shipments with different levels of safety/security, and many other reasons.

There are many valid reasons why wireless, computer-networked, sensory-based part identification methods, tools, and technologies are being researched and deployed in industry. The application fields and opportunities are vast. The key driver is that even in chaotic, largely distributed, more stochastic than deterministic business environments, adaptive organizations and enterprises must react to demands quickly, else a competitor will take the business. Therefore they must reduce waste and improve efficiency at all fronts. The most important aspect of this strategy is to know exactly what parts they have in stock, exactly where these parts are, and in what condition/state of assembly or preparedness they are. Furthermore, major distributors dealing with complex, global supply chains must be able to trace their shipments in detail because of cost, security, safety, quality degradation (as in the case of temperature-, humidity-, and/or shock-sensitive components or drugs), or other reasons.

RFID technologies with the appropriate IT infrastructure help major distributors and manufacturers as well as other logistics operations such as the health care system, defense industries, and others deal with complex, global supply chains in which products and product shipments must be traced and identified in a noncontact, wireless fashion using a computer network.

All of the above-listed requirements point to an automated, wireless-readable, sensory-based identification method and network that offer more functionalities, and are significantly “smarter” than the well-known bar code or the UPC—hence the new popularity of RFID technology.

RFID tags carry a serialized tag data construct. As an example, a 64-bit class 0 tag offered by a supplier includes 64 bits of total user memory on the tag itself,

including a unique serial number. This number is encoded by the manufacturer and uniquely identifies up to 264 = 18,446,744,073,709,551,616 tagged items.

RFIDs are available as passive and/or active radio read/write sensor packages with active read (and often write) capabilities in relatively large areas (like a large distribution center warehouse or a containership), all performed automatically, supervised by computers, and communicated in a wireless fashion. The attraction to an assembly factory or a supply chain manager here is that when the RFID network is integrated with the factories' material resource IT management systems, accurate information can be obtained on all tagged parts at close to real time, all throughout the entire supply chain. This can include the globally distributed factories as well as information about parts and assemblies in shipment/in transit.

This is why RFID represents excellent research, technology, as well as big business opportunities. To illustrate this, consider research challenges such as remotely scanning and tracing products and parts in boxes on a cargo ship as it approaches national waters from international waters; tracing parts that are subject to corrosion and being used in agricultural or military equipment; medical drugs that are being counterfeited and repackaged and then shipped and imported illegally; or laptop computers that are dropped and damaged by accident. As a clear sign of the business opportunity, consider that according to a U.S. Department of Defense published presentation, RFID-enabled supply chain savings reached over U.S. $460 million in 2004 and the projections for 2010 are in excess of $4 billion!

3.1.14 RFID SYSTEM INTEGRATION MODELS FOR DIGITAL PHARMACEUTICAL MANUFACTURING AND ASSEMBLY SUPPLY CHAINS

In the U.S. manufacturing and assembly industry, many of the RFID pilot projects focus on achieving 100% read rates at speeds set by the widely used bar code technology. The focus for these projects is to achieve proper tag placement on cases and pallets as well as the proper configuration of pallets to enable 100% RFID tag read rates. This is a huge issue in pharmaceutical manufacturing and assembly in the fight to eliminate fake products and packages reaching the market! (See Figures 8–15.)

Based on the above-described requirements analysis, network planning, and server balancing reasoning, for our purposes, we have decided to follow a simple but powerful network architecture. In this architecture, we have included subnetworks. In terms of the way OPNET IT-Guru handles subnetworks, a subnetwork contains other network objects and abstracts them into a single object. A subnetwork can encompass a set of nodes and links to represent a physical grouping of objects (this can be a local-area network of CNC machines or robot PC controllers) or it can contain other subnetworks (e.g., including the material-handling system control of the line) [32, 34, 63, 66, 69–77].

Subnetworks within other subnetworks form the hierarchy of the network model. This hierarchy can then be extended as required to model the structure of the network. A subnetwork is considered the parent of the objects inside of it, and the objects are the children of the subnetwork. The highest level subnetwork in the network hierarchy does not have a parent, and therefore it is the top subnetwork,

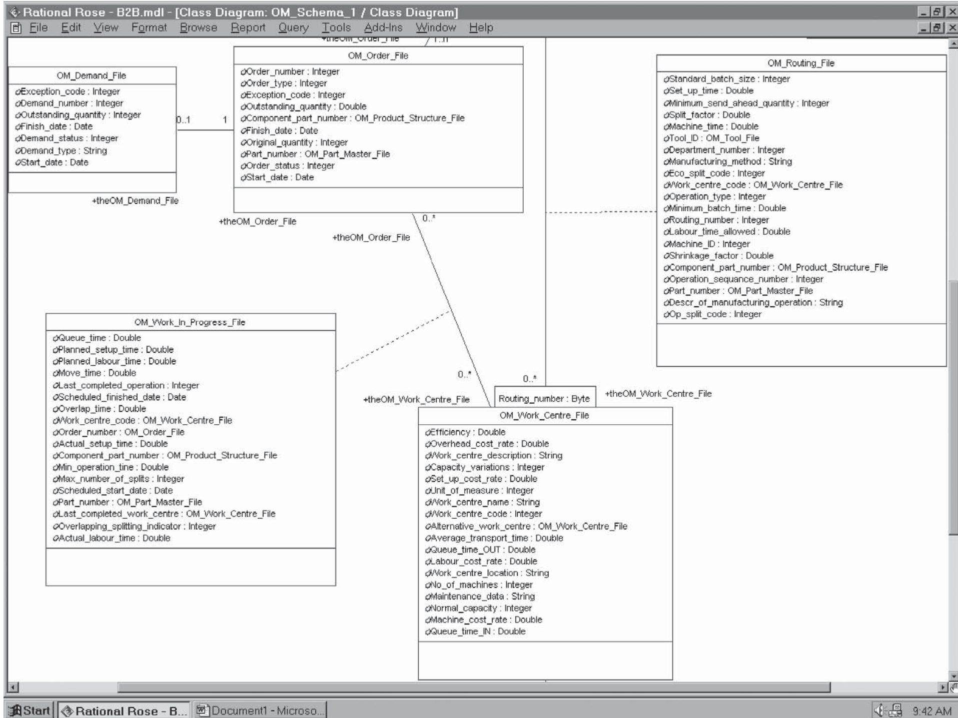


FIGURE 8 UML model segment illustrating the way the stock file is integrated with the routing and tooling files, assuming that all parts and all tools are RFID tagged. UML models like this should be used prior to any implementation work to assess requirements, technology needs, and RFID integration challenges with the rest of the factory's IT infrastructure.

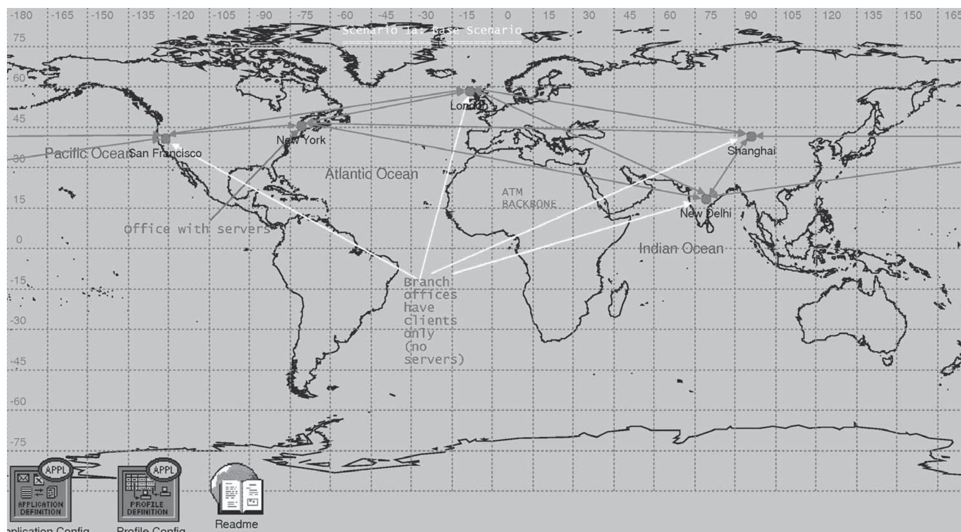


FIGURE 9 Simulation network for distributed pharmaceutical manufacturing systems and their warehouses in U.S., Europe, India, and Asia. Model focuses on information and data management, the way the servers can cope with the task of tracking pharmaceutical product, and RFID data on a world wide basis. As a modeling tool we use OPNET, a professional network simulation tool.

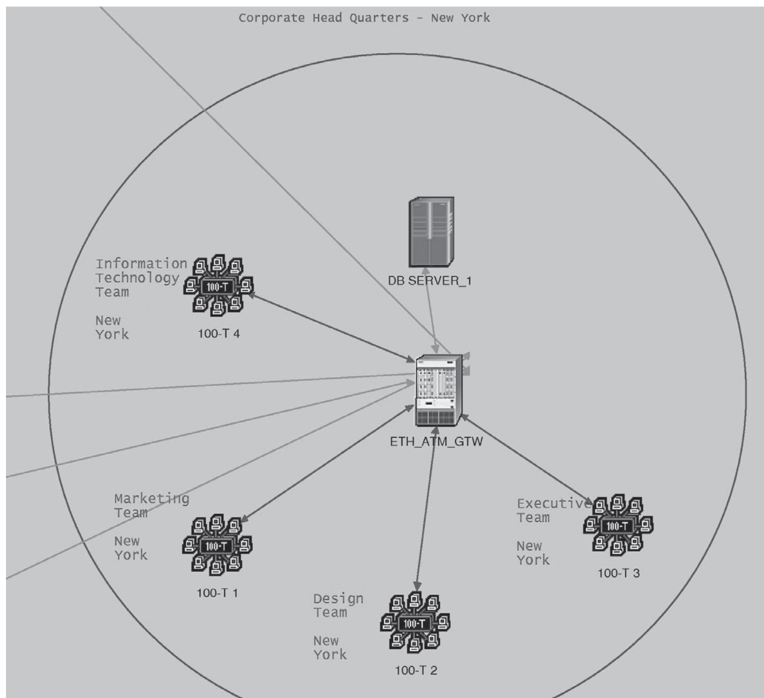


FIGURE 10 Segment of simulation model illustrating corporate headquarters in New York. This is where we have our main servers in our distributed system. Modeling tool is OPNET.

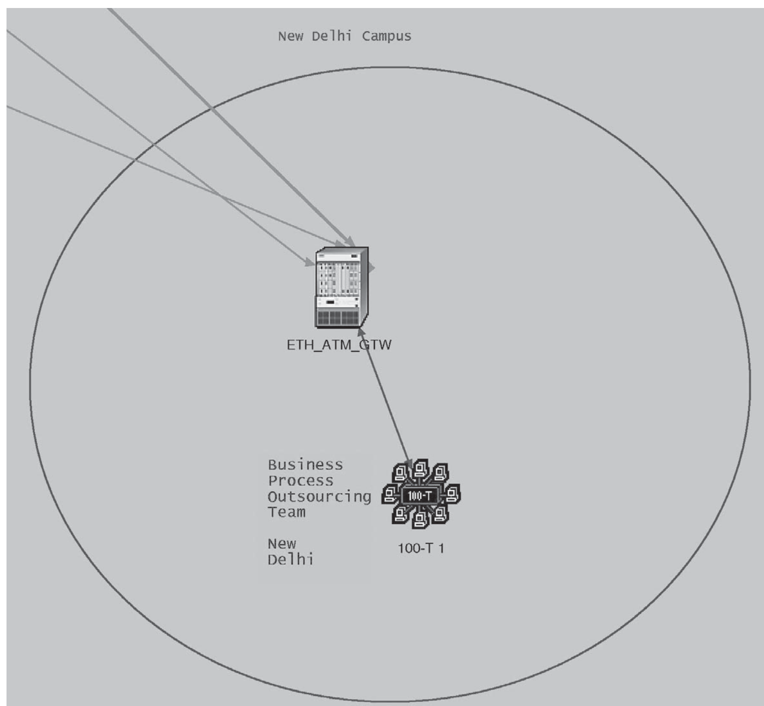


FIGURE 11 Segment of simulation model illustrating New Delhi campus network. This is where the business process outsourcing team and related servers in our distributed system are located. Modeling tool is OPNET.

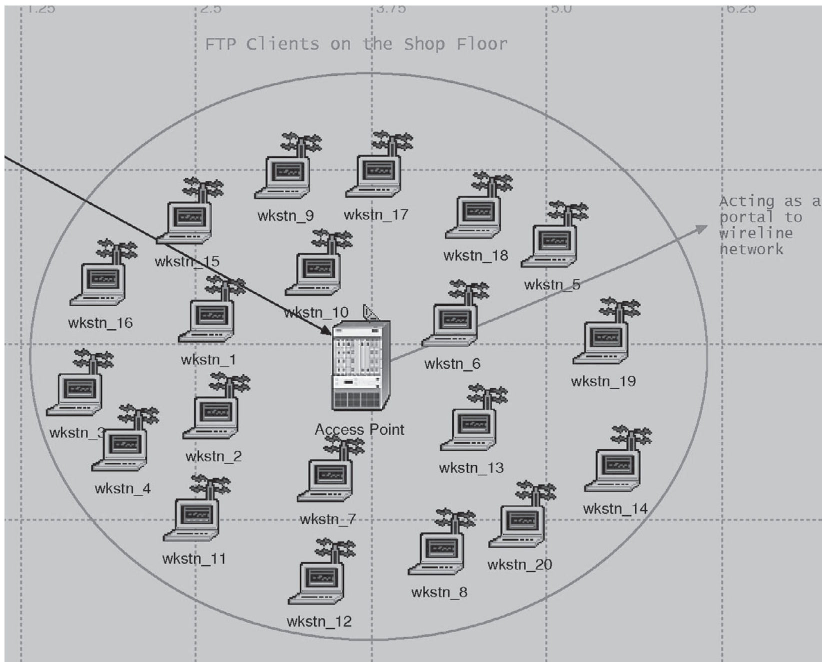


FIGURE 12 Pharmaceutical company portals as a wireless network of a pharmaceutical manufacturing system. The power of the model is that we can simulate a shop-floor request, comment, or warning throughout the entire international network of globally distributed pharmaceutical companies, with all important functions and processes. This means that before any pharmaceutical manufacturing system is actually built, we can simulate the entire system in the digital domain, saving huge expense and time. Modeling tool is OPNET.

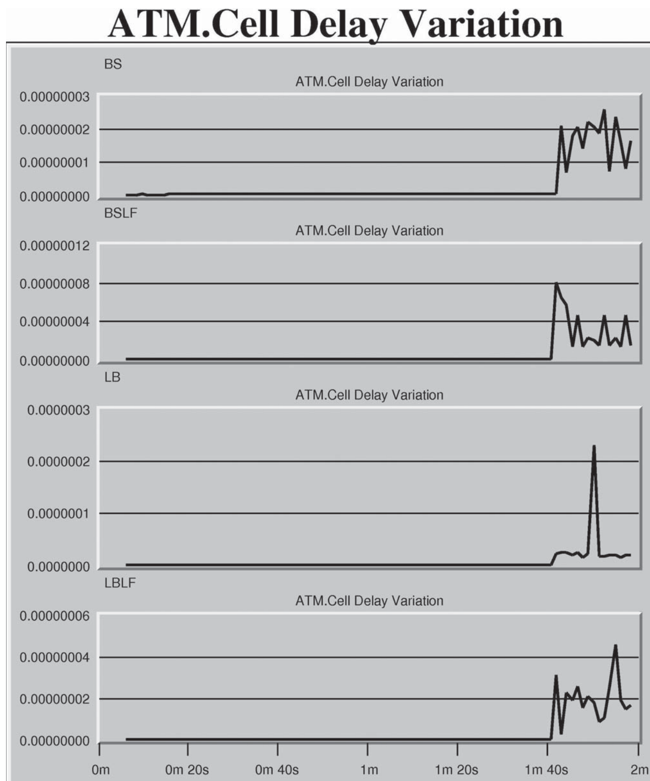


FIGURE 13 Simulation diagram illustrating and confirming that the network system design from an ATM variation–response time point of view can cope with the demand. Modeling tool is OPNET.

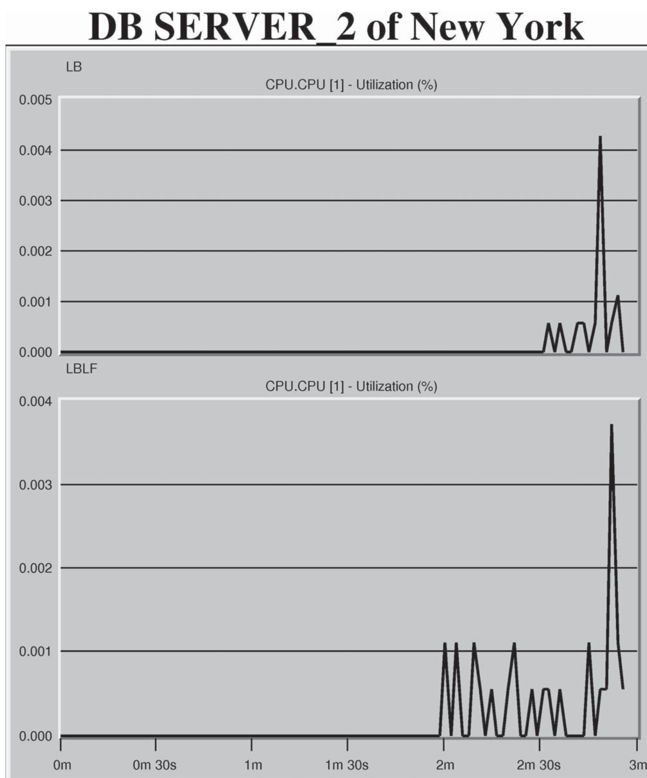


FIGURE 14 Simulation diagram illustrating and confirming that the server balancing aspects of the network system design can cope with the demand. Modeling tool is OPNET.

or global subnetwork. Subnetworks can be created and interconnected within this top level or within other subnetworks. Subnetworks provide a powerful mechanism for manipulating complex networks by breaking down the system's complexity through abstraction.

Since in our pharmaceutical network simulation models we deal with packets, let us explain a few aspects of packet formats. Packets carry information and can be sent between transmitters and receivers. In our example, packets can carry robot programs when uploaded from the design/programming office servers to the robot lines and then to the individual CNCs, or robots, or parts of them if there is a need for an update, edit, quality control, production control, maintenance, and other data. (Packets can include mission-critical, "panic" related real-time data between the robot controller PCs and the line servers.)

Packets are data structures consisting of storage areas called fields and can either be formatted or unformatted. Formatted packets have fields designed according to a packet format which specifies the packets' field names, data types, sizes, and default values. Formatted packets can be read by corresponding communication protocols only. Unformatted packets have no predefined fields. In IT-Guru, packet formats are predefined and typically named according to the model in which they are intended to be used.

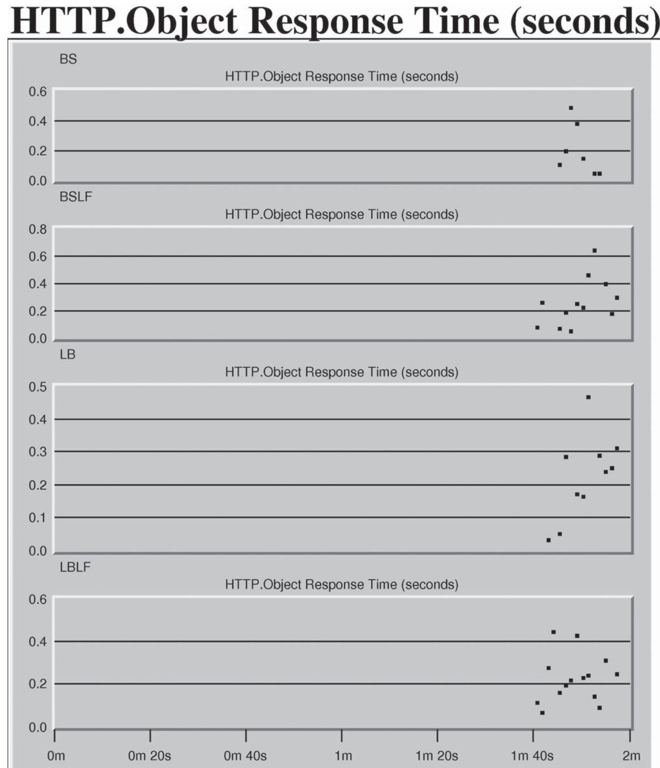


FIGURE 15 Simulation diagram illustrating and confirming that the network system design from an object variation–response time point of view can cope with the demand. As an example, this is important if a pharmaceutical manufacturing system line manager in India wants to notify a manager in New York by sending an image object, a sound object, or a multimedia object of a machine in the line for quality evaluation. Modeling tool is OPNET.

3.1.15 EVALUATION OF NETWORK SIMULATION RESULTS

The goal of most simulation scenarios is to evaluate some aspect of a system's behavior or performance and to quantify, typically in terms of statistics, the results and then use the results for decisions. This requires a simulation environment with software tools that provide insight into a model's dynamic operation.

Based on IT-Guru's in-depth analysis, the pharmaceutical manufacturing system network engineering analyst can collect object, scenariowide, and global statistics as follows:

- Object statistics are collected from individual objects. They allow the network engineering analyst to evaluate the performance of specific network nodes or links (a single hub's Ethernet delay or a server balancing change, as in our example).
- Scenariowide object statistics are collected from all relevant objects in a network (e.g., Ethernet delay for every node). They allow the network engineering analyst to easily monitor the performance of all objects of a specific type.

- Global statistics are collected from the entire network. They represent results that apply to the network as a whole (such as global end-to-end delay) and let the designers and management analyze aspects of the network's overall performance.
- More specifically, IT-Guru offers the following types of statistics when analyzing networks:
  - Queue size
  - Available space
  - Overflow occurrences
  - Delay
  - Interarrival times
  - Packet sizes
  - Throughput
  - Utilization
  - Error rates
  - Collisions
  - Application-specific statistics defined by a model developer

Because there are many possible statistics to collect, the data files would quickly grow past practical use if the simulation program recorded them all. Therefore, the analyst must specifically select the statistics that are valuable for the particular study before running a simulation [71–79].

3.1.16 SUMMARY

In this chapter, we have presented the foundations of an analytical and simultaneously computational lean and flexible pharmaceutical manufacturing system design approach based on total quality standards. We have discussed why this approach is essential for pharmaceutical product, process, and manufacturing system designs.

As illustrated, based on simulation results, using the plotted graphs and screens, management can easily evaluate different design alternatives, machine and human behavior models, control systems, sensory feedback processing, and the need of a balanced server architecture, and even investigate "what if" scenarios further, without committing to major upfront investment.

We can clearly state that the time has come when pharmaceutical manufacturing systems can be designed and built in an entirely digital domain, saving huge amounts of capital and other related cost, and simultaneously increasing quality.

3.1.17 COMPLIMENTARY VIDEO ON DVD

To show real-world high-technology examples of pharmaceutical product, process, manufacturing, assembly, and packaging system designs, in action, something we cannot do in static, printed books, we have created a supplementary video, in high definition, and compressed onto a DVD. This professionally edited DVD supports

this chapter as an independent, self-contained publication illustrating advanced pharmaceutical and medical product, process, and manufacturing system designs, related quality assurance processes and solutions, and others, explained by industry experts. To find out more about this DVD, refer to Ranky, P. G., Ranky, G. N., and Ranky, R. G. (2006), *Design Principles and Examples of Pharmaceutical Manufacturing Systems (Product, Process, Lean and Flexible Manufacturing, Assembly and Packaging System Designs)*, Video on DVD, available: www.cimwareukandusa.com.

REFERENCES

1. Ranky, P. G., A generic, analytical method to assess process-related risk with case studies, The Project Management Institute (PMI) Risk SIG and the Institute for International Research (IIR), paper presented at the Annual US National Project Risk Symposium, Houston, TX, May 22–May 26, 2006.
2. Ashley, S. (2004), Penny-wise smart labels, *Sci. Am*. 291(2), 30–31.
3. Ashton, P., and Ranky, P. G., (1999, Feb.), An advanced concurrent engineering research toolset and its application at Rolls-Royce motor cars, *ADAM (Adv. Des. Manufacturing),* available: http://www.cimwareukandusa.com, listed and indexed by the Association of Research Libraries, Washington, DC, and the Edinburgh Engineering Virtual Library, United Kingdom, Vol. 1.
4. Bradbrook, R. (2004), Wal-Mart and RFID, folding carton industry, *Printing News*, 31(4), 30–33.
5. Bradbrook, R. (2004), Procter and Gamble aim to be among the world leaders in RFID implementation, *Int. Paper Board Ind*., 47(8), 20–23.
6. Brzozowski, C. (2004), Tags, tickets & labels: New technologies emerge, *Printing News*, 153(3).
7. Ranky, P. G., An integrated PM approach, including: Process modeling, requirements analysis, risk analysis, statistical tools and 3D multimedia, presented at the Project Management Institute (PMI), New Jersey Chapter. Jan. 2006.
8. Ranky, P. G., Focus on RFID (radio frequency identification) methods, technologies and education, presented as part of the NCME Mission (National Center for Manufacturing Education), sponsored by NSF (National Science Foundation, USA) and industry, Jan. 2006.
9. Flaherty, M., Ranky, P. G., Ranky, M. F., Sands, S., and Stratful, S. (1999, Mar.), An engineering multimedia approach to servo pneumatic positioning, ADAM (Adv. Des. Manufacturing), available: http://www.cimwareukandusa.com, listed and indexed by the Association of Research Libraries, Washington DC, and the Edinburgh Engineering Virtual Library, United Kingdom, Vol. 1.
10. Glidden, R., Bockorick, C., etal. (2004), Design of ultra-low-cost UHF RFID tags for supply chain applications, *IEEE Commun. Mag*. 42(8), 140–151.
11. Graham-Rowe, D. (2004), Tags to banish forgetfulness, *New Scientist*, 183(2460), 19.
12. Graham-Rowe, D. (2004), Who's keeping tabs on your tags? *New Scientist*, 183(2462), 22.
13. Ho, K. L., and Ranky, P. G. (1999, Mar.), An object oriented approach to flexible conveyor system design, ADAM (Adv. Des. Manufacturing), available: http://www.cimwareukandusa.com, listed and indexed by the Association of Research Libraries, Washington DC, and the Edinburgh Engineering Virtual Library, United Kingdom, Vol. 1.

14. Knights, P. F., Henderson, E., and Daneshmend, L. K. (2004), Drawpoint control using radio frequency identification systems, *CIM Bull.*, 89(1003), 53–58.

15. Loose, D. C., and Ranky, P. G. (2007), *A Case-Based Introduction to IBM's Telematics Solutions*, interactive multimedia eBook with 3D objects, text, and videos in a browser-readable format on CD-ROM/intranet, available: http://www.cimwareukandusa.com, CIMware USA, Inc., and CIMware Ltd., United Kingdom; multimedia design and programming by P. G. Ranky and M. F. Ranky.

16. Mongeon, D. G. (2005, Feb.), RFID: A tool for the 21st century distribution, USA Department of Defense Conference Presentation, RFID Media Briefing, Washington DC.

17. Nadler, S. F., Ranky, P. G., and Ranky, M. (2002–2003), A 3D multimedia approach to the diagnosis of low back pain (Vol. 1, 18- and 40-year-old males), interactive 3D multimedia presentation on CD-ROM with off-line Internet support (650 Mbytes, approx. 150 interactive screens, 50 minutes of digital videos, 3D internal and external body tour, animation, and 3DVR objects), by CIMware (IEE and IMechE Approved Professional Developer); also in Multimedia design and programming by P. G. Ranky and M. F. Ranky.

18. Ranky, G. N., and Ranky, P.G. (2005), Japanese service robot R&D trends and examples, *Ind. Robot*, 32(6), 460–467.

19. Ranky, P. G., Caudill, R. J., Limaye, K., Alli, N., ChamyVelumani, S., Bhatia, A., and Lonkar, M. (2002, May), A Web-enabled virtual disassembly manager (web-VDM) for electronic product/process designers, disassembly line managers and operators, a UML (Unified Modeling Language) model of our generic digital factory, and some of our electronic support system analysis tools, ADAM with IT (Adv. Des. Manufacturing), available: http://www.cimwareukandusa.com, listed and indexed by the Association of Research Libraries, Washington DC, and the Edinburgh Engineering Virtual Library, United Kingdom, Vol. 3.

20. Ranky, P. G. (2006), Introduction to RFID—Radio frequency identification methods and solutions, *Assembly Automation*, 26(1), 28–33.

21. Ranky, P. G. (1999, Apr.), New trends in flexible, lean and agile manufacturing cells and systems, *ADAM (Adv. Des. Manufacturing)*, available: http://www.cimwareukandusa.com, listed and indexed by the Association of Research Libraries, Washington DC, and the Edinburgh Engineering Virtual Library, United Kingdom, Vol. 1.

22. Ranky, P. G. (2002), A method for planning industrial robot networks for automotive welding and assembly lines, *Ind. Robot: Int. J.*, 29(6), 530–537.

23. Ranky, P. G. (2003), A real-time manufacturing/assembly system performance evaluation and control model with integrated sensory feedback processing and visualization, *Assembly Automation*.

24. Ranky, P. G. (2003), A simulation method and distributed server balancing results of networked industrial robots for automotive welding and assembly lines, *Ind. Robot: Int. J.*, 30(2), 192–197.

25. Ranky, P. G. (2002), Advanced digital automotive sensor applications, *Sensor Rev.: Int. J.*, 22(3), 213–217.

26. Ranky, P. G. (2003), Advanced machine vision systems and application examples, *Sensor Rev.: Int. J.*, 23(3), 242–245.

27. Ranky, P. G. (2003), Collaborative synchronous robots serving machines and cells, *Ind. Robot: Int. J.*, 30(3), 213–217.

28. Ranky, P. G. (2004), Digital, Internet-enabled assembly line and factory modeling, *Assembly Automation*, 24(3), 247–253.

29. Ranky, P. G. (2004), Novel automated inspection methods, tools and technologies, *Assembly Automation: Int. J.*, 23(3), 252–257.

30. Ranky, P. G. (2003), Reconfigurable robot tool designs and integration applications, *Ind. Robot: Int. J*., 30(4), 338–344.
31. Ranky, P. G. (2002), Smart sensors, *Sensor Rev.: Int. J*., 22(4), 312–318.
32. Ranky, P. G. (1999, Jan.,) Some generic algorithmic solutions to the problem of dynamic scheduling in flexible manufacturing systems that operate globally, ADAM (Adv. Des. Manufacturing), available: http://www.cimwareukandusa.com, listed and indexed by the Association of Research Libraries, Washington DC, and the Edinburgh Engineering Virtual Library, United Kingdom, Vol. 1.
33. Ranky, P. G. (2001), Trends and R&D in virtual and robotized product disassembly, *Ind. Robot*, 28(6), 454–456.
34. Ranky, P. G. (2000, May), Some analytical considerations of engineering multimedia system design within an object oriented architecture, *Int. J. CIM*, 13(2), 204–214.
35. Ranky, P. G., and ChamyVelumani, S. (2003), A method, a tool (CORA), and application examples for analyzing disassembly user interface design criteria, *Int. J. CIM*, 16(4–5), 317–325.
36. Ranky, P. G., and ChamyVelumani, S. (2003), An analytical approach, a tool (DFRA) and application examples for assessing process-related failure risks, *Int. J. CIM*, 16(4–5), 326–333.
37. Ranky, P. G., and Nadler, S. F., A novel multimedia approach to low back pain diagnosis with internal and external 3D interactive body tours, paper presented at the 29th Annual Northeast Bioengineering Conference, New Jersey Institute of Technology, University Heights, Newark, NJ, Mar. 2003.
38. Ranky, P. G., and Nadler, S. F., A new, Web-enabled multimedia approach with 3D virtual reality internal and external body tours to support low back pain diagnosis, paper presented at the 4th Annual Faculty Best Practices Showcase in Kean University, NJ, Mar. 2003.
39. Ranky, P. G., and Ranky, M. F. (2000), A Dynamic Operation control algorithm with multimedia objects for flexible manufacturing systems, *Int. J. CIM*, 13(2), 245–263.
40. Ranky, P. G., Lonkar, M., and ChamyVelumani, S. (2003), eTransition models of collaborating design and manufacturing enterprises, *Int. J. CIM*, 16(4–5), 255–266.
41. Ranky, P. G., Morales, C., and Caudill, R. J., Lean Disassembly line layout, process and network simulation models and cases, based on real-world data, paper presented at the IEEE (USA) International Symposium on Electronics and the Environment and the IAER Electronics Recycling Summit, Boston, MA, May 19–22, 2003.
42. Ranky, P. G., Ranky, G. N., and Ranky, R.G. (2006), Examples of pharmaceutical product/process/manufacturing/assembly and packaging system designs, video on DVD, available: www.cimwareukandusa.com.
43. Ranky, P. G., Subramanyam, M., Caudill, R. J., Limaye, K., and Alli, N., A dynamic scheduling and balancing method and software tool for lean and reconfigurable disassembly lines, paper presented at the IEEE (USA) International Symposium on Electronics and the Environment and the IAER Electronics Recycling Summit, Boston, MA, May 19–22, 2003.
44. Ranky, P. G., 3D engineering multimedia cases. A customizable 3D Web-enabled library with reusable objects, paper presented at the ASEE (American Society of Engineering Educators) Mid-Atlantic Conference, Kean University, NJ, Apr. 2003.
45. Ranky, P. G., A 3D multimedia approach to biomedical engineering: Low back analysis, paper presented at the ASEE, American Society of Engineering Educators, U.S. National Meeting, Biomedical Engineering Division, Nashville, TN, June 2003.
46. Ranky, P. G., Ranky, G. N., and Ranky, R. G. (2006), Design principles and examples of pharmaceutical manufacturing systems (product, process, lean & flexible manufacturing,

assembly and packaging system designs), video on DVD, available: www.cimwareukandusa.com.

47. Ranky, P. G. (2001–2006), A 3D multimedia case: Component oriented disassembly failure risk analysis, an interactive multimedia publication with 3D objects, text and videos in a browser-readable format on CD-ROM/intranet available: http://www.cimwareukandusa.com, CIMware USA, Inc., and CIMware Ltd., United Kingdom; Multimedia design and programming by P. G. Ranky and M. F. Ranky (published 6 volumes of this main title with different risk analysis challenges explained).
48. Ranky, P. G. (2001–2005), A 3D multimedia case: component oriented disassembly user requirements analysis, an interactive multimedia eBook publication with 3D objects, text and videos in a browser-readable format on CD-ROM/intranet available: http://www.cimwareukandusa.com, CIMware USA, Inc., and CIMware Ltd., United Kingdom, Multimedia design and programming by P. G. Ranky and M. F. Ranky (published 7 volumes of this main title with different requirements analysis challenges explained).
49. Ranky, P. G., A 3D Web collaborative concurrent automotive engineering Method Based on our "distributed digital factory" and "digital car" models, paper presented at the Society of Automotive Engineers World Congress, Detroit, MI, Mar. 2003.
50. Ranky, P. G. (2003), A 3D Web-enabled, case based learning architecture and knowledge documentation method for engineering, information technology, management, and medical science/biomedical engineering, *Int. J. CIM*, 16(4–5). 346–356.
51. Ranky, P. G., A Biomedical Engineering case with 3D lower back interactive virtual anatomy tours inside and outside the human body with automated post-test student assessment, paper presented at the ASEE (American Society of Engineering Educators) Mid-Atlantic Conference, Kean University, NJ, Apr. 2003.
52. Ranky, P. G., A new approach for teaching and learning about engineering process failure risk analysis with IE (industrial engineering) case studies, paper presented at the ASEE, American Society of Engineering Educators, US National Meeting, Industrial Engineering Division, Nashville, TN, June 2003.
53. Ranky, P. G., A novel 3D Internet-based multimedia method for teaching and learning about engineering management requirements analysis, paper presented at the ASEE, American Society of Engineering Educators, US National Meeting, Engineering Management Education Division, Nashville, TN, June 2003.
54. Ranky, P. G., An interactive 3D multimedia problem-based library for manufacturing engineering technology education with Internet support, paper presented at the ASEE, American Society of Engineering Educators, US National Meeting, Engineering Technology Division, Nashville, TN, June 2003.
55. Ranky, P. G. (2003–2005), An introduction to alternative energy sources: Hybrid & fuel cell vehicles; an interactive multimedia eBook publication with 3D objects, text, and videos in a browser-readable format on CD-ROM/intranet, available: http://www.cimwareukandusa.com, CIMware USA, Inc., and CIMware Ltd.; United Kingdom, Multimedia design and programming by P. G. Ranky and M. F. Ranky, (2003–2005), Customer needs, wants & requirements analysis: Automotive exterior rearview mirror, an interactive multimedia eBook publication with 3D objects, text, and videos in a browser-readable format on CD-ROM/intranet, available: http://www.cimwareukandusa.com, CIMware USA, Inc., and CIMware Ltd., United Kingdom.
56. Ranky, P. G. (2003–2005), An introduction to digital factory & digital telematic car modeling with R&D and industrial case studies, an interactive multimedia eBook publication with 3D objects, text, and videos in a browser-readable format on CD-ROM/intranet, available: http://www.cimwareukandusa.com, CIMware USA, Inc. and CIMware Ltd., United Kingdom, Multimedia design and programming by P. G. Ranky and M. F. Ranky.

57. Ranky, P. G. (2005), An introduction to RFID, radio frequency identification methods and applications, DVD video, available: www.cimwareukandusa.com (approximately 30 min).
58. Ranky, P. G. (2005–2006), An introduction to RFID, radio frequency identification methods and applications with a total quality management and control focus, interactive browser-readable 3D eBook, available: www.cimwareukandusa.com, (approximately 30 min).
59. Ranky, P. G. (1999, Apr.), An object oriented system analysis and design method (CIMpgr) and an R&D case study, Adv. Des. Manufacturing, available: http://www.cimwareukandusa.com, listed and indexed by the Association of Research Libraries, Washington DC, and the Edinburgh Engineering Virtual Library, United Kingdom, Vol. 1.
60. Ranky, P. G., Computerized engineering assessment method based on 3D interactive multimedia, That students enjoy, paper presented at the ASEE, American Society of Engineering Educators, US National Meeting, Continuing Professional Development Division, Nashville, TN, June 2003.
61. Ranky, P. G. (1999), Design, manufacturing and assembly automation trends and strategies in China, *Assembly Automation*, 19(4), 301–305.
62. Ranky, P. G. (2003, Feb.), Designing a lean infrastructure; advanced machining cell design concepts, methods, architectures and cases, *Manuf. Eng., J. IEE*, 22–24.
63. Ranky, P. G. (2000), Engineering multimedia in CIM (computer integrated manufacturing), *Int. J. CIM*, 13(2), 169–171.
64. Ranky, P. G. (2003), eTransition in the multi-lifecycle CIM (computer integrated manufacturing) context, *Int. J. CIM*, 16(4–5), 229–234.
65. Ranky, P. G., Interactive 3D multimedia cases for engineering education with Internet support, ASEE, American Society of Engineering Educators, paper presented at the U.S. National Meeting, Computers in Education Division, Nashville, TN, June 2003.
66. Ranky, P. G., Interactive 3D multimedia cases for manufacturing engineering education with Internet support, paper presented at the ASEE, American Society of Engineering Educators, US National Meeting, Manufacturing Engineering Education Division, Nashville, TN, June 2003.
67. Ranky, P. G. (2002, Dec.), Introduction to concurrent engineering, an NSF (National Science Foundation, USA) sponsored Gateway Coalition streamed multimedia narrated web presentation, New Jersey Intitute of Technology, Public Research University, Newark, New Jersey.
68. Ranky, P. G. (2003–2005), Key R&D and eTransition trends in US and international collaborative design & manufacturing enterprises, an interactive multimedia eBook publication with 3D objects, text, and videos in a browser-readable format on CD-ROM/intranet, available: http://www.cimwareukandusa.com, CIMware USA, Inc., and CIMware Ltd., United Kingdom; Multimedia design and programming by P. G. Ranky and M. F. Ranky.
69. Ranky, P. G. (2000, Jan.), Modular fieldbus designs and applications, *Assembly Automation*, 20(1), 40–45.
70. Ranky, P. G. (2003), Network simulation models of lean manufacturing systems in digital factories and an intranet server balancing algorithm, *Int. J. CIM*, 16(4–5), 267–282.
71. Ranky, P. G., Rapid prototyping cases for integrated design and manufacturing engineering education with 3D Internet support, paper presented at the ASEE, American Society of Engineering Educators, US National Meeting, Design in Engineering Education Division, Nashville, TN, June 2003.
72. Roman, H. T., and Ranky, P. G. (2003–2005), A case-based Introduction to Service robotics, an interactive multimedia eBook publication with 3D objects, text, and videos in a browser-readable format on CD-ROM/intranet, available: http://www.cimwareukandusa.

com, CIMware USA, Inc., and CIMware Ltd., United Kingdom. Multimedia design and programming by P. G. Ranky and M. F. Ranky.

73. Romero, C., Department of logistics passive RFID initial implementation, paper presented at the USA Department of Defense Conference, RFID Media Briefing, Washington DC, Feb. 2005.
74. Sangoi, R., Smith, C. G., et al. (2004), Printing radio frequency identification (RFID) tag antennas using inks containing silver dispersions, *J. Dispersion Sci. Technol*. 25(4), 513–521.
75. Smith, K., Enabling the supply chain, paper presented at the USA Department of Defense Conference, RFID Media Briefing, Washington, DC, Feb. 2005.
76. Sugimoto, M., Kusunoki, F., Inagaki, S., Takatoki, K., and Yoshikawa, A. (2004), A system for supporting collaborative learning with networked sensing boards, *Syst. Comput. Jpn*., 35(9), 39–50.
77. Wilke, P., and Braunl, T. (2001), Flexible wireless communication network for mobile robot agents, *Ind. Robot*, 28(3), 220–233.

3.2

ROLE OF QUALITY SYSTEMS AND AUDITS IN PHARMACEUTICAL MANUFACTURING ENVIRONMENT

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By regulation, appropriate practice, and common sense, quality assurance (QA) is a critical function in the pharmaceutical manufacturing environment. The need for an independent unit to audit and comment on the appropriate application of

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standard operating procedures, master batch records, procedures approved in product applications, and the proper functioning of the quality control (QC) unit is paramount. This helps assure that products are manufactured reliably, with adherence to approved specifications, and that current good manufacturing practices (cGMP) are maintained in conformance to regulation, both in the facility in general and the microenvironment of each product's manufacturing sequence.

Quality assurance personnel must have the appropriate training, experience, familiarization with the manufacturing facility and products, enforced independence from the production chain of command, and the ability to review adherence to procedures, policies, and agreed-upon approaches to manufacturing quality pharmaceuticals. This helps to provide both an environment and a manufactured product that can withstand Food and Drug Administration (FDA) inspection and support a firm's reputation for quality products.

The cGMP regulations establish requirements that are intended to provide a high level of assurance that the pharmaceutical products produced satisfy the strength, purity, potency, and other quality requirements established for the finished product to assure that it is fit for its intended use. Manufacturers must establish a quality control unit that is responsible for many of the quality-related activities required by the regulations. These regulations have not been substantially updated since 1978. Since then, the science and practice of quality assurance have substantially evolved to include the development of quality management systems and risk management approaches to better assure product quality and fitness for use. Pharmaceutical product manufacturers are increasingly interested in implementing a comprehensive quality management system (QMS) and employing risk management approaches because they allow them to apply newer quality management principles that they believe enable them to more effectively assure product quality and better allow harmonization with evolving international regulatory quality system requirements. The FDA has not changed the cGMP regulations but, as part of its Pharmaceutical CGMPs for the 21st Century Initiative, encourages this quality systems approach to cGMP compliance.

This Chapter describes outlines and discusses the regulations applicable to the QA function and unit, structure, function, charter, and application of the unit in the pharmaceutical manufacturing environment. In addition, it discusses additional quality-related responsibilities that may result when manufacturers move toward a quality systems approach to quality that incorporates current quality system models to further improve quality and harmonize with international quality system requirements.

The justification for, and execution of, the QA audit are also described, including preparation, key items of interest, a typical checklist of the audit itself, corrective and preventive actions following the audit, and suggested measures for assuring successful operation of the unit.

3.2.1 cGMP REGULATIONS

The cGMP regulations for the manufacture of pharmaceutical products are contained in Parts 210 and 211 of Title 21 of the *Code of Federal Regulations* (CFR) [1]. These regulations, as well as guidance documents and other FDA documents

pertaining to the regulation and FDA inspection of pharmaceutical product manufacturers, may be accessed on the FDA website at www.fda.gov. Part 210 specifies the scope and applicability of the cGMP regulations and defines terms used in the regulations. Part 210 also indicates that the regulations establish "minimum" cGMP requirements and that products that are not manufactured under cGMP are adulterated. Adulterated products and the persons responsible for the adulteration are subject to regulatory action by the FDA.

Part 211 contains specific good manufacturing practice requirements for finished pharmaceuticals and is divided into Subparts A–K as follows:

A. Scope
B. Organization and Personnel
C. Buildings and Facilities
D. Equipment
E. Control of Components and Drug Product Containers and Closures
F. Production and Process Controls
G. Packaging and Labeling Control
H. Holding and Distribution
I. Laboratory Controls
J. Records and Reports
K. Returned and Salvaged Drug Products

The cGMP regulations are written to address the primary potential sources of product variability. Subpart B establishes the quality control unit and the duties of that unit, establishes personnel requirements and addresses personnel practices (e.g. sanitation) intended to reduce the likelihood of product contamination. Subparts C and D establish requirements for buildings and facilities and equipment used in the manufacture, processing, packing, or holding of a drug product. Subparts E through H establish controls over the major processes associated with the production of a finished and packaged drug product that is ready to be shipped for distribution to users. Controls are established for incoming raw materials and components and continue through manufacturing, packaging, labeling, holding, and distribution of finished, packaged, labeled, and released drug product. Subpart I requires the establishment of scientifically sound and appropriate specifications, standards, sampling plans, and test procedures; requires instrument specifications and calibration; and establishes lot or batch testing and release requirements. Subpart J establishes documentation requirements including master and batch records, and Subpart K addresses the control and disposition of returned drug products and places limitations on the salvage of drug products that have been subjected to improper storage conditions (e.g., smoke, heat, fire, moisture).

3.2.1.1 Duties of Quality Control Unit under cGMP Regulations

The cGMP regulations assign specific duties to the quality control unit. The unit is required to have the responsibility and authority to approve or reject all components, drug product containers, closures, in-process materials, packaging material,

labeling, and drug products and the authority to review production records to assure that no errors have occurred or, if errors have occurred, that they have been fully investigated. The responsibilities of the unit extend to approving or rejecting drug products manufactured, processed, packed, or held by contract manufacturers. The organization must assure that the quality control unit has adequate laboratory facilities for the testing and approval (or rejection) of components, drug product containers, closures, packaging materials, in-process materials, and drug products.

In addition to duties associated with the approval of materials and finished products, the unit is also responsible for approving or rejecting all procedures or specifications impacting on the identity, strength, quality, and purity of the drug product. This includes review and approval of procedures for production and process control, including any changes to these procedures. These procedures, and the responsibilities and procedures applicable to the quality control unit within the organization, must be written and followed.

All specifications, standards, sampling plans, test procedures, or other laboratory control mechanisms, including any changes, must be in writing and reviewed and approved by the quality control unit.

Written procedures describing the handling of all written and oral complaints regarding a drug product are required. The quality control unit is responsible for reviewing any complaint involving the possible failure of a drug product to meet any of its specifications and, for such drug products, making a determination as to the need for an investigation in accordance with cGMP requirements. The review should include a determination if the complaint represents a serious and unexpected adverse drug experience, which is required to be reported to the FDA. A written record of each complaint must be maintained in a complaint file.

3.2.2 QUALITY ASSURANCE FUNCTION

The term *quality* is used in many industries and in everyday life and can have various meanings depending on context. For the purposes of discussion here quality means the product requirements or attributes that have a bearing on the product's specified requirements. Quality assurance activities are those processes and activities conducted to assure that a product or service consistently satisfies its requirements and is fit for its intended use. In the pharmaceutical manufacturing environment, this means the activities conducted to assure that the pharmaceutical product's identity, strength, purity, potency, and other quality attributes conform to approved specifications.

In the United States, cGMP requirements for the manufacture of drugs were established by regulation in 1978 and have not been substantially updated since then. The science and practice of quality assurance has substantially evolved since then to include the development of quality systems [2, 3] and risk management approaches [4] to better assure product quality and fitness for use. Pharmaceutical product manufacturers are increasingly interested in implementing these approaches because they allow the manufactures to apply newer quality management principles that they believe enable them to more effectively assure product quality and better allow harmonization with evolving international regulatory quality system requirements.

3.2.3 QUALITY SYSTEMS APPROACH

The systems approach to quality involves a coordinated approach to the management of quality-related activities as processes that work in conjunction with one another to provide assurance that the product meets its specified requirements. It involves:

- A management commitment to quality that is communicated throughout the organization
- Identifying quality requirements using risk management and other methods as appropriate
- Developing a quality policy, plan, objectives
- Establishing an organizational structure with identified responsibilities and authorities that allows quality objectives to be met
- Providing the resources needed to meet quality objectives
- Developing the required systems and processes
- Establishing methods for the ongoing objective evaluation of the performance of systems and processes including quality auditing
- Initiating corrective and preventive actions as needed to assure that quality objectives are consistently and reliably met

The use of risk management techniques in identifying product requirements, establishing processes and process control and monitoring methods, evaluating quality data, identifying appropriate corrective and preventive actions to address quality problems, and for other quality-related activities can increase the overall efficiency and effectiveness of the quality system.

The FDA has recognized the value of and encourages a risk based quality systems approach for the manufacture of pharmaceutical products. This is reflected in its Pharmaceutical CGMPs for the 21st Century Initiative. In association with this initiative the FDA has published reports and guidance documents that collectively provide information that can be used by pharmaceutical product manufacturers in implementing a quality systems and risk management approach to pharmaceutical cGMP regulations compliance [5–8]. In implementing this initiative, the FDA has made it clear that it does not impose new regulatory requirements on manufacturers. The FDA has provided information and guidance that is intended to serve as a bridge between the 1978 regulations and current quality systems by explaining how manufacturers implementing such systems can do so in full compliance with the cGMP regulations. This approach differs from that used by the FDA when it updated the cGMP regulations for medical devices to employ a quality systems approach. The 1996 Quality System Regulation updated the GMP requirements for finished medical device manufacturers to reduce the risk of inadequate device design and to harmonize them with international quality system standards that were in effect at that time [9]. These international standards have since been updated [10]; however the device quality system regulation remains consistent with modern quality system models.

In a modern quality system, the organizational unit responsible for quality-related activities within the organization generally has a central role in the

development and management of the overall quality system. These activities can include quality control, quality assurance, quality planning, and quality improvement. The cGMP regulations do not define or employ these terms, but the activities the regulations assign to the quality control unit fall within these definitions as currently defined [2, 8, 11].

Current quality system models involve quality-related activities and terms that are not included in the cGMP regulations. Further, quality as a professional discipline is evolving. It is, therefore, important for organizations adopting a quality systems approach to unambiguously define the terms and quality concepts they will be using and to include these definitions as appropriate in training all staff in the organization who will be involved in quality-related activities. This will help assure effective communication throughout the organization and with vendors and others (e.g., regulatory agencies, third-party auditors) who interact with the organization on quality-related matters. Regulatory definitions should be recognized, and the use of nonstandard or outdated terminology should be avoided to the extent possible. Incorporating terms and definitions by reference from pertinent standards and FDA guidance documents may be helpful. FDA guidance on the quality systems approach to pharmaceutical cGMP regulations (pharmaceutical QS guidance) includes the following definitions:

Quality Assurance (QA) Proactive and retrospective activities that provide confidence that requirements are fulfilled.

Quality Control (QC) The steps taken during the generation of a product or service to ensure that it meets requirements and that the product or service is reproducible.

Quality Management (QM) Accountability for the successful implementation of the quality system.

Quality System (QS) Formalized business practices that define management responsibilities for organizational structure, processes, procedures, and resources needed to fulfill product/service requirements, customer satisfaction, and continual improvement.

Quality Unit (QU) A group organized within an organization to promote quality in general practice.

The FDA notes in its pharmaceutical QS guidance document that many current quality system concepts correlate very closely with the cGMP regulations and that the activities required by the regulations are generally consistent with a quality systems approach. In this and other guidance documents, the FDA uses the term *quality unit* rather than *quality control unit* as defined in the cGMP regulations to refer to the organizational unit with responsibility for quality-related activities. In a modern quality systems model these quality-related activities may go beyond, but are not necessarily inconsistent with, those required by the cGMP regulation.

Use of the term quality unit is consistent with current quality management system models [2, 10], which are intended to assure that the various operations associated with all systems are appropriately planned, approved, conducted, and monitored, and because the cGMP regulations specifically assign the QU the authority to create,

monitor, and implement a quality system. The FDA cautions that such activities do not substitute for, or preclude, the daily responsibility of manufacturing personnel to build quality into the product. The FDA has specifically indicated that the overarching philosophy articulated in both the cGMP regulations and in robust modern quality systems is that quality should be built into the product, and testing alone cannot be relied on to ensure product quality.

Other cGMP-assigned responsibilities of the QU that are consistent with modern quality system approaches include the following:

- Ensuring that controls are implemented and completed satisfactorily during manufacturing operations
- Ensuring that developed procedures and specifications are appropriate and followed, including those used by a firm under contract to the manufacturer
- Approving or rejecting incoming materials, in-process materials, and drug products
- Reviewing production records and investigating any unexplained discrepancies

The FDA has stressed that the release of the pharmaceutical QS guidance document does not impose new regulatory requirements on manufacturers but encourages manufactures to adopt a quality systems approach to cGMP compliance because of the potential benefits. An appropriately designed and implemented quality system can do the following:

- Reduce the number of (or prevent) recalls, returned or salvaged products, and defective products entering the marketplace
- Harmonize the cGMP regulations to the extent possible with other widely used quality management systems, which is desirable because of the globalization of pharmaceutical manufacturing, and the increasing prevalence of drug–device and biologic–device combination products
- When coupled with manufacturing process and product knowledge and the use of effective risk management practices, handle many types of changes to facilities, equipment, and processes without the need for prior approval regulatory submissions
- Potentially result in shorter and fewer FDA inspections by lowering the risk of manufacturing problems
- Provide the necessary framework for implementing *quality by design* (building in quality from the product development phase and throughout a product's life cycle), continual improvement, and risk management in the drug manufacturing process

This suggests that even without making changes in the cGMP regulations, the FDA may be looking at them from a "new" quality systems perspective. The regulations include terms such as *adequate* and *appropriate* that may be subject to interpretation based on relevant technical or scientific capabilities and state-of-the-art knowledge. As these improve, the interpretation of what is adequate or appropriate

can change as well. Practically, most manufacturers are more than willing to adopt methods that can improve the quality and safety of their pharmaceutical products because it is cost effective in the long run [11] but may be reluctant to do so for fear of being considered out of compliance with the cGMP regulations. Current FDA efforts in this regard should serve to allay manufacturers' concerns in this area.

The major elements of the quality system model described in the FDA's pharmaceutical QS guidance document are consistent with existing quality system standards. These elements are as follows:

- Management responsibilities
- Resources
- Manufacturing operations
- Evaluation activities

3.2.4 MANAGEMENT RESPONSIBILITIES

Current quality system models assign management a major role in the deployment and operation of a successful quality system. In such systems, major management responsibilities include the following:

- Provide leadership by establishing a commitment to quality that is supported by all levels of management and is communicated throughout the organization
- Create an organizational structure with clearly defined responsibilities and authorities to perform quality functions associated with achieving quality objectives
- Building and documenting a quality system to meet specified quality and regulatory requirements and achieve quality objectives
- Establishing a quality policy and objectives, and quality plans that are aligned with the organization's strategic plans and communicate this throughout the organization
- Reviewing the system by establishing appropriate accountability systems within the organization to monitor and report quality data and system status to management and assure that appropriate corrective and preventive actions are taken in response to quality problems using effective change control procedures and documented

The cGMP does not specifically assign management responsibility for these actions, although actions of this nature are required by the regulation. Table 1 from the pharmaceutical QS guidance document shows this relationship.

Under a comprehensive quality system the QU can expect an expanded and more visible role within the organization with greater accountability to and interaction with upper management. The QU should ideally be independent of the other organizational units to assure clear delineation of responsibility and authority and avoid conflicts. In certain instances, such as auditing, independence or objectivity is central

TABLE 1 21 CFR cGMP Regulations Related to Management Responsibilities

Quality System Element	Regulatory Citations
1. Leadership	
2. Structure	Establish quality function: §211.22(a) [see definition §210.3(b)(15)[Notification: §211.180(f)
3. Build QS	QU procedures: §211.22(d) QU procedures, specifications: §211.22(c), with reinforcement in: §§211.100(a), 211.160(a) QU control steps: §211.22(a), with reinforcement in §§211.42(c), 211.84(a), 211.87, 211.101(c)(1), 211.110(c), 211.115(b), 211.142, 211.165(d), 211.192 QU quality assurance; review/investigate: §§211.22(a), 211.100(a–b) 211.180(f), 211.192, 211.198(a) Record control: §§211.180(a–d), 211.180(c), 211.180(d), 211.180(e), 211.186, 211.192, 211.194, 211.198(b)
4. Establish policies, objectives, and plans	Procedures: §§211.22(c–d), 211.100(a)
5. System review	Record review: §§211.100, 211.180(e), 211.192, 211.198(b)(2)

to the effectiveness of the audit process, and auditors therefore should not have direct responsibility over the areas being audited.

The cGMP regulations do not specify how the QU should be integrated into the overall organization but, in general, the QU should be structured to reflect management's strong commitment to quality and to facilitate achieving quality objectives. The structure (e.g., organizational relationship to other organizational units, reporting relationships) should provide clear lines of responsibility and authority that support the production, quality, and management activities necessary to achieve quality objectives. Different organizations may accomplish this in different ways; however, experience has been that placement of the quality function on the same level within the organizational hierarchy as other major organizational units (e.g., production) sends a clear message both within and outside the organization that top management has a strong commitment to quality.

The cGMP regulations require quality-related activities to be conducted during all phases of manufacturing from the acceptance of raw materials through batch release, packaging, and labeling. The regulations also require that all personnel, including those engaged in quality-related activities, have sufficient education, training, and experience or any combination thereof to enable them to perform their assigned functions. In a quality systems approach to cGMP compliance, the role of quality personnel can be significantly expanded to include internal quality auditing, expanded review and analysis of quality data, investigation of nonconformance, root cause analysis, risk analysis, and other quality-related activities. Many of these activities are likely to be conducted with personnel from other organizational elements such as manufacturing, material control, facilities, product development, or engineering staff. Quality staff should have sufficient scientific and technical knowledge and training (e.g., statistical methods, risk analysis) and knowledge of the product and manufacturing processes to effectively perform their assigned functions

and competently interact with personnel from other organizational elements as necessary.

3.2.5 RESOURCES

The appropriate assignment of resources is essential to the success of any endeavor, and this is particularly critical in a pharmaceutical manufacturing environment. Inadequate staffing, training, manufacturing equipment and facilities, environmental controls, analytical equipment, and other resources can be sources of variability leading to the production of product that does not meet specified requirements. Modern quality system standards specifically address the issue of resources by requiring the organization to determine and provide the human, infrastructure, and work environment resources necessary for the quality system. The cGMP regulations address the resource issue in provisions that are intended to assure the adequacy of personnel (including consultants), manufacturing facilities including contract facilities, equipment, and laboratory facilities. The QU has significant responsibility in this regard.

The FDA, in its pharmaceutical QS guidance document, discusses the need for adequate resources in developing, implementing, and managing a quality system that complies with the cGMP regulations. Management is responsible for identifying resource requirements and providing resources accordingly, including providing training that is appropriate to the assigned activities. Personnel should understand the impact of their activities on their assigned duties and be familiar with cGMP requirements and the organization's quality system. This is consistent with the generally accepted idea that a culture of quality within an organization requires personnel to understand quality concepts, the organization's quality and regulatory objectives, and how their assigned activities contribute to the achievement of these objectives and fit into the overall quality system. Management should establish a working environment that encourages problem solving and communication in identifying and acting upon quality-related issues. While the provision of resources is generally considered a management function, the QU and other organizational units should be involved in the identification of the resources required to achieve quality objectives, including regulatory compliance, the assessment of the adequacy of existing resources, evaluating the effect of personnel, facility, product, process, regulatory, and other changes on resource needs, and generally providing management the information needed to make necessary and appropriate resource decisions.

Current quality system models employ a risk-based and data-driven approach to the development of QS system requirements to assure their adequacy. The FDA notes that the cGMP regulations place as much emphasis on processing equipment as testing equipment and contain specific requirements for the qualification, calibration, cleaning, and maintenance of production equipment that may be a higher standard than most nonpharmaceutical quality system models. Organizations should always keep in mind that, while the FDA may be encouraging the adoption of a comprehensive quality system, any system developed must satisfy the requirements of the cGMP regulations.

TABLE 2 21 CFR cGMP Regulations Related to Resources

Quality System Element	Regulatory Citation
1. General arrangements	
2. Develop personnel	Qualifications: §211.25(a) Staff number: §211.25(c) Staff training: §211.25(a–b)
3. Facilities and equipment	Buildings and facilities: §§211.22(b), 211.28(c), 211.42–211.58, 211.173 Equipment: §§211.63–211.72, 211.105, 211.160(b)(4), 211.182 Lab facilities: §211.22(b)
4. Control outsourced operations	Consultants: §211.34 Outsourcing: §211.22(a)

Under a quality system model, the specification of facility and equipment requirements may be performed by technical experts (e.g., engineers, development scientists) who have an understanding of the pharmaceutical science, manufacturing processes, and risk factors associated with the product and its manufacture. The cGMP regulations require the QU to be responsible for reviewing and approving all initial design criteria and procedures pertaining to facilities and equipment and any subsequent changes. These requirements are not mutually exclusive; while they place ultimate responsibility for review and approval of these activities with the QU, the regulations do not preclude a cross-functional review involving persons with relevant expertise from multiple areas of the organization. A requirement of both the cGMP and current quality system models is that such review and approval be conducted by persons who are qualified by education, training and experience to do so.

In the control of outsourced operations, the cGMP regulations require that the QU approve or reject products or services provided under a contract. Under current quality system models, the organization must follow a formal vendor qualification process to qualify outsource providers and verify through inspection or other appropriate means that the provider is capable of meeting the requirements of the organization. To comply with the regulation, these operations should be conducted by the QU.

Table 2 compares the major elements of a quality systems approach to addressing resource issues with corresponding requirements in the CGMP regulations.

3.2.6 MANUFACTURING OPERATIONS

There is significant commonality between the requirements contained in current quality system models such as ISO 9001-2000 and the cGMP regulation requirements for manufacturing operations. The FDA has identified four major elements of a QS approach to manufacturing operations. These are identified and compared to the cGMP requirements in Table 3.

TABLE 3 21 CFR cGMP Regulations Related to Manufacturing Operations

Quality System Element	Regulatory Citation
1. Design and develop product and processes	Production: §211.100(a)
2. Examine Inputs	Materials: §§210.3(b), 211.80–211.94, 211.101, 211.122, 211.125
3. Perform and monitor operations	Production: §§211.100, 211.103, 211.110, 211.111, 211.113 QC criteria: §§211.22(a–c), 211.115(b), 211.160(a), 211.165(d), 211.188 QC checkpoints: §§211.22 (a), 211.84(a), 211.87, 211.110(c)
4. Address nonconformities	Discrepancy investigation: §§211.22(a), 211.100, 211.115, 211.192, 211.198Recalls: 21 CFR Part 7

3.2.6.1 Design, Develop, and Document Product and Processes

In a modern quality systems manufacturing environment, the significant characteristics of the product being manufactured should be defined and verified as meeting requirements from design to delivery, and control should be exercised over all changes. This is consistent with the requirements of the cGMP regulation that require quality and manufacturing processes and procedures, and changes to them, to be defined, approved, and controlled. The idea of controlling the design of both product and process is consistent with concepts included in the FDA Pharmaceutical cGMPs for the 21st Century Initiative to assure product safety that focus on the entire product life cycle. No amount of "downstream" control and testing can compensate for a design that results in a product or production process that is incapable of meeting the requirements necessary to assure that the product is safe and effective for its intended use. Documentation is required and can include the following:

- Resources and facilities used
- Procedures to carry out the process
- Identification of the process owner who will maintain and update the process as needed
- Identification and control of important variables
- Quality control measures, necessary data collection, monitoring, and appropriate controls for the product and process
- Any validation activities, including operating ranges and acceptance criteria
- Effects on related process, functions, or personnel

The cGMP regulations include specific packaging and labeling controls, so packaging and labeling requirement, processes, and controls should be included in a QS-based approach to product and process design and development.

Manufacturers and the FDA have expressed concern that existing regulatory requirements (e.g., the need to effect manufacturing process changes through the regulatory submission process) may be excessively rigid and not conducive to innovation regardless of the potential benefits. The FDA acknowledges that the reluctance to pursue potentially innovative changes in pharmaceutical manufacturing can be undesirable from a public health perspective and has published a process analytical technology (PAT) guidance document that is intended to address this by promoting the use of analytical tools to gain process understanding and meet regulatory requirements for validating and controlling manufacturing processes [7].

The PAT guidance document describes a voluntary approach to the design, analysis, and control of manufacturing processes that involves the timely (e.g., in-process) measurement of critical quality and performance attributes of raw and in-process materials and processes, with the goal of ensuring final product quality. The term *analytical* in PAT is broadly interpreted to include the integrated application of chemical, physical, microbiological, mathematical, and risk analysis as appropriate. One goal of PAT is to design and develop well-understood processes that will consistently ensure predefined quality at the end of the manufacturing process. This is consistent with a quality systems approach. PAT should ideally be initiated during the development stage and is intended to be integrated into existing regulatory processes with timely communication with the FDA a key element. The FDA has published the guidance document and other pertinent PAT information on its website at www.fda.gov. Companies interested in PAT methods should contact the FDA. FDA internal implementation of PAT includes the following:

- A PAT team approach of CMC review and cGMP inspections
- Joint training and certification of FDA PAT review, inspection, and compliance staff
- Scientific and technical support for the PAT review, inspection, and compliance staff

Process analytical technology is consistent with the quality systems approach in that it is based on science and engineering principles for assessing and mitigating risks related to poor product and process quality. In the PAT guidance, the FDA indicates that the desired state for pharmaceutical manufacturing may be characterized as follows:

- Product quality and performance are ensured through the design of effective and efficient manufacturing processes
- Product and process specifications are based on a mechanistic understanding of how formulation and process factors affect product performance
- Continuous real-time quality assurance
- Relevant regulatory policies and procedures are tailored to accommodate the most current level of scientific knowledge
- Risk-based regulatory approaches recognize the following:

- ◦ The level of scientific understanding of how formulation and manufacturing process factors affect product quality and performance
- ◦ The capability of process control strategies to prevent or mitigate the risk of producing a poor-quality product

Process analytical technology is consistent with a modern risk-based data-driven quality systems approach to cGMP compliance.

3.2.6.2 Inputs

Current QMS models adopt a process-oriented approach to the design and operation of a QMS as a system of interrelated processes, each with inputs and outputs, which are designed to function in a defined way. Some process outputs are inputs to other processes. This concept is easily applied and understood within the manufacturing environment because it is process oriented. Inputs to manufacturing processes include any material that goes into the final product, including materials purchased from vendors for use in manufacturing and in-process materials. Manufacturing operations generally involve multiple processes conducted in a defined manner to produce the finished product. Each process has a set of inputs and produces one or more outputs that may, in turn, be an input to a subsequent process. Each process has an input–output relationship such that changes or variation in one or more inputs will produce an attendant change in the output. Input specifications are established to assure that the final product meets its requirements. A robust quality system will ensure that all inputs to the manufacturing process are suitable for use by establishing quality controls for the receipt and acceptance from qualified vendors, production, storage, and use of all inputs.

The cGMP regulations require either testing or use of a certificate of analysis (COA) plus an identity analysis for the release of materials for manufacturing. The quality systems approach additionally calls for initial supplier qualification based on an objective evaluation and periodic auditing of suppliers based on risk assessment to verify the adequacy of suppliers' quality systems. During the audit, a manufacturer can observe the testing or examinations conducted by the supplier to help determine the reliability of the supplier's COA. Under a QMS model, the QU would normally be responsible for auditing suppliers as part of its overall responsibility for materials acceptance.

Change control involves the evaluation of proposed changes in a systematic way to determine how they would affect process outputs and ultimately the finished product and is an important element of current quality system models. The cGMP regulations require the QU to approve specifications, and certain changes require review and approval by the QU. Under a quality system model, changes to materials (e.g., specification, supplier, or materials handling) should be implemented through a formal change control system involving the documented competent review and approval of the proposed change prior to implementation and communication of changes as appropriate throughout the organization. Manufacturers should also consider how best to assure that changes made by suppliers in supplied materials that may affect the quality of the finished product can be identified and appropriately evaluated by the manufacturer. Such provisions should be included in supplier agreements where possible.

3.2.6.3 Perform and Monitor Operations

Both the cGMP regulations and quality system models call for the monitoring of critical processes that may be responsible for causing variability during production. The cGMP regulations require written production and process control procedures and specify process control activities that must be performed and documented. Current quality system models also require written procedures, process verification and validation as appropriate, the establishment of appropriate process control measures and documentation. Risk analysis methods and design and development data may be used to establish process control and monitoring requirements. A quality systems approach allows the manufacturer to more efficiently and effectively validate, perform, and monitor operations and ensure that the controls are scientifically sound and appropriate. Production and process controls should be designed to ensure that the finished products have the identity, strength, quality, and purity they purport or are represented to possess. A systems approach will consider all sources of variability from inputs, through manufacturing processes, packaging, labeling, and shipping to assure that the product that is delivered to the user meets quality requirements.

One important aspect of the quality systems approach is the ongoing collection and analysis of quality data to continuously evaluate quality system effectiveness. Historical data, process knowledge, and risk analysis methods can be applied to identify specific data requirements. Trending and other data analysis methods can allow identification of actual and potential sources of nonconformity so that appropriate corrective and preventive actions can be taken in accordance with established change control procedures.

The entire product life cycle should be addressed by the establishment of monitoring and continual improvement mechanisms in the quality system. Even well-defined or mature manufacturing processes may "drift" due to a host of factors including equipment and facility aging, changes or variation in raw materials, electrical power fluctuations, and environmental changes. Thus, process validation is not a one-time event but an activity that continues throughout a product's life. One major quality system objective should be to identify emerging quality problems before nonconformities occur. Trending of periodically collected environmental monitoring data may, for example, identify a slow but steady increase in airborne particulate levels that, if left unaddressed and the trend continues, could exceed a firm's internal environmental standards and adversely affect the product. Early identification of such problems allows an investigation to be initiated to identify the cause so that appropriate corrective and preventive actions can be taken in accordance with established change control procedures. After a change is implemented, its effectiveness should be objectively verified and affected processes revalidated if necessary.

3.2.6.4 Address Nonconformities

A key component in any quality system is appropriately responding to nonconformities (i.e., deviations from requirements established under the quality system for in-process material or final product quality attributes, process control parameters, records, procedures, etc.). Nonconformities may be detected during any stage of the

manufacturing process or during quality control activities. The cGMP regulations require an investigation to be initiated and that the investigation, conclusion, and follow-up be documented. A primary objective of any manufacturing quality system is to prevent nonconforming product from being produced and distributed. The complete response to nonconformities should be risk based and can include the following components:

- Assessment of how the nonconformity will affect the quality of the finished product (i.e., determination if the nonconformity has resulted, or could result, in product that does not meet its specified purity, potency, and quality characteristics).
- Determine any actions necessary to assure that product that does not meet its specified requirements is not produced and that appropriate steps are taken with regard to any nonconforming product that has been produced to assure that consumers are not harmed and that regulatory requirements are satisfied.
- Determine the cause of the nonconformity.
- Identify any actions needed to correct the cause and to prevent recurrence.
- Document the investigation, findings, and follow-up actions.
- Assess the effectiveness of follow-up actions.
- Repeat the cycle as needed.

A nonconformity may not result in the finished product failing to meets its requirements; however, investigation of the nonconformity may identify process or quality system deficiencies that require attention. For example, a small but unexpected deviation from a process control requirement (e.g., temperature, blending time) may not exceed the limit for which the process was initially validated and thus not be expected to adversely effect the finished product but could suggest an emerging process control or equipment issue that if not corrected could result in future product nonconformities. Similarly, nonconformities in the form of errors or omissions in production records or deviations from written procedures may not always result in product nonconformity but could suggest training, process design, or other issues that ought to be addressed. Thus the response to nonconformities should not be limited to a determination of the immediate impact on the finished product, but also consider its implications regarding overall quality system performance.

3.2.7 EVALUATION ACTIVITIES

The evaluation component of a QMS is intended to provide objective information and data that allow the organization to assess the conformity of the product, evaluate the performance of its quality system, and maintain and improve its effectiveness [10]. The cGMP regulations similarly require evaluation activities as shown in Table 4.

TABLE 4 21 CFR cGMP Regulations Related to Evaluation Activities

Quality System Element	Regulatory Citation
1. Analyze data for trends	Annual review: §211.180(e)
2. Conduct internal audits	
3. Risk assessment	
4. Corrective action	Discrepancy investigation: §§211.22(a), 211.192
5. Preventive action	
6. Promote improvement	§211.110

3.2.7.1 Trend Analysis

The cGMP regulations require review and analysis of certain quality data annually at least. Current quality system models emphasize data-based decision making and the use of appropriate statistical analysis methods [2, 11]. Trend analysis is one statistical tool specifically recommended by the FDA in its pharmaceutical QS guidance document that can be very valuable in monitoring processes and quality system performance to identify emerging problems and to assess the effectiveness of improvement efforts. Traditional statistical process control and other methods also provide valuable support in the objective and ongoing analysis of quality data and can be helpful in implementing real-time quality assurance practices as recommended by the FDA [7].

3.2.7.2 Conduct Internal Audits

Internal auditing is not specifically required by the cGMP regulations, but manufacturers have traditionally used internal audits as a self-assessment tool and to prepare for FDA inspections. The FDA has for some time recognized the value of internal auditing and encourages firms to conduct audits by, as a matter of policy, not reviewing internal audit results during inspections [12].

Current quality system models call for audits to be conducted at planned intervals to evaluate effective implementation and maintenance of the quality system and to determine if processes and products meet established parameters and specifications. International standards provide guidance on auditing [13]. Audit procedures should be developed and documented to ensure that the planned audit schedule takes into account the relative risks of the various quality system activities. Factors that can be incorporated into a risk-based approach to planning audit frequency and scope include the following [6]:

- Existing legal requirements (e.g., cGMPs)
- Overall compliance status and history of the company or facility
- Robustness of a company's quality risk management activities
- Complexity of the site
- Complexity of the manufacturing process
- Complexity of the product and its therapeutic significance
- Number and significance of quality defects (e.g., recall)

- Results of previous audits/inspections that can include prior internal audit results as well as regulatory (e.g., state, federal, or other regulatory agencies) and third-party audits
- Major changes of building, equipment, processes, and key personnel
- Experience with manufacturing of a product (e.g., frequency, volume, number of batches)
- Test results of official control laboratories

In general, auditors should not have direct responsibility over the matters being audited. Auditors should be trained in auditing methods and have sufficient technical knowledge to be able to evaluate the systems being audited using objective audit criteria [14]. Audit criteria may be based on applicable regulatory requirements, standards to which the quality system is intended to conform (e.g., ISO 9001-2000), and the specific requirements of the quality system being audited as indicated in quality system documents. Auditing criteria should be defined prior to the initiation of the audit.

Different audit approaches may be applied depending on the intended purpose and scope of the audit. A top-down approach first evaluates the overall structure of the quality system and its subsystems. Selected subsystems may be chosen for review. Systems identified and developed by the FDA in a six-system inspection model for the inspection of drug manufacturers [15] include the following:

- Overall quality system
- Facilities and equipment
- Materials system
- Production system
- Packaging and labeling
- Laboratory controls

Subsystems must be pertinent to the specific quality system being audited and may coincide with major elements of a standard to which the quality system is intended to conform or the major elements identified in the FDA pharmaceutical QS guidance. When using the top-down approach, the auditor will first review each subsystem to determine if the requirements that apply to that subsystem (e.g., regulatory requirements, the requirements of the standard) are met by defining, documenting, and implementing appropriate procedures. Once the auditor has verified that the requisite procedures are in place, he or she will review the associated records and other documents to verify that the procedures have been followed and documented and that the quality system is functioning effectively as designed and conforms to applicable regulatory requirements and standards. This approach allows for a systematic evaluation of each subsystem and can be as detailed as needed.

A bottom-up approach may be used to follow up on a specific quality problem identified from trend analyses, product nonconformities, adverse experiences, customer complaints, or other sources of quality data. Starting with quality records associated with the problem, the auditor will work his or her way up through the

quality system, examining the quality processes having a bearing on the quality problem. This approach is helpful in identifying quality system issues that may be associated with specific quality problems but does not readily allow evaluation of the entire quality system.

A combination approach may also be used that employs elements of top-down and bottom-up audits. This allows some level of assessment of the effectiveness of the overall quality system while evaluating the cause of specific quality problems.

Auditors should select the audit method most appropriate for their intended audit purpose. Initial quality system audits or regularly scheduled audits are likely candidates for the top-down approach, while audits conducted as part of a root cause analysis, for example, may best employ a bottom-up approach. The FDA employs a similar approach to inspections. Regular scheduled biennial inspections are more likely to employ a top-down methodology. *For cause* inspections conducted in response to a specific product issue such as a recall are more likely to employ a bottom-up approach. FDA investigators may employ a combination approach during biennial inspections if investigators are aware of specific quality problems that they wish to include in the inspection.

Auditing as described in QMS models is intended to assess the effectiveness of the overall quality system as designed and conformance to applicable standards. The overall quality system does not have to be covered in a single audit. Manufacturers may choose to employ a *rolling audit* approach in which specifically identified subsystems are chosen for evaluation in accordance with an approved audit schedule. Audit plans should be designed to effectively perform this assessment.

Compliance with cGMP requirements is also a major concern, and audit planning should include assessment of conformance to cGMP requirements and readiness for FDA inspections. Existing FDA guidance documents and compliance policy guides describe FDA inspectional approaches and policy and can be used for reference in developing audit plans [15–17]. It can be helpful to include mock FDA audits as part of an overall auditing regimen. Some firms prefer to use outside auditors for mock audits to better simulate the FDA inspection process. Mock audits are also useful for training purposes to prepare the organization for FDA inspections.

The audit plan should be consistent with written quality auditing procedures included in the quality manual or other quality system documentation. The plan should include or refer to the objective criteria to be used to evaluate conformance to requirements. The plan should include or refer to other documents that will be used during the audit, including previous audit reports. If the audit is to include the review of batch or production records, such review should be conducted in accordance with a specified sampling plan or other appropriate statistical rationale as specified in a firm's quality system procedures.

Manufacturers implementing a quality system that conforms to an existing standard may find it helpful to create a table or some other document that shows the relationship between cGMP requirements, requirements of the standard, and the element(s) of the manufacturer's quality system. Such a tool can help assure that all pertinent requirements are covered in the quality system design and that audit plans designs include assessment of all pertinent requirements.

Since current quality system models employ a systems approach, an audit checklist that is organized by subsystem may be helpful, as described in Table 5. The form would include appropriate document control information such as form

TABLE 5 Example Audit Checklist

[Company Name] Quality System Audit Checklist			
Form:	Rev:	Date:	Approved:
Audit Date(s):	Refs:		
Auditor:		Title	Signature:
Requirement	cGMP Section Cross Reference	Conforms (Y/N/NA)	Objective Evidence and Comments
Subsystem 1			
Requirement 1.1			
Requirement 1.2			
Subsystem 2			
Requirement 2.1			
Requirement 2.2			
Subsystem 3			
Requirement 3.1			
Requirement 3.2			
Subsystem N			

identification, revision, and approval information. Companies may also wish to include reference information used in planning the audit such as previous audit reports, completed FDA Form 483 Inspectional Observations, third-party audit reports, and pertinent internal QS documents (e.g., audit procedures). Depending on the purpose of the audit, the subsystems may correspond to the six subsystems identified by the FDA for use by investigators in conducting cGMP inspections (i.e., quality, production, facilities and equipment, laboratory controls, materials, packaging and labeling) or the major elements of a quality system standard. Cross references between elements of the standard being used and the pertinent sections of the cGMP regulations may be included as appropriate. The audit form should allow entry of information regarding conformance or nonconformance to each requirement and have space for a description of pertinent findings.

The QMS models require periodic audits but do not specify audit frequency. Audit frequency must be determined based on the risk associated with the matters to be audited and other factors including results of previous audits and other quality data. Periodic audits should be conducted over the entire product life cycle and follow-up audits conducted as appropriate to verify that previously identified quality problems have been corrected in accordance with applicable quality system and regulatory requirements.

3.2.7.3 Quality Risk Management

The FDA has endorsed quality risk management as part of an overall quality systems approach to compliance with the cGMP regulations and achieving overall

quality system objectives [6]. Risk management methodologies permit management to assign priorities to activities or actions based on an assessment of the risk including both the probability of occurrence of harm and the severity of that harm.

Implementation of quality risk management includes assessing the risks, selecting and implementing risk management controls commensurate with the level of risk, and evaluating the results of the risk management efforts. In a manufacturing quality systems environment, risk management is used as a tool in the development of product specifications and critical process parameters. Used in conjunction with process understanding, quality risk management helps manufacturers effectively manage and control change.

A formal risk management process consists of several components:

- Risk assessment
  - Risk identification
  - Risk analysis
  - Risk evaluation
- Risk control
  - Risk reduction
  - Risk acceptance
- Risk communication
- Risk review

Risk assessment starts with risk identification, a systematic use of available information to identify hazards (i.e., events or other conditions that have the potential to cause harm). Information can be from a variety of sources including stakeholders, historical data, information from the literature, and mathematical or scientific analyses. Risk analysis is then conducted to estimate the degree of risk associated with the identified hazards. This is estimated based on the likelihood of occurrence and resultant severity of harm. In some risk management tools, the ability to detect the hazard may also be considered. If the hazard is readily detectable, this may be considered a factor in the overall risk assessment. Risk evaluation determines if the risk is acceptable based on specified criteria. In a quality system environment, criteria would include impact on the overall performance of the quality system and the quality attributes of the finished product. The value of the risk assessment depends on how robust the data used in the assessment process is judged to be. The risk assessment process should take into account assumptions and reasonable sources of uncertainty. Risk assessment activities should be documented.

Risk control starts with risk reduction, which includes any actions taken to eliminate or reduce the risk. Actions taken should be commensurate with the significance of the risk. If the risk has been reduced to an acceptable level, an affirmative decision can be made to accept the risk (risk acceptance). One question to ask is if new risks have been introduced as a result of the identified risks being controlled. Risk control measures should generally be conducted in accordance with change control procedures and documented.

Risk communication involves the communication of appropriate information about the risk to stakeholders (e.g., others involved in or affected by the quality

system including management, users, regulatory agencies). Risk communication should be documented. The included information might relate to the existence, nature, form, probability, severity, acceptability, control, treatment, detectability, or other aspects of risks to quality. Communication should be as appropriate and does not necessarily need to be carried out for each and every risk acceptance.

Risk review should be conducted to evaluate the outputs of the risk management process and repeated as necessary, based on new quality data or if there are process or product changes.

The Q9 Quality Risk Management guidance document [6] identifies a number of risk management tools that manufacturers can apply, including failure mode effects and criticality analysis (FMECA), hazard analysis and critical control points (HAACP), and preliminary hazard analysis (PHA), and provides examples of how quality risk management might be applied to quality management, development, materials management, production, and other operations within the organization.

3.2.7.4 Corrective and Preventive Actions

Corrective and preventive action (CAPA) is the term commonly used to describe the subsystem of a comprehensive quality system that deals with the systematic investigation, understanding, and response to quality issues including nonconformities. A corrective or preventive action may be initiated based on review and analysis of quality data from a variety of sources including adverse experiences, product complaints, quality audits, FDA inspections, third-party inspections, nonconforming materials reports, process control information, trend analyses, and other sources.

A corrective action is initiated to correct the cause of an identified nonconformity and to prevent it or similar problems from reoccurring. It may include initial and follow-up actions (e.g., conducted after root cause analysis). Current quality system models and the cGMP regulations emphasize corrective actions and require that actions be documented. Under current quality system models, preventive actions include actions taken in response to quality data to address the cause of potential nonconformities to prevent their occurrence. An effective CAPA system therefore includes both reactive and proactive components. The effectiveness of corrective and preventive actions should be evaluated using objective criteria when possible and the evaluation documented.

A firm's CAPA system and processes should be designed to analyze and respond to quality issues in a systematic way that is commensurate with the risk. The system should provide for the verification or validation of corrective and preventive actions to assure their effectiveness and to assure that actions do not adversely affect the finished product. The system should also assure that pertinent CAPA information is appropriately disseminated throughout the organization as necessary to assure the effective operation of the quality system and for management review.

3.2.7.5 Promote Improvement

Continual improvement is a requirement of existing quality system models such as ISO 9001-2000 in which the organization is required to continually improve the effectiveness of the quality management system through the use of the quality

policy, quality objectives, audit results, analysis of data, corrective and preventive actions, and management review. In adapting the ISO 9001-2000 standard to serve as a regulatory standard for medical device quality management systems, drafters of the ISO 13485 standard altered the requirement slightly to require the organization to "identify and implement any changes necessary to ensure and maintain the continued suitability and effectiveness of the quality management system through the use of the quality policy, quality objectives, audit results, analysis of data, corrective and preventive actions, and management review." The word *improvement* was deleted as not an objective of current regulatory standards, but the concept of continually monitoring the performance of the quality system and appropriately responding to quality data was retained.

The cGMP regulation does not specifically require continual improvement; however, the regulations are specific with regard to the sampling and testing of in-process materials and drug products, and failure to take reasonable action to reduce identified sources of variability may be of concern to FDA investigators. The FDA in its pharmaceutical QS guidance document encourages organizations to promote improvement through quality system activities and notes that it is critical for senior management to be involved. Process improvement, along with improvement of in-process controls, can render a manufacturing process more efficient and more robust. The end result can reduce costs and further prevent product failures and defects from occurring.

3.2.8 TRANSITIONING TO QUALITY SYSTEMS APPROACH

The cGMP regulations assign significant responsibilities to the organizational unit responsible for quality-related activities. Organizations implementing a quality system model will be responsible for additional quality-related activities including, but not necessarily limited to, conducting quality audits, analysis of quality data, risk assessment, and preventive actions based on review and analysis of quality data to prevent the occurrence of product nonconformities. In addition, management is required to provide requisite leadership by actively participating in the quality system and assuring that the quality system functions as intended. This is accomplished by establishing a quality policy and associated objectives, planning for quality, establishing an appropriate organization structure with designated responsibilities and authorities to appropriately carry out quality system requirements, providing appropriate resources and training, and periodically reviewing quality information and data, and assuring that the organization responds appropriately.

The organizational unit responsible for quality-related activities will in all likelihood have an even greater role within the organization, and roles and responsibilities throughout the organization are likely to change. Careful planning will be required to assure that the transition is effected smoothly with no adverse impact on product quality. Following are some points to consider in planning the transition:

- Create a transition team: A cross-functional team should be developed involving key managers and staff from throughout the organization to plan and

execute the transition. The transition team should have a clear understanding of its mission and the organizational objectives associated with the transition.

- Train the transition team: The decision to make the transition must come from management and management should assure that all individuals on the transition team receive proper training on quality systems requirements, risk management, and FDA's recommended approach to quality systems.
- Develop a transition plan: A transition plan, based on clearly defined objectives, should be developed by the transition team.
- Identify staffing requirements: The transition will likely affect individual job descriptions and create additional duties that will have to be addressed through the reassignment of staff, hiring new staff, and providing necessary training to all affected staff.
- Identify other resource needs: The plan should include a definition of resource requirements for planning and executing the plan.
- Define roles and responsibilities: the plan should clearly define the roles and responsibilities of those responsible for development and execution of the plan for quality system implementation as well as staff roles and responsibilities under the quality system.
- Consider organizational structure requirements: In order to function properly, persons responsible for quality-related activities must have the responsibility and associated authority defined and appropriately communicated within the organization.
- Conduct a gap analysis: The plan should conduct a gap analysis that identifies how the quality system model chosen can be effectively integrated with existing processes to create a quality system that conforms to the organization's quality objectives, meets regulatory requirements, and is consistent with other organizational requirements. The quality systems approach is intended to be somewhat flexible in application and can be tailored to specific organizational requirements. In order to function properly the quality system must be effectively integrated into the organization so that it is not viewed as an "add-on" or a set of extra requirements that prevent the "real" work from getting done.
- Consider benchmarking: If possible, arrange with other organizations that have successfully made the transition to meet with them, review their system, and discuss transition issues and how they were solved.
- Consult with experts: In addition to benchmarking, seeking assistance from persons familiar with quality systems can be very helpful, particularly when existing staff are relatively inexperienced with quality systems. It may be useful for one or more outside experts to work with the transition team on a regular basis as a coach or facilitator.
- Communicate regularly: Clear and ongoing communication within the transition team and with management is essential to effectively coordinate plan activities, report progress, resolve issues, and identify evolving resource needs.
- Sell the system: Successful implementation of a QS requires the active and informed participation of many individuals within the organization. Manage-

ment commitment should be clearly communicated and training provided so that affected staff understand basic quality system concepts and their role in the quality system.

- Validate the system.
- Maintain regulatory compliance.

3.2.9 AUDIT CHECKLIST FOR DRUG INDUSTRY

The checklist provided in Table 6 [15] is intended to aid in the systematic GMP audit of a facility that manufactures drug components or finished products.

The adequacy of any procedures is subject to the interpretation of the auditor. Therefore, the author accepts no liability for any subsequent regulatory observations or actions stemming from the use of this audit checklist.

3.2.9.1 Instructions for Using Audit Checklist

Before starting an on-site audit, plan the audit. Review past audits, note indications of possible problem areas and items, if any, that were identified for corrective action in a previous audit. If you are not already familiar with this facility, learn the type of product produced and how it is organized by personnel and function. What does your "customer," that is, your superior or senior facility management, expect to learn from this audit?

1. The checklist is to be used with a notebook into which detailed entries can be made during the audit.
2. While the checklist is to guide the auditor, it is not intended to be a substitute for knowledge of the GMP regulations.
3. Although a single question may be included about any requirement, the answer will usually be a multipart one since the auditor should determine the audit trail for several products that may use many different components. Enter details in you notebook and cross reference your comments with the questions.
4. At least three production batches should be selected for thorough analysis to include: (a) traceability of all components or materials used in the subject batches, (b) documentation of raw material or component, in-process, and finished goods testing for the subject product batches, and (c) warehousing and distribution records as they would relate to a possible recall.
5. Responses entered on the checklist should be consistent. "X" is recommended for "No"; a checkmark for "Yes"; "N/A" for not applicable to questions that do not apply. An asterisk and notebook page number should be entered on the checklist to identify where relevant comments or questions are recorded in your notebook.
6. The notebook used should be a laboratory-type notebook with bound pages. The notebook should be clearly labeled as to the audit type, date, and auditor(s). Many auditors prefer to use a notebook for a single audit so it may be filed with the checklist and the final report.

TABLE 6 Audit Checklist

Question	Instructions/Questions (note any exceptions and comments in notebook)	Yes, No, or NA
1.0	**General Controls**	
	Does the facility and its departments (organizational units) operate in a state of control as defined by the GMP regulations?	
1.1	**Organizational & Management Responsibilities**	
1.101	Does this facility/business unit operate under a facility or corporate quality policy?	
1.102	§211.22(a) Does a Quality Assurance unit (department) exist as a separate organizational entity?	
1.103	§211.22(a) Does the Quality Assurance unit alone have both the authority and responsibility to approve or reject all components, drug product containers and closures, in-process materials, packaging materials, labeling, and drug products?	
1.104	§211.22 Does the QA department or unit routinely review production records to ensure that procedures were followed and properly documented?	
1.105	§211.22(b) Are adequate laboratory space, equipment, and qualified personnel available for required testing?	
1.106	If any portion of testing is performed by a contractor, has the Quality Assurance unit inspected the contractor's site and verified that the laboratory space, equipment, qualified personnel, and procedures are adequate?	
1.107	Date of last inspection: —	
1.108	§211.22(c) Are all QA procedures in writing?	
1.109	§211.22(c) Are all QA responsibilities in writing?	
1.110	Are all written QA procedures current and approved? (Review log of procedures)	
1.111	Are the procedures followed? (Examine records to ensure consistent record-keeping that adequately documents testing.)	
1.112	§211.25 Are QA supervisory personnel qualified by way of training and experience?	
1.113	§211.25 Are other QA personnel (e.g., chemists, analysts, laboratory technicians) qualified by way of training and experience?	
1.2	**Document Control Program**	
1.201	§211.22(a) Does the QA unit have a person or department specifically charged with the responsibility of designing, revising, and obtaining approval for production and testing procedures, forms, and records?	
1.202	§211.22(d) Does a written SOP, which identifies how the form is to be completed and who signs and countersigns, exist for each record or form?	
1.203	§211.165(a)(b)(c) Is the production batch record and release test results reviewed for accuracy and completeness *before* a batch/lot of finished product is released?	

TABLE 6 ***Continued***

Question	Instructions/Questions (note any exceptions and comments in notebook)	Yes, No, or NA
1.3	**Employee Orientation, Quality Awareness, and Job Training**	
1.301	Circle the types of orientation provided to each new employee: (1) Company brochure. (2) Literature describing GMP regulations and stressing importance of following instructions. (3) On-the-job training for each function to be performed (*before* the employee is allowed to perform such tasks). (4) Other: enter in notebook.	
1.302	§211.25(a) Does each employee receive retraining on an SOP (procedures) if critical changes have been made in the procedure?	
1.303	Indicate how ongoing, periodic GMP training is accomplished.	
1.304	§211.25 is all training documented in writing that indicates the date of the training, the type of training, and the signature of both the employee and the trainer?	
1.305	§211.25 Are training records readily retrievable in a manner that enables one to determine what training an employee has received, which employees have been trained on a particular procedure, or have attended a particular training program?	
1.306	Are GMP trainers qualified through experience and training?	
1.307	§211.25(a) Are supervisory personnel instructed to prohibit any employee who, because of any physical condition (as determined by medical examination or supervisory observation) that may adversely affect the safety or quality of drug products, from coming into direct contact with any drug component or immediate containers for finished product?	
1.308	§211.28(d) Are employees required to report to supervisory personnel any health or physical condition that may have an adverse effect on drug product safety and purity?	
1.309	§211.25(a) Are temporary employees given the same orientation as permanent employees?	
1.310	§211.34 Are consultants, who are hired to advise on any aspect of manufacture, processing, packing or holding, of approval for release of drug products, asked to provide evidence of their education, training, and experience?	
1.311	§211.34 Are written records maintained stating the name, address, qualifications, and date of service for any consultants and the type of service they provide?	
1.4	**Plant Safety and Security**	
1.401	Does this facility have a facility or corporate safety program?	
1.402	Are safety procedures written?	
1.403	Are safety procedures current?	

TABLE 6 ***Continued***

Question	Instructions/Questions (note any exceptions and comments in notebook)	Yes, No, or NA
1.404	Do employees receive safety orientation *before* working in the plant area?	
1.405	Is safety training documented in a readily retrievable manner that states the name of the employee, the type of training, the date of the training, and the name of the trainer and the signature of the trainer and the participant?	
1.406	Does this facility have a formal, written security policy?	
1.407	Is access to the facility restricted?	
1.408	Describe how entry is monitored/restricted:	
1.409	Is a security person available 24 hours per day?	
1.5	**Internal Quality/GMP Audit Program**	
1.501	Does this business unit/facility have a written quality policy?	
1.502	Is a copy of this quality policy furnished to all employees?	
1.503	If “yes” to above, when provided? —	
1.504	Is training provided in quality improvement?	
1.505	Does a formal auditing function exist in the Quality Assurance department?	
1.506	Does a written SOP specify who shall conduct audits and qualifications (education, training, and experience) for those who conduct audits?	
1.507	Does a written SOP specify the scope and frequency of audits and how such audits are to be documented?	
1.508	Does a written SOP specify the distribution of the audit report?	
1.6	**Quality Cost Program**	
1.601	Does this facility have a periodic and formal review of the cost of quality?	
1.602	Does this facility have the ability, through personnel, software, and accounting records, to identify and capture quality costs?	
1.603	Does this facility make a conscious effort to reduce quality costs?	
2.0	Design control	
	Not directly related to the drug regulation	
3.0	Facility control	
3.1	**Facility Design and Layout**	
3.101	§211.42(a) Are all parts of the facility constructed in a way that makes them suitable for the manufacture, testing, and holding of drug products?	
3.102	§211.42(b) Is there sufficient space in the facility for the type of work and typical volume of production?	
3.103	Does the layout and organization of the facility prevent contamination?	
3.2	**Environmental Control Program**	
3.201	The facility is NOT situated in a location that potentially subjects workers or product to particulate matter, fumes, or infestations?	

TABLE 6 ***Continued***

Question	Instructions/Questions (note any exceptions and comments in notebook)	Yes, No, or NA
3.202	Are grounds free of standing water?	
3.203	§211.44 Is lighting adequate in all areas?	
3.204	§211.46 Is adequate ventilation provided?	
3.205	§211.46 Is control of air pressure, dust, humidity, and temperature adequate for the manufacture, processing, storage, or testing of drug products?	
3.206	§211.46 If air filters are used, is there a written procedure specifying the frequency of inspection and replacement?	
3.207	Are drains and routine cleaning procedures sufficient to prevent standing water inside the facility?	
3.208	§211.42(d) Does the facility have separate air-handling systems, if required, to prevent contamination? (MANDATORY IF PENICILLIN IS PRESENT!)	
3.3	**Facility Maintenance and Good Housekeeping Program**	
3.301	§211.56(a) Is this facility free from infestation by rodents, birds, insects, and vermin?	
3.302	§211.56(c) Does this facility have written procedures for the safe use of suitable (e.g., those that are properly registered) rodenticides, insecticides, fungicides, and fumigating agents?	
3.303	Is this facility maintained in a clean and sanitary condition?	
3.304	Does this facility have written procedures that describe in sufficient detail the cleaning schedule, methods, equipment, and material?	
3.305	Does this facility have written procedures for the safe and correct use of cleaning and sanitizing agents?	
3.306	§211.58 Are all parts of the facility maintained in a good state of repair?	
3.307	§211.52 Is sewage, trash, and other refuse disposed of in a safe and sanitary manner (and with sufficient frequency)?	
3.4	**Outside Contractor Control Program**	
3.401	§211.56(d) Are contractors and temporary employees required to perform their work under sanitary conditions?	
3.402	Are contractors qualified by experience or training to perform tasks that may influence the production, packaging, or holding of drug products?	
4.0	Equipment control	
4.1	**Equipment Design and Placement**	
4.101	§211.63 Is all equipment used to manufacture, process, or hold a drug product of appropriate design and size for its intended use?	
4.102	Are the following pieces of equipment suitable for their purpose: blender(s), conveyor(s), tablet, presses, capsule fillers, bottle fillers, other (specify)?	
4.103	Are the following pieces of equipment suitable in their size/capacity: blender(s), conveyor(s), tablet, presses, capsule fillers, bottle fillers, other (specify)?	

TABLE 6 ***Continued***

Question	Instructions/Questions (note any exceptions and comments in notebook)	Yes, No, or NA
4.104	Are the following pieces of equipment suitable in their design: blender(s), conveyor(s), tablet, presses, capsule fillers, bottle fillers, other (specify)?	
4.105	Are the locations in the facility of the following pieces of equipment acceptable: blender(s), conveyor(s), tablet, presses, capsule fillers, bottle fillers, other (specify)?	
4.106	Are the following pieces of equipment properly installed: blender(s), conveyor(s), tablet, presses, capsule fillers, bottle fillers, other (specify)?	
4.107	Is there adequate space for the following pieces of equipment: blender(s), conveyor(s), tablet, presses, capsule fillers, bottle fillers, other (specify)?	
4.108	§211.65(a) Are machine surfaces that contact materials or finished goods nonreactive, nonabsorptive, and nonadditive so as not to affect the product?	
4.109	§211.65(b) Are design and operating precautions taken to ensure that lubricants or coolants or other operating substances do NOT come into contact with drug components or finished product?	
4.110	§211.72 Fiber-releasing filters are NOT used in the production of injectable products.	
4.111	§211.72 Asbestos filters are NOT used in the production of products.	
4.112	Is each idle piece of equipment clearly marked “needs cleaning” or “cleaned; ready for service”?	
4.113	Is equipment cleaned promptly after use?	
4.114	Is idle equipment stored in a designated area?	
4.115	§211.67(a)(b) Are written procedures available for each piece of equipment used in the manufacturing, processing, or holding of components, in-process material, or finished product?	
4.116	Do cleaning instructions include disassembly and drainage procedure, if required, to ensure that no cleaning solution or rinse remains in the equipment?	
4.117	Does the cleaning procedure or startup procedure ensure that the equipment is systematically and thoroughly cleaned?	
4.2	**Equipment Identification**	
4.201	§211.105 Are all pieces of equipment clearly identified with easily visible markings?	
4.202	§211.105(b) Are all pieces of equipment also marked with an identification number that corresponds with an entry in an equipment log?	
4.203	Does each piece of equipment have written instructions for maintenance that includes a schedule for maintenance?	
4.204	Is the maintenance log for each piece of equipment kept on or near the equipment?	

TABLE 6 ***Continued***

Question	Instructions/Questions (note any exceptions and comments in notebook)	Yes, No, or NA
4.3	**Equipment Maintenance & Cleaning**	
4.301	§211.67(b) Are written procedures established for the cleaning and maintenance of equipment and utensils?	
4.302	Are these procedures followed?	
4.303	§211.67(b)(1) Does a written procedure assign responsibility for the cleaning and maintenance of equipment?	
4.304	§211.67(b)(2) Has a written schedule been established and is it followed for the maintenance and cleaning of equipment?	
4.305	Has the cleaning procedure been properly validated?	
4.306	§211.67(b)(2) If appropriate, is the equipment sanitized using a procedure written for this task?	
4.307	§211.67(b)(3) Has a sufficiently detailed cleaning and maintenance procedure been written for each different piece of equipment to identify any necessary disassembly and reassembly required to provide cleaning and maintenance?	
4.308	§211.67(b)(3) Does the procedure specify the removal or obliteration of production batch information from each piece of equipment during its cleaning?	
4.309	Is equipment cleaned promptly after use?	
4.310	Is clean equipment clearly identified as "clean" with a cleaning date shown on the equipment?	
4.311	§211.67(b)(5) Is clean equipment adequately protected against contamination prior to use?	
4.312	§211.67(b) Is equipment inspected immediately prior to use?	
4.313	§211.67(c) Are written records maintained on equipment cleaning, sanitizing, and maintenance on or near each piece of equipment?	
4.4	**Measurement Equipment Calibration Program**	
4.401	§211.68(a) Does the facility have approved written procedures for checking and calibration of each piece of measurement equipment? (Verify procedure and log for each piece of equipment and note exceptions in notebook with cross reference.)	
4.402	§211.68(a) Are records of calibration checks and inspections maintained in a readily retrievable manner?	
4.5	**Equipment Qualification Program**	
4.501	§211.63 Verify that all pieces of equipment used in production, packaging, and quality assurance are capable of producing valid results.	
4.502	§211.68(a) When computers are used to automate production or quality testing, have the computer and software been validated?	
4.503	Have on-site tests of successive production runs or tests been used to qualify equipment?	
4.504	Were tests repeated a sufficient number of times to ensure reliable results?	

TABLE 6 ***Continued***

Question	Instructions/Questions (note any exceptions and comments in notebook)	Yes, No, or NA
4.505	§211.63 Is each piece of equipment identified to its minimum and maximum capacities and minimum and maximum operating speeds for valid results?	
4.506	Have performance characteristics been identified for each piece of equipment? (May be provided by the manufacturer but must be verified under typical operations conditions.)	
4.507	Have operating limits and tolerances for performance been established from performance characteristics?	
5.0	Material/component control	
5.1	**Material/Component Specification and Purchasing Control**	
	Although purchasing is not specifically addressed in the current GMP regulation, incumbent upon user of components and materials to ensure quality of product, material, or component.	
5.101	Has each supplier/vendor of material or component been inspected/audited for proper manufacturing controls? (Review suppliers and audits and enter names, material supplied, and date last audited in notebook.)	
5.2	**Material/Component Receipt, Inspection, Sampling, and Laboratory Testing**	
5.201	§211.80(a) Does the facility have current written procedures for acceptance/rejections of drug products, containers, closures, labeling, and packaging materials? (List selected materials and components in notebook and verify procedures.)	
5.202	§211.80(d) Is each lot within each shipment of material or components assigned a distinctive code so material or component can be traced through manufacturing and distribution?	
5.203	§211.82(a) Does inspection start with visual examination of each shipping container for appropriate labeling, signs of damage, or contamination?	
5.204	§211.82(b) Is the number of representative samples taken from a container or lot based on statistical criteria and experience with each type of material or component?	
5.205	§211.160(b) Is the sampling technique written and followed for each type of sample collected?	
5.206	Is the quantity of sample collected sufficient for analysis and reserve in case retesting or verification is required?	
	Verify that the following steps are included in written procedures unless more specific procedures are followed:	
5.207	§211.84(c)(2) Containers are cleaned before samples are removed.	
5.208	§211.84(c)(4) Stratified samples are not composited for analysis.	
5.209	§211.84(c)(5) Containers from which samples have been taken are so marked indicating date and approximate amount taken.	

TABLE 6 *Continued*

Question	Instructions/Questions (note any exceptions and comments in notebook)	Yes, No, or NA
5.210	Each sample container is clearly identified by material or component name, lot number, date sample taken, name of person taking sample, and original container identification.	
5.211	§211.84(d)(1)(2) At least one test is conducted to confirm the identity of a raw material (bulk chemical or pharmaceutical) when a Certificate of Analysis is provided by supplier and accepted by QA.	
5.212	If a Certificate of Analysis is not accepted for a lot of material, then additional testing is conducted by a written protocol to determine suitability for purpose.	
5.213	§211.84(d)(6) Microbiological testing is conducted where appropriate.	
5.3	**Material Component Storage and Handling**	
	Verify that materials and components are stored and handled in a way that prevents contamination, mixups, and errors.	
5.301	§211.42(b) Are incoming material and components quarantined until approved for use?	
5.302	Are all materials handled in such a way to prevent contamination?	
5.303	Are all materials stored off the floor?	
5.304	Are materials spaced to allow for cleaning and inspection?	
5.305	§211.122(d) Are labels for different products, strengths, dosage forms, etc., stored separately with suitable identification?	
5.306	Is label storage area limited to authorized personnel?	
5.307	§211.89 Are rejected components, material, and containers quarantined and clearly marked to prevent their use?	
5.4	**Inventory Control Program**	
5.401	§211.142 Are inventory control procedures written?	
5.402	Does the program identify destruction dates for obsolete or out-dated materials, components, and packaging materials?	
5.403	§211.150(a) Is stock rotated to ensure that the oldest approved product or material is used first?	
5.404	§211.184(e) Is destruction of materials documented in a way that clearly identifies the material destroyed and the date on which destruction took place?	
5.5	**Vendor (Supplier) Control Program**	
5.501	Are vendors periodically inspected according to a written procedure?	
5.502	Is the procedure for confirming vendor test results written and followed?	
6.0	Operational control	

TABLE 6 ***Continued***

Question	Instructions/Questions (note any exceptions and comments in notebook)	Yes, No, or NA
6.1	**Material/Component/Label Verification, Storage, and Handling**	
6.101	§211.87 Do written procedures identify storage time beyond which components, containers, and closures must be reexamined before use?	
6.102	§211.87 Is release of retested material clearly identified for use?	
6.103	Are retesting information supplements originally obtained?	
6.104	Do written procedures identify steps in the dispensing of material for production?	
6.105	Do these procedures include (1) release by QC, (2) documentation of correct weight or measure, and (3) proper identification of containers?	
6.106	Does a second person observe weighing/measuring/dispensing and verify accuracy with a second signature?	
6.107	§211.101(c) Is the addition of each component documented by the person adding the material during manufacturing?	
6.108	§211.101(d) Does a second person observe each addition of material and document verification with a second signature?	
6.109	§211.125(a) Does a written procedure specify who is authorized to issue labels?	
6.110	§211.125(a) Does a written procedure specify how labels are issued, used, reconciled with production, returned when unused, and the specific steps for evaluation of any discrepancies?	
6.111	§211.125(d) Do written procedures call for destruction of excess labeling on which lot or control numbers have been stamped or imprinted?	
6.2	**Equipment/Line/Area Cleaning, Preparation, and Clearance**	
6.201	§211.67(b)(5) Do written procedures detail how equipment is to be checked immediately prior to use for cleanliness, removal of any labels, and labeling from prior print operations?	
6.202	§211.67(b)(3) Do written procedures detail any disconnection and reassembly required to verify readiness for use?	
6.3	**Operational Process Validation and Production Change Order Control**	
6.301	Have production procedures been validated? (Review selected procedures for validation documentation. Adequate?)	
6.302	§211.100(a) Does the process control address all issues to ensure identity, strength, quality, and purity of product?	
6.303	§§211.101(a) Does the procedure include formulation that is written to yield not less than 100% of established amount of active ingredients?	

TABLE 6 ***Continued***

Question	Instructions/Questions (note any exceptions and comments in notebook)	Yes, No, or NA
6.304	§211.101(c) Are all weighing and measuring preformed by one qualified person and observed by a second person?	
6.305	§211.101(d) Have records indicated preceding policy been followed by presence of two signatures?	
6.306	§211.103 Are actual yields calculated at the conclusion of appropriate phases of the operation and at the end of the process?	
6.307	§211.103 Are calculations performed by one person? Is there independent verification by a second person?	
6.4	**In-Process Inspection, Sampling, and Laboratory Control**	
6.401	§211.110(a) Are written procedures established to monitor output and validate the performance of manufacturing procedures that may cause variability in characteristics of in-process materials and finished drug products?	
6.402	§211.110(c) Are in-process materials tested at appropriate phases for identity, strength, quality, purity, and are they approved or rejected by Quality Control?	
6.403	§211.160(b) Are there laboratory controls including sampling and testing procedures to assure conformance of components, containers, closures, in-process materials, and finished product specifications?	
6.5	**Reprocessing/Disposition of Materials**	
6.501	§211.115(a) Do written procedures identify steps for reprocessing batches?	
6.502	§211.115(b) Are quality control review and approval required for any and all reprocessing of material?	
6.503	Does testing confirm that reprocessed batches conform to established specification?	
6.504	Does a written procedure outline steps required to reprocess returned drug products (if it can be determined that such products have not been subjected to improper storage conditions)?	
6.505	Does Quality Control review such reprocessed returned goods and test such material for conformance to specifications before releasing such material for resale?	
7.0	Finished product control	
7.1	**Finished Product Verification, Storage, and Handling**	
7.101	§211.30 Do written procedures indicate how and who verifies that correct containers and packages are used for finished product during the finishing operation?	
7.102	§211.134(a) In addition, do written procedures require that representative sample of units be visually examined upon completion of packaging to verify correct labeling?	
7.103	§211.137(a) Are expiration dates stamped or imprinted on labels?	
7.104	§211.137(b) Are expiration dates related to any storage conditions stated on the label?	

TABLE 6 *Continued*

Question	Instructions/Questions (note any exceptions and comments in notebook)	Yes, No, or NA
7.105	§211.142(a) Are all finished products held in quarantine until QC has completed its testing and releases product on a batch-to-batch basis for sale?	
7.106	§211.142(o) Is finished product stored under appropriate conditions of temperature, humidity, light, etc.	
7.2	**Finished Product Inspection, Sampling, Testing, and Release for Distribution**	
7.201	§211.166 Has the formulation for each product been tested for stability based on a written protocol? (Containers must duplicate those used in final product packaging.)	
7.202	§211.166 Are written sampling and testing procedures and acceptance criteria available for each product to ensure conformance to finished product specifications?	
7.203	§211.170(a) Is a quantity of samples equal to at least twice the quantity needed for finished product release testing maintained as a reserve sample?	
7.204	§211.167(a) Are sterility and pyrogen testing performed as required?	
7.205	§211.167(b) Are specific tests for foreign particles or abrasives included for any ophthalmic ointments?	
7.206	§211.167(c) Do controlled release or sustained release products include tests to determine conformance to release time specification?	
7.3	**Distribution Controls**	
7.301	§211.150(a) Does a written procedure manage stocks to ensure that oldest approved product is sold first?	
7.302	§211.150(a) Are deviations to the policy above documented?	
7.303	§211.150(a) Does a written procedure identify the steps required if a product recall is necessary?	
7.304	Is the recall policy current and adequate?	
7.4	**Marketing Controls**	
7.401	The current regulation does not address marketing controls per se except that all finished products must meet their specifications.	
7.5	**Complaint Handling and Customer Satisfaction Program**	
7.501	§211.198(a) Are complaints, whether received in oral or written form, documented in writing, and retained in a designated file?	
7.502	§211.198(a) Are complaints reviewed on a timely basis by the Quality Control unit?	
7.503	§211.198(b)(1) Is the action taken in response to each complaint documented?	
7.504	§211.198(b)(3) Are decisions not to investigate a complaint also documented and the name of the responsible person documented?	
7.505	§211.198(b)(2) Are complaint investigations documented and do they include investigation steps, findings, and follow-up steps, if required? Are dates included for each entry?	

7. The references to sections in the GMP regulation are for your convenience should a question arise. In some instances, two or more sections within the GMP regulation may have bearing on a specific subject. The headings in the GMP regulation will usually offer some guidance on the areas covered in each section.
8. A general suggestion for a successful audit is to spend most of your time on major issues and a smaller portion of your time on small issues. There may be observations that you may wish to point out to supervisory personnel that deserve attention but do not belong in an audit report because they are relatively insignificant. By the same token, too many small items suggests a trend of noncompliance and deserve attention as such. When citing these, be specific.

REFERENCES

1. *U.S. Code of Federal Regulations* (CFR), Title 21, Part 211, Current good manufacturing practice for finished pharmaceuticals, available: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CRFSearch.cfm?CFRPart=211, accessed Dec. 5, 2006.
2. American National Standards Institute (ANSI) (2000), Quality management system—Requirements, ANSI/ISO/ASQ Q9001-2000, ANSI, New York.
3. American National Standards Institute (ANSI) (2000), Quality management system—Fundamentals and vocabulary, ANSI/ISO/ASQ Q9000-2000, ANSI, New York.
4. International Organization for Standardization (ISO), Application of risk management of medical devices, ISO 14971:2000, ISO, Geneva.
5. U.S. Department of Health and Human services, U.S. Food and Drug Administration, Pharmaceutical cGMPs for the 21st century—A risk -based approach, Final Report—Fall 2004, September 2004, available: http://www.fda.gov/cder/gmp/gmp2004/GMP_finalreport2004.htm, accessed Dec. 5, 2006.
6. U.S. Department of Health and Human Services (DHHS), Food and Drug Administration (2006, June), Guidance for industry: Q9 Quality risk management, DHHS, Rockville, MD.
7. U.S. Department of Health and Human Services (DHHS), Food and Drug Administration (2004, Sept.), Guidance for industry: PAT—A framework for innovative pharmaceutical development, manufacturing, and quality assurance, DHHS, Rockville, MD.
8. U.S. Department of Health and Human Services (DHHS), Food and Drug Administration (2006, Sept.), Guidance for industry: Quality systems approach to pharmaceutical CGMP regulations, DHHS, Rockville, MD.
9. *U.S. Code of Federal Regulations* (CFR), Title 21, Part 820, Quality system regulation for medical devices, available: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=820, accessed Dec. 5, 2006.
10. International Organization for Standardization (ISO), (2003), Medical devices—Quality management systems—Requirements for regulatory purpose, ISO 13485:2003, ISO, Geneva.
11. Juran J. M., and Godfrey, A. B., Eds. (1999), *Juran's Quality Handbook*, 5th ed., McGraw-Hill, New York.
12. FDA compliance policy guide section 130.000, FDA access to results of quality assurance program audits and inspections (CPG 7151.02), available: http://www.fda.gov/ora/compliance_ref/cpg/cpggenl/cpg130-300.html, accessed Dec. 5, 2006.

13. International Organization for Standardization (ISO), (2002), Guidelines for quality and/or environmental management systems auditing, ISO 19011:2002, ISO, Geneva.
14. The Global Harmonization Task Force, SG4, Training requirements for auditors (guidelines for regulatory auditing of quality systems of medical device manufacturers—Part 1: General requirements—Supplement 3), available: http://www.ghtf.org/sg4/inventorysg4/trainingfinal.pdf, accessed Dec. 5, 2006.
15. FDA compliance program guidance manual for FDA staff: Drug manufacturing inspections program (7356.002), 2/1/2002, available: http://www.fda.gov/cder/dmpq/compliance_guide.htm, accessed Dec. 5, 2006.
16. U.S. Department of Health and Human Services (DHHS), Food and Drug Administration (2004, Sept.), Guidance for industry: Sterile drug products produced by aseptic processing—Current good manufacturing practice, DHHS, Rockville, MD.
17. U.S. Department of Health and Human Services (DHHS), Food and Drug Administration (2001, Aug.), Guidance for industry: Q7A good manufacturing practice guidance for active pharmaceutical Ingredients, DHHS, Rockville, MD.

3.3

CREATING AND MANAGING A QUALITY MANAGEMENT SYSTEM

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3.3.1 INTRODUCTION

The world's population continues to grow and the average life expectancy continues to increase. Pharmaceutical and biopharmaceutical products are more in demand as the population expands, requiring novel and specialized medications to treat common and debilitating diseases. The industry is challenged to rapidly discover and commercialize products to treat existing unmet medical needs and emerging threats as viruses mutate into new diseases that threaten the stability of the world as we know it.

At the same time, the global marketplace continues to increase its demand on the industry. Government, consumer, and wholesale buying pressures demand lower prices. Higher quality standards are expected by regulators and consumers. Competition continues to increase from generic, biosimilar, and counterfeit producers. Developing nations, with lower cost overheads, are developing economical production capabilities. Meanwhile, research and development costs are increasing.

This chapter will outline the concepts, benefits, and practical implementation steps for developing a comprehensive quality management system (QMS) that supports pharmaceutical and biopharmaceutical manufacturing operations. The material presented is universal in its utility, applicable to small and large companies, development, and commercial enterprises. A QMS is a proactive, structured approach

to supporting development and manufacturing operations. It includes all processes, metrics, management review, and continuous improvement activities. The QMS, as described in this chapter, is further supported through an active change management program and application of annual quality plans to ensure ongoing system sustainability.

A well-designed QMS, with mature, developed processes, provides the required infrastructure and support necessary for successful manufacturing operations. Integrated processes, proactively managed, that can be quickly modified to meet changing business and regulatory demands will support ongoing manufacturing operations and provide competitive advantage. This chapter provides guidance on creating and managing a robust QMS that supports manufacturing operations in the pharmaceutical and biopharmaceutical industry.

3.3.2 UNDERSTANDING A QUALITY MANAGEMENT SYSTEM

Every development, testing, manufacturing, packaging, warehouse, or distribution facility has its own unique role in producing an output or product for consumption by a customer somewhere in the pharmaceutical or biopharmaceutical supply chain. Each facility and organization is critically dependent upon several different processes that function interdependently producing the desired output. Organizations' survival and profitability are directly linked to the efficiency of design, execution, performance, and interrelational attributes of these processes. Throughout a product life cycle, from early discovery through development, scale up, clinical testing, product technology transfer, registration, approval, commercialization, and eventually product discontinuance, robust processes are the foundation supporting the successful enterprise.

Manufacturing support processes are discrete in their output, but interrelated in their overall effect. Weak or ill-defined processes have a diminishing overall effect on the organization and its product. It manifests itself as increased rework, rejected material, extended cycle times, delayed disposition, high nonconforming performance metrics, complaints, recalls, or other inabilities to meet customer or market demands. A comprehensive QMS may encompass all the processes supporting development and manufacturing. It includes the standards, policies, and procedures required to measure those processes for performance and maturity. It provides metrics necessary for leadership to perform risk-based prioritization and focus resources for business improvement and regulatory compliance.

Robust processes will have owners that have defined roles, responsibilities, and accountabilities. These process owners must be fully dedicated to their process. They must know their process capabilities and expectations, the interrelationship between their process and other processes and manage them like a business unto themselves. Functional management must support process owners, and leadership must understand and lead the QMS effort as an ongoing program, treating it as the integral part of the business that it is.

A QMS is an organizational approach consisting of people, interrelated processes, process inputs and outputs, and structured review programs that lead to ongoing continuous improvement. This complexity of processes requires a programmatic organization and management to effectively interrelate its components. A

QMS program office is required to provide the organizational benefits expected from well-managed processes and should be one of the first elements established when instituting the program.

A QMS and the processes comprising it are not the sole responsibility of the quality function or a single functional group. Inherently, these processes have no bounds in the organization. The concept must be owned, managed, or executed by all staff from leadership to the most entry-level manufacturing associate. A quality mindset must be part of every employee that contributes to the discovery, manufacture, packaging, testing, warehousing, and shipping of a product or output. A culture of quality and understanding of the processes in which personnel work are essential to advance the QMS to maximize benefits to the enterprise and remain competitive.

Instituting a QMS through a holistic approach that supports manufacturing operations has the potential to meet and exceed customer, patient, shareholder, and employee expectations. It requires a cross-functional team approach, with proactive management of all the processes responsible for manufacture, including functional support from development, manufacturing, analytical, engineering, and quality assurance. The development and maintenance of a tested, robust QMS requires time and resources. Full maturation of processes and organizational culture change may take, in some cases, years to fully implement and realize benefits, but worth the effort and time. Significant QMS issues should not be addressed as one-off fixes. Rather, action taken to remediate deficient processes should be approached as long-term corrections, addressing the root cause of the failed process, so they do not repeatedly plague the organization.

The ultimate responsibility for a robust, functional QMS lies with top management. The organization follows the leadership, and therefore, leadership must support a QMS that is specifically designed for the organization, be aware of and monitor its progress and contribution to the organization, and frequently support, guide, and maintain it. Doing so ensures viability of the QMS, and in turn the QMS will provide leadership the data and guidance necessary to effectively manage the organization.

3.3.2.1 Defining Quality Management Systems

The term *system* or *quality system* is used with surprising inconsistency throughout the pharmaceutical and biopharmaceutical industry and by government regulators. Even within a single company or within a department, the terms can be nebulous in their use and interpretation. System is often used to describe an individual process or unit operation. Often, the term system is used so narrowly as to describe an individual policy, standard, or even a single procedure.

Recent initiatives by global organizations such as ISO (International Organization for Standardization, www.iso.org) and ICH (International Conference on Harmonization, www.ich.org) are attempting to bring consistency in concept and standardization in definition to the QMS. In 2004, the Pharmaceutical Inspection Co-Operation Scheme (PIC/S, www.picscheme.org) issued its recommendation on Quality System Requirements for Pharmaceutical Inspectorates. The U.S. Food and Drug Administration (FDA) initiated inspection surveillance approaches based upon QMS organization and is another source of definition and interpretation.

Inconsistency in language and expectations continues to exist; however, efforts are progressing to minimize distinctions and globally harmonize efforts, structure, and language concerning quality systems.

According to Webster's dictionary, *system* is defined as a regularly interacting or interdependent group of items forming a unified whole; a group of interacting bodies under the influence of related forces . . . an assemblage of substances that is in or tends to equilibrium . . . a group of organs that, when together, perform one or more vital functions . . . an organization forming a network especially for distributing something or serving a common purpose . . . an organized set of doctrines, ideas, or principles usually intended to explain the arrangement or working of a systematic whole [1].

The vocabulary and definitions used in this chapter defines a quality management system as the compilation of all the processes required to support the manufacture, packaging, testing, release, and distribution of an active pharmaceutical ingredient (API) or drug product. It is aligned with that of the FDA Center for Drug Evaluation and Research (CDER) compliance program 7356.002, issued to investigators for the inspection of pharmaceutical and biopharmaceutical manufacturing plants (www.fda.gov/IOM 7356.002). The CDER inspection program subdivides the processes comprising the QMS into six subsystems: quality, facilities/equipment, production, materials control, laboratory controls, and packaging and labeling.

There are no specific CDER requirements as to which processes belong under each subsystem; however, one can easily follow the outline provided in 21 CFR Part 211, the regulations applicable to human drug product manufacture, to aid in the determination of processes likely to be inspected during a regulatory inspection (www.fda.gov). The FDA subdivides all the processes comprising a company's QMS into six subsystems to ensure adequate and varied coverage during inspections. See Figure 1. Using the same process organization structure and vocabulary as regulators provides an enterprise the advantage of more efficient inspection preparation and avoidance of miscommunication during and after regulatory inspections.

The CDER subsystem organization provides regulators and management the ability to focus attention to specific functional areas. Table 1 is an example of the processes, organized under appropriate subsystems, supporting a typical API or drug fill-and-finish operation. These subsystems are organized according requirements found in regulations used by investigators during inspections, 21 CFR Part 210, 211, and the unit operations and support processes necessary for production.

One size does not fit all situations. Each enterprise has the responsibility and latitude to design a QMS to meet its specific needs. Even facilities with very similar manufacturing operations may require different processes to support the business. Each manufacturing organization requires a customized set of processes which will comprise its QMS. The management group responsible for the QMS should be able to identify and justify the processes comprising the system. There is not a single set of processes that can be universally applied to all operations, as each organization is unique in its business, product output, organization, culture, as well as local and global regulatory and customer requirements.

Processes identified as part of the QMS can be organized into the appropriate CDER subsystems for the purpose of aligning with the methodology used during inspections. It also provides management the ability to determine areas of strength or opportunities for improvement within the QMS. Regulators will always include

FIGURE 1 Subsystems and management relationship.

TABLE 1 Quality Management System Subsystems and Processes

Subsystem	Processes
Quality	Audits and inspections
	Management review
	Risk management
	Organization and personnel
	Training
	Document management
	Change control
	Nonconformances
	Corrective and preventative actions
	Biological product deviation
	Product disposition
	Validation
Production	Manufacturing
	Process monitoring
	Environmental and gowning monitoring
	In-process controls
	Gowning
Facilities/equipment	Facility and equipment design
	Equipment maintenance
	Equipment cleaning
	Calibration
Materials control	Supplier quality management
	Sampling and inspection
	Receiving, warehouse, and storage
	Inventory management
	Transport
	Return and salvage
Laboratory controls	Laboratory testing
	Sample management and sample plans
	Stability program
Packaging and labeling	Labeling controls and approvals
	Package development

a focus on the processes within the quality subsystem. Other subsystems will be reviewed during inspections based upon the type of inspection and compliance history of the enterprise. More information on how the FDA focuses inspections based on quality system and subsystem organization is available at the FDA website (www.fda.gov) or articles written on this subject [2].

To maximize the effect of a QMS, it should be designed to be scalable and transferable throughout the enterprise and easy to understand and execute. An adequately designed QMS results in increased efficiency, a compliant operation, and staff satisfaction.

3.3.2.2 Synthesis versus Analysis

With systems thinking, the whole is greater than the sum of its parts. Systems rely upon the interaction of several processes. An individual process has limited value on its own, regardless of the level of development it has achieved. Processes provide value to the system through synthesis with other processes.

In the early twentieth century, researchers began to recognize the existence of interdependent relationships and organizational patterns among seemingly discrete parts. It is the relationships that allow parts to function as a whole. The "perceived whole" is a system. Systems thinking involves considering the parts in the context of that whole. In systems thinking:

- Everything in a system is related to everything else in the system.
- The parts of a system work together to achieve the overall objective of the whole system.
- In addition to the immediate effects of an action, there will be other consequences that ripple through the system.
- Every change brings benefits and consequences.
- Changing or reinforcing patterns and relationships within a system is as necessary to achieving the goals of the system as changing or retaining the parts of the system.
- Systems are "living" entities that sustain themselves through self-regulating dynamic equilibrium and organize to respond to externally imposed change.

Viewing a QMS in this context is beneficial to organizational leadership and management responsible for the system. It puts into perspective the overall effect on an organization that is achievable by individual processes alone and what can be achieved and sustained through active management and the interaction between those processes.

3.3.2.3 System versus Process

Traditional industry paradigm has the Quality Department responsible for quality and the Manufacturing Department responsible for producing product. Inherent conflict exists in this model due to competing functional priorities. By building quality concepts and accountabilities into production processes responsible for production, quality becomes infused into the organization. Both Quality and

Manufacturing therefore share the common goal of supplying high-quality product through the efficient execution of their processes.

Historically, very few processes were regarded as "quality systems," and they were viewed as something owned by the Quality Department. These "systems" were in fact ill defined and nonrelated processes used to monitor or detect individual actions and activities occurring in the manufacturing environment. These systems were based on quality control (QC) type of responsibilities for testing quality into the product. Examples include raw material testing, in-process and finished-product testing, nonconforming material review, environmental monitoring, and release and distribution. Few were interrelated with other processes, actively supported by management, or reviewed by leadership for performance or compliance.

The QC monitoring processes described above, if supported, were limited in their ability to support improvements and could only lead to action that was reactive in nature. Process integration is weak or nonexistent. Neither process maturity and development nor proactive system management is achievable. In the past, QMS enhancement was viewed as an expense and not seen as a relational contributor to the value chain. Aware management now realizes, through regulatory action, penalty and fines, delayed product approvals, recalls, and the like that establishment of a comprehensive QMS is essential to survive in the current regulatory environment and remain competitive in the business environment.

With the advancement of quality assurance (QA) principles and concepts at the end of the last century, QMSs have evolved to be more proactive to include change control, supplier and internal auditing, risk management, lagging and leading metric collection, and review. Review of predictive metrics has become the basis for preventive action and continuous improvement programs. Today's competitive environment obligates leading manufacturers and world-class organizations to apply proactive system thinking to expand their focus to include all processes that support product quality, irregardless of the stage of development or manufacture. Early implementation of appropriate processes supports quality-by-design concepts and practice, within the framework of a QMS and ensures quality in all processes and provides the foundation for good investigations and continuous improvement.

A QMS should be comprised of all the processes supporting that business and include an effective management review of those process metrics. Management needs to be aware of and understand process performance through structured metrics review programs in order to take appropriate action, providing resources and capital to improve the QMS. This hierarchy is illustrated in Figure 2.

Processes supporting and applicable to pharmaceutical and biopharmaceutical manufacturing are easily determined by examining the business needs of the organization and the regulations governing them. A carefully designed QMS will consider the needs of the enterprise as a whole, as well as that of the individual unit operations comprising the enterprise. If the QMS design is comprehensive, it will provide significant value to global and local management. It will support staff by standardizing processes, requirements, and expectations and provide leadership meaningful and comparable metrics on system and process performance. Changes can be quickly facilitated and implemented when process modifications are required. A consistent representation of processes to regulators builds confidence and trust that the enterprise is capable to produce the product for which approval has been granted.

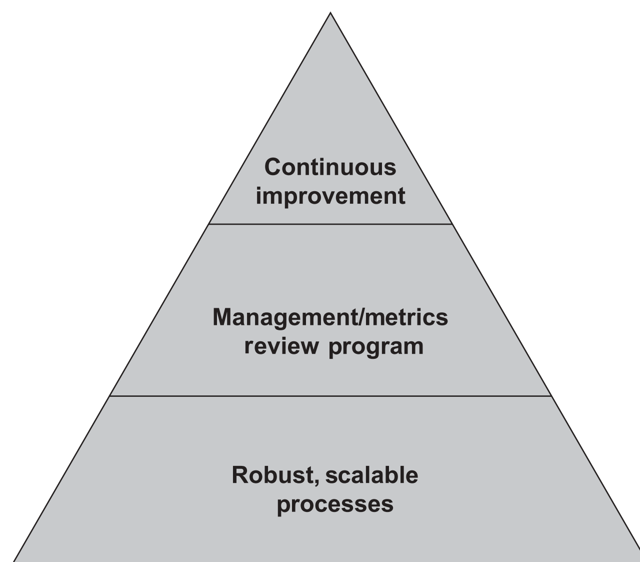

FIGURE 2 Quality management system hierarchy.

3.3.2.4 Business Benefits of Establishing a Robust Quality Management System

The competitive nature of the pharmaceutical business demands capable and efficient processes supporting discovery, development, technology transfer and scale-up, and commercial manufacturing and distribution. Execution of efficient processes is the foundation for new and ongoing enterprises to be successful. It is the basis for successful manufacture and the bedrock upon which management and regulators can gauge the capability level of the enterprise. Providing patients with needed medicines in a timely, cost-efficient manner, without delay due to manufacturing or compliance issues, should be a primary driving force behind the pharmaceutical and biopharmaceutical industry.

Leadership may ask the question: Why implement a quality management system? The answer is that a well-designed system is necessary to establish a state of control to ensure that a high quality, safe, and efficacious product is produced and available for patients. Quality systems as described in the forthcoming ICH Q10 guidance is the logical complement to its predecessors, ICH Q8 (Product Development) and ICH Q9 (Risk Management) (www.ich.org). These three guidance documents build upon each other from quality-by-design activities in development through the entire product life cycle. When used together, the guidance documents maximize their benefits to the enterprise through better process understanding, less regulatory scrutiny, and increased freedom to operate. Together, these guidance's support more efficient product life-cycle management from discovery through development and commercialization.

Inefficient operations cost businesses untold amounts in financial and human capital. A poorly designed system coupled with inefficient processes may result in rework of development and commercialization activities, data integrity issues, inefficient use of resources, and delay in approval. Poorly designed processes may also

lead to loss of future revenue with business partners and have a negative regulatory consequence.

A recent study conducted by the Pharmaceutical Manufacturing Research Project, a joint venture by Georgetown University and Washington University in St. Louis business schools, collected data from 42 manufacturing facilities owned by 19 companies to determine factors that affected industry performance. The Final Benchmarking Report assessed performance in terms of manufacturing times, frequency of deviations from manufacturing standards, reasons for deviations, manufacturing yield, and rates of improvement for those metrics.

The study determined that improvements in manufacturing process could save industry more than $50 billion in manufacturing costs, which the researchers believe could result in lower drug prices and more money for R&D. The report received no industry or government funding [3].

Leadership is both challenged and rewarded for supporting the development of a robust QMS. On one hand, it takes time and resources to design and develop a comprehensive program. Immediate return on this investment is not usually forthcoming. Management is typically under pressure to deliver aggressive results in a short time period, which is counterintuitive to careful planning and long-range development. Conversely, proactively formalizing and supporting a robust QMS will, in the long run, ensure the operations freedom to operate (regulatory compliance) and deliver business efficiencies.

In the pharmaceutical and biopharmaceutical manufacturing industry, the perception of quality has dramatically changed over the past several years, and loss of market capitalization can be a direct correlation to this perception. Large pharmaceutical companies have gone from some of the world's most admired companies to losing significant percentage of their value, based on consumer, media, and investor perceptions of quality and ethics. Speaking at a recent Parenteral Drug Association (PDA)/FDA joint regulatory conference, Daniel Diermeier, IBM Distinguished Professor of Regulation and Competitive Practice, Northwestern University stated: "The perception of quality on the pharmaceutical value chain is greater than in other industries (auto, furniture, etc.). Patients cannot assess the quality of drugs as they can a car or hotel room. In healthcare, the 'value proposition' is higher than other industries and the Quality [Management] System is a critical subset of that perception" [4]. Dr. Diermeier goes on to suggest a QMS include processes for decision and detection to further protect the "value proposition" of the enterprise.

Enterprises lacking individual capable processes experience degrees of negative effects throughout the organization. This is true for processes that support discovery, development, manufacturing, or marketing. Recent examples of fines imposed by regulators for poor processes supporting the QMS are increasing (see Table 2). These costs are only indicative of the fine itself and do not include lost revenue, cost of consultancy for remediation, decreased shareholder value, and diminished staff morale and support. These costs are typically an order of magnitude or more greater than the fine itself.

A common misconception of pharmaceutical and especially smaller biopharmaceutical companies is that the implementation of a robust QMS is not required in areas other than commercial manufacturing. Small, biotech start-up companies also tend to delay the implementation of well-designed processes until they near the approval stage, focusing the organization instead for product approval or sale. This

TABLE 2 Potential Financial Impacts

Company	Compliance Issue	Type of Impact	Cost to Business ($Mil)
A	Failure to follow procedure Inadequate training	Multiple 483 observations	<1
B	Inadequate process definition, controls, and oversight	Warning letter	>1
C	Repeat observations—direct product impact Failure to meet warning letter commitments	Consent decree	>100
D	Plant shutdown	Direct fines product stock-out	>500

can become a costly miscalculation, as speed to market and limited capital demand processes supporting efficient development, clinical and regulatory submission processes be executed with minimal waste or rework.

Although QMSs are routinely identified with commercial manufacturing, it is critical to establish process parameters for discovery, development, and technology transfer, including scale-up, characterization of process, analytical methodology, and validation. Development activities are executed more efficiently through the application of robust processes and ultimately become the foundation for robust manufacturing. Failed development studies, inadequate comparability reports, clinical studies requiring repeated, or poorly supported analytical and process characterization contribute to delayed submissions and weak regulatory submission and inspection presentation. The identification of processes supporting these activities, owner identification and accountability and support will ensure success of the enterprise and reduce the anxiety and uncertainty that is inherent in development and approval activities.

Several opportunities exist for pharmaceutical and biopharmaceutical manufacturing plants to improve efficiency and cost savings, which ultimately validate the program's benefits and supports leadership in achieving their financial goals. Traditionally, the industry environment is heavily regulated and has been very risk adverse. These two elements combine to offer countless opportunities to improve inefficient and ill-defined processes, clarify process scope, define process owner accountabilities and responsibilities, and remediate process duplication or gaps. Performing inefficient processes for the sake of avoiding regulatory scrutiny or attempting to defend poorly characterized processes without adequate data and interpretation becomes self-defeating to the industry. Poor prioritization of work, ill-defined process relationships, and functional management interference or neglect may also contribute to inefficiency. Staff requires processes that are easy to execute, well integrated, and result in value-added activities. This can only be accomplished through the design and execution of efficient processes that are interrelated, bringing value to the enterprise, process owners, and stakeholders.

An example of a robust process is the design, development, and operation of a nonconformance process. Regulations require an operational process to identify, document, and correct nonconformances occurring in licensed pharmaceutical manufacturing facilities for approved products. Companies spend significant human

capital identifying, documenting, and tracking nonconformances. But how much is actually being done to remediate these nonconformances? Can the nonconformances be related to previously completed development or commercialization studies? Is the nonconformance process sufficiently related to an effective corrective or preventive action (CAPA) process? Does the preventive action interrelate efficiently with an efficient change control process to ensure proposed changes remain in compliance with registrations? Are the documentation and training processes sufficient to support approved changes? An adequately designed QMS will ensure the supporting processes are present and that functional and interrelationships established. A systems implementation provides a holistic approach, which results in both building effective individual processes and interrelating those processes to maximize their effect on the business, driving efficient and science-based activities.

Maintaining good manufacturing practice (GMP) compliance is essential for pharmaceutical and biopharmaceutical companies. Results of noncompliance are costly fines, loss of revenue, higher overhead costs, delayed approvals, and poor customer and regulatory perceptions. Poor compliance results from an inadequately designed QMS that lacked the processes and management review required to support the enterprise. Processes supporting compliance include self-audits, change control, document revision and approval, and staff training programs. Regular management review of these processes will ensure resources are allocated to appropriate initiatives and there should be no surprises during inspections. A well-designed QMS should prevent negative regulatory consequences. Efficient and compliant processes support lean manufacturing efforts through the documentation and understanding of processes. Management review of these processes ensures that leadership awareness, support, and action is taken by the organization when appropriate.

Figures 3 and 4 illustrate how a biennial document review process and document processing cycle time metrics faltered in their early stages due to lack of process ownership, definition, and management review. This situation presented a compliance risk to the organization and resulted in poor business efficiencies. Improve-

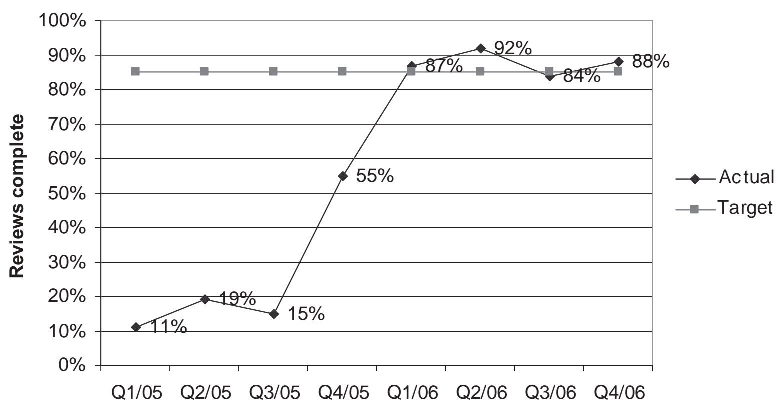

FIGURE 3 Biennial document review process.

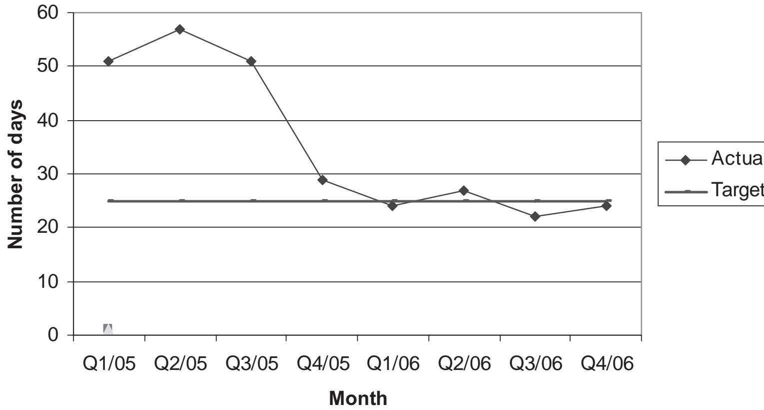

FIGURE 4 Document review cycle time.

ment was attained by assigning a process owner who defined and improved each process, developed meaningful metrics, and presented those metrics to management. Management became aware of process performance, understood the compliance risk and business impact and took appropriate action to focus staff efforts to meet process requirements. Results were improved document review cycles, proactive compliance with internal procedures and regulatory requirements, and the satisfaction of knowing that no additional effort was required to achieve better business results and regulatory compliance.

3.3.2.5 Industry and Regulatory Expectations

While there are no requirements for a "quality system" in current FDA regulations applicable to pharmaceutical and biopharmaceutical manufacturing, regulatory agencies and industry trade organizations are increasingly recognizing the importance of robust, functioning quality systems in support of manufacturing the world's medicinal products. The FDA realizes not all quality principles are represented in current GMP regulations for drug products (21 CFR Part 211), which were last updated in 1978.

Quality management system issues and their association with risk management are common topics discussed in trade and regulatory seminars and conferences. Recent guidelines such as FDA "Quality Systems Approach to Current Good Manufacturing Practice Regulations" found on the FDA website and part of FDA's initiative titled "GMP's for the 21st Century" was written to complement existing regulations. While the FDA guidance may change or even become redundant with the issuance of ICH Q10, there is common intent among industry and government to advance quality management systems. According to Joe Famulare, Director DMPQ, FDA, the "FDA wanted to write a comprehensive Quality System model that would support and correlate with CGMP regulations. The guidance is consistent with defining a state of control; facilitate quality efforts, change control, Quality by Design, and risk management" [4].

In discussing quality systems at a recent industry conference on GMPs, Chris Joneckis of the FDA CBER (Center for Biological Evaluation and Research) had

this to say: "A robust Quality Management System makes a strong case for quality product. It is a win, win, win—for patient, industry and regulators. It benefits technology transfer, process control, monitoring, capability, improves manufacturing, fewer nonconformances and better quality of investigations. Regulatory benefits include enhanced Chemistry, Manufacturing, Controls (CMC) review, change control, and submission of postapproval changes" [5].

Regulatory and industry guidance documents have been generated in support of developing and organizing quality systems. In the late 1990s, the system-based inspection approach was formalized by the Center for Devices & Radiological Health (CDRH) of the FDA [6]. These regulations were codified as QSR, Quality Systems Regulations, and are included in Part 820 of the *Code of Federal Regulations* (CFR).

The CDER and CBER soon followed the CDRH approach and issued their own Compliance Program Guidance Manuals, 7356.002 [7] and 7345.848 [8], respectively, which were modeled on the CDRH QSR approach. The CDER and CBER are responsible for ensuring the biennial inspection of pharmaceutical and biopharmaceutical manufacturing facilities. The guidelines listed here are used by investigators during manufacturing inspections. Process owners and stakeholders as well as management and leadership should be familiar with these compliance manuals and how investigators plan to use them during inspections.

Current FDA inspectional surveillance, based on the models described above, requires investigators evaluate the processes within the subsystems defined by the QMS to determine compliance and risk to patient safety. This is different than the traditional approach of reviewing individual products during inspections. There is subtle, yet significant advantage to both the regulating agencies and compliant companies by using a system approach, as the inspections are designed to be faster and cover many product types during one inspection. Companies with compliant histories can benefit with nominal inspections, whereas companies with noncompliant histories will receive more regulatory scrutiny and possible regulatory action.

The movement by industry groups such as the ISO, which attempts to provide recognized standards for many industries, was also grounded in a systems approach with the publication and certification of ISO 9000 series and later with ISO 2000:9004 (www.iso.org), which is based on QMS establishment and eventually continuous improvements once processes become stable.

The ICH, a joint regulatory–industry initivative on international harmonization for drug development and approval, also recognizes the value and contribution of a quality systems approach through its guidance development on this topic (ICH Q10). The pharmaceutical and biopharmaceutical industry and regulatory agencies are collaborating to finalize the guidance sometime in 2008. ICH Q10 is focused on pharmaceuticals and is intended to align GMP requirements with a quality system approach. It will be applicable to drug substance and drug product, large and small molecule products, and harmonize one approach to quality systems. It also will complement ICH Q8 and ICH Q9. ICH Q10 contains a pharmaceutical context emphasizing a comprehensive approach; key elements included are management response and continuous improvement. Several ICH guidance documents are already adopted by regulatory agencies, such as ICH Q7A, for the manufacture of APIs. As these guidance documents are adopted, they often become the basis for regulatory expectations and inspections.

3.3.3 MANAGEMENT AND STAFF: LEADERSHIP AND SUPPORT

All manufacturing operations operate, to some extent, with elements and components of a quality management system. Those elements and processes may not be recognized or managed as though they are an integral part of a larger system and may be primarily reactive in nature. Significant time and resources are required to change an organization's culture and practices to move existing elements from a fragmented, reactive program to a defined structure that is proactively managed. The degree to which a program is proactively managed and supported by its leadership is directly related to the benefits experienced by the organization.

Three distinct levels of support are required for successful implementation of a QMS program: executive leadership, functional management, and operational staff. All three levels of the organization must support the effort to attain success. Delivering program understanding and benefits to each should be a priority to ensure acceptance and continuity. Motivating staff and leadership, through benefits and business results, is important to ongoing program sustainability.

Leadership requires capable and dedicated staff to design and maintain a dynamic QMS program. Leadership must embrace the program and support it throughout the organization. Functional management must understand the program in order to support it and direct its staff in execution of the program. Staff must understand what the program means to them and experience and realize the benefits in order to support it.

The quality organization must be seen as a partner in assuring product quality, not the department that disseminates quality. Within a QMS, certain processes are owned by the quality function, just as manufacturing, engineering, development, technical support, and facilities own processes within the system. All functional groups should have defined roles and responsibilities to ensure quality product is produced. Cross-functional support and delineation of responsibilities ensure quality is built into every process, and each process owner is ultimately responsible for his or her process output. Leadership that understands and embraces this concept will support and infuse a culture of quality throughout the organization, maximizing the probability of success and competitive advantage.

The organizations leadership, management, staff, and QMS program group must work together to develop and progress the QMS. A successful program should detail expected benefits for all stakeholders in the organization and provide ongoing results demonstrating functionality and utility.

3.3.3.1 Outlining Benefits to the Enterprise

Establishing a formal, structured QMS for an organization requires leadership approval, resources, and capital. Leadership support and approval is the place to initiate the program to ensure all program efforts are supported and the proposed system meets the business needs. This includes having dedicated resources that can focus their efforts to design and manage the program and operate and manage the processes.

Leadership has visibility to present business needs and budget and the vision and insight for the organizations' future. Quality management system design needs to fulfill present and future needs to be robust and value added. A gap analysis on

current business processes can help leadership understand where opportunities exist for improving processes. These gaps can be determined by analyzing the purpose of the organization and its ability to deliver quality results on time and on budget.

Manufacturing areas to examine for operational improvement are regulatory compliance, audit findings, rework, nonconformances, document revisions, disposition timeliness, complaints received, inventory on hand, equipment failures, manufacturing cycle times, employee turnover, and training opportunities. Additional areas targeted for improvement may come from benchmarking key manufacturing parameters against industry peers. Results of a gap analysis begin the dialogue regarding process performance and the need for process improvements. Leadership must be convinced there is opportunity for financial and competitive gain, and the resource investment to operate the QMS will be outweighed by the program benefits received.

Management at the highest level in the organization must understand, support, and lead the strategy to implement systems across the enterprise. More often than not, this requires some level of business transformation, a cultural and behavioral shift, and a certain level of risk. The risk associated in implementing change is minimal compared to that of not having a robust system, as outlined in the benefits section.

3.3.3.2 Speaking Management Language

Without upper management championing the establishment of systems, midlevel management will not support the effort, dedicate the time required, nor practice the behaviors essential to establish and maintain the processes. Leadership needs to be cognizant of the benefits and consequences of nonimplementation and be clear and unwavering in its support, delivering frequent consistent messaging to management and staff. Leadership requires tangible and intangible benefits to be convinced that the efforts are worthwhile and working and to regularly convey results to staff.

Tangible benefits should include metrics and improvements demonstrating process and system cost savings, compliant inspections and customer audits, faster product approvals and manufacturing throughput, less rejected material, reduced nonconformance issues, and more efficient continuous improvement and project implementation. Intangible benefits include improved staff morale, faster, more accurate transparent decision making, less employee turnover, increased staff accountability, and an enhanced culture of quality throughout the organization. The "feeling" conveyed by an organization that is reactive, stressed, and without well-structured processes is much different than that of a proactive organization with simple processes that are easily and successfully executed by trained staff.

Systems thinking allows decision making and process management to occur at the process owner level, not the functional management level. This is a cultural shift for many organizations but brings with it many benefits. Faster decision making, by subject matter experts is valuable to organizations. It can benefit both on a day-to-day, lot-to-lot basis as well as provide long-term strategic direction to leadership. Taking the burden off functional management and defining process owner responsibilities allows functional management to manage resource and personnel issues and not split time and attention between resources, personnel, technical, and process issues.

3.3.3.3 Translating Benefits to Staff

Similar to leadership and management requirements regarding system understanding and benefits, staff requires understanding prior to accepting the cultural changes that a system-based approach will bring to the organization. Once the program is initiated, tangible and intangible benefits must be realized and appreciated in order for staff to continually support the program. Staff support, through benefit realization and management direction, will ensure program execution, ultimately delivering the expected business results.

Transforming disparate processes into processes that are simple to understand, easy to execute, and provide a sense of accomplishment meet one of management's obligations to staff. Staff interest lies in the ability to perform their work, contribute to continuous improvement, and have a reasonable work–life balance. Finally, they want to be able to contribute to their careers, have defined career paths, and have attainable development goals for advancement. A well-designed quality management system can contribute to provide all these employee benefits.

Staff benefits should be designed into the QMS. An outline of expected benefits should be presented to staff to gain their support of the system initiative. Accomplishments should be advertised and rewarded. Establishing well-defined processes empowers employee involvement, participation, and contribution to the organization. It reinforces a culture of quality throughout the organization, and provides a conduit for their contribution.

3.3.3.4 Ensuring Staff Support and Management Leadership

Management's responsibility includes providing staff robust tools and processes necessary to accomplish their jobs efficiently. Complex, missing, or fragmented processes do not allow for easy operational execution, the ability to leave work at reasonable times, and may result in poor-quality output or rework. This type of environment quickly becomes dissatisfying to employees and results in poor morale, low efficiency, and ultimately lack of interest and loss of staff.

Staff empowerment allows pride in workmanship. Well-designed quality systems make clear to staff where decision authority and process accountability lies, provide clear expectations of the process and process owners, and provide personnel a clear development path to process ownership.

Clearly identified process attributes provide organizations more than tribal knowledge to pass onto the next process owner. They provide clear structure, process, and other attributes critical to the ongoing success of the enterprise. The organization becomes reliant on their system and processes not people's personal knowledge, which can be lost with staff turnover.

Ensuring leadership and staff support requires that a well-defined plan be designed and shared throughout the organization. A long-range plan, spanning several years may benefit the organization to maintain perspective and govern expectations. An annual quality plan should encompass all aspects of the QMS and contain detailed periodic goals and objectives. Progress against the quality plan needs to be advertised and celebrated. Quality plan leadership should be recognized for its efforts and accomplishments. Advertising wins and accomplishments in both small group and large settings should be designed into the communication and change management program.

TABLE 3 Long-Term Strategic Vision

Year 1	Year 2	Year 3	Year 4	Year 5
Gain management support Create QMS office Identify site processes and resources Develop communication and change management plan	Implement program Train management, process owners, QA, and support staff Focus on maturing high-risk/impact processes Reward and recognize QMS efforts	Indoctrinate remaining processes into program Document and communicate cost/resource savings Begin integrating processes across the organization	Focus on key projects based on QMS portfolio and management review Provide ongoing training, communications, and change management	Adapt to changing business and regulatory environment Provide leadership to industry on QMS paradigm

Table 3 provides an outline of a long-term vision and goals for a quality management system. A long-term strategy provides leadership, management, and staff with an understanding of the program and anticipated timelines for implementation and benefit expectations. Annual quality plans become the short-term strategic milestone vehicle to achieve the long-term strategic vision.

3.3.3.5 Traps to Avoid

Several challenges and requirements present themselves when establishing a formal QMS. A primary requirement is a skilled team that understands the needs of the organization, regulatory, and customer requirements. It should have the skill, experience, and expertise to design a robust system and identify processes that support the enterprise. A mismatch of team skills with enterprise needs may result in a nonviable system that is not supported by leadership and staff, leading to failure and disuse over time.

Quality management system design must be well thought out and tested. Pilot programs are crucial to test system robustness and reliability, staff and management acceptance, and the ability to produce the desired results. Time spent in system design will pay dividends for years to come and increase staff support and critical mass throughout the enterprise supporting the program efforts. Avoid implementing any system or process design that has not been well thought out, does not have input from the stakeholders using the system, or has not been piloted prior to a full-scale implementation. Typically, a single opportunity exists to introduce a new program before staff and management either accept it or reject the ideas and concepts. Rebuilding interest and trust of a failed system is difficult. The probability for successful reintroduction is minimized. Taking sufficient precaution for correct implementation the first time is important.

Change management is another very important consideration when implementing a QMS because of the culture change required from the organization. Several resources can assist in managing change, and these should be incorporated into the system design. It is important to be cognizant that successful implementation requires change at all three levels of the organization; leadership, functional management, and operational staff. Each will need different messages, encouragement, rewards, and benefits. Consideration to deliver both tangible and intangible benefits to stakeholders is necessary.

Leadership support from the highest level is required. Middle management will not support an effort that is not supported by its leadership. Leadership must provide unwavering support, not provide mixed messages, continue to advertise and celebrate success, and support the program through rough times. Consistency in language and deeds from management supports understanding and appropriate risk taking by management and staff.

Functional management must also support system efforts and long-term strategies, to ensure that staff, who are critical to execution of the processes, know that their support and efforts are expected. Functional management send powerful messages to staff, and their support of the long-term plan and annual quality plan are essential. Specific system objectives, included in leadership, management, and staff goals reinforce the commitment and help ensure success of the program.

The system needs to remain flexible. Having a long-term plan and vision is necessary to provide a roadmap to the future. That roadmap may need to change as the business environment and enterprise needs change. The long-term plan and vision should be written at the level that it changes very little, but flexibility is maintained through the preparation of an annual quality plan that is capable of addressing temporal issues and business needs.

Prior to implementing any QMS initiative, one must understand what leadership, management, staff, and customers require. Knowing which processes are required to support customer needs and the impact of those processes upon each other is essential to system design. Developing process owners that understand their roles and deliverables in the organization, eliminating constraints so they may meet their goals is essential for success. Process owners must understand product and process priorities so significant benefits may be realized. These are important considerations in designing system and processes that support the organization, produce meaningful metrics, and demonstrate progress. Consideration of these important points prevents system initiatives from failing and interpreted as another burden to the already overburdened work and demands placed upon the organization.

3.3.4 ESTABLISHING QUALITY MANAGEMENT SYSTEM SCOPE

In many pharmaceutical and biopharmaceutical manufacturing operations, duplicity exists in some processes and gaps are present between others. Often it is unclear exactly what boundaries or scope constitute a process, the expected outputs, who are the customers, who is the owner, and who is responsible for continuous improvement. Duplicity is inefficient and costly. Examples include multiple layers of an organization performing data reviews as documentation or information moves through the value chain. Regulatory submissions for analytical validation are an

example, where raw data may be checked at the laboratory, supervisor, quality assurance, compliance, and regulatory group levels. On the other hand, gaps may exist where each functional group listed above assumes data verification is occurring with another group, and in fact there are gaps in data integrity. In this case, the result can be tremendously expensive if, upon regulatory inspection, errors are found and it appears data integrity issues are ubiquitous in a submission.

This section will discuss the importance of defining business requirements to ensure processes comprising the QMS are designed to support the enterprise, integrated into a quality plan, sufficiently defined to provide adequate resolution and are transferable and scalable throughout the enterprise.

3.3.4.1 Defining Business Requirements

The QMS and the processes that comprise it must be custom designed for the needs of the business. One size does not fit all situations. The requirements of an enterprise vary across sites and the phases of a product life cycle. A comprehensive system will ensure a holistic programmatic approach in its support to the enterprise. This does not mean that every phase of the product life cycle (discovery, development, commercial manufacturing) will utilize all the processes that comprise the system. Nor does it require that all commercial manufacturing sites will necessarily implement all processes. It does, however, provide a common platform and expectation for all processes, owners, metrics review programs, continuous improvement efforts, and the like when they are implemented.

The first step in designing a QMS is determining business needs and the processes required to support the enterprise. Important consideration must be given to ensure that all processes are included in the assessment. The assessment must include all activities that affect product quality at corporate, business, manufacturing, distribution, contractors, or joint venture sites. Processes controlling incoming materials from vendors, laboratory services, contractual support, and other inputs should also be included in the initial assessment.

Upon identification of the processes required to support the enterprise, the next step is to define exactly what is in and out of scope for each process. Mapping all the processes and their inter relationship with other processes will determine if any gaps or duplication exists in the system. Duplication may be warranted or eliminated. Gaps between processes require remediation. For example, a nonconformance process should have direct linkage into a corrective action process. A well-operating nonconformance process without an active, integrated corrective/preventive action process will yield little benefit to the organization and efforts expended on the nonconformance process will be nominal in their overall positive business impact.

This comprehensive approach allows for efficient integration between processes, different phases of product life cycle, and integration between different sites in the supply chain. This integration provides opportunity for efficiency in that process owners are integrated with each other's needs and expectations. Duplication of effort is avoided and efficiencies gained. Quality outputs from one process become reliable inputs into the next process. Management and leadership will have access and insight into compliance, infrastructure, and performance metrics of all processes on a comparable basis. This provides leadership the opportunity for risk-based resource allocation to appropriate areas of the enterprise.

Process mapping of the enterprise's requirements to supply product enables design of the processes required for the system. Staff, management, and leadership input into the business needs provide additional guidance into processes attributes.

3.3.4.2 Integrating Quality Management System into Quality Plans

A quality plan is required by the regulations governing medical devices (QSR) but can readily be adopted as a useful tool for pharmaceutical and biopharmaceutical manufacturing operations. A quality plan is the documented plan and goals for enhancing and advancing the QMS. It can provide the outline and requirements of the organization's purpose, mission, product, and business practices used to produce a quality product. A quality plan can detail the processes that comprise the QMS, the maturity level required for each process, organizational structure, and other requirements needed to meet the organization's purpose. Included in the quality plan are the elements of the business including location, size, products, and expectations. It also includes its structure and support functions, values, and other attributes of the organization.

An annual quality plan can be the detailed execution plan of the organization's long-term quality vision for the QMS. It provides staff and management the outline and goals for improving the QMS. It enables employees to see the big picture, how they fit into the organization, and the organization's expectations. Within the quality plan attributes of the QMS should be described, including functional management responsibility. This then becomes the foundation for further definition of processes, description of management review and responsibility, and continuous improvement programs. The preparation of a quality plan begins defining what is assumed to be known by all levels of the organization. It is the mechanism for ensuring requirements are addressed and gaps in the organization do not exist.

A quality plan may outline the organization's long-term (several years) and short-term (annual) goals through a risk-based approach to improving product quality. It is the foundation for the manufacturing structure and support processes. A quality plan ensures integration of personnel, their qualifications, product requirements, quality management system, and regulatory and compliance infrastructure. An example of an outline of a quality plan is in Table 4. Leadership review and approval of the quality plan is required to ensure that mission, scope, expectations, and division of labor in the organization is consistent and supported.

In larger organizations, site or suborganization-based quality plans can be designed to support the scaling of the QMS across all components of the enterprise. The individual site plans provide focus on process challenges that are more critical than at other sites due to variations in business and compliance environments. While the specific plans emphasize goals based on site priorities, they also connect the members of an organization to the mission of the greater QMS, as shown in Figure 5.

3.3.4.3 Determining Process Resolution Requirements

Leadership expects cost-efficient reliable results from their manufacturing operations. Management requires a capable workforce, equipment, facilities, and materials to manufacture the product. Employees require robust processes that are easy to

TABLE 4 Elements of QMS Annual Plan

Element	Definition
Introduction	Purpose of plan and definitions for clarity
Plan	Planned activities for the calendar year
Goals	Specific/cascading goals of the site
Projects	Major projects in support of the goals
Metrics	Key metrics with defined targets
Approvals	Site/plant management

FIGURE 5 Scaling the QMS through site quality plans.

execute to perform their jobs. All this needs to be considered in the design of the processes that comprise the quality management system.

Complex processes may need to be managed as distinct subprocesses in order to provide process owners the ability to accomplish their work with specific focus and expertise. Management and leadership may require data and metrics on specific areas of the process that are not available if the process is too complex and large. Dividing a complex process into simpler, more manageable processes also allows for scalability and transferability throughout the organization.

Once processes have been defined for the enterprise, sufficient system resolution should be determined. This is accomplished by evaluating the ability of the process owner to manage and execute the process requirements. Another factor in this determination is the data and metrics needed from the process by management and leadership. An example of a complex process that benefits the organization by being managed through distinct subprocesses is validation.

Validation is a regulatory requirement and has become an industry standard for ensuring product consistently meets quality attributes and regulatory requirements. Validation requirements are woven throughout the manufacturing supply chain encompassing many different subprocesses. The validation process may best be

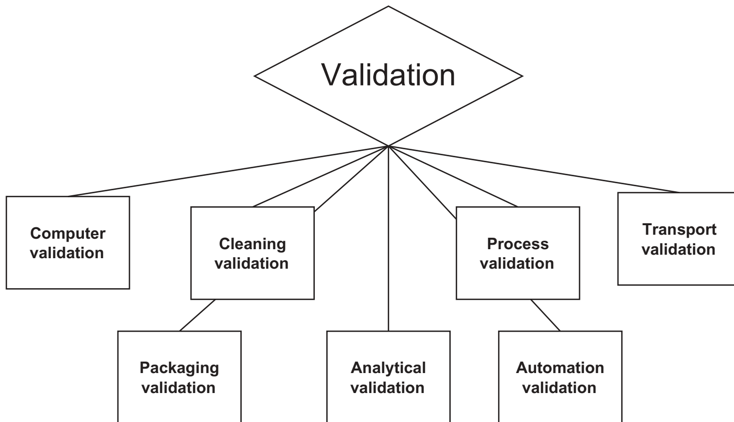

FIGURE 6 Validation subprocesses.

managed by dividing it into manageable subprocesses. This allows for efficient management and execution of the subprocesses, and the metrics reported for those subprocesses are meaningful and specific. See Figure 6, which illustrates one potential organization of the validation subprocesses. Subprocesses contained within the validation system could be cleaning, computers, automation, analytical, packaging, process, transport validation, etc. Manufacturing is another example of a large, complex process that may best be subdivided to support better management and more meaningful metrics to management.

By dividing a larger process into manageable and specific subprocesses, management can assign appropriate subject matter expertise to lead and manage each subprocess. The metrics measuring subprocess performance can be uniquely reviewed, evaluated, and compared to similar subprocess metrics at other sites or companies. Valuable, meaningful comparisons can be obtained for process and subprocess performance that would otherwise be blinded or diluted, if they were summarized within the higher level process metrics.

An additional advantage of establishing subprocesses is that it affords the opportunity for rapid assimilation and transfer of the subprocess at various sites within the enterprise. An example of this is the comparison of a bulk manufacturing facility with that of a distribution center. Both will need to implement aspects of the subprocess "transport validation," however, the distribution center will not need to implement other subprocesses such as process or packaging validation. As the subprocess transport validation is designed and implemented at one site, that infrastructure and knowledge transfer to the other site is rapid, avoiding duplication of efforts. Sharing of information and expectations of the two sites becomes a common goal and format. Management can, therefore, compare transport validation needs and maturity levels between sites equally.

Once all the processes and subprocesses supporting an operation have been defined, another gap analysis may be conducted to ensure that there are no assumptions, and all required processes and subprocesses required to support the business are included in the scope of the QMS. This can easily be accomplished by listing all the business drivers for an operation and comparing that against the processes

established. Written definition of the process and subprocess scope is required. Stakeholders, owners, and users of the processes should be involved to ensure clear definition and understanding of process scope. Questions to be asked are: Are all the business needs addressed? Have all our activities and operations been included in the assessment? Is there any duplication in process expectations? Are there any gaps between process outputs and inputs? Once this evaluation has been concluded, it can be easily determined if any existing work process have been overlooked and the system requires further modification.

3.3.4.4 Scalability to Enterprise

Well-designed processes and subprocesses are scalable to the enterprise. A comprehensive design will allow for replication and comparison of processes and subprocesses between multiple sites. This allows for rapid implementation of new technology, sharing of best practices, and comparison of similar metrics to determine compliance, infrastructure, and performance. A comprehensive system allows for each unit operation or site within the enterprise to have the flexibility to apply applicable processes and subprocesses, yet continue operating within the defined structure of the QMS. For example, a manufacturing site may utilize almost all of the processes discussed in the validation process, whereas a distribution site may only utilize the transport process. Both sites, however, implement the same structure for the transport process, allowing for meaningful comparison of data and metrics and rapid implementation of any required changes to that process.

Well-designed quality management systems support structured organic growth and are valuable in evaluating and integrating manufacturing acquisition opportunities. Business and manufacturing management should utilize the QMS and its standards whenever evaluating external facilities for appraisal, approval, integration, or expansion. Meaningful metrics obtained from a QMS provides the standard to make critical decisions affecting multiple internal or external manufacturing capabilities.

Documented process structure provides rapid employee assimilations when transferring employees between sites. New employees, replacing existing process owners, are enabled to rapidly execute process responsibilities due to the abbreviated learning curve when processes have been well defined and documented. Systems designed as described here provide meaningful and comparable metrics for leadership to evaluate progress, compliance, and performance.

3.3.5 SYSTEM AND PROCESS OWNERSHIP: ROLES AND RESPONSIBILITIES

A well-designed QMS and the processes that comprise it require competent ownership with defined roles and responsibilities for program success. This combination ensures that the system and processes are established, maintained, improved, and remain current with industry practices and business expectations. Operational execution of the QMS and the processes comprising it will engage stakeholders, management, and leadership, provide business results, and support and ensure compliance.

3.3.5.1 Quality Management System Ownership and Management

The QMS is best owned at the highest level in the organization. At a minimum it should be owned at a level in the organization above manufacturing and quality. The owners' main responsibility is to champion the program and ensure organizational alignment. Regulatory investigators expect processes supporting manufacture are fully incorporated into the QMS. They also expect leadership to have significant knowledge of the operations and interact with investigators during inspections with some degree of familiarity with the processes supporting manufacture. At the conclusion of an inspection, regulators issue inspectional findings and, if appropriate, take regulatory action against the most senior member of the leadership group. Through high-level leadership's active involvement and ownership, the QMS program and enterprise will be successful.

As mentioned previously, the QMS is best managed by a group dedicated to the program. The QMS program office should have defined roles and responsibilities. In the FDA regulation 21 CFR Part 211.22, the responsibility of the quality unit is described. It is the only functional group in a manufacturing organization that has its job description codified in federal regulations. These responsibilities should not enable or dilute the responsibility for ensuring quality of other functional groups in the organization. All functional groups supporting manufacture should be applying their trade to the GMP world. Regulators expect the Quality Department to have oversight and approval of all processes affecting product quality. Program management is important because of the need for coordination and accountability to bring individual processes, long-term system strategy, yearly quality plans, and goals together to accomplish the program's objectives. These activities and benefits cannot be realized from individual process owners.

The program management of the QMS can be managed just as a process, with predefined expectations, metric collection, and management review, culminating in risk management application to continuous improvement programs. These metrics and improvement initiatives need to be vetted through leadership review and input to ensure alignment throughout the organization.

An outline for the roles and responsibilities for the QMS program office is illustrated in Table 5. By establishing the roles and responsibilities of the program office a defined point of contact and accountability is established for program execution. It establishes strong linkage and focus on the program objectives for process owners, training, functional management, and leadership. Similar program management structures are required at manufacturing sites and corporate functions to maximize benefits of the program through establishing common and specific goals and

TABLE 5 QMS Program Office Roles and Responsibilities

Subject matter expert for QMS program
Develop and execute communication plan
Initial and ongoing training
Facilitate management review process
Identify process maturity goals and metrics
Develop long-term strategic vision
Create and execute annual action plan

providing a platform for sharing best practices and knowledge. As organizations grow in complexity, additional management may be required to ensure that the elements of the QMS are integrated, functioning, and delivering the results expected.

3.3.5.2 Process Ownership

Designing a QMS that mandates and assigns process ownership to designated individuals is a significant strategic decision in the establishment of a successful quality management system. It provides efficiency, expertise, dedication to the process, and focused ownership for documentation, improvements, benchmarking, and compliance. Without defined and assigned process ownership functional management becomes the de facto process owner. This is problematic in that functional management is already overburdened with personnel and business management issues, unable to adequately focus and deliver the demanding process owner requirements required in today's manufacturing environment. Several processes typically are organized under an individual functional manager, further diluting focus, attention, and expertise if functional management is relied upon as a process owner.

3.3.5.3 Process Owner Selection

Process owner selection requires program management to establish defined criteria for the selection process. Criteria include the capability to perform process owner roles and responsibilities, including self-development and decision making. Empowered process owners are accountable for maintaining and executing the processes that management relies upon to deliver business results. This accountability ensures that staff, management, and leadership know who to solicit for answers to process-related questions and issues. It also provides the best representation to regulators, clients, and customers. Efficiencies are gained and current trends maintained with an active owner, with defined responsibilities.

Selection criteria may include attributes of technical, interpersonal, and management skills. The capabilities needed for different process will vary and should be considered in the selection process. At the end of the selection process, functional management and the process owner may consider inclusion of the process owner's roles and responsibilities into the process owner's job description. Personal goals and development activities should be based on improving the process owner's capabilities to manage the process and develop future process owners through active mentoring and talent development programs.

Process owners need to be dedicated to their process. They must be empowered and held accountable for all the attributes listed in their roles and responsibilities. Process owners may have ownership of more than one process and may have other job responsibilities, but it must be clear throughout the organization as to who has full authority for the process.

Process owners require a defined set of responsibilities to maintain a vibrant and effective process that continues to support product quality deliverables. Having roles and responsibilities defined provides owners with the structure and parameters

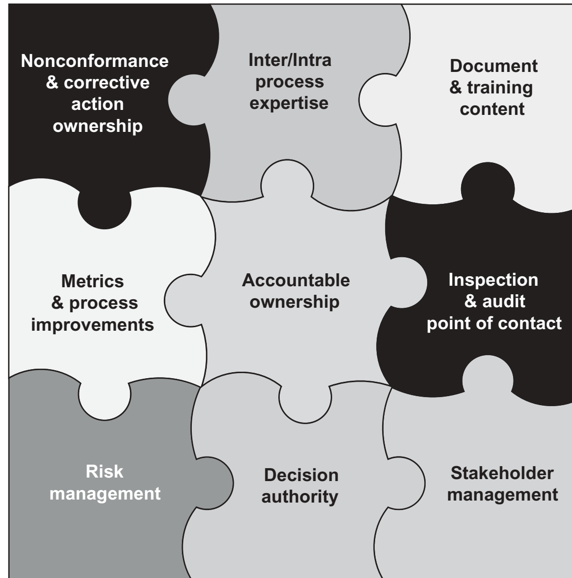

FIGURE 7 Process owner roles and responsibilities.

needed to be effective. Examples of owner responsibilities include identification of stakeholders, defined decision authority, document ownership, nonconformance ownership, knowledge of regulations and industry trends, subject matter expertise, training content, metric ownership, and representation to internal auditors and external regulators. Identifying, training, and development of the process owner on his or her roles and responsibilities is similar to assembling the piece of a puzzle. If one piece is missing, the effectiveness of the process owner will be minimized (see Figure 7). Developing a sound methodology for process owner selection ensures objectivity and is critical to the success of the program. A brief discussion on each process owner roles and responsibilities follows.

3.3.5.4 Stakeholder/Process Owner Integration

Process owners must identify the stakeholders of their process and ensure design and output of the process meets stakeholder needs. Regular communication, interaction, and support are maintained with stakeholders through scheduled meetings to discuss process status and improvements. Any changes to the process are vetted through the stakeholder group. Typical stakeholders include the QA unit that is responsible for review and approval of the process components, suppliers to and receivers of the process (i.e., owners of other processes that interact with the process), customers, management, and leadership. Each stakeholder roles and responsibilities also require definition.

Including the key stakeholders in decisions affecting process design or changes ensures efforts by the process owner are applied correctly. Process robustness is dependent upon meeting business and customer needs, and the process owners require input and support from the stakeholder group.

3.3.5.5 Decision Authority

Each process owner requires a defined level of decision authority. This authority level delineates the bounds of decision making granted by the organization to the process owner. Business needs and risk assessment must be incorporated into the design of the decision authority granted to a process owner. Table 6 is an example of a decision authority matrix design for a process owner. It requires cross-functional management support to be effective.

Preparing a decision matrix that is shared and agreed upon by the stakeholder group and functional management ensures decisions are made and communicated quickly by appropriate persons. It removes the burden of making every technical process decision from functional management. It is important to outline the process owner's role in the decision-making process, as well as conditions for escalation. Effective process management is realized when the culture of an organization can support the outline of the decision matrix and not continually rely upon functional

TABLE 6 Decision Authority Matrix

Decision Category	Definition	Decision Maker	Decision Support Required	Informed of Decision
Company standards	Process-related global standards relevant to all manufacturing sites	Corporate process owner	Site process owners Site quality assurance counterpart	Process stakeholders Impacted staff
Standard operating procedures	SOPs related to the specific process	Site process owner	Process stakeholders Site quality assurance counterpart	Management review Corporate quality assurance counterpart Impacted staff
Training	Training on processes or procedures	Corporate and site process owners	Training Technical system matter expert	Process stakeholders Corporate quality assurance counterpart Impacted staff
Site projects	All projects related to the existing process or the projected improved state of the process at a specific site	Site head	Process owner Leadership team Site project portfolio manager	Process stakeholders Corporate quality assurance counterpart QMS office

management. If functional management continues to be relied upon and seen as the process decision makers, efforts and progress by the process owner will be nominal. The organization's culture must support the process owner at all levels of the enterprise for the owners to be successful.

3.3.5.6 Industry Knowledge

The *c* in *c*GMP represents the notion of current industry practices. Process owners must work to remain current with industry and regulatory trends affecting their process and its overall effect on the QMS and the business. Awareness of process capability and comparability with other like processes within and outside the pharmaceutical and biopharmaceutical industry is essential. Regulators will compare an owner's process against other similar processes in which they have experience when formulating value judgments. Benchmarking against similar processes provides process owners the data needed to determine adequacy of their process with industry peer groups.

Where technology, efficiency, performance, or compliance can be enhanced, it should be considered by an aware and informed process owner. Functional management cannot keep pace with the changes occurring with all the processes supporting manufacturing. Ensuring process owners dedicate sufficient time to keeping current with process-related external events will ensure process success. This may include review of industry periodicals, attendance at seminars and regulatory presentations, and routine self-evaluation and benchmarking against relative processes.

Often, the best examples of process efficiency can be found outside the pharmaceutical and biopharmaceutical industries. Other fields such as electronics, space, and software industries have evolved their documentation, training, quality, and change control systems to the point of best in class. These industries are more time sensitive to get product to market and have often evolved their processes to be efficient and decision processes to be very quick. Process owners may expand their knowledge by investigating other industries to find best practices and apply them internally.

3.3.5.7 Regulatory Inspection and Audit Lead

Process owners play a critical role during regulatory inspections and customer audits. Process owners are the best choice to represent process attributes and performance to interested parties. Process owners provide regulators and auditors with a capable, knowledgeable resource to represent the process and answer detailed questions. The process owner should be aware of process history, requirements, operations, exceptions, changes, and nonconformances. The process owner will have detailed knowledge of process operations, compliance, and be able to defend the process. Providing an accurate answer the first time to regulators and auditors is essential in building trust and representing competence.

Each process owner is required to work closely with his or her QA counterpart. This ensures design and operational issues are clearly reviewed and approved by a representative from the quality assurance function, a regulatory expectation. The quality assurance counterpart must be familiar with the process, understand documentation supporting the process, and able to convey what approval the Quality

Department has conveyed on the process and meaning of that approval. The QA counterpart to a process should also have defined and documented roles and responsibilities.

When teamed together, the process owner and the quality assurance counterpart for a process will make a favorable impression upon regulators, be able to explain all operations involving the process, supporting documentation, and any ongoing projects or process improvements. This pair is the best to evaluate and consider any deviations to the process or recommendations for continuous improvement.

In most all cases, the process owner and QA contact will posses more information about the process than regulators and be capable to defend the process design and operation. Should regulators have suggestions on process design or functionality, the process owner may consider them. If appropriate, any recommendations or observations made by regulators or auditors can be incorporated into the process design. However, it is critical for the process owner and stakeholders to evaluate proposed changes to avoid reactive management commitments, which could be deleterious to the efficient operation and output of the process.

3.3.5.8 Subject Matter Experts

Process owners, through their selection and development, become the subject matter experts for the process. It is more efficient for an enterprise to focus its expertise on individuals that have the authority and accountability described in this section, rather than dilute those attributes and accountabilities, thereby risking poor process execution and management.

As a subject matter expert on the process, the owner has the capacity to deliver results outlined in the list of owner responsibilities, mentor future process owners, assist in staff development, and accurately guide management in its strategy related to the process. The process owner's personal development of his or her process expertise is essential in delivering operational results and providing direction for future strategic changes to the process.

3.3.5.9 Metric Ownership

Process owners' responsibilities include determining appropriate metrics for their process. These metrics should include lagging and leading metrics that are meaningful to the process owner and management in determining performance, compliance, and infrastructure of the process.

The process owner should represent and interpret these metrics to the organizations leadership. The metric output from a process is the basis for management and leadership's action in resource deployment and approval of continuous improvement projects. Key operating parameters such as number of nonconformances and regulatory observations against the process should be tracked and factored into the maturity of the process.

Every process owner needs to base their continuous improvement plan for the process based upon metrics collected from the process output. The metrics must be designed to assist in these decisions and be readily available for review, presentation, and interpretation.

3.3.5.10 Documentation Ownership

Process owners are the most appropriate owners for all documentation supporting their processes. This includes having either direct ownership or controlling influence over guidance and execution documentation such as corporate policies and standards, local requirements and standard operating procedures (SOPs), logs, and records.

For the manufacturing process owner, this means owning the master manufacturing records and executed batch records, SOPs, use logs, and related training documents for their process. Combining responsibilities for process management and process ownership results in true accountability for the process owner. It also allows for progress and continuous improvement of the QMS. Removing questions of responsibility and accountability ensures integration between requirements (standards, policies, procedures) and execution (training, performance, and documentation).

3.3.5.11 Training

Assurance of adequate training for process users is an important responsibility of a process owner. Process owners must have a clear understanding of the requirements of their process and its operation. This understanding requires translation into executable training. Users must be able to understand and apply the training. Complicated processes coupled with ambiguous training will lead to confusion and an inability to properly execute a process, which eventually constitutes failure for the organization. A simple process, with easy to understand process steps, that are consistent with instructions and documentation requirements will support success, reduce production costs, minimize nonconforming events, and allow for employee satisfaction.

Process owners are subject matter experts and should influence and provide consulting for training on the process. They may also participate in training delivery. Ensuring adequate training on a process is a key goal for system efficiency and regulatory compliance. Process owners, capable of explaining the reasoning behind the process requirements, enhance the training experience for process users. Process owners should include effective presentation and training skill development into their personal development programs.

3.3.5.12 Risk Management

Process owners require basic understanding of risk management and its application to process design and continuous improvement prioritization. Several industry and regulatory resources exist, such as ICH Q9, that provide understanding on risk assessment, identification, control, methodology, and the overall risk management process. Process owners should be familiar with risk management techniques and tools and apply them to their process management when designing, executing, or managing improvement efforts for their process.

Risk management is especially important for the presentation of process improvement proposals to management where resources are required. The ability to quantify risk and demonstrate continuous improvement benefits is essential to project

and resource approval. Risk analysis, management, and presentation constitute guiding leadership to work on the right things at the right time and then improve it.

3.3.5.13 Continuous Improvement and Project Management

Instituting quality-by-design efforts early in the design of a process should negate the need for major process improvements. However, over time, due to business needs, regulatory changes, or technology improvements, processes will require some form of change to ensure compliance or performance enhancement. As part of executing and maintaining their processes, owners need to collect and report performance metrics to management and staff. These metrics will inevitably direct attention to opportunities for improvement that require capital and human resources. Process owners are the best leaders of continuous improvement projects due to their intimate knowledge of the process and accountability for process output.

Managing or leading a continuous improvement project requires process owners be knowledgeable in project management and team-leading skills. Improvement projects typically require cross-functional support and expertise from areas such as information systems, project management, manufacturing, engineering, and development. It is essential that continuous improvement efforts name the process owner as the project lead to ensure the required output of the project meets the process owner, stakeholders, and enterprise needs. Often, projects are completed and declared a success, delivering a substandard result that the process owner, users, and stakeholders find inadequate to meet process requirements.

Upon completion of continuous improvement projects, process owners' responsibilities include monitoring the changes made to the process to determine the impact of improvements. Metrics monitoring changes to the process, pre- and post-implementation, should be incorporated into existing performance metrics and reported during regular management reviews.

3.3.5.14 Non Conformance/CAPA/Planned Deviation Ownership

An important barometer of process performance is the number of nonconformances, corrective and preventive actions taken, and planned deviations initiated against a process. These types of process artifacts must be known and owned by the process owner and stakeholders. The process owner must consider these process metrics for evaluation of and changes to process design, training, documentation, and performance.

Nonconformances may fall into the category of manpower, machinery methods, materials, etc. Employees not following procedures or unable to execute required steps of the process indicate a poorly designed process requiring modification and/or improved training. Machinery failures often indicate poor qualification, validation, calibration, or maintenance programs. Unexpected results or outcomes are indicative of poor process design, characterization, or a break down between processes.

Although planned deviations are frowned upon by many in the industry and regulators, there are times when temporary changes to a process must be employed to support the business. Permanent changes must be made through a formal change

control process. When the use of a planned deviation is required, the affected process owner should be aware of and own the change. This provides owner control over the duration and extent of the change to the process and provides data for possible consideration in making a permanent change to the process. A planned deviation should be rare and monitored closely as it affects previously established standards, expectations, and training.

Process owners must be capable to evaluate and interpret the effect of nonconformances and planned deviations on their systems. Process owners can evaluate the need and lead efforts for corrective or preventive action, ensuring adequate corrections and improvements are implemented. An effective QMS ensures deviations from approved processes are owned and adequately investigated by the process owner's and ultimately approved by their quality assurance counterpart. The knowledge of these events is the basis and foundation for the process owners to make a risk-based evaluation on whether or not process changes are required, documentation or training require modification, or continuous improvement efforts are warranted.

A well-designed QMS will include identified process owners with defined roles and responsibilities. Process owners require support from management, their customers, stakeholders, and quality assurance. Accountability and decision-making parameters will empower process owners to drive execution and improvements to their process, delivering the business results expected. Without these process owner attributes and support, minimal results will be achieved, and functional management will be burdened with and assume the responsibility for making decisions that should be in the hands of capable process owners.

3.3.6 CHANGE MANAGEMENT/COMMUNICATION

Establishing and maintaining an effective QMS, as this chapter describes, requires a significant cultural shift. Many employees and functional management will find the business transformation of defining processes, assigning ownership, delegating authority, and responsibility for process performance within the QMS is a significant change in business conduct. The most significant change results from the shift of control in process expertise and decision-making authority from functional management to process owner. Significant business transformation may result by assigning responsibility and accountability to the process owner, and management's support of process owners who drive continuous process improvement.

In *The Second American Revolution*, Rockefeller describes the conservatism of organizations: "An organization is a system, with a logic of its own, and all the weight of tradition and inertia. The deck is stacked in favor of the tried and proven way of doing things and against the taking of risks and striking out in new directions" [9]. If an organization is not already practicing principles of delegation, process ownership, established metric collection, management review, and continuous improvement, barriers within the organization will need to be addressed and broken down in order to establish new behaviors. These barriers to change will exist within and between functions, functional management and staff, and possibly between companies and regulators.

Although expected benefits are significant when implementing a QMS and the end result desirable for employees and management, describing the desired state and motivating personnel to change and implement new behaviors contains significant challenges. A successful business transformation requires a robust change management and communication plan that includes support for all staff affected.

3.3.6.1 Managing Organizational Change

Integration of the skill sets of human resources, training, and change management groups will significantly augment efforts toward cultural change and acceptance. Often personality profiling tools are effective to gauge the organization's preferences, learning styles, and adoption tendencies. These types of tools should be considered in the overall change management program, used where applicable, and program modifications made based upon their results.

The first and critical step in developing a successful change management plan is to obtain initial support from the corporation's leadership and functional management. Without this support the QMS will not gain critical mass and may not deliver the desired effects or changes. To acquire this support, the implementation team must put together a strong business case that speaks to the leadership's needs and wants. The business case must include a risk assessment against compliance and the benefits of the financial gains. It is important to be honest, consider current system status and future requirements, and include a long-term strategy that addresses costs and benefits. The change management plan must include frequent and repetitive communications, to all levels of the organization, of the cost/benefits and successes expected and realized by the program.

Functional management support is also critical to the success of a new program such as a quality management system. Any time a staff member is asked to take on a new role or responsibility, he or she needs to be supported by the functional manager as well as leadership within the organization. Corporations are resource limited and necessarily need to continually prioritize where to allocate resources. Staff will only take on roles or responsibilities that they believe are supported by their functional manager in an effort to successfully meet their perceived immediate goals. Quantifiable support from leadership and functional management can be directly correlated to the success or failure of the QMS program.

Significant work is involved in training new process owners, functional managers, leadership, support organizations, and actualizing their new behaviors. A support system must be in place for the process owners, stakeholders, and management to guide and reinforce the new behaviors and maintain the process effectiveness. It is preferable that this support system be established through a dedicated team that can be fully attentive to all their needs. Without a single source to lead the efforts, diversity in interpretation and implementation will dilute the program, within different functions and sites, and its effectiveness and outcomes will be diminished.

Establishing an organization to lead the systems initiative is important. That organization requires management, standards, and parameters similar to managing an individual quality process. It requires roles and responsibilities be established,

metrics be determined, collected, reviewed and acted upon, and receive management and leadership visibility and support. The QMS program is best organized as a function within the Quality Department and be regarded as an ongoing program, not a short-term project or effort with limited shelf life. The group must be led by competent persons who are familiar with quality concepts and applications, regulatory expectations and requirements, needs of the enterprise, good communication and influencing skills, and are flexible and enduring.

3.3.6.2 Communication

Trying to get people to comprehend a vision of an alternative future is also a communications challenge of a completely different magnitude from organizing them to fulfill a short-term plan. It is much like the difference between a football quarterback attempting to describe to his team the next two or three plays versus his trying to explain to them a totally new approach to the game to be used in the second half of the season. Aligning the organization to accept and implement a system-based approach requires careful messaging coupled with management support and results.

Messages are not necessarily accepted just because they are understood. Another big challenge for leadership is credibility and getting people to believe the message. Aligning words and deeds supports the worthiness and credibility of the messaging. People have learned from experience that even if they correctly perceive important external changes and then initiate appropriate actions, they are vulnerable to someone higher up who does not like what they have done. Reprimands can take many forms: "That's against policy," or "We can't afford it," or "Shut up and do as you're told" [10].

Having established a dedicated team that provides overall program management, it is imperative that the team outline a strategic plan for presentation to leadership. Without a vision and long-term plan, which is supported by the enterprise leadership, quality system initiatives will become difficult. The plan needs to be comprehensive in nature, yet broad enough to convey purpose, mission, and benefits at a high enough level to be understood and supported. An outline such as this provides framework and direction for the program management team and leadership. It also guides the program management team to developing annual goals and quality plans that fit into the overall strategy and provide momentum and results to the organization.

Annual quality plans should be prepared by the QMS program office that address the long-term strategy and intermediate goals that come to surface during program implementation. Training, changes in regulatory requirements, metric-driven projects, and special circumstances warranting process changes such as implementation of new technology or programs should be included into the annual quality plan.

Long-term strategy documents and annual quality plans require leadership and functional management support and approval. These documents must be reviewed and discussed with the leadership of the organization, modified to meet the business and regulatory requirements, and then have full support through upper management approval. In this way, the goals are being led by top leadership and management and not any individual group in the organization. Once top leadership signs

onto the program, it can be shared throughout the organization in a number of ways.

If leadership can support the long-term strategy and annual quality plans to accomplish the vision, then the foundation for change management and cultural shift is in place. Leadership will need to continually discuss the need for systems implementation, in front of a variety of audiences. This includes leadership staff meetings, management, and employee meetings. The importance of leadership support cannot be overlooked. Without consistent visible leadership and management support process owners and staff will revert to old behaviors, become reactive, and perhaps unrelated in their process integration efforts. Leadership needs to require aspects of the program be included into functional management annual objectives with defined deliverables outlined and evaluated. Likewise, functional management should require staff to include appropriate aspects and objectives of the QMS program into their individual goals and work to accomplish them.

3.3.6.3 Feedback and Alignment

Managing the changes required to fully implement a QMS can include several forms of communication and feedback. A detailed annual communications plan can aid the QMS's group in identifying specific target groups, methods, and frequencies of communications, messaging types, and feedback mechanisms to monitor progress for program modifications. Table 7 is an example of an annual communications plan that supports efforts to keep internal audiences informed, aligned, and engaged.

Each target audience requires specific messaging that connects with its needs. Failure to get the appropriate message, that is, what is the program bringing to them, will minimize support for the program. This plan should include face-to face and written communications addressing multiple audiences and media types. Face-to-face meetings can include presentations to steering committees, process owners, functional departments, and all staff meetings. Written communications can include sitewide communications, poster sessions, and newsletters. The communications should speak to all audiences—"what's in it for me?" Topics can include leadership's commitment (direct quotes or actions taken); spotlight on successes (real-life stories from process owners); impact to the site (process improvements or risk mitigation); and progress to the program (metrics and successes). The progress and success of the QMS cannot be overcommunicated.

Another useful tool to help the message and modify the program is the use of a feedback survey. If properly designed and distributed to a defined set of stake-

TABLE 7 Communication Plan

Vehicle	Communication Type	Frequency	Date
Functional metrics meeting	Face to face	Monthly	First week of month
Management interviews	Face to face	Annually	January 1–31
QMS newsletter	Written	Quarterly	First week of quarter
All staff meeting	Presentation	Semiannually	March & September
Poster session	Written/face to face	Annually	July

holders and employees, the survey can provide valuable insight into how staff and management view the program, its progress, and suggestions for modifications. If surveys are distributed electronically and offer only one-way communication, the benefits may be limited as the respondents are limited in their ability to fully convey their impressions or offer effective feedback. An electronic feedback survey may be a first good step in understanding the thoughts and concerns of the stakeholders.

Another suggestion or follow-up to the electronic survey is to utilize focus groups that have the ability to interact with the program questioners. This two-way conversation, verbal dialogue, allows further understanding of the program by the participants that follows with more meaningful feedback to the program administrators. Focus groups should be selected at different levels within the organization, including process owners, stakeholders and users of the system, leadership, functional management, and the general populace of employees. Focus groups provide valuable input into programs that the program administrators may be unaware of and can provide program redirection.

Once suggestions are received on the program, it is essential to consider and incorporate those ideas and modifications that make sense to implement. Those changes need to be communicated and seen by the focus group members to ensure that their time and effort has not been wasted and their suggestions have been heard. This is one of the best ways to spread the word about the QMS program and garner grassroots support.

3.3.6.4 Training

A training plan should be developed to identify the needs of the staff and affected functional areas required to support the successful implementation of a QMS. It is the responsibility of the corporation to adequately support staff with training and tools when staff is expected to take on new roles, responsibilities, or behaviors. The training plan should consist of targeted training for general staff, process owners, and functional management of the process owners.

At a minimum, all staff should be introduced to the purpose, goals, and requirements of the QMS. This training should be a high level explanation of the program looking to gain understanding and support for the program by communicating why it is important and what are the risks of not adopting the program. This can be accomplished by instructor-led training or an electronic, Web-based learning module depending on the size of the corporation.

Process owners require more comprehensive levels of training to fully understand their role and responsibilities within the program. Process owner training should teach key concepts and tools that owners will need to evaluate and support their processes. This training can be done in a phased approach to support the elevation and advancement of a process within the organization's chosen maturity model.

Training should be provided to offer functional managers supervising process owners a thorough understanding of the QMS. Training should address new roles and responsibilities of staff, time demands on process owners, overall program timelines, and impact to functional areas. The acceptance and support of functional management is critical to successful implementation of a QMS.

Managing organizational change demands a well-written strategy, skill set, and resources to ensure changes that come with system implementation are understood, supported, and maintained. Starting with high-level overviews of system design, benefits and timelines for implementation are the foundation for management understanding and support. Detailed annual quality plans can be the tactical vehicle for program implementation. Leadership support through understanding and approval of the annual quality plan, inclusion of program objectives into management goals, and frequent verbal and visual support of the program are essential to success. Building the program infrastructure is a significant undertaking. Inclusion of a comprehensive training, communication, and change management plan should be built into the overall goals of the program and routinely evaluated and delivered.

3.3.7 MEASURING SUCCESS THROUGH MEANINGFUL METRICS

Successful implementation of a comprehensive QMS can be determined by the establishment of a meaningful metrics program. The purpose of a metrics program is twofold: first, to allow an organization to evaluate its progress toward meeting its goals in an objective, data-driven manner and, second, to monitor the performance of each process to ensure continuous improvement. By evaluating metrics for the QMS and its processes, the enterprise has the knowledge and understanding of the overall health of its system and processes and can develop strategies based on risk for continuous improvement of the system and processes.

Once the metrics program is in place, the system and process metrics require visibility to process owners, upper management, and stakeholders. Process owners require understanding of the metrics' trends, issues, and associated risks. Stakeholders must work with the process owner to identify and propose process improvement opportunities. Leadership is accountable to understand the issues and associated risks and responsibly apply resources for remediation efforts.

3.3.7.1 Performance Metric Development

Quality and business indicating metrics should also be reviewed on a routine basis. These may include the following:

- Quality indicating: ability to meet quality standards and procedures
- Supply: ability to meet demand
- Cost: savings as well as avoidance
- Safety: near misses and incidents against process

The guiding principle of metric development is to have a stable system or process to collect, review, and draw conclusions. All metrics should be developed with stakeholders input taking into account the requirements and needs of the customers. This includes the touch points of the downstream quality processes. Without this input and understanding metrics may be developed within a silo and hold little value, causing both frustration at the leadership as well as the staff level. Without proper

design, metrics may become a check box activity that results in minimal or no action by management to support efforts by a process owner.

Metrics can either fall into one of two categories: lagging or leading indicators. Both types are important to the process owner and management. Lagging indicators are metrics that represent the process's ability to deliver results or outputs. They indicate the performance of the system in the past. They can assist process owners, management, and leadership in determining if goals have been met, objectives attained, or existing standards or expectations have been met. Leading metrics focus on the inputs and suppliers of a process. These metrics are important indicators to proactively allow owners and management to take action on a process prior to violating a standard, objective, or goal. A successful process owner will understand the relationship of leading metrics and their affect on the lagging metrics and process. Metrics need to be designed to meet the needs of the organization, be simple to track and present, and be regularly reviewed.

3.3.7.2 Metric Review

Ignorance of system and process performance leads to inefficiency, poor compliance, and low employee morale. It is good business practice to have regular review of process metrics to gauge the health and output of the system and processes that drive the organization.

Process owners should be aware of all the metrics affecting their process and have a conduit to present the critical metrics to upper management. There are examples in the industry where process owners responsible for execution of a process are not aware of the metrics being collected, if any are, and have no basis for judging the adequacy of their process or its performance.

Regulatory agencies hold management accountable for the operations of an organization. It is the fiduciary responsibility for process owners to share the output and performance of the operation with management and be able to explain and interpret those metrics. Management has the responsibility to know the operations, its performance, and take appropriate action to ensure compliance with government, industry, and company policies and regulations.

Regulations require an annual product review be conducted of pharmaceutical products to determine and assess changes made to processes that may affect product quality. However, good industry practices would mandate quarterly or monthly review for faster detection, decision, and action. Reviews need to include metrics on key operating parameters and critical quality attributes to ensure product safety and efficacy. Several other key business metrics also benefit the organization and should be included in the metrics review program. The metrics collected should easily provide the process owner and management with an indication if the process is in control and delivering the desired results. If not, the process owner needs to present management a proposal to pursue continuous improvement opportunities and be able to describe required changes necessary to realize process enhancement.

3.3.7.3 Maturity Model

A maturity model is a useful management tool to determine process status and provides a standard in which to value processes. It provides a standard in determin-

ing the overall robustness and progression of a process and assists in the determination of resource prioritization. It provides the basic framework to apply risk management in determination of process development. For example, development of high-risk systems, such as aseptic filling where high patient and business risk exist, should be developed to a higher maturity than other processes with less patient or regulatory risk. Business demands placed on the pharmaceutical and biopharmaceutical industry limit resources in development, quality, and manufacturing requiring wise deployment of these resources to the areas that can best benefit the organization.

An example of a maturity model can be seen in Figure 8. This example provides the QMS program group and leadership the ability to evaluate processes based on an objective standard. It is divided into five general levels, moving from informal, unstructured to best in class. It includes specific deliverables for each level of the model to be completed before a process can be considered to have achieved that level. This model can also be divided into distinct subcategories, for instance, infrastructure, performance, and compliance, which are depicted in Table 8. Each subcategory can be designed to provide meaningful information to the process owners

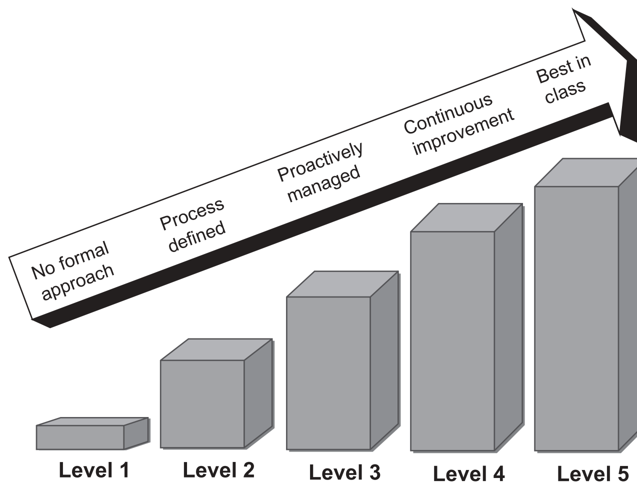

FIGURE 8 Maturity model overview. (*Source*: Adapted from Capability Maturity Model Integration, www.sei.cmu.edu.)

TABLE 8 Example Maturity Model

Theme	Level 1 (No Formal Approach)	Level 2 (Process Defined)	Level 3 (Proactively Managed)	Level 4 (Continuous Improvement)	Level 5 (Best in Class)
Compliance	→	→	→	→	→
Infrastructure	→	→	→	→	→
Performance	→	→	→	→	→

Source: Adapted from Capability Maturity Model Integration, www.sei.cmu.org.

and management. The maturity model is an excellent metric to measure development of the QMS and focus leadership in deployment of resources.

The subcategories of the model demonstrate, through defined attributes that must be in place, specific areas required of a robust process. The infrastructure category includes a capable owner of the process is in place and a quality assurance counterpart is identified, the process owner has a strong understanding of the process flow, scope, process boundaries, suppliers, customers, and roles and responsibilities. The goal is to develop a highly integrated process that is fully transferable and scalable.

Compliance is a key process attribute for a process in the pharmaceutical and biopharmaceutical industry. Process maturity determination related to compliance can include documentation such as standards and SOPs, number of observations written against the process from internal audits, supplier audits, regulatory inspections, nonconformances, and a risk assessment on the process against patient safety and efficacy. Training programs are also required as part of the process compliance. Audit and inspection observations written against a process are key metrics indicating maturity. Processes that can meet high maturity level for compliance represents a well-managed process that is consistently delivering a compliant and quality output.

Performance metrics determine process performance, preferably against predetermined standards or expectations. Performance metrics should be indicators as to the health and robustness of the system. Performance metrics may include cycle turn around time, time to disposition from end of manufacture, and a risk assessment against business drivers. The purpose is to raise target performance objectives, developing a strategic approach, reducing variability, and improving efficiencies. Efficiencies gained in process performance contribute to the business needs.

Advantages to utilizing a maturity model are that it provides a useful methodology for the QMS program group and leadership to evaluate, grade and provide process owners a goal for process development. Again, using a risk-based mindset, the entire inventory of process can be evaluated by leadership to determine where to place resources and to what maturity level each process is best positioned to support the enterprise.

Maturity-level goals are best made by process owners, the QMS program group, and leadership. It is recommended that all processes are assessed against risk to the customer and the business. This allows the QMS to prioritize the processes and identify which of the processes need to be elevated to a higher maturity level in the maturity model. Upon completion of the risk assessment, results should be reviewed by leadership to determine if the processes have been prioritized appropriately and meet the corporation's goals. This feedback forum will ensure that leadership supports process owners as they endeavor to achieve a higher level of process maturity. A well-designed QMS will allow for two-way conversation between the leadership and the process owners. It is as important for the leadership to communicate priorities to the process owners as well as having the process owners communicate issues and concerns that need to be addressed to the leadership. This will improve the alignment of priorities between the leadership and process owners. This integration ensures that the corporation is working on the right things at the right time with the right people.

3.3.7.4 Meeting Process Maturity Requirements

A dedicated team or review board should be developed to review and approve all maturity-level deliverables upon completion of the attributes for the current level. This review board's purpose is to ensure that all deliverables meet a consistent level of quality and documentation. This board can provide feedback to process owners or QMS program group to communicate best practices and lessons learned.

A well-designed metric review program is essential to the success of the QMS. The program should include metrics for the QMS, process maturity-level assessment and process performance, infrastructure, and compliance metrics. These metrics are the basis for evaluating system progress against long-term vision and annual quality plans. The metrics provide leadership and process owners specific and objective data to determine program goal achievement. Leadership will have visibility and comparability of process performance within and between sites and have risk-based data to support their deployment of resources in addressing business issues.

3.3.8 DRIVING CONTINUOUS IMPROVEMENT: PROJECTS

Pharmaceutical and biopharmaceutical companies are under significant pressure to deliver consistent quality product as well as drive the overall product cost down. The goal of implementing ICH Q8, Q9, and ultimately Q10 is to characterize processes based on risk assessments and improve them through a well-designed QMS. There are regulatory and business drivers to continually improve the QMS processes by building in quality and improve process efficiency. The regulatory agencies are now focused on ensuring systems are in place that protect the public health by assuring both the safety and efficacy of products. Understanding manufacturing processes, through well-designed characterization studies, is one of the most efficient and effective methods to ensure process efficiency. To meet business and consumer demands as well as regulatory guidance and expectations, the implementation of continuous improvement through risk-managed evaluations of manufacturing processes is expected.

3.3.8.1 Process Improvements

A quality management system's process should follow a standard Six Sigma process improvement life cycle that includes the following steps: define (process and metrics), measure and control (identify problems and issues), analyze (analyze problems and issues), and improve (implement) circling back to measure and control [11]. An example of a process improvement life cycle can be seen in Figure 9.

The basic foundation of continuous improvement begins with a process owner who fully understands the process and recognizes how the process impacts other processes within the QMS. Understanding this cause-and-effect relationship between processes requires close integration between process owners and stakeholders. This integration is critical throughout the entire life cycle of a process, from design through development and management.

Prior to process improvements the process must be well-defined and predictable. This does not mean that the process or output is desirable but instead well under-

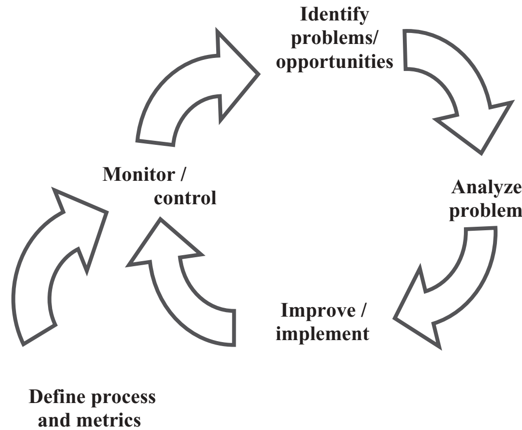

FIGURE 9 Continuous improvement process.

TABLE 9 Nine-Block Risk Assessment Matrix

		Severity		
		Minor	Major	Severe
Frequency	Probable	Medium	High	High
	Occasional	Low	Medium	High
	Remote	Low	Low	Medium

stood and predictable. It is through metrics, trends, and risk assessments that issues and concerns should be evident. Process owners can use the management review forum to present a proposal for process improvements.

3.3.8.2 Process Improvement Proposal

The process owner with stakeholders will need to provide a process improvement proposal if the issue or change requires prioritization due to funds or additional resources from the enterprise. The proposal should include, at minimum, the problem and or opportunity statement, impact to the site based on risk, and proposal of an action and/or project, including both cost and resource requirements.

During the development of the proposal, the process owner should consider requesting subject matter experts to assist with the development of the problem statement, risk assessment, and cost avoidance or savings. Many times a process owner's core competencies align closely with the process but may lack business or project management skills. The process owner may need assistance to clearly articulate to the leadership what the benefits are to accept the proposed change versus the risks for not adopting the proposal.

Risk assessment tools such as a nine-block risk assessment (Table 9) or a failure mode and effect analysis (FMEA) are available to assist the process owner with the evaluation of the process or issue to better understand and communicate the

probability of failure and the severity of a process issue. These analyses assist with the prioritization of issues and identification of specific actions required to mitigate risks or the identification of contingency plans for issues that are not mitigated. In a pharmaceutical and biopharmaceutical environment it is important that all risk tools are completed assessing impact to product safety and efficacy as well as the business drivers. Any steps or issues in the process that can negatively impact the safety and efficacy of the product require immediate elevation to leadership and must be addressed immediately. If the proposal requires prioritization, the process owners should clearly identify potential cost savings or avoidance through a cost of quality model to further engage senior leadership. The combination of risk and costs is an effective way to gain leadership support and attention.

Leadership's role in the process improvement proposal is to understand the issue or opportunity, understand associated risk(s), and approve or redirect a proposed action or project and provide appropriate funding and or resources. As action items and proposals are approved and initiated, the progress should be monitored on a routine basis to ensure appropriate progress is made.

3.3.8.3 Task versus Project

Process improvements may be conducted by the completion of a task or a project. A task is an activity that can be completed by the process owner with minimal cost and/or resources over a short period of time. A project is defined as temporary work to provide a product or service that is beyond the process owner's support. In general, a project requires more than one full-time equivalent (FTE), crosses over multiple functional organizations, and the duration of the effort spans over a longer period of time. Improvement status, updates, and issues should be discussed on a regular basis by a management forum or steering committee. Tasks and projects should be prioritized based on the risk against patient safety and efficacy and compliance.

If the process improvement meets the requirements of a project, a project manager should be identified. Formal project management allows for a holistic and integrated approach to the change. The project manager should not replace the process owner but ensure that the issues are identified, prioritized, and resources are applied, milestones are met, issues escalated and resolved, and progress reported. The process owner needs to be the project lead with the stakeholders or steering committee, providing support and guidance. This allows the process owner to focus on the issues and improvements (their core competencies) and allows the project manager to move the project forward in a methodical manner. During the project it is critical that success is defined and measured.

3.3.8.4 Project Metrics

Project metrics should be identified to measure the actual benefit of the change versus the expected result following the implementation. Many times, corporations implement a change and move on to the next project without fully understanding whether or not the changes achieved the desired result. A project that does not achieve the expected benefits can lead to an ineffective process, conflicts with associated touch points with other processes, or frustration from staff and customers.

Applying a systems-based approach to continuous improvement of the QMS, utilizing formal risk management tools benefits the overall efficiency of the organization. Process owners are accountable and empowered to drive continuous improvements. Metrics are utilized to identify trends, issues, and opportunities. Stakeholders are engaged throughout the process, and management is involved in the prioritization and staffing of the task or project. The processes are continually managed and evaluated. Continuous improvements based on risk allow the organization to apply resources and money to the most critical projects that will make the most impact. As process improvements are implemented, staff will benefit from a predictable, lean process allowing them to focus on the proactive nature of their work as opposed to the high stress of reacting to the issue of the day. The process owner will gain credibility as he or she demonstrate the ability to ensure that the right people are making the right decisions in a timely manner and that process improvements are addressing systemic process problems and not superficially addressing issues that will resurface again.

3.3.9 ENSURING ONGOING SUCCESS

Building infrastructure to establish and maintain a quality management system requires resources and resolve from leadership and staff. The current pharmaceutical and biopharmaceutical global and regulatory environment requires an organization invested in developing and maintaining a robust system and processes meeting the organization's requirements for producing quality product. Future competition, shorter time to market, efficient development, and first-pass approval expectations exacerbate the need for robust processes. The global marketplace continues its pressure on industry to deliver lifesaving and life-style changing medicines faster and cheaper.

3.3.9.1 Establishing Mutual Goals

Companies that have designed, developed, and established QMSs and processes that are simple to execute, easy to understand, and deliver the business and regulatory results will have competitive advantage over their industry peers. They will be faster and more efficient at adapting new technologies, assimilating new organizations through merger and acquisitions, able to apply adequate resources to appropriate business needs, and most importantly quickly modify and adapt to changing marketplace demands. Dependence on people, fragmented procedures, or tribal knowledge, rather than integrated, functional processes, will bring undesirable results to all levels of the organization.

Ensuring ongoing success requires establishing mutual goals for the organization from the beginning. These goals must satisfy the needs of the business, the employees, and the shareholders. Well-designed processes with accountable ownership that have been established through discussion, design, and support of leadership, functional management, operational stakeholders, and general staff provide the foundation for common shared needs (Figure 10). If anyone of these groups is not considered, nominal support and eventually failure of the program can be expected.

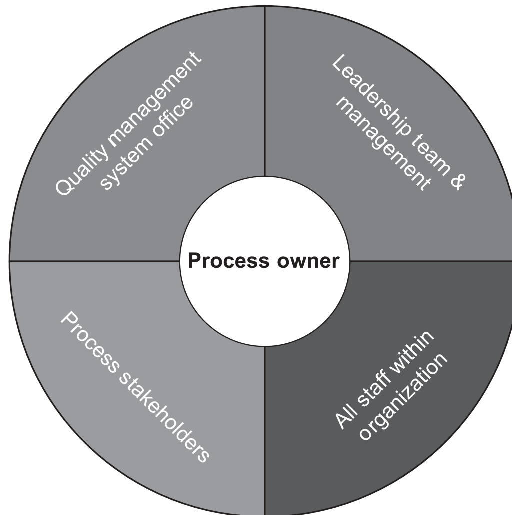

FIGURE 10 Process owner support model.

These shared goals need to be memorialized through documentation of the program. This includes outlining the long-term objectives of the program, benefits required to be achieved for stakeholders, and annual quality plans for achieving milestone goals and success. Program success comes from leadership support, robust system design, adequate training for employees, and meaningful metrics to measure performance and continuous improvement efforts.

Mechanisms to determine stakeholder feedback on program acceptance, clarity, and improvement opportunities need incorporation into the ongoing maintenance of the QMS. Focus groups are one method of obtaining this type of information. Another opportunity exists with regulatory inspections and customer audits. Taking appropriate action to implement program changes and enhancements while recognizing contributors will ensure stakeholder support and participation.

Mutual goals will drive success of the program and provide the reference for why the system approach is needed and the benefits it can bring. There is no better situation than having an entire organization aligned around the business design, and executing against it, while supporting each other.

3.3.9.2 Rewards and Recognition

Process owners' responsibilities are significant. Owners need to be selected from predetermined criteria that are discriminatory in nature. Process owners are the drivers of the operations and therefore need to be recognized for their special efforts and responsibilities. This recognition can take many forms. A significant distinction in base qualifications and rewards is a valuable incentive to becoming a process owner. Ongoing development for process owners is another incentive and reward for the process owner. In addition to the financial and tangible rewards, being recognized by the organization to have the confidence of management is also another form of reward and recognition. Inevitably, processes contain waste and

inefficiencies, thereby providing another opportunity for owners to improve their process and be recognized for that improvement.

Public recognition of system program and process owner accomplishments is essential. This can easily be accomplished through regular review sessions, at metric review meetings, through staff meetings and updates, poster sessions, newsletters, and departmental meetings. Simple recognition and small gifts are appreciated and reinforce management's support and commitment to the program. Process owners and stakeholders are the most influential group to spread the word on the usefulness of the program and must be cultured to ensure ongoing success.

Studies indicate financial rewards alone cannot provide employee satisfaction and retention. High employee turnover costs companies tremendous financial and competitive resources. Many employees faced with equal or higher pay but unsatisfying work will move onto another company or position. A poorly integrated QMS with complicated processes is often the foundation for that dissatisfaction. To repeat work, lose valuable time, or deliver substandard product does not satisfy today's highly educated and competitive worker in the pharmaceutical and biopharmaceutical industry. The cost to recruit, replace, relocate, and retrain employees is significant. Avoidance of these costs can be used as a partial basis for support of the program.

3.3.9.3 Ensuring Ongoing Program Continuity

Accomplishments of a comprehensive QMS program should be shared between locations and be consistent. Common, competent leadership for the enterprise will ensure consistency. A consistent QMS program also allows for transfer of staff between sites with little or no training and assimilation requirements. Divergent evolution will dilute the QMS effort and support. Flexibility to execute is important, however, caution must be exercised to restrict diverging language, interpretation, and philosophy. Within a short time of a global execution, efficiencies will be quickly realized. Ensuring consistency also increases the number of process users with similar experiences and leverages focus for process improvements and therefore support.

Regulators and customers require assurance in consistency of pharmaceutical and biopharmaceutical manufacturing operations. Today's manufacturing supply chains require multiple sites in varying locations to produce a product. Quality systems must be perceived as an integral part of the value chain. This requires that all sites be compliant in their operations and systems. Strong areas in one location do not make up for weak or absent systems in another location. Fines are levied and business is made or lost based on the individual site or weakest link in the supply chain. Management must have a mechanism to measure its processes, and a comprehensive QMS is the mechanism to demonstrate capability.

3.3.9.4 Program Institutionalization

Program institutionalization is realized with time. All levels of the organization need to recognize and verbalize that the quality management system approach is the way business is conducted. This way of doing business will become part of the culture to the point at which it is second nature to leadership, management,

and staff. Regulators and customers will recognize the benefits, as do the shareholders and patients.

REFERENCES

1. *Webster's New Collegiate Dictionary*, ninth edition, 1986, p.1199.
2. Arling, E. R. (2004), Integrating QSIT into quality plans, *Biopharm. Int.*, June, 44–46, 48, 50–52.
3. *Drug Industry Daily*, Oct. 12, 2006, Vol. 5, No. 200, Washington Business Information.
4. 2006 PDA/FDA joint regulatory conference, Sept. 11, 2006, Washington, DC.
5. Joneckis, C. (2006), Ph.D. presentation at 11th Annual GMP by the Sea, Aug. 28–30, Cambridge, MD.
6. Quality systems regulations CDRH, available: www.fda.gov.
7. CPGM 7356.002, CDER, available: www.fda.gov.
8. IOM biological inspections 7345.848 CBER, available: www.fda.gov.
9. Rockefeller, J. D., III (1973), *The Second Revolution*, Harper-Row, New York.
10. Kotter, J. P. (2001), Leadership insights, *Harvard Bus. Re.*, p. 29.
11. George, M. (2004), *Lean Six Sigma Pocket Tool Book*, McGraw-Hill Professional, New York, p. 4.

3.4

QUALITY PROCESS IMPROVEMENT

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3.4.1 DIAGNOSING A PROCESS

3.4.1.1 Introduction

Quality process improvement starts with a diagnostic journey where problems are identified. Remedial activity will be taken and the process will be continuously monitored afterward. The common activities taken in the diagnostic journey are analyzing symptoms, formulating hypotheses, testing hypotheses, and identifying causes. Table 1 describes basic tools for the diagnostic journey. A description of them is given in Section 3.4.1.2.

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TABLE 1 Basic Quality Process Improvement Tools during Process Diagnosis

Common Activities to Diagnose Cause	Basic Tools for Quality Process Improvement								
	Cause–Effect Diagram	Pareto Chart	Histogram	Scatter Diagram	Normal Probability Plot	Flow Diagrams	Data Collection	Box Plot	Stratification
Analyzing symptoms	•	•	•	•	•	•	○	•	○
Formulating hypotheses	•	○	•	○	•	○	○	•	○
Testing hypotheses	•	•	○	•	•	○	•	○	•
Identifying cause(s)	•	•	•	•	○	○	○	○	•

Note: (•) major; (○) minor.

3.4.1.2 Basic Tools for Diagnosing a Process

Cause-and-Effect Diagram A cause-and-effect diagram relates potential causes of a problem to their effects. This is a tool that could be very useful in diagnosing a process. It focuses on the possible causes of a specific problem in a structured and systematic way. The following steps are suggested for constructing a cause-and-effect diagram:

1. Define the problem (effect).
2. Write problem on the right side and draw an arrow from the left to the right side.
3. Brainstorm the main categories of causes of problems and draw major branch arrows to the main arrow.
4. For each major branch, detailed causal factors (subcauses) are drawn as subbranches.
5. Write sub-subcauses branching off the subcauses.
6. Ensure all the items that may be causing the problem are indicated in the diagram.

Figure 1 shows a cause-and-effect diagram which is used to identify causes to yield a problem in a biopharmaceutical manufacturing process. Possible main causes and subcauses are identified. Once the causes are identified, other tools are employed to determine the contribution of various causes to the effect. Actions are taken to eliminate or minimize the impact of these causes.

Pareto Chart The Pareto principle suggests a problem (effect) can be attributed to relatively few causes. In quantitative terms, 80% of the problems come from 20% of the causes (machines, raw materials, operators, etc.); therefore effort aimed at the right 20% can solve 80% of the problems. A Pareto chart includes three basic elements: (1) the causes to the total effect, ranked by the magnitude of the contribution; (2) the frequency of each cause; and (3) the cumulative-percent-of-total effect of

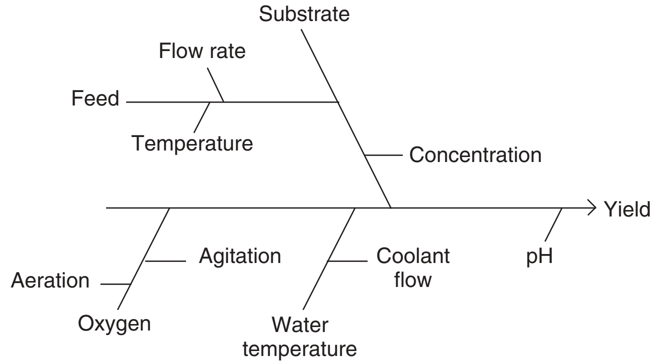

FIGURE 1 Cause-and-effect diagram.

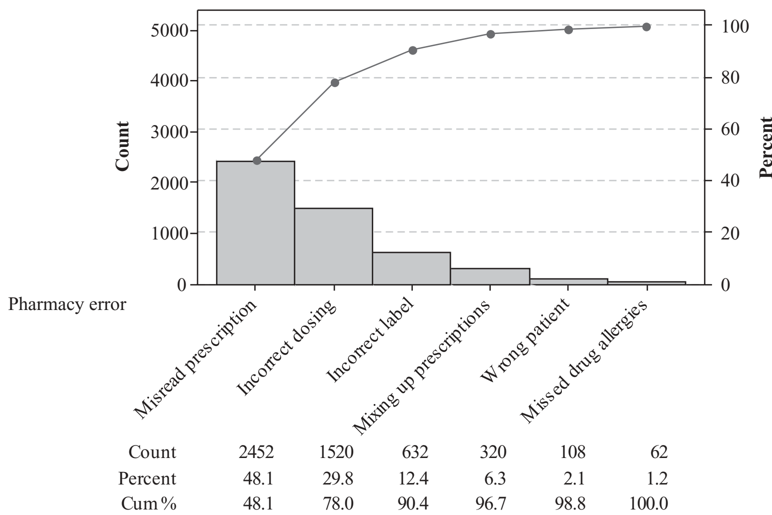

FIGURE 2 A Pareto chart showing pharmacy errors.

the ranked causes. Figure 2 gives an example of a Pareto chart which exhibits errors found in a pharmacy store chain in one month.

Histogram A histogram is a graphic summary of variation in a set of data. Data are clustered into categories and the values of individual clusters are plotted to give a series of bars. For illustration, Table 2 presents 40 observations on the shelf life of a certain drug and their frequency distribution. Figure 3 gives a histogram for the drug shelf life data.

Scatter Diagram A scatter diagram is a basic tool to identify the potential relationship between two variables. Scatter diagrams are similar to line graphs in that they use horizontal and vertical axes to plot data points. However, they have a very specific purpose. Scatter diagrams show how much one variable is affected by another. The relationship between two variables is called their correlation. The

TABLE 2 Drug Shelf Life (days)

102.2	104.1	103.5	104.5	103.2	103.7	103.0	102.6
103.4	101.6	103.1	103.3	103.8	103.1	104.7	103.7
102.5	104.3	103.4	103.6	102.9	103.3	103.9	103.1
103.3	103.1	103.7	104.4	103.2	104.1	101.9	103.4
104.7	103.8	103.2	102.6	103.9	103.0	104.2	103.5

Range	Midpoint	Frequency	Cumutative %
$101.5 \leq x < 102.0$	101.75	2	5.00
$102.0 \leq x < 102.5$	102.25	2	10.00
$102.5 \leq x < 103.0$	102.75	5	22.50
$103.0 \leq x < 103.5$	103.25	15	60.00
$103.5 \leq x < 104.0$	103.75	8	80.00
$104.0 \leq x < 104.5$	104.25	6	95.00
$104.5 \leq x < 105.0$	104.75	2	100.00

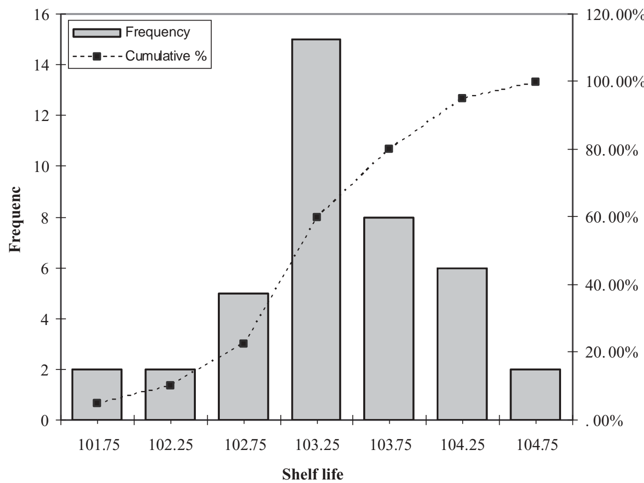

FIGURE 3 Histogram of drug shelf life.

closer the data points come when plotted to making a straight line, the higher the correlation between the two variables. If the data points make a straight line going from the origin out to high x and y values, then the variables are said to have a positive correlation. If the line goes from a high value on the y axis to a high value on the x axis, the variables have a negative correlation. Figure 4 gives a few examples of scatter diagrams.

Normal Probability Plot The normal probability plot is a graphical technique for assessing whether or not a data set is approximately normally distributed. The data are plotted against a theoretical normal distribution in such a way that the points form an approximate straight line. Departures from this straight line indicate departures from normality. The normal probability plot is important for quality process improvement since many other tools require the normality assumption. A normal

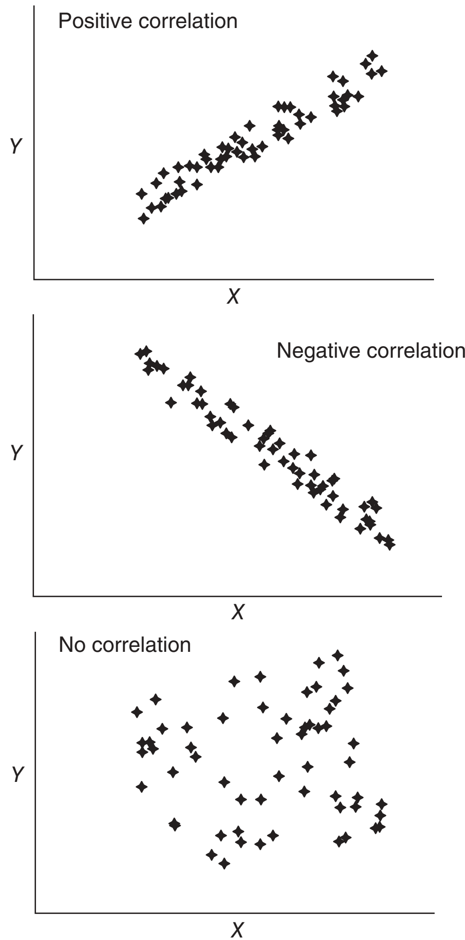

FIGURE 4 Scatter diagrams.

probability plot of the drug shelf life in Table 2 is given in Figure 5. As seen from the plot, the observations follow a straight line and are contained in the 95% confidence interval. It can thus be said that the shelf life of this drug follows a normal distribution.

Other Tools

Box Plot This plot is useful when analyzing the pattern of the data. It displays several important features of data such as central tendency, variability, departure from symmetry, and presence of outliers.

Flow Diagrams A process flow diagram can be used to study and understand the process.

Data Collection Data are essential for making a proper evaluation of the current process. Tools for data collection include checklists and data sheets.

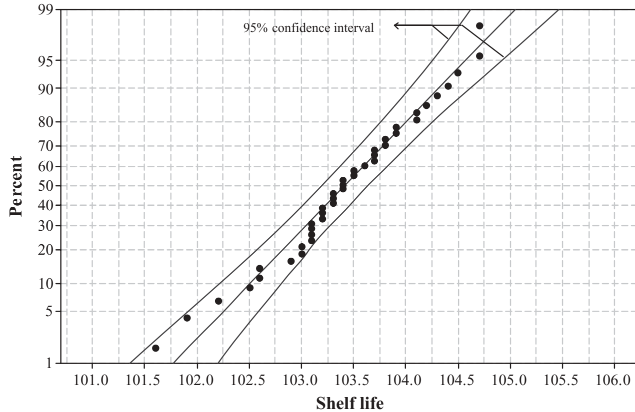

FIGURE 5 Normal probability plot of drug shelf life with 95% confidence interval.

Stratification This technique is used to separate data into groups based on categories or characteristics. It is the basis for the application of other tools or it can be used with other data analysis tools such as scatter diagrams.

3.4.2 STABILIZING AND IMPROVING A PROCESS

3.4.2.1 Introduction

Basic Concepts of a Control Chart The control chart is one of the main tools for quality process improvement. It is used to assess the nature of variation in a process and to facilitate the forecasting and management of a process. Values of the quality characteristic are plotted against the sample number or time, as shown in Figure 6. The centerline represents the process average. The upper and lower control limits (UCL and LCL) are usually chosen as three standard deviations (SDs) above and below the centerline so they can be used to detect "out-of-control" situations without causing mamy false alarms. An out-of-control situation is usually signaled by a plotted point falling outside the control limits or a cluster of plotted points forming an abnormal pattern.

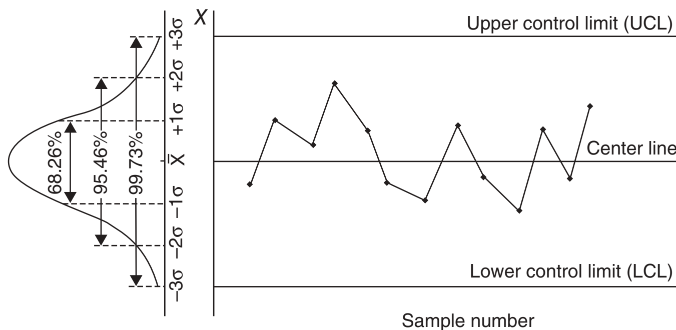

FIGURE 6 Normal curve-based control chart.

Plotted points on a control chart are usually based on data collected from samples in a process. After a sufficient number of samples are collected and the data are plotted on a control chart, the stability of the process can be evaluated. A stable process is "in control" while an out-of-control process is unstable. Depending on the type of quality characteristic, control charts can be divided into two groups: variable control charts and attribute control charts. Variable control charts are used to monitor quality characteristic that are continuously varying in nature; attribute control charts are used to monitor those quality characteristics that are not numerically measurable. The determination of the centerline and control limits are described in Sections 3.4.2.2 and 3.4.2.3 with respect to different types of control charts.

Applications of Control Charts Control charts serve to direct management attention toward special causes of variation in a process when they appear. In evaluating control charts, the following symptoms could indicate a process that is out-of-control:

- *Outlier* One or more point(s) that fell outside the control limits.
- *Run* A series of plotted points above or below the centerline.
- *Trend* A continual rise or fall of plotted points.
- *Cyclicity* A pattern that repeats itself over time.

The following steps are usually followed in a control chart's development and application:

- Determine a "base period" for initial control chart development.
- Collect sample data from the base period.
- Calculate the parameters for the control chart, that is, centerline and control limits.
- Plot collected sample points on the chart with the centerline and control limits.
- Determine whether the chart parameters can be used to monitor the process; revise the parameters if necessary.
- Collect ongoing samples and continue monitoring the process using the developed control chart.
- Conduct periodic audits on the parameters of the control chart.

Variable control charts are widely applied in many manufacturing and nonmanufacturing settings. They can be used to monitor, for example, the inside diameter of an aircraft bearing, the moisture content of a drug tablet, the net weight of a pharmaceutical product, the processing time of phone inquiries, and the satisfaction level of customers. The latter two are examples of nonmanufacturing applications.

Attribute control charts are less used compared to variable control charts. When it is not possible or practical to measure the quality characteristic of a product, attribute control charts are often applied. Examples of their application include monitoring the fraction of nonconforming of a certain sensor production, the number of defective diodes in an electronic assembly, the number of imperfections in textile

production, the fraction of defective batches in a biomedical manufacturing production, and the number of errors found in a pharmacy store.

In most applications, the choice between a variable control chart and an attribute control chart is clear-cut. In some cases, the choice will not be obvious. For instance, if the quality characteristic is the softness of an item, such as the case of pillow production, then either an actual measurement or a classification of softness can be used. Quality managers and engineers will have to consider several factors in the choice of a control chart, including cost, effort, sensitivity, and sample size. Variable control charts usually provide more information to analysts but cost more to implement and use. Attribute control charts are less sensitive and expensive but usually requires large samples to reach certain statistical significance.

3.4.2.2 Control Charts for Attributes

Control charts based on attribute data include the p chart, np chart, c chart, and u chart. The former two are applied when fraction nonconforming or number of nonconforming is a concern, and the latter two are used to deal with the nonconformities. Most pharmaceutical manufacturing industries employ one or more of these charts.

p *Control Chart* A p chart can be used to monitor the fraction nonconforming of a process. *Fraction nonconforming* is defined as the ratio of the number of nonconforming items in a population to the total number of items in that population. In pharmaceutical manufacturing, an item will be classified as nonconforming if it fails to conform to standards on one or more attributes, for example, fill volumes of vials, moisture content, hardness, and solubility.

Let us suppose a random sample of n items is selected and examined from a process running with a stable nonconforming rate p and D units of nonconforming items are found; then D is a random variable following a binomial distribution with parameters n and p. If the true fraction nonconforming, p, is known, then the parameters of the p chart are

$$\begin{aligned} \text{UCL} &= p + 3\sqrt{\frac{p(1-p)}{n}} \\ \text{Centerline} &= p \\ \text{LCL} &= p - 3\sqrt{\frac{p(1-p)}{n}} \end{aligned} \tag{1}$$

In practice, the fraction nonconforming, p, is unknown most of the time and is thus needed to be estimated from the sample data. An estimated p_i can be calculated for the ith sample collected and an average $\bar{p}$ value can be obtained as an arithmetic average of those individual p_i found from the m samples:

$$\bar{p} = \frac{\sum_{i=1}^{m} D_i}{mn} = \frac{\sum_{i=1}^{m} p_i}{m} \tag{2}$$

The $\bar{p}$ can then be used in place of p in Equation (1) in the application. It should be noted that the $\bar{p}$ value needs to be assessed periodically to assure its representativeness of the average process fraction nonconforming.

np *Control Chart* An np chart is used to monitor the number of nonconforming items produced in a process. Very similar to the p chart, the parameters of an np chart are

$$\begin{aligned} \text{UCL} &= np + 3\sqrt{np(1-p)} \\ \text{Centerline} &= np \\ \text{LCL} &= np - 3\sqrt{np(1-p)} \end{aligned} \tag{3}$$

As in the p chart, if the actual p value is not available, $\bar{p}$ can be used in the calculation.

c *Control Chart* A c chart can be used to monitor the number of nonconformities (defects) per inspection unit. An inspection unit can be a single unit of product, a batch of multiple products, or a certain measured volume (weight) of product. Many pharmaceutical manufacturing processes are lot based where raw material or semi-product passes from one process to the next. For example, an inappropriately coated tablet in a coating process can be considered as a nonconformity (defect) where an inspection unit might be defined as 1 kg of the tablet.

Suppose an inspection unit of a certain product is selected and examined from a process running with a stable nonconformity rate c per inspection unit and X nonconformities are found. Then X is a random variable following a Poisson distribution with parameter c. If the true nonconformity level c is known, then the parameters of the c chart are

$$\begin{aligned} \text{UCL} &= c + 3\sqrt{c} \\ \text{Centerline} &= c \\ \text{LCL} &= c - 3\sqrt{c} \end{aligned} \tag{4}$$

If the actual nonconformity level c is unknown, it can be estimated by using average c values obtained from m inspection units collected in a base period:

$$\bar{c} = \frac{\sum_{i=1}^{m} C_i}{m} \tag{5}$$

The $\bar{c}$ can then be used in place of c in Equation (4) in the application. Since it is possible to obtain a negative LCL using Equation (4), a value of zero should be used in that case.

u *Control Chart* A u chart is used to monitor the rate of nonconformities. The rate of nonconformities (u) is the number of nonconformities (x) in an inspection unit divided by the number of physical units (n) inspected (e.g., 100 ft of pipe, 100 items in a batch). Similar to the c chart, the parameters of a u chart are

$$\text{UCL} = u + 3\sqrt{\frac{u}{n}}$$
$$\text{Centerline} = u \tag{6}$$
$$\text{LCL} = u - 3\sqrt{\frac{u}{n}}$$

If the actual u value is not available, ū can be used in Equation (6).

Example 1 A medical device manufacturer is concerned about the nonconforming (defective) and the nonconformity (defect) produced in its recently set-up production line. Twenty batches of this medical device were randomly selected from the production line. Each batch contained 100 units. Each unit is inspected and is classified as either "conforming" or "nonconforming." During the inspection, the number of nonconformities (defects) was also counted. The data collected are shown in Table 3.

TABLE 3 Nonconforming and Nonconformity Counts of 20 Batches of Medical Device

Batch number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Nonconformings	3	2	4	2	5	2	1	2	0	5	2	4	1	3	6	0	1	2	3	2
Nonconformities	9	7	13	8	6	8	10	10	7	10	12	9	11	15	8	12	11	8	7	15

Based on the data in Table 3, the average fraction nonconforming, $\bar{p}$, can be obtained as 2.5%; the average nonconformity per batch, $\bar{c}$, is 9.8; and the average nonconformity per unit, $\bar{u}$, is 0.098. The resulting control charts are shown in Figures 7–10. These charts indicate that the process is in control and thus the parameters established here can be used to monitor future productions.

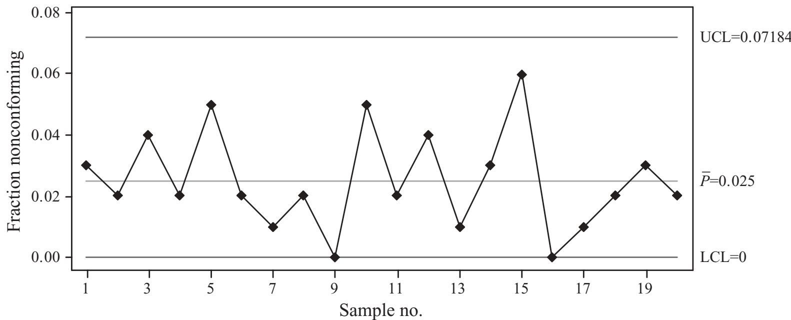

FIGURE 7 *p* Chart for medical device manufacturing example.

3.4.2.3 Control Charts for Variables

Control charts based on variable sample data include the $\bar{x}$ chart and the s chart. When dealing with a numerically measurable quality characteristic, the $\bar{x}$ chart is usually employed to monitor the process average and the s chart is used to monitor the process variability. When there is only one observation in each sample, the individual measurement chart (I chart) and moving range chart (MR chart) are used to monitor the process average and variability. It should be noted that due to the poor

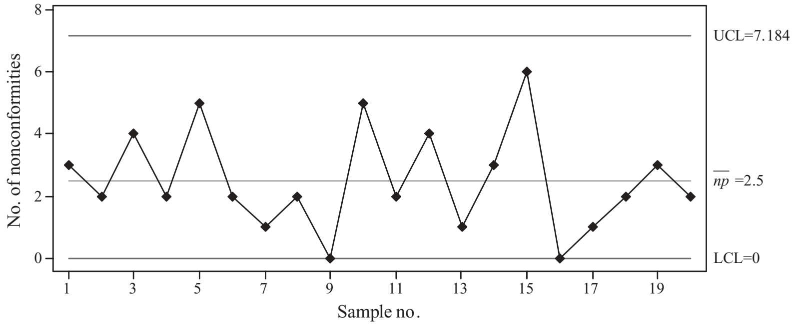

FIGURE 8 *np* Chart for medical device manufacturing example.

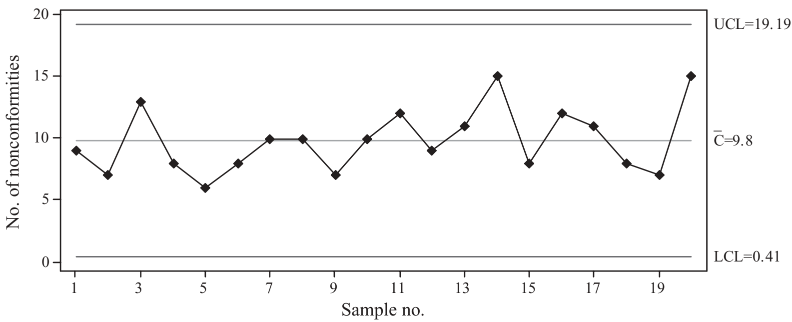

FIGURE 9 *c* Chart for medical device manufacturing example.

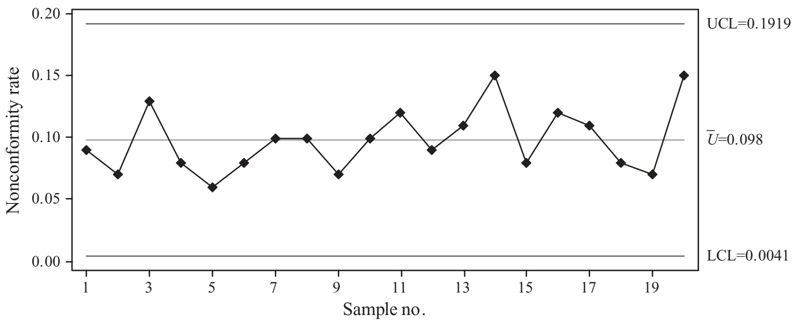

FIGURE 10 *u* Chart for medical device manufacturing example.

sample size, the *I* and MR charts are less sensitive in detecting if the process is out of control than the $\bar{x}$ and *s* charts.

$\bar{\mathbf{x}}$ *Control Chart* An $\bar{x}$ chart is used to monitor the process average. It is usually used in pharmaceutical manufacturing where multiple units are collected in each sample (e.g., a sample of multiple tablets formed by dry powders or wet granules.) Due to contamination risk and cost of sampling (including product loss due to

sample volumes and incurred labor cost of laboratory analysis), the sample size is usually kept small.

Sample means $\bar{x}$ are plotted on the $\bar{x}$ chart. Assume that random samples of n items are collected and examined from a stable process with a process mean μ and standard deviation σ. Then $\bar{x}$ can be considered as a random variable following a normal distribution with mean $\mu_{\bar{x}}$, and standard deviation $\sigma_{\bar{x}}$, where

$$\mu_{\bar{x}} = \mu \qquad \text{and} \qquad \sigma_{\bar{x}} = \frac{\sigma}{\sqrt{n}} \tag{7}$$

If the true process mean μ and standard deviation σ are known, then the parameters of the $\bar{x}$ chart are

$$\begin{aligned} \text{UCL} &= \mu_{\bar{x}} + 3\sigma_{\bar{x}} = \mu + 3\frac{\sigma}{\sqrt{n}} \\ \text{Centerline} &= \mu_{\bar{x}} = \mu \\ \text{LCL} &= \mu_{\bar{x}} - 3\sigma_{\bar{x}} = \mu - 3\frac{\sigma}{\sqrt{n}} \end{aligned} \tag{8}$$

Since μ and σ are not usually known, estimators of them can be obtained from the sample means ($\bar{x}$) and sample standard deviations (s) of the m samples collected in the base period:

$$\begin{aligned} \text{Estimator of } \mu &= \bar{\bar{x}} = \frac{\sum_{i=1}^{m} \bar{x}_i}{m} \\ \text{Estimator of } \sigma &= \frac{\sum_{i=1}^{n} s_i / m}{4(n-1)/4n-3} = \frac{\bar{s}}{c_4} \end{aligned} \tag{9}$$

Using the estimators, the parameters of the $\bar{x}$ chart are now

$$\begin{aligned} \text{UCL} &= \bar{\bar{x}} + 3\frac{\bar{s}/c_4}{\sqrt{n}} = \bar{\bar{x}} + A_3\bar{s} \\ \text{Centerline} &= \bar{\bar{x}} \\ \text{LCL} &= \bar{\bar{x}} - 3\frac{\bar{s}/c_4}{\sqrt{n}} = \bar{\bar{x}} - A_3\bar{s} \end{aligned} \tag{10}$$

The common values of constants c_4 and A_3 are tabulated in Table 4 for sample sizes from 2 to 10. Like other control charts, the values of $\bar{\bar{x}}$ and $\bar{s}$ should be periodically verified to assure that they can be used to derive good estimators for the process average and process standard deviation.

s *Control Chart* An s chart is used to monitor the process variability. Since it is equally important to ascertain that the variability and the mean of a process are in control, an s chart is usually used in conjunction with the $\bar{x}$ chart. Sample standard deviations are plotted on the s chart. Consider s as a random variable with mean μ_s and standard deviation σ_s. Then the parameters of the s chart can be stated as

TABLE 4 Values of Constants in Variable Control Chart Parameters

n	A_3	c_4	B_3	B_4	d_2	d_3
2	2.659	0.798	0	3.267	1.128	0.853
3	1.954	0.886	0	2.568	1.693	0.888
4	1.628	0.921	0	2.266	2.059	0.880
5	1.427	0.940	0	2.089	2.326	0.864
6	1.287	0.952	0.030	1.970	2.534	0.848
7	1.182	0.959	0.118	1.882	2.704	0.833
8	1.099	0.965	0.185	1.815	2.847	0.820
9	1.032	0.969	0.239	1.761	2.970	0.808
10	0.975	0.973	0.284	1.716	3.078	0.797

$$\begin{aligned} \text{UCL} &= \mu_s + 3\sigma_s \\ \text{Centerline} &= \mu_s \\ \text{LCL} &= \mu_s - 3\sigma_s \end{aligned} \tag{11}$$

In practice, the parameters of the s chart can be estimated using $\bar{s}$ as

$$\begin{aligned} \text{UCL} &= \bar{s} + 3\frac{\bar{s}}{c_4}\sqrt{1-c_4^2} = B_4\bar{s} \\ \text{Centerline} &= \bar{s} \\ \text{LCL} &= \bar{s} - 3\frac{\bar{s}}{c_4}\sqrt{1-c_4^2} = B_3\bar{s} \end{aligned} \tag{12}$$

If the LCL calculation results in a negative value, use zero as the LCL.

Example 2 In a Pet Tabs (pet vitamin tablets) production, the pharmaceutical manufacturer is using milling and micronizing machines to pulverize raw materials into fine particles. These finished particles are combined and processed further in mixing machines. The mixed ingredients are then pressed into tablets, dried, and sealed in packages. A normally distributed quality characteristic, moisture content, is monitored. Samples of $n = 4$ tablets are taken from the manufacturing process every hour. The data after 25 samples have been collected are shown in Table 5.

From these data, it is found that $\bar{\bar{x}} = 10.254$ and $\bar{s} = 0.926$. Using Equations (10) and (12), the parameters of the $\bar{x}$ and s charts are found as:

	$\bar{x}$ Chart	s Chart
UCL	11.761	2.098
Centerline	10.254	0.926
LCL	8.747	0

The control charts are shown in Figure 11. The $\bar{x}$ and s charts show that the process is in control and thus the parameters established here can be used to monitor future productions.

TABLE 5 Moisture Content (%) of 25 Samples of Pet Tabs

	Observations					
Sample Number	1	2	3	4	$\bar{x}$	s
1	7.84	11.01	10.14	9.41	9.600	1.343
2	10.51	9.1	9.52	10.83	9.990	0.814
3	9.74	10.39	9.62	11.16	10.228	0.708
4	10.71	11.41	10.71	8.63	10.365	1.203
5	9.93	10.95	8.99	10.73	10.150	0.889
6	9.94	10.27	9.35	9.42	9.745	0.438
7	12.11	9.72	8.89	9.75	10.118	1.387
8	9.61	8.93	11.12	8.75	9.603	1.077
9	9.17	10.87	9.97	10.79	10.200	0.798
10	11.41	10.39	8.83	12.19	10.705	1.451
11	8.43	9.48	10.56	10.2	9.668	0.939
12	9.92	10.13	9.66	8.21	9.480	0.868
13	8.39	9.94	10.4	8.69	9.355	0.967
14	10.42	10.27	10.94	10.91	10.635	0.341
15	10.98	12.57	11.14	8.97	10.915	1.481
16	9.73	10.05	12.82	12.43	11.258	1.592
17	11.36	8.91	10.08	10.55	10.225	1.024
18	9.42	11.12	9.01	10.52	10.018	0.973
19	10.15	10.08	10.12	9.88	10.058	0.122
20	11.73	11.1	10.75	9.94	10.880	0.746
21	11.52	9.11	9.88	11	10.378	1.087
22	11.29	10.43	11.6	11.74	11.265	0.588
23	9.39	12.96	11.42	10.28	11.013	1.541
24	10.26	9.59	9.33	9.26	9.610	0.456
25	11.25	10.65	11.06	10.63	10.898	0.307

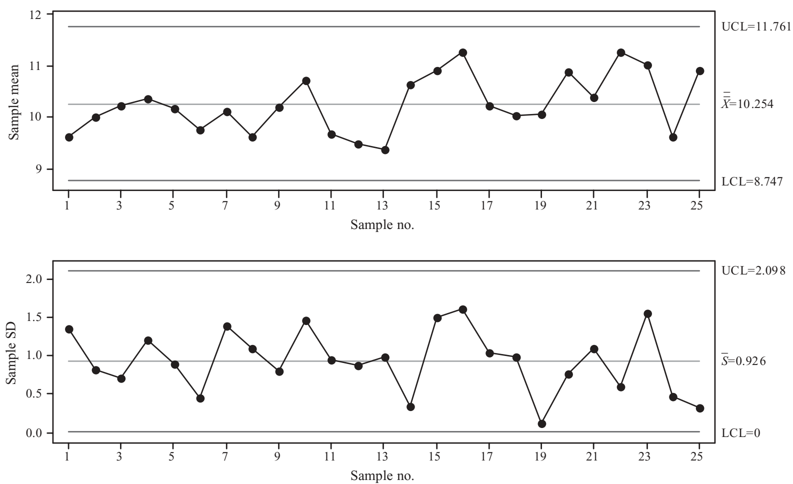

FIGURE 11 $\bar{x}$ and s charts for Pet Tab manufacturing example.

Individuals Control Charts In some chemical and biopharmaceutical manufacturing processes involving lengthy and expensive procedures, it is not feasible to form a sample of size greater than one because only one product or one batch is available each time. When the sample size used for statistical process monitoring is limited to one, individual control charts, *I* and MR charts, are needed.

The *I* chart is serving the same function as the $\bar{x}$ chart except that now x is the value of the individual measurement. Assuming that x follows a normal distribution with mean μ and standard deviation σ, the theoretical parameters of the *I* chart are

$$\begin{aligned} \text{UCL} &= \mu + 3\sigma \\ \text{Centerline} &= \mu \\ \text{LCL} &= \mu - 3\sigma \end{aligned} \tag{13}$$

The process average μ can be estimated by $\bar{x}$, which is

$$\hat{\mu} = \bar{x} - \frac{\sum_{i=1}^{m} x_i}{m} \tag{14}$$

Since only individual measurements are available, moving ranges need to be calculated for the estimation of process standard deviation σ. A k-point moving range, MR_k, can be calculated as

$$\text{MR}_k = \max(x_i, \ldots, x_{i+k}) - \min(x_i, \ldots, x_{i+k}) \tag{15}$$

For m individual measurements, there are $m - k$ MR_k available, and the process standard deviation σ can be estimated as

$$\hat{\sigma} = \frac{\overline{\text{MR}}_k}{d_2} = \frac{\sum_{i=1}^{m-k} \text{MR}_{ki} \big/ m - k}{d_2} \tag{16}$$

The estimated process mean and standard deviation can be used to calculate the practical parameters for the *I* chart in Equation (13). The constant d_2 value is determined by k and can be found by using k as n in Table 3. Common k values can range from 2 to 5.

The MR chart is used to monitor process variability. Considering MR_k as a random variable with a mean of μ_{MR_k} and a standard deviation of σ_{MR_k}, the theoretical parameters of the MR chart can then be stated as

$$\begin{aligned} \text{UCL} &= \mu_{\text{MR}_k} + 3\sigma_{\text{MR}_k} \\ \text{Centerline} &= \mu_{\text{MR}_k} \\ \text{LCL} &= \mu_{\text{MR}_k} - 3\sigma_{\text{MR}_k} \end{aligned} \tag{17}$$

Since μ_{MR_k} and σ_{MR_k} are not usually available, they can be estimated as

$$\hat{\mu}_{\text{MR}_k} = \overline{\text{MR}}_k \qquad \text{and} \qquad \hat{\sigma}_{\text{MR}_k} = \frac{d_3}{d_2}\overline{\text{MR}}_k \tag{18}$$

The constant value of d_3 can also be found in Table 3. If a negative LCL was obtained, use zero.

3.4.2.4 Special Control Charts

The control charts discussed earlier are very useful in the diagnostic aspects of quality process improvement. They can be used to stabilize a process by identifying out-of-control situations. After the process is stabilized and brought in control, further improvement of the process can be achieved by using some special control charts such as the cumulative sum (CUSUM) control chart and the exponentially weighted moving average (EWMA) control chart. These control charts can be used when "small shifts" in a process are of interest.

CUSUM Control Chart A CUSUM chart provides an efficient way of detecting small shifts in the mean of a process (<1/2 σ). For larger shifts (>1/2 σ), the $\bar{x}$ chart is usually used. The CUSUM chart incorporates information contained in a sequence of sample points. It keeps track of the cumulative sum of the deviations between each sample point (a sample mean) and a target value. Unlike the $\bar{x}$ chart, which often bases its out-of-control decision on just the most recently collected sample, the CUSUM calculated for a sample point carries the "history" prior to that sample. For example, a sequence of sample points above the centerline can trigger an out-of-control signal although all of them stayed well below the UCLs of the $\bar{x}$ chart.

There are two forms of the CUSUM chart, the tabular form and the V-mask form. Due to its practicality, the tabular form is more preferred in industrial settings. The tabular CUSUM accumulates deviations from a target value (or a known process mean μ_0). Deviations above that target value are cumulated as a one-sided upper CUSUM (C^+) and deviations below the target value are cumulated as one-sided lower CUSUM (C^-):

$$\begin{aligned} C_i^+ &= \max[0, \bar{x}_i - (\mu_0 + k\sigma_{\bar{x}}) + C_{i-1}^+] \\ C_i^- &= \max[0, (\mu_0 - k\sigma_{\bar{x}}) - \bar{x}_i + C_{i-1}^-] \end{aligned} \tag{19}$$

where $C_0^+ = C_0^- = 0$.

The parameter k is called the allowance and is usually determined as the magnitude of the shift to be detected in terms of $\sigma_{\bar{x}}$. If either C_i^+ or C_i^- exceeds a decision interval h, the process is considered out of control. In other words, the value of h is considered a UCL and $-h$ is considered an LCL. Its centerline is always at zero. A reasonable value for h is five times the process standard deviation σ.

EWMA Control Chart An EWMA control chart plots weighted moving average values for variables data. A weighting factor is chosen by the user to determine how older data points affect the mean value compared to more recent ones. Because the EWMA chart uses information from all samples, it is a good alternative to the CUSUM chart in detecting smaller process shifts.

The EWMA for sample i (z_i) is plotted on the chart and is defined as $z_i = \lambda\bar{x}_i + (1 - \lambda)z_{i-1}$, where $z_0 = \mu_0$. The constant λ defines the weight assigned to the current

sample ($0 < \lambda \le 1$) and $1 - \lambda$ is the weight assigned to earlier samples. Parameters of the EWMA are

$$\begin{aligned} \text{UCL} &= \mu_0 + L\sigma_{\bar{x}}\sqrt{\frac{\lambda}{2-\lambda}[1-(1-\lambda)^{2i}]} \\ \text{Centerline} &= \mu_0 \\ \text{LCL} &= \mu_0 - L\sigma_{\bar{x}}\sqrt{\frac{\lambda}{2-\lambda}[1-(1-\lambda)^{2i}]} \end{aligned} \tag{20}$$

where L is a design parameter that defines the width of the control limits. The choice of $L = 3$ and $0.05 < \lambda \le 0.25$ is reasonable. The control limits will become wider when the sample number i is getting larger and finally reach constant values as

$$\begin{aligned} \text{UCL} &= \mu_0 + L\sigma_{\bar{x}}\sqrt{\frac{\lambda}{2-\lambda}} \\ \text{Centerline} &= \mu_0 \\ \text{LCL} &= \mu_0 - L\sigma_{\bar{x}}\sqrt{\frac{\lambda}{2-\lambda}} \end{aligned} \tag{21}$$

Example 3 The data in Example 2 are now analyzed by CUSUM and EWMA charts. Table 6 shows calculated CUSUM and EWMA values. The value of h in CUSUM is chosen as 5 times the standard deviation of $\bar{x}$ ($\hat{\sigma}_{\bar{x}} = 0.5027$) and the value of k is chosen as 0.5. The C_i^+ and C_i^- are calculated using a target value $\mu_0 = 10$. The CUSUM chart is shown in Figure 12. The value of λ in EWMA is chosen as 0.2 and L is chosen as 3. The UCL and LCL for individual samples are shown in Table 6 and the EWMA chart is shown in Figure 13. Although the $\bar{x}$ and s charts in Figure 6 indicate that the process is in control, both CUSUM and EWMA gave out-of-control signals at sample point 22. A small process shift has occurred after sample 21.

3.4.3 IMPROVING PERFORMANCE OF A PROCESS

3.4.3.1 Introduction

Basic Concepts After a process is diagnosed, corrected, and brought into statistical control, the next question is "How can the performance of a process be improved?" To answer this question, quality managers and engineers need first measure the present process performance. This measurement can be achieved through a process capability study which gauges the ability of a process to produce products according to the specifications. A process can achieve a state of statistical control but still exhibit a poor capability due to the variability in the process. It will be necessary to reduce variability to improve the process capability. Designed experiments based on statistical principles can offer helps toward reduction of variability and optimization of the process. Employing designed experiments, intentional changes can be made in various places in the process; results gathered from these experiments can lead to further process improvement and bring it to the next level. This section presents commonly

TABLE 6 CUSUM and EWMA Values for Pet Tabs Example

		CUSUM		EWMA		
Sample Number	$\bar{x}$	C_i^+	C_i^-	z_i	UCL	LCL
1	9.600	0.000	0.149	9.920	10.302	9.698
2	9.990	0.000	0.000	9.934	10.386	9.614
3	10.228	0.000	0.000	9.993	10.432	9.568
4	10.365	0.114	0.000	10.067	10.458	9.542
5	10.150	0.012	0.000	10.084	10.475	9.525
6	9.745	0.000	0.004	10.016	10.485	9.515
7	10.118	0.000	0.000	10.036	10.491	9.509
8	9.603	0.000	0.146	9.950	10.495	9.505
9	10.200	0.000	0.000	10.000	10.498	9.502
10	10.705	0.454	0.000	10.141	10.500	9.500
11	9.668	0.000	0.081	10.046	10.501	9.499
12	9.480	0.000	0.350	9.933	10.501	9.499
13	9.355	0.000	0.744	9.817	10.502	9.498
14	10.635	0.384	0.000	9.981	10.502	9.498
15	10.915	1.047	0.000	10.168	10.502	9.498
16	11.258	2.054	0.000	10.386	10.502	9.498
17	10.225	2.027	0.000	10.354	10.502	9.498
18	10.018	1.794	0.000	10.286	10.502	9.498
19	10.058	1.600	0.000	10.241	10.502	9.498
20	10.880	2.229	0.000	10.368	10.502	9.498
21	10.378	2.355	0.000	10.370	10.503	9.497
22	11.265	3.369	0.000	10.549	10.503	9.497
23	11.013	4.130	0.000	10.642	10.503	9.497
24	9.610	3.489	0.139	10.435	10.503	9.497
25	10.898	4.135	0.000	10.528	10.503	9.497
		$h = 2.513$		$\lambda = 0.2$		
		$k = 0.5$		$L = 3$		

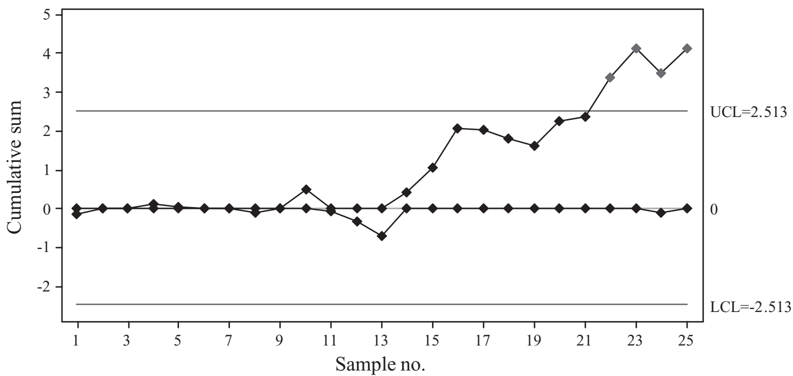

FIGURE 12 CUSUM chart for Pet Tabs manufacturing example.

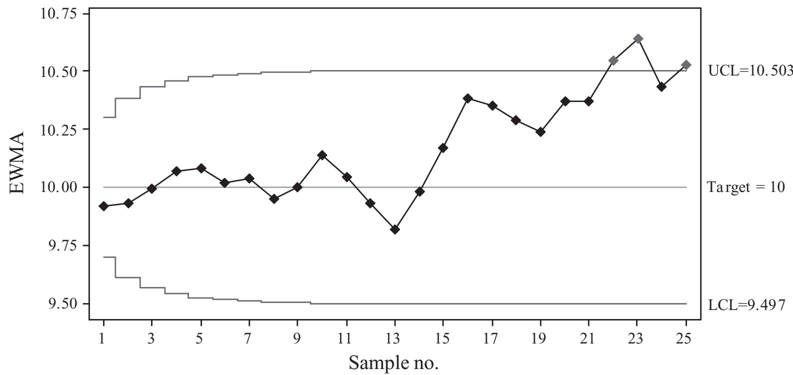

FIGURE 13 EWMA chart for Pet Tabs manufacturing example.

used methods in process capability studies. Design-of-experiment techniques can be found elsewhere in this handbook and in many other textbooks.

Specification Limits, Control Limits, and Natural Tolerance Limits To conduct a process capability study, it is important to distinguish the *specification limits* of a product, the *control limits* of the process producing the product, and the *natural tolerance limits* (NTLs) of the product. In general, specification limits are given by customers or prescribed by in-house design engineers before production. A product that failed to meet the specifications is a nonconforming product. Control limits are usually determined by samples collected from a process during a base period. A sample point that fell outside the control limits will trigger an out-of-control state; however, a product produced in the out-of-control state is not necessarily a nonconforming product. It should also be noted that a sample point plotted in a control chart usually represents a statistic of the sample such as the sample mean. In other words, a single product that fell outside of the control limits will neither cause the process to be out of control nor become nonconforming. The variability of products produced can usually be described by its natural tolerance limits. It is commonly acceptable that the ±3 standard deviations from the process mean be used as the natural tolerance limits.

Example 4 Following Example 2, the specification limits are specified as 10.00 ± 2.00, where:

Nominal or target value (μ_0) = 10.00
Upper specification limit (USL) = 10.00 + 2.00 = 12.00
Lower specification limit (LSL) = 10.00 – 2.00 = 8.00

The control limits for the $\bar{x}$ chart are:

Center line ($\bar{\bar{x}}$) = 10.254
Upper control limit (UCL) = 11.761
Lower control limit (LCL) = 8.747

Using the $\bar{\bar{x}} \pm 3\hat{\sigma}$ natural tolerance limits, they can be obtained as:

Process mean ($\bar{\bar{x}}$) = 10.254
Upper natural tolerance limit (UNTL) = 13.270
Lower natural tolerance limit (LNTL) = 7.238

The relationships among the three sets of limits are illustrated in Figure 14. As can be seen from this figure, the current process is not centered at its nominal value and its specification limits are tighter than its natural tolerance limits. Due to this, a portion of manufactured products (~5.4%) will not be able to conform to the specifications.

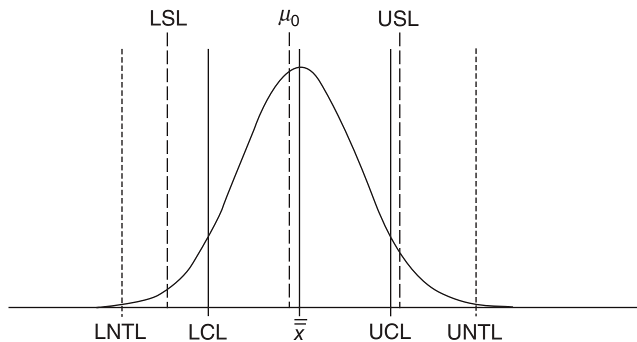

FIGURE 14 Specification limits, control limits, and natural tolerance limits for Pet Tabs manufacturing example.

3.4.3.2 Process Capability and Improvement Studies

Process Capability Indices Process capability indices provide a quantitative measure to assess the ability of a process to produce products that meet the specifications. A commonly used process capability index, denoted as C_p, can be calculated as

$$C_p = \frac{\text{USL} - \text{LSL}}{6\sigma} \tag{22}$$

where USL is the upper specification limit, LSL is the lower specification limit, and σ is the process standard deviation. Since σ is not usually known, it can be estimated by $\hat{\sigma} = \bar{s}/c_4$. A $C_p = 1$ means that the process is just capable. If the process is centered at its nominal value, it will produce 2,700 nonconforming products out of one million (PPM). The target value C_p is usually set at 1.33 for an existing process and 1.50 for a new process.

It should be noted that the C_p value could not indicate the proper process capability if the process is not centered since C_p does not account for where the process mean is with respect to the specifications. To alleviate this issue, another process capability index, C_{pk}, is used:

$$C_{pk} = \min(C_{pu}, C_{pl})$$

where

$$C_{pu} = \frac{\text{USL} - \mu}{3\sigma} \qquad \text{and} \qquad C_{pl} = \frac{\mu - \text{LSL}}{3\sigma} \tag{23}$$

The μ value can be estimated by $\bar{\bar{x}}$ and the σ value can be estimated as discussed earlier. In general, a process is considered "centered" at the nominal value of the specifications when $C_p = C_{pk}$ and "off centered" when $C_p < C_{pk}$. The relationships between C_p and C_{pk} are further illustrated in Figure 15 where the process mean has shifted from μ_0 to $\mu_0 + 2\sigma$ to $\mu_0 + 4\sigma$. As noted from the figure, C_p remains the same regardless of the shift but C_{pk} is significantly reduced.

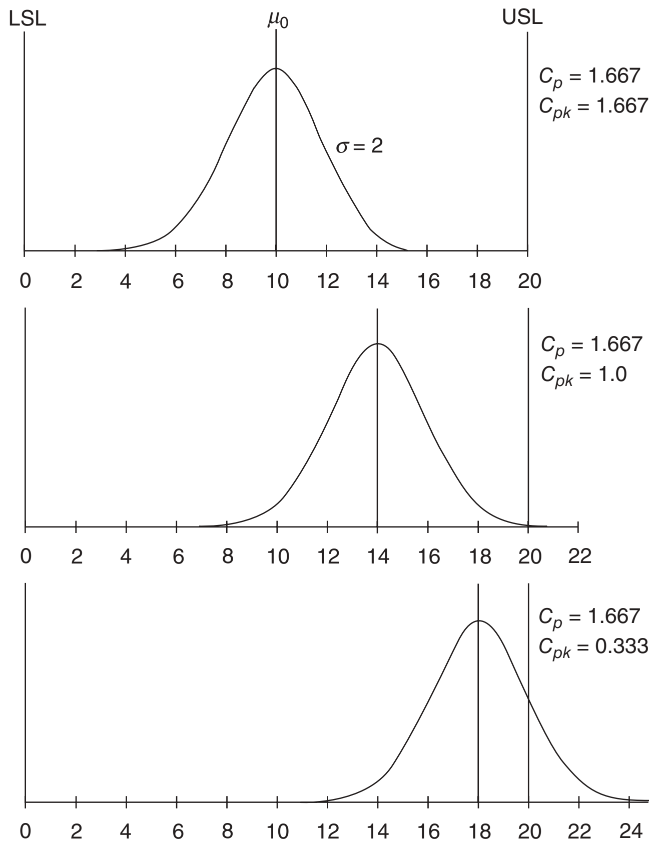

FIGURE 15 Relationships between C_p and C_{pk}.

If one-sided specifications are used, one-sided process capability can also be defined by Equation (23) where C_{pu} is for upper specification and C_{pl} for lower specification.

Interpretation and Improvement of Process Capability Evaluation and interpretation of process capability represent an important step in process quality

improvement. A process must have its source of instability eliminated before it can be improved. Results obtained from process capability studies can help determine whether the process is stable and meeting its specifications. It should be noted that a valid process capability study is based on the normality assumption of the process. The normality assumption will need to be checked before proceeding to the next step.

Conclusions regarding whether the process is centered at the target and is meeting the specifications can be drawn from the process capability study. When $C_p = C_{pk}$, the process is centered. When C_p has a value of 1.0 or greater, the process is capable of producing products meeting specifications; otherwise, it is not capable.

Example 5 Following Example 2, C_p and C_{pk} are calculated as

$$C_p = \frac{\text{USL} - \text{LSL}}{6\sigma} = \frac{12 - 8}{6 \times 1.0054} = 0.6633$$

$$C_{pk} = \min(C_{pu}, C_{pl}) = \min(0.5790, 0.7476) = 0.5790$$

where

$$C_{pu} = \frac{\text{USL} - \mu}{3\sigma} = \frac{12 - 10.254}{3 \times 1.0054} = 0.5790$$

$$C_{pl} = \frac{\mu - \text{LSL}}{3\sigma} = \frac{10.254 - 8}{3 \times 1.0054} = 0.7476$$

Figure 16 shows the histogram of the data in relation to the specifications. The $\bar{x}$ and s charts in Figure 11 show that the process is in statistical control. However, since $C_p < C_{pk}$, the process is not centered. With a C_{pk} value of 0.579, it is expected to have 53,711 nonconforming Pet Tabs manufactured out of one million parts in this production line.

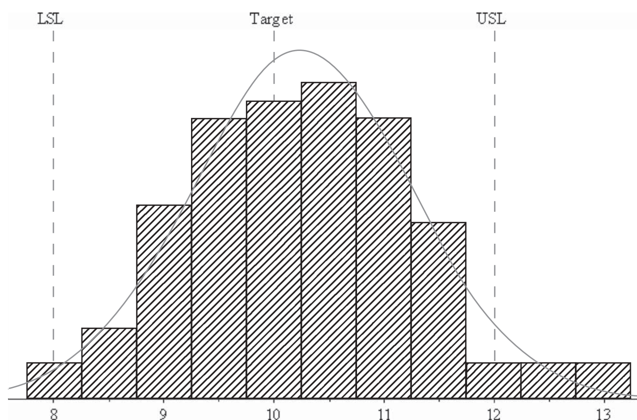

FIGURE 16 Process capability plot for Pet Tabs manufacturing example.

To improve the process capability, the process needs to be centered first. This usually involves adjusting the process settings. Cause-and-effect diagrams, Pareto charts, and other tools discussed earlier in the chapter can be employed to find causes to the "off-centering" problems. After the process is brought back to its nominal (10), the total nonconforming Pet Tabs produced will be dropped to 46,673 PPM. This is still far from the 2700 PPM for a "just capable" process ($C_p = 1$), not to mention reaching the goal of 63 PPM at $C_p = 1.33$.

To further improve the process capability, the variability needs to be reduced. This can be achieved by designed experiments. Design of experiment (DOE) is a systematic approach that allows engineers and managers to make intentional changes in some process settings and assess the effects of those changes. An experiment can be designed in this example by varying a few key process settings such as drying time, mixing time, and temperature. Through a series of experimentations, optimum settings are found for these process variables and the variability of the process is reduced by 50%. With this reduction in process variability, the process is now exhibiting a C_p of 1.265 with 694 PPM. This example highlights the benefits of process improvement. The move from an off-centered state to a centered state resulted in a reduction of process fall-out by 13.1%. With designed experiments, the process variability was cut in half and the process fall-out was significantly reduced by 98.5%.

BIBLIOGRAPHY

Aft, L. S. (1997), *Fundamentals of Industrial Quality Control*, 3rd ed., CRC Press, Boca Raton, FL.

DeVor, R. E., Chang, T. H., and Sutherland, J. W. (2006), *Statistical Quality Design and Control*, 2nd ed., Prentice-Hall, Upper Saddle Brook, NJ.

Gitlow, H., Gitlow, S., Oppenheim, A., and Oppenheim, R. (1989), *Tools and Methods for the Improvement of Quality*, Irwin, Boston.

Grant, E. L., and Leavenworth, R. S. (1996), *Statistical Quality Control*, 7th ed., McGraw-Hill, New York.

Ishikawa, K. (1982), *Guide to Quality Control*, 2nd ed., Asian Productivity Organization, Tokyo, Japan.

Juran, J. M., and Godfrey, A. B. (1998), *Juran's Quality Handbook*, 5th ed., McGraw-Hill, New York.

Ledolter, J., and Burrill, C. W. (1998), *Statistical Quality Control: Strategies and Tools for Continual Improvement*, Wiley, New York.

Montgomery, D. C. (2004), *Design and Analysis of Experiments*, 6th ed., Wiley, New York.

Montgomery, D. C. (2001), *Introduction to Statistical Quality Control*, 4th ed., Wiley, New York.

Ryan, T. P. (2000), *Statistical Methods for Quality Improvement*, 2nd ed., Wiley, New York.

Smith, G. M. (2003), *Statistical Process Control and Quality Improvement*, 5th ed., Prentice-Hall, Upper Saddle Brook, NJ.

Summers, D. C. S. (2006), *Quality*, 4th ed., Prentice-Hall, Upper Saddle Brook, NJ.

Tague, N. R. (2005), *Quality Toolbox*, ASQ Quality Press, Milwaukee.

Thompson, J. R., and Koronacki, J. (2001), *Statistical Process Control: The Deming Paradigm and Beyond*, 2nd ed., Chapman & Hall, New York.

SECTION 4

PROCESS ANALYTICAL TECHNOLOGY (PAT)

4.1

CASE FOR PROCESS ANALYTICAL TECHNOLOGY: REGULATORY AND INDUSTRIAL PERSPECTIVES

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4.1.7 Perspectives on the Impact of PAT
References

4.1.1 INTRODUCTION

The implementation of process analytical technology (PAT) is occurring in what is perhaps the most exciting period of change in pharmaceutical manufacturing of the past three decades. A host of technological, regulatory, and market forces have converged during the last five years, yielding new opportunities for innovation in the development and operation of pharmaceutical production processes. A major driving force for change is the Food and Drug Administration (FDA) initiative to implement a modern, risk-based framework for regulation and oversight of pharmaceutical manufacturing [1]. The objectives of this section are to outline the historical background of process analytics, to provide an overview of PAT in the pharmaceutical industry and the business drivers for change, to summarize the FDA's new initiative and the PAT guidance [2], and to present a basic plan for PAT implementation. While the focus of this chapter is PAT, it should be kept in mind that PAT is an important part of the much broader and risk-based paradigm introduced by the twenty-first-century current good manufacturing practices (cGMPs) initiative.

4.1.2 BASIS FOR PROCESS ANALYTICAL TECHNOLOGY

Despite the fact that the FDA's PAT framework (and guidance) began to take form just ahead of the creation of the twenty-first-century cGMPs initiative in 2001, it is well known that several of the core concepts were pioneered decades ago by other manufacturing industries such as fine chemicals, semiconductors, petroleum, and consumer products. The main concepts that differentiate PAT from the traditional industrial pharmacy skill set (including pharmaceutical and materials science, chemistry, and engineering) are process analytical chemistry (PAC) and advanced manufacturing science (Figure 1).

For the purpose of this discussion, the term *manufacturing science* is meant to describe the science and technology related to modern innovations in the design and management of manufacturing processes. Since it is neither practical nor necessary to cover all aspects of modern pharmaceutical manufacturing science in detail, the following sections are intended to introduce two specific topics which are popular in the current industrial vernacular but are not covered in detail in the pharmaceutical literature: quality management systems and "lean" manufacturing.

4.1.2.1 Process Analytical Chemistry

Process analytical chemistry generally describes the science and technology associated with displacement of laboratory-based measurements with sensors and instrumentation positioned closer to the site of operation. Although industrial process analyzers have been in use for more than 60 years [3], the modern period of PAC essentially began with the formation of the Center for Process Analytical Chemistry (CPAC) in 1984 [4]. As described by Callis, Illman, and Kowalski [5], the goal of

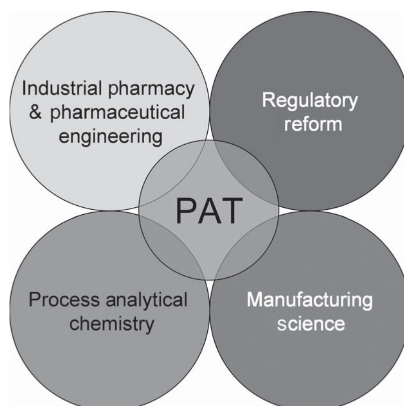

FIGURE 1 Multidisciplinary components of PAT in pharmaceutical manufacturing.

PAC is to "supply quantitative and qualitative information about a chemical process" for monitoring, control, and optimization: they went on to define five "eras" of PAC: (1) off line, (2) at line, (3) online (4) inline, and (5) noninvasive, which describe the evolution of sensor technologies. In addition, they discussed the importance of issues beyond chemical sensing, such as sampling, extraction of information from data (chemometrics), integration with process controls, as well as the sociological aspects of PAC deployment (e.g., gaining the trust of plant operators).

The industrial PAC movement has been bolstered by two decades of advances in materials science, electronics, and chemometrics. Since the inception of CPAC, the pace of innovation in sensors, instrumentation, and analytics has quickened dramatically. The development of more robust, sensitive photodetector materials, microelectromechanical systems (MEMSs), and fiber optics and the perpetual advancement of computing power (as predicted by Moore's law) have both increased the performance and reduced the cost of PAC. As a result, PAC is now a critical part of routine operations within the realm of industrial chemistry. Many general reviews on the subject of PAC (and PAT) have been published [6–10]. A series of literature reviews on the subject of PAC have been published regularly in *Analytical Chemistry*.

The first review [11] listed manuscripts published between 1987 and 1992, covering seven specific topics (general PAC, chromatography, optical spectroscopy, fiber optics, mass spectrometry, chemometrics, and flow injection analysis), along with a section on needs for the future of PAC; in all, the first review included 507 references. Subsequent reviews were published in 1995 [12], 1999 [13], 2001 [14], 2003 [15], and 2005 [16]. The review series is an essential resource for scientists seeking information on specific PAC methods; in total, 2650 references covering more than 16 topics were catalogued by the authors.

Currently there are three major consortia involving university, government, and industrial partners—CPAC, the Measurement & Control Engineering Center (MCEC), and the Control Theory and Applications Centre (CTAC)—along with an annual conference, the International Forum on Process Analytical Chemistry (IFPAC), and numerous online resources that are devoted to issues related to process analytics [16]. In parallel with the FDA's initiative, the term *process*

analytical chemistry is gradually being replaced in the industrial vernacular by *process analytical technology*. This reflects the expansion of the field as the importance of physical characterization, risk analysis, and manufacturing science is recognized.

4.1.2.2 Quality Management

Many of the quality improvement goals for implementation of PAT in the pharmaceutical industry have been achieved by companies in other industries, such as automobile production and consumer electronics, as a direct result of adopting principles of quality management. The lineage of modern quality management can be traced to the work of Walter Shewhart, a statistician for Bell Laboratories in the mid-1920s [17]. His observation that statistical analysis of the dimensions of industrial products over time could be used to control the quality of production laid the foundation for modern control charts. Shewhart is considered to be the father of statistical process control (SPC); his work provides the first evidence of the transition from product quality (by inspection) to the concept of quality processes [18, 19].

Shewhart's methodologies were adopted and expanded by W. Edwards Deming [20] and Joseph M. Juran [21], who are credited with the birth of the "total quality" (TQC) approach in Japan following World War II. Successors to the total quality movement include management by objectives (MBO) (1960's), Crosby's zero-defects (ZD) movement (1970s), the American incarnation of total quality management (TQM) (1970s–1980s), quality circles (1970s), quality function deployment (QFD) (1980s), the International Organization for Standardization (ISO) 9000 series (1987), and the Malcolm Baldridge National Quality Award (1987–present). The most recent major quality management methodology, Six Sigma (6σ) [22, 23], pioneered by Motorola, has become immensely popular because of the litany of corporate CEOs (e.g., Thomas Galvin, Jack Welch) who have openly credited their internal 6σ initiatives for dramatic improvements in bottom-line performance. All of these quality movements [24], however, as well as PAT, are related to the principles of Shewhart, Deming, Juran, Crosby, Taguchi [25, 26], and others, in that they are based on systematic methods for understanding the sources of variability in processes and minimizing their impact on product quality.

The so-called DMAIC (define, measure, analyze, improve, and control) methodology is a common framework used by improvement teams in many industries to apply the concepts of quality management to systematically identify, prioritize, and eliminate the root cause of quality problems. A variant of DMAIC, known as DMADV (define, measure, analyze, design, and verify), is sometimes used when a process or operation requires complete redesign to bring about the desired quality improvement and is a central concept of the DFSS (design for six sigma) movement. The origins of DMAIC, DMADV, DFSS, and other various quality management cycles can be traced to the "Shewhart cycle" of (1) plan, (2) do, (3) study, and (4) act [24].

Arguably, the most important aspects of quality management for PAT are the concepts of quantitative process performance characterization using process capability indices as universal descriptors, which form the basis of the "measure" and "analyze" portions of the DMAIC model. Process capability indices consider simultaneously both process variability and process specifications to determine whether

the process is "capable" [27]. A process is said to be capable if the quality measurements for nearly all samples are within the specification limits. A common version of the process capability index, C_{pk}, is calculated according to

$$C_{pk} = \min\left[\frac{\text{USL}-\mu}{3\sigma}, \frac{\mu-\text{LSL}}{3\sigma}\right]$$

where μ and σ are the mean and standard deviation and USL and LSL are the upper and lower specification limits, respectively, for a product quality measurement. Process capability indices are useful for process improvement studies because they transform diverse measures of quality (e.g., weight, concentration, rate) into dimensionless units, thereby allowing investigators to pinpoint major sources of variation in a process (operations which have the lowest C_{pk} scores) when many measurement systems and quality attributes are involved.

The process capability index, C_{pk}, is related to the so-called "process sigma" such that a 6σ process corresponds to a C_{pk} of exactly 2.00, or 2.0 defective parts per billion (PPB), assuming ~N (0, σ) quality variance distribution (can alternative calculation for process sigma estimates 3.4 defective parts per million for a 6σ process). Examples of the correspondence between C_{pk}, process sigma, and defect rate for ~N(0, σ) distributions are shown in Figure 2. The process capability (based on observed yield) of pharmaceutical manufacturers has been cited by some benchmark studies to be roughly 0.7 (2.1σ) [28].

While industrial benchmarks clearly indicate that pharmaceutical manufacturers have many opportunities to improve quality control, direct comparison with other industries may be somewhat misleading. As opposed to such industries as semiconductor manufacturing, where defective parts are often readily apparent at some

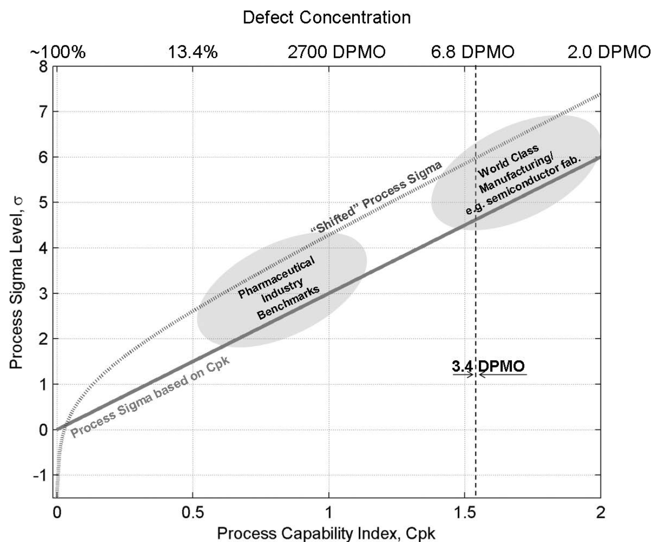

FIGURE 2 Graphical illustration of the correspondence between the defects per million opportunity (DPMO) process capability (C_{pk}) and process sigma (assuming normally distributed quality variation).

point in the value chain (i.e., the device built from the part will fail), drug products suffer from a high degree of ambiguity in their quality specifications.

For example, finished-product release specifications such as content uniformity are rarely correlated to clinical evidence; rather, they are set according to compendial test standards. Furthermore, the functional relationship between in-process material characteristics and finished-product quality is seldom known at a high level; hence, the assigned in-process specifications for some operations may over- or underestimate the true level of process capability. As the level of process understanding in the pharmaceutical industry increases, development of science- and evidence-based in-process and release specifications will improve the reliability of C_{pk} as a tool for process characterization.

For further information, the NIST/SEMATECH *Handbook of Engineering Statistics*, which is freely available online [23], and the American Society for Quality (www.ASQ.org), are excellent sources for background information and technical details related to quality management.

4.1.2.3 Lean Manufacturing

In contrast to quality management systems, which have clear parallels with PAT (i.e., reduction of quality variation), the links between PAT and lean manufacturing are less direct. In fact, while quality management systems are concerned with process analysis of quality variation, lean flow path management is concerned with process analysis of production time variation. Furthermore, the core concepts of lean manufacturing, however, provide the technology platform which the pharmaceutical industry will use to derive gains in production efficiency from the adoption of PAT. Without considering the impact of PAT on production efficiency [i.e., the return on investment (ROI) from implementing PAT], industry would have very little impetus to voluntarily embrace PAT. The following paragraphs are intended to provide a brief introduction to lean manufacturing; later portions of this chapter will discuss the business drivers for implementation of PAT.

Lean manufacturing, or "lean," is often misunderstood (not unlike TQM or 6σ); for some people, lean business initiatives conjure "slash-and-burn" management tactics to reduce workforce levels or shut down low-productivity operations. In fact, lean manufacturing has been characterized as "an amalgam of methodologies including industrial engineering, just-in-time (JIT) (Osadas's) 5-S's, TQC, continuous quality improvement (CQI), Visual Control, Total Productive Maintenance (TPM), Quality Circles, and Kaizen" [24].

The origins of lean manufacturing are often ascribed to the creation of the Toyota Production System (TPS) by the Toyota Motor Corporation. However, the history of lean manufacturing can be traced back to industrial developments which occurred more than 150 years before TPS. The foundation for modern manufacturing was laid by Eli Whitney in 1798; while Whitney is best known for his invention of the cotton gin, it is his invention of interchangeable parts and uniform production which revolutionized mass production (www.EliWhitney.org).

Nearly a century later, Frederick W. Taylor introduced the concepts of time study and standardized work, coining the term *scientific management*. It was not until 1908, with Henry Ford's introduction of the Model T, that the value of lean manufacturing was recognized worldwide. Henry Ford is considered by some to be the first practi-

tioner of JIT manufacturing; furthermore, his manufacturing system has been described as the inspiration for TPS [29]. More recently Ford Motor Company has developed a modernized version of Henry Ford's original system, the Ford Production System [24], which borrows heavily from TPS.

As a discipline of manufacturing science, lean manufacturing is a technical philosophy focused on the reduction of seven types of waste, or "muda," in manufacturing: overproduction, waiting, transport, inappropriate processing, unnecessary inventory, excess motion, and defects. The transformation of a process to lean operation is accomplished using many tools and strategies. Arguably, the most important mechanism for change is to replace traditional "make to forecast" or "push" production scheduling with "pull" strategies, such as "kanban" cards. The principles of lean have been applied with success in manufacturing and service industries, as well as governmental entities. Not unlike quality management, there are literally hundreds of books describing the various tools and techniques used to apply lean methodologies. The Society of Manufacturing Engineers (www.SME.org) maintains publications, conferences, and a technical community devoted to production management and is a good first source for more information on lean manufacturing.

Compared with other industries, pharmaceutical manufacturers have been relatively late to adopt lean manufacturing; consequently, pharmaceutical cycle times are extremely long when compared with other industries [30, 31]. By comparing the ratio of total cost of goods sold (COGS) to inventory value for the top 22 publicly traded branded, generic, and biotech pharmaceutical companies to the reported figures for other process industries, a rough indication can be gained of how much less effectively pharmaceutical manufacturers manage their supply chains (Figure 3). Furthermore, it would not be difficult for most industrial pharmaceutical scientists to find common examples of each of the "seven wastes" in a typical pharmaceutical manufacturing facility.

Admittedly, there are some constraints intrinsic to the industry which may ultimately prevent pharmaceutical manufacturers from achieving "world-class" supply chain and manufacturing performance. Furthermore, application of lean and quality management tools to pharmaceutical manufacturing is proving to be a unique challenge. A recent survey of 1500 pharmaceutical manufacturing professionals indicated that, while more than half of the companies surveyed have implemented lean, 6σ, or operational excellence, less than half of those programs have yielded satisfactory results [32].

While these data seem to suggest that lean manufacturing is not suited to pharmaceutical manufacturing, it is important to consider that most lean methodologies (e.g., TPS) were developed for high-volume production of uniform products. Although many "blockbuster" drugs are produced in dedicated facilities or in plants specializing in only a few products, it is quite common for pharmaceutical manufacturers to produce many products in a single plant, having a high proportion of shared equipment. Traditional lean methods, such as kanban cards, are difficult to manage in a complex, "high-mix" production environment. In order to solve these limitations, innovative software algorithms for "flow path management" [33] have been developed to simulate, design, and optimize pharmaceutical production processes according to lean manufacturing principles.

Furthermore, the effectiveness of lean manufacturing is limited by variability in the cycle time (C/T) for individual unit operations as well as by the finite risk of

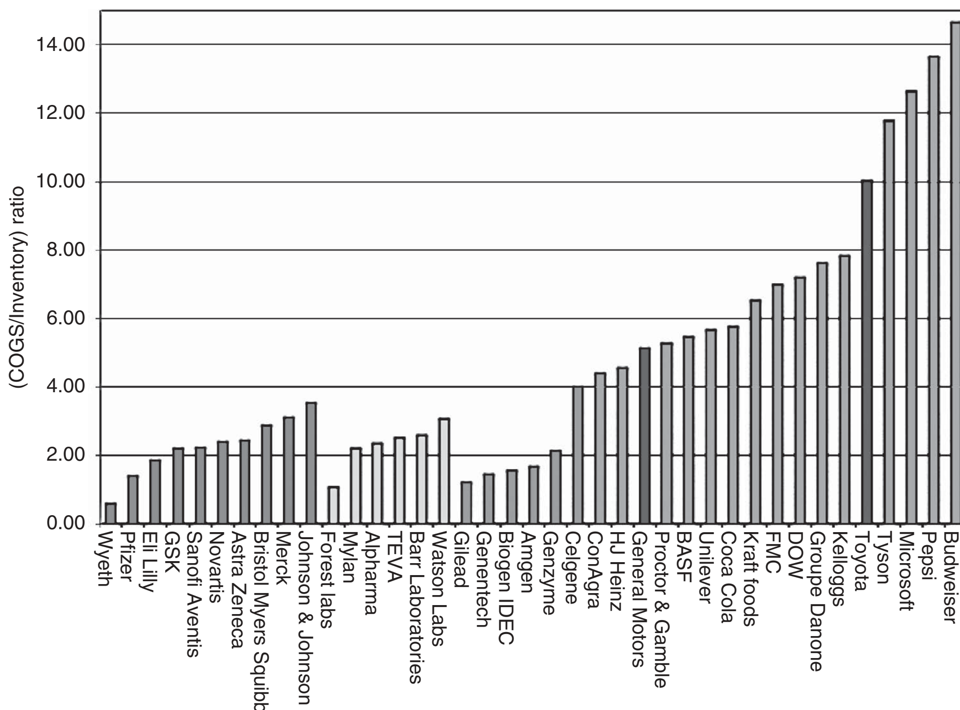

FIGURE 3 Ratio of total COGS to reported inventory value. The ratio of COGS to inventories is a rough indicator of supply chain velocity. A large ratio implies that inventories are small relative to COGS and are turned over frequently. Toyota Motors, for example, which is well known for effective supply chain management, has a much higher ratio of COGS to inventories than General Motors.

batch failure during production; this is true regardless of the complexity of the flow path or of the degree to which equipment is shared. Pharmaceutical manufacturers cope with such risks by building up long production queues to accumulate work in process (WIP) ahead of unit operations. While this helps to improve capacity utilization and overall equipment effectiveness (OEE), it decreases efficiency by consuming working capital and increasing the intensity of overhead operations (required to finance, transport, and warehouse WIP). In order to gainfully implement lean, pharmaceutical manufacturers must first minimize C/T variation and risks to product quality.

Finally, it is well known that a significant portion of the typical production C/T reported by industry is consumed by the delay between completion of a unit operation, sampling, analysis, reporting, and in-process or finished-product release. In some scenarios, PAT will enable manufacturers to release finished products to the market immediately, with no delay for manual, offline testing; this is the so-called real-time release (RTR) benefit of PAT. Without PAT and RTR, the effectiveness of lean strategies in reducing C/T will be limited by the maximum rate of product inspection and release. Thus, it is critical for pharmaceutical manufacturers to deploy PAT and lean in parallel if real gains in process performance are to be realized. The lean–PAT concept is quite similar to lean–6σ, or "fusion management" [24].

4.1.3 HISTORICAL FACTORS LIMITING IMPLEMENTATION OF PAT

Despite the evidence of fiscal and competitive benefits enjoyed by the various industries which have embraced process analytics, pharmaceutical companies have been notoriously restrained in their efforts to deploy PAT. Indeed, the pharmaceutical industry has slipped so far behind peer industries that a well-known *Wall Street Journal* article from 2003 [34] characterized the manufacturing prowess of drug makers as lagging "far behind potato-chip and laundry-soap makers." While the declaration was shocking to many, it was, nonetheless, an accurate assessment. Before indicting the industry for gross negligence, however, it is important to consider the various factors which have acted over time to create the current state of affairs.

Over the years, dozens of excuses have been provided for the industry's lack of manufacturing innovation; many of the reasons are well known and have been published elsewhere [35]. For the sake of simplicity, the factors limiting the adoption of PAT can be distilled into three categories: real and perceived technological barriers, lack of economic incentive, and regulatory disincentives.

4.1.3.1 Real and Perceived Technological Barriers

Despite the fact that near infrared spectroscopy (NIR) has been used industrially for decades [36], there has been hesitance to accept and trust "new" process analytical measurement technologies as equivalent or superior to traditional methods. For example, when a discrepancy between online NIR and laboratory analyses is observed, it is rare that the destructive reference methods are ever targeted as the source of error, despite the fact that NIR is often the more precise method. The hesitance to trust more advanced, multivariate tools (which are perhaps less directly understood) has certainly been a detriment to progress in deploying PAT.

Similar concerns persist with regard to chemometrics (multivariate data analysis), information technology (IT), and advanced controls. One reason for such behavior may be the practice of calibrating and validating PAT sensors by correlating their signals to traditional, laboratory-based reference methods and characterizing performance in terms of prediction error [37–39]. It is a truism of statistics that, no matter how sensitive or accurate the PAT sensor may be in detecting quality variation, the performance of the reference method will always limit the level of perceived accuracy. A much more accurate depiction of the performance of PAT sensors compared to reference techniques would be to compare analytical figures of merit, such as signal-to-noise ratio (S/N) or analytical sensitivity, which explicitly account for measurement precision [40, 41].

Even though the perceptions of PAT instrumentation have begun to improve, companies continue to worry that the intensity of product quality sampling afforded by PAT sensors will result in negative consequences, such as increased inspection and investigations. In other words, many companies continue to "fear what they will find" if they begin to analyze their operations more closely. Prior to the introduction of rapid, nondestructive quality monitoring tools, there were few alternatives for efficient quality assurance except to rely on batch release criteria, such as the well-known U.S. Pharmacopeia (USP) ⟨905⟩ procedure, which were based on extremely limited sampling (i.e., assay 10 individual dosage units from a 30-unit sample of a production-scale batch).

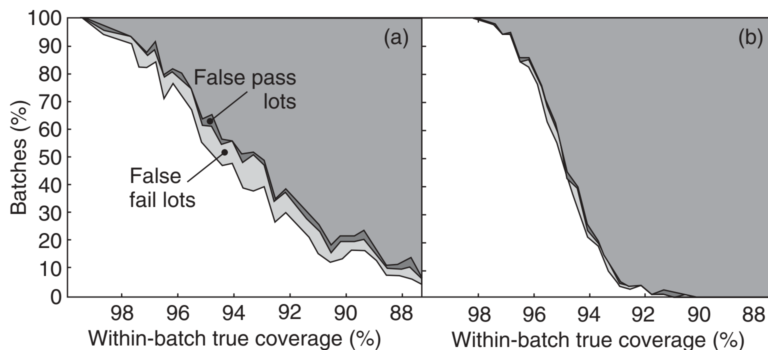

FIGURE 4 Comparison of operational characteristic (OC) curves for the USP ⟨905⟩ (*a*) and PAT-based (*b*) release strategies generated by Monte Carlo simulation. The USP OC curve (*a*) is based on the assumption of 2% RSD measurement precision; the PAT OC curve (*b*) assumes NIR measurement of 800 tablets with 0.9% measurement precision; both curves were estimated using the same simulated populations of one million tablets having varying levels of quality uniformity. Each curve consists of four regions: the regions above and below the sigmoid curve correspond to proportions of batches accurately passed or rejected based on the release criteria. Along the sigmoid curve are regions related to the rates of false batch failure (lower side of curve) and false batch acceptance (upper side of curve). The jagged nature of the curves is related to the limitations imposed by finite iterations. The slope of the curves demonstrates the superior specificity (or "tunability") of release tests optimized for PAT systems.

Despite the fact that the operating characteristic (OC) curve of the USP ⟨905⟩ test guarantees a significant portion of each batch will have poor quality before batch rejection is probable [42, 43], companies have become comfortable with their odds. Process analytical monitoring tools such as NIR spectroscopy, which are capable of high-speed sampling in line, online, or at line, have been perceived as an additional burden on the rate of successful batch release.

By forgoing real-time, pervasive quality monitoring, however, companies incur significant opportunity costs in at least three ways. First, without continuous monitoring there are few feasible opportunities for implementing RTR; time delays related to offline release testing are one of the most significant factors limiting supply chain velocity in pharmaceutical manufacturing. Second, while there is some potential for "discovering" a greater number of batches which do not meet release criteria, statistical simulations suggest that potentially fewer batches will be rejected when larger sample sizes are considered. In other words, when the impact of measurement imprecision and the true distribution of quality characteristics are considered, traditional release testing methods pose finite risks of failing passable batches (which otherwise should have passed) because the limited sample does not adequately represent the characteristics of the population (Figure 4). Finally, and perhaps most importantly, traditional sampling techniques are an effective barrier to continuous improvement; based on fundamentals of statistical theory, it can be shown that samples of at least hundreds of individuals are required to detect incremental changes in process capability (Figure 5). Hence, even if a company were to investigate potential process improvements, only process capability changes of improbable magnitude would be recognized with statistical confidence.

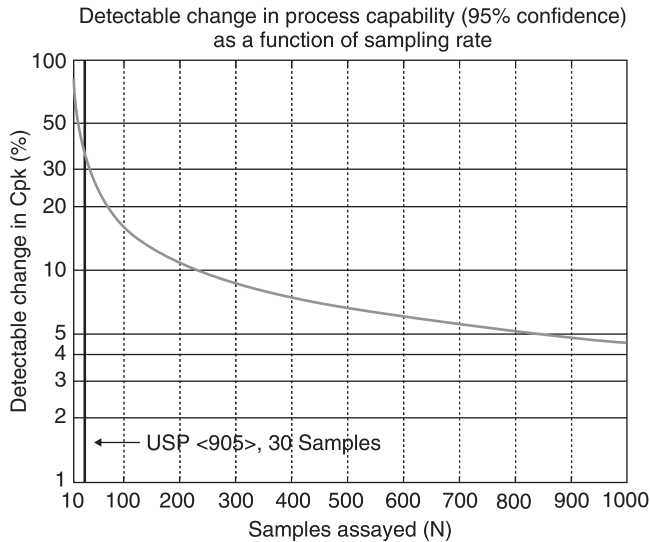

FIGURE 5 Relationship between sampling rate and effective resolution of process capability assessment. The curve is based on the width of the confidence intervals for estimation of mean and variance. The relationship shown does not consider the effect of reference measurement precision, which would further reduce the ability to discern changes in process capability.

4.1.3.2 Lack of Economic Incentive

A common refrain within the industry has been that there simply is not sufficient financial return from investment in process analytics or manufacturing technology upgrades to justify spending. In some respects, this is a valid argument. Historically, many of the industries which have justified significant investment in process analytics utilized continuous manufacturing; it is far more difficult to efficiently control continuous processes (relative batch production systems) without real-time process analytics [35]. Hence, while the pharmaceutical industry has been able to choose, many other manufacturers have been forced to integrate PAT into their operations.

Since pharmaceutical investment in PAT continues to be an option rather than a priority for most companies, arguments justifying PAT spending are forced to compete with other spending initiatives for capital. During each planning cycle, company managers must decide whether to allocate additional capital toward diverse opportunities, such as greater research and development (R&D), improvements in manufacturing capabilities, or additional forces in sales and marketing [i.e., selling, general and administrative (SG&A)]. For any particular project to be funded, expected returns must not only exceed the company's cost of capital [i.e., weighted average cost of capital (WACC)], winning projects may be required to exceed the company's expected return on invested capital (ROIC) or at least provide expected returns in excess of other investment alternatives. A recent academic case study of the potential financial returns on investment (ROI) in PAT and lean manufacturing in the pharmaceutical industry show, however, that many pharmaceutical manufacturers could ultimately benefit tremendously by improving manufacturing performance [44].

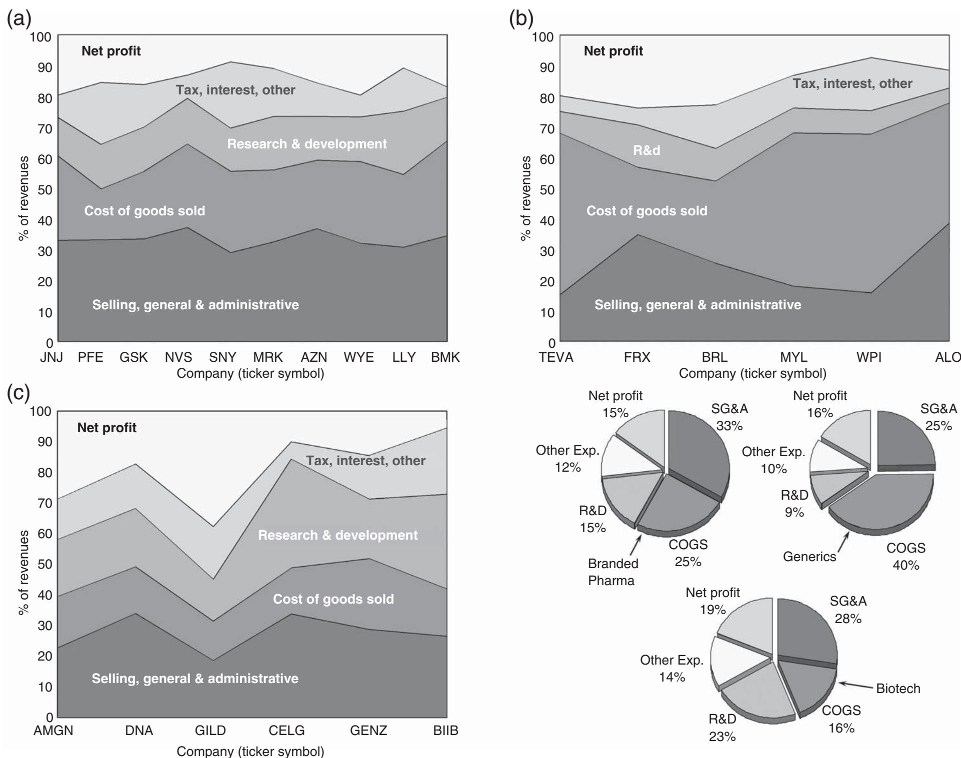

FIGURE 6 Distribution of the components of revenue (FY2005 annual data) for branded (*a*), generic (*b*), and biotech (*c*) drug manufacturers. Companies are arranged according to market capitalization (as of November 2006).

Unfortunately, proponents of PAT are only just beginning to develop the methods to quantify all of the potential opportunities for ROI. Furthermore, it is important to consider the relative level of risk posed by investment in PAT (as opposed to other alternatives). Unlike investments in sales or marketing, there remains considerable uncertainty in the industry regarding the likelihood of achieving ROI projections or the prospect of PAT investment creating new problems. For these reasons, management teams have typically found it easier to justify spending in R&D and marketing instead of PAT or manufacturing reforms.

Besides concerns over the likelihood and magnitude of returns on PAT investments, it is often cited that manufacturing and optimizing the cost of production have simply not been a priority in the industry; manufacturing has often been viewed as a cost rather than a value-generating component. The distribution of corporate expenditures has been provided as evidence in support of this theory (Figure 6). Based on corporate annual income statements from 2005, the average expenditure on R&D and SG&A among the top-10 branded pharmaceutical companies (by market capitalization, November 7, 2006) was nearly double their reported cost of goods sold. Another take on this theory is that institutional and individual investors (who own the pharmaceutical companies and supply the capital for their operation) and the boards of directors elected by them look favorably on the expansion of R&D and marketing investment while taking a more myopic view on the importance of manufacturing. It has sometimes been said that Wall Street rewards

(pharmaceutical companies) for innovation in discovery and replication in manufacturing [45]. It is not completely coincidence, for example, that Merck's appointment of its president of manufacturing, Richard T. Clark, to chief executive in May 2005, which, according to financial journalists, "disappointed investors" who apparently would have preferred someone with a "research and development background" [46], marked the beginning of a nearly 25% loss in market capitalization over the next six months.

While the various reasons discussed for the pharmaceutical industry's tepid approach to PAT and manufacturing reform are plausible, they are likely secondary to the real and perceived risks posed by the regulatory uncertainty surrounding innovation in manufacturing. For example, it is well known that many companies were beginning to use PAT tools long before the FDA's initiative, which suggests that the economic benefits of process analytics have been recognized internally for some time. In response to the fear that their use of new technologies would spur additional investigations by the FDA, however, some of these companies operated in a "Don't use, or don't tell" manner with regard to PAT [45].

4.1.3.3 Regulatory Disincentives

The real and perceived fear of regulatory noncompliance has arguably been one of the most important factors explaining the industry's reluctance to pursue manufacturing innovation [1, 2]. While the first 25 years of pharmaceutical GMP have been effective in ensuring the safety of prescription drug products for consumers, it has been achieved at the expense of innovation and flexibility. Without the ability to adjust processes to account for changes in materials, operating conditions, or the level of process understanding, process analytics are of nearly no value since there is no capacity to act on new information (besides material/batch rejection).

Furthermore, companies who dared to make changes or implement new technologies, whether conventional process improvements, new unit operations, or process analytics, were met with extensive supplemental documentation, FDA inspection, and the finite risk of production delays. Ultimately, the potential for regulatory action stifled the industry's desire to pursue technologies which might have seemed extraordinary, such as real-time analytics or chemometrics. Finally, without the benefits conferred by the PAT guidance and risk-based cGMPs initiative, industry rarely had incentive to formally analyze the risk of established processes out of fear that what they might discover would be used against them in regulatory or legal actions.

4.1.4 FDA TWENTY-FIRST-CENTURY cGMPs INITIATIVE

The observation that the state of cGMP at the beginning of the twenty-first-century was stifling innovation in pharmaceutical manufacturing did not go unnoticed by the FDA, which also saw opportunity in remodeling the regulatory framework. Since many changes, even minor operational modifications, required prior approval from the agency prior to implementation, regulators were swamped with thousands of supplements every year. Resources were stretched between processing of supplements, review and approval of new facilities, processes and documentation, and inspection; all the while, the FDA was being squeezed by external constraints on

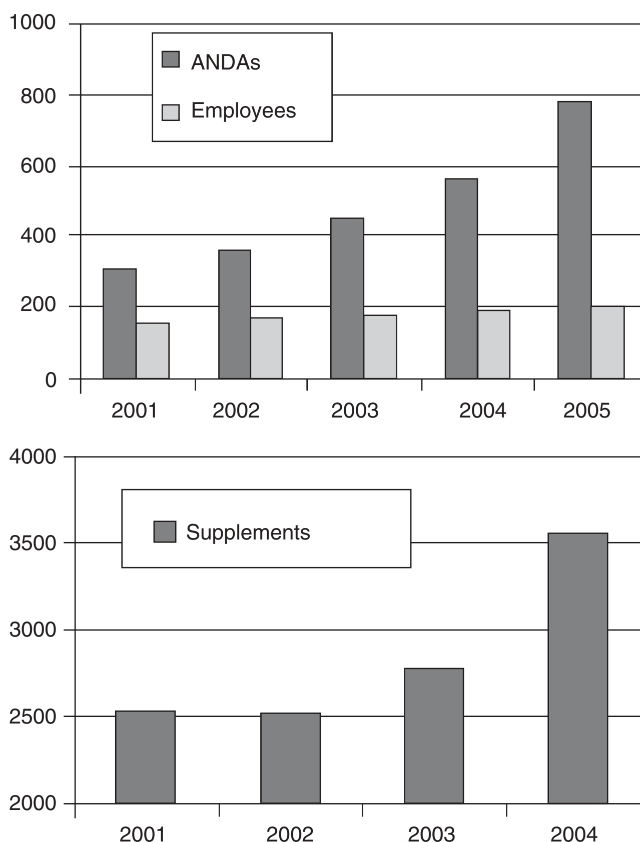

FIGURE 7 Trends in FDA workload and staffing resources. (Adapted from L. X. Yu, Implementation of quality-by-design: Question-based review, Drug Information Association (DIA) 42nd Annual Meeting, Philadelphia, PA, 2006.)

budget growth (Figure 7). As of 2001, FDA regulators were so burdened that they were unable to meet statutory biennial GMP inspections. Finally, the load of supplements, reviews, and inspections were acting as a significant drag on the advancement to market of new pharmaceutical therapies.

4.1.4.1 Conception of the Initiative

The agency began a public dialogue on the state of pharmaceutical manufacturing and FDA regulation during discussions with the Advisory Committee for Pharmaceutical Science (ACPS) in July 2001, followed by further discussion within the FDA Science Board meetings in November 2001 and April 2002 [47]. A significant focus of the discussions was the impact of the regulatory framework on innovation, quality, and efficiency as well as opportunities for change. A new, risk-based paradigm which rewards innovative producers through opportunities for "regulatory relief" began to take shape, displacing the notion of regulatory compliance as a force for innovation. The new paradigm offered advantages to the FDA, as well, in that the level of inspection resources could be prioritized and allocated according to risk, thereby easing

the strain on FDA resources. These changes signaled an evolution of what seemed to be an adversarial FDA–industry relationship toward greater cooperation.

While the pharmaceutical incarnation of the term PAT was formally introduced during these meetings [48], a significant portion of the concepts which define the core of PAT in pharmaceutical science were presented by industrial and academic scientists, many of whom had been building support for and working on these issues within their organizations for years. Industrial and academic presentations included topics such as total quality management [49], new technologies for pharmaceutical manufacturing [50], and QbD [51], among others.

In August 2002, the agency announced the Pharmaceutical cGMPs for the 21st Century initiative (or "the initiative"), which began a two-year effort undertaken by a number of multidisciplinary working groups within the FDA, as well as the cGMP steering committee, to assess the current regulatory structure and define the agency's new vision for risk-based regulation of manufacturing and product quality. The new initiative, which was intended to modernize the FDA's regulation of pharmaceutical quality for human, veterinary, and select human biological products, sought to reform the pharmaceutical as well as the chemistry, manufacturing, and controls (CMC) programs, with the following specific objectives:

- Encourage the early adoption of new technological advances by the pharmaceutical industry.
- Facilitate industry application of modern quality management techniques, including implementation of quality systems approaches, to all aspects of pharmaceutical production and quality assurance.
- Encourage implementation of risk-based approaches that focus both industry and agency attention on critical areas.
- Ensure that regulatory review, compliance, and inspection policies are based on state-of-the-art pharmaceutical science.
- Enhance the consistency and coordination of the FDA's drug quality regulatory programs, in part, by further integrating enhanced quality systems approaches into the agency's business processes and regulatory policies concerning review and inspection activities.

The result of the working groups' assessment enabled the development of the new framework embodied by the finalized twenty-first-century cGMPs as well as the associated components, such as the PAT guidance. Throughout the assessment and development, and continuing during the "implementation phase" of the initiative, the following set of guiding principles has been maintained:

- Risk-based orientation
- Science-based policies and standards
- Integrated quality systems orientation
- International cooperation
- Strong public health protection

The final report on the results and future plans for the initiative were released in September 2004. The report efficiently describes the motives, origins, development

process, and mechanisms for implementing and evaluating the initiative and can be found posted on the Center for Drug Evaluation and Research (CDER) Office of Pharmaceutical Science (OPS) website (http://www.fda.gov/cder/OPS/).

Since it would be impractical to accurately describe all of the important aspects of the report within this space, the following sections are intended to detail some of the concepts and guiding principles of the initiative which are particularly important for understanding PAT. The organization of this summary is intended to efficiently describe selected concepts of the agency's twenty-first-century cGMPs and is not intended to mirror the structure or totality of the associated FDA documentation. All who are actively engaged in pharmaceutical manufacturing or are interested in PAT are encouraged to read the final report [1], which should be considered a primary source for direction.

4.1.4.2 Risk-Based Orientation

The FDA's adoption of a risk-based orientation for regulation is the most important aspect of the twenty-first-century cGMPs. It is a common misconception that the agency's initiative describes a new set of practices for the industry. In fact, while the FDA is committed to encouraging innovation in the industry, the twenty-first-century cGMPs initiative is entirely focused on changing the agency's regulatory framework so that quality and innovation are rewarded with reduced oversight. Now that the agency has entered the implementation phase of the initiative, many of the previous regulatory disincentives have been eliminated. In other words, pharmaceutical companies are currently free to voluntarily choose whether or not to pursue innovative changes in their development, operation, and quality assurance of manufacturing processes such as PAT.

Risk-Based Prioritization of cGMP Inspections The mechanism by which the FDA will encourage the industry to join in implementing the new methods is provided by the risk-based algorithm for prioritizing cGMP inspections. Incidentally, risk-based site selection is the same mechanism which will allow the agency to optimally allocate its limited oversight resources to achieve the greatest public health impact. Operational efficiency is a major component of the FDA's plans for the future. The key to the risk-based site selection program is the agency's risk-ranking model, which has been deployed as a pilot program since the beginning of its 2005 fiscal year.

The model is based on a hierarchical risk-ranking and risk-filtering method whereby a *site risk potential* (SRP) is estimated as a function of the weighted potentials for each of three *top-level components* of site risk—product, facility, and process (Figure 8). The risk potential for each of the three top-level components is calculated as a function of selected risk factors which are relevant to the component (specific to the site). A set of subcategories are defined for each top-level component; each subcategory is comprised of individual risk factors. The initial model weights (the actual risk scores at the lowest level) were optimized using a combination of empirical evidence and expert judgment. Examples of potential risk factors for each top-level component (and associated subcategories) were provided in a report which describes the first iteration of pilot risk-ranking model in detail [52].

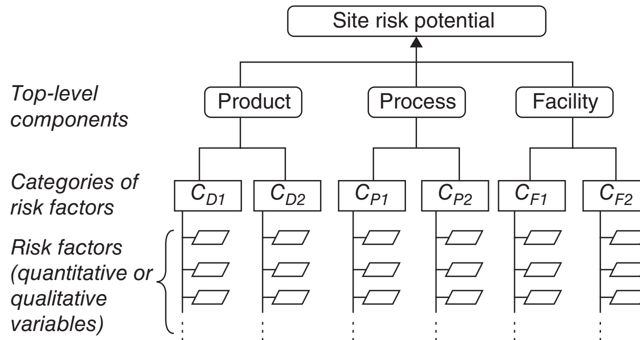

FIGURE 8 Schematic of FDA's pilot risk-ranking model for calculation of site risk potential.

The results from the first iteration of the risk-ranking model demonstrated the capability of the model to spread SRP scores for the purpose of filtering. Future iterations of the risk-ranking model will be generated by correlating predicted site risk potentials with data gathered by traditional oversight activities (e.g., cGMP compliance inspections) and adjusting the risk factor weights to maximize the effectiveness of SRP prediction (similar to multivariate linear regression). The selection of risk factors included in the first iteration of the model was based on the availability of data. Some proposals for future iterations of the model include incorporating factors such as systems for continuous assessment of process capability as indicators of the site's level of process understanding and control. Certainly, as the model is updated to capture the benefits of new best practices in manufacturing, such as PAT, the risk ranking will begin to provide effective incentive for producers to pursue innovation.

4.1.4.3 Quality Systems Approach

According to the FDA staff manual guide [53], a *quality system* is a "set of formal and informal business practices and processes that focus on customer needs, leadership vision, employee involvement, continual improvement, informed decision making based on real-time data and mutually beneficial relationships with external business partners to achieve organizational outcomes." Based on this description, PAT should be considered to be an important tool for supporting a quality management system. As stated earlier, one of the FDA's objectives in undertaking the initiative was to integrate quality systems and risk management approaches into its existing programs with the goal of encouraging industry to adopt modern and innovative manufacturing technologies, including industrial deployment of quality management systems such as those described earlier in this chapter (e.g., ISO 9000).

In September 2006, the FDA released "Guidance for Industry: Quality Systems Approach to Pharmaceutical cGMP Regulations" [54]. The guidance is intended to "help manufacturers implementing modern quality systems and risk management approaches to meet the requirements of the Agency's cGMP regulations," in particular, Parts 210 and 211. In developing the guidance, the Quality System Guidance

FIGURE 9 FDA's six-system inspection model.

Development (QS) working group "mapped" the relationship between cGMP regulations and various quality system models both internal and external to the FDA. Their result is a comprehensive model which allows producers seeking to implement their own quality management systems to quickly identify those aspects of quality systems which are, and are not, correlated with cGMP.

The QS guidance begins by defining critical concepts of modern quality systems, including quality, QbD and product development, quality risk management, corrective and preventative action (CAPA), change control, the "quality unit," and the six-system inspection model. The discussion of the quality unit describes its relationship with the concepts of quality control (QC) and quality assurance (QA) and the relationship between the quality unit and the other units within the pharmaceutical manufacturing organization. The six-system inspection model is described as a blueprint for how compliance inspections will be organized under the new quality systems approach and should be considered a template for internal verification of compliance within pharmaceutical organizations adopting quality management systems (Figure 9).

The majority of the QS guidance is devoted to describing the essential components of modern quality systems, including four major factors which must be addressed: management responsibilities, resources, manufacturing operations, and evaluation activities. Each factor is described in detail, including aspects which overlap with cGMP regulations (for each factor there is a table listing the related regulatory citations). In particular, the manufacturing section describes aspects of quality systems (and related cGMPs) which are closely related to PAT, including raw materials analysis, operations monitoring, and procedures for addressing nonconformities. Finally, the guidance includes many important references and related guidance documents which should be considered by companies seeking to implement a quality management system.

4.1.4.4 Science-Based Policies

Continuous improvement, which the agency describes as an "essential element in a modern quality system," is aimed toward improving efficiency by "optimizing a process and eliminating wasted efforts in production" [1]. One of the unintended consequences of the regulatory system (prior to the new initiative) had been the suppression of nearly all opportunities for continuous improvement in manufacturing once a pharmaceutical product has been approved for market. Changes to formulations and processes needed to be justified regarding their impact on product quality, often requiring time-consuming postapproval supplements. Producers in most other modern industries (many of whom deal with public safety risks on par with or exceeding those managed by the pharmaceutical industry) make it a practice to continuously fine tune and adjust their operations to maximize quality and efficiency. Pharmaceutical manufacturers, on the other hand, have largely been constrained to treat demonstrated processes as if they were set in stone.

While there is some logic to limiting the scope and pace at which changes can be made to processes, there is obvious fallacy in the idea that the first approved configuration for a drug manufacturing operation will be optimal, especially considering the enormous financial and ethical pressures on process development teams to quickly bring new drug therapies to market. This realization spurred the agency to begin the process of developing *science-based policies and standards to facilitate innovation*, which currently includes three new updated guidance documents: "Sterile Drug Products Produced by Aseptic Processing—cGMP" [55], the PAT guidance, and the draft guidance on comparability protocols. Each guidance document encourages voluntary adoption of new technologies in pharmaceutical manufacturing by defining modern, science-based regulatory mechanisms which enable producers to implement strategic improvements with opportunities for more efficient regulatory compliance.

Comparability Protocols In fact, pharmaceutical manufacturers have always had the option to explore changes to their production processes. The difference between the old regulatory paradigm and the twenty-first-century cGMPs initiative is that producers who seek to improve the quality and efficiency of their processes will be able to implement changes much more quickly while spending significantly fewer resources to maintain compliance. The key to achieving these benefits is demonstrating that there is sufficient understanding of the process and changes to be made and that implementation of the improvements poses very little risk to consumers.

A new mechanism for implementing process changes, which reflects the inclination for science-based policies, is detailed in the FDA's draft guidance "Comparability Protocols—Chemistry, Manufacturing, and Controls (CMC) Information". A *comparability protocol* (CP) is a "well-defined, detailed, written plan for assessing the effect of specific CMC changes in the identity, strength, quality, purity, and potency of a specific drug product as these factors relate to the safety and effectiveness of the product" [56]. Submission of a CP by a producer is optional and may be used to facilitate changes in a manufacturing process, analytical procedures, manufacturing equipment or facilities, or container closure systems or for implementation of PAT.

The benefit for producers submitting a CP is that, upon approval of a CP, "the FDA can designate, where appropriate, a reduced reporting category for future

reporting of CMC changes covered by the approved CP". For example, changes that otherwise would require submission, review, and acceptance of a *postapproval supplement* (PAS) might be designated as *annual report* (AR) changes if they were provided for in an approved CP. The CP is one of the mechanisms by which the FDA intends to reduce the number of supplements requiring review. Additionally, the CP was designed to facilitate free flow of communication with the agency, thereby reducing the risk that process changes will lead to unexpected regulatory shutdown or delay.

Process Validation In agreement with the pursuit of science-based policies, the FDA has begun to revise the 1987 "Guideline of General Principles of Process Validation" and in March 2004 released a revision of the compliance policy guide (CPG) (Section 490.100) "Process Validation Requirements for Drug Products and Active Pharmaceutical Ingredients Subject to Pre-Market Approval" [52]. The current revisions are designed to support continuous improvement and replace the notion of "three-batch" validation. The CPG describes the concept that, after having identified and established control of all critical sources of variability, conformance batches are prepared to demonstrate that under normal conditions and operating parameters the process results in the production of acceptable product. However, the CPG does not describe how many conformance batches are required; rather, the manufacturer is expected to provide "sound rationale" for the procedure they choose to follow in demonstrating validation.

The ambiguity in the revised (CPG) regulations may seem to signify that manufacturers would need to undertake even more extensive validation exercises when in fact the CPG contains language providing a pathway for batch release to market distribution concurrent with the manufacture of initial conformance batches or with a single conformance batch [57]:

> Advanced pharmaceutical science and engineering principles and manufacturing control technologies can provide a high level of process understanding and control capability. Use of these advanced principles and control technologies can provide a high assurance of quality by continuously monitoring, evaluating, and adjusting every batch using validated in-process measurements, tests, controls, and process endpoints. For manufacturing processes developed and controlled in such a manner, it may not be necessary for a firm to manufacture multiple conformance batches prior to initial distribution.

Interpretation of the CPG suggests that implementation of PAT can be an important consideration for streamlining process validation. Finally, a quotable interpretation of the new science-based paradigm suggests that (instead of validating the process) producers should "control the process, and validate the controls." Beyond revision of the CPG, FDA is expected in the near future to release draft guidance on process validation, which will be closely aligned with concepts associated with PAT, QbD, and the rest of the 21st century cGMPs.

4.1.4.5 International Collaboration

Recognizing the current realities of the global marketplace, the FDA has made coordination with international regulatory partners a priority of the twenty-first-century cGMPs initiative. By increasing its collaboration with international health and regulatory partners, the FDA has been able to leverage its resources through

increased sharing of information and harmonization of activities. The International Conference on Harmonization of the Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) (www.ich.org) has been the dominant mechanism for international cooperation among pharmaceutical regulatory authorities in Europe, Japan, and the United States.

A consensus vision statement was drafted at the July 2003 ICH meeting with regard to the objective of the ICH in harmonizing the efforts of regulatory bodies to establish quality systems approaches in their operations: "Develop a harmonized pharmaceutical quality system applicable across the life cycle of the product emphasizing an integrated approach to quality risk management and science."

Three consensus guidelines define the core of the ICH's involvement in harmonization of pharmaceutical quality systems—Q8: Pharmaceutical Development, Q9: Quality Risk Management, and Q10: Pharmaceutical Quality Systems (in addition, each of the guidance documents cites critical areas of overlap with Q6A: Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances).

Q8: Pharmaceutical Development According to the ICH Q8 guideline [58], the aim of pharmaceutical development is to "design a quality product and the manufacturing process to deliver the product in a reproducible manner." While QbD is not specifically mentioned in the guideline, the intent of the ICH Q8 expert working group (EWG) was to describe a system that would provide incentive for manufacturers to incorporate aspects of QbD and continuous improvement throughout the product life cycle. In achieving this goal, the guideline they produced describes the suggested contents for Section 3.2.P.2 of a regulatory submission in the ICH M4 common technical document (CTD) [59] and the FDA electronic common technical document (eCTD) [60].

The pharmaceutical development and quality overall summary (QOS) sections of the CTD (Figure 10) provide pharmaceutical scientists with dedicated channels to present regulators with the relevant knowledge and process understanding gathered during the development of a new product (which can be updated to support new knowledge gained over the life cycle of the product following approval). The knowledge communicated within these sections are important considerations for justification of a lower site risk potential (i.e., SRP, with regard to risk-based inspection) and for facilitation of efficient, question-based review (QbR) [61]. Question-based review is another mechanism by which the agency intends to streamline the regulatory process as well as reward producers for adopting best practices in quality management.

In addition to facilitating risk-based oversight, the content of the pharmaceutical development and QOS sections of the CTD are critical to enabling continuous improvement and flexible operation. The information and knowledge communicated within these sections provide scientific understanding to support the establishment of a manufacturing design space, in-process and release specifications, and manufacturing controls.

As described within the Q8 guideline, a design space is the "multidimensional combination and interaction of input variables and process parameters that have been demonstrated to provide assurance of quality." So long as process control is maintained within the bounds of the design space, operating parameters can be adjusted to improve product quality or manufacturing efficiency. Based on the

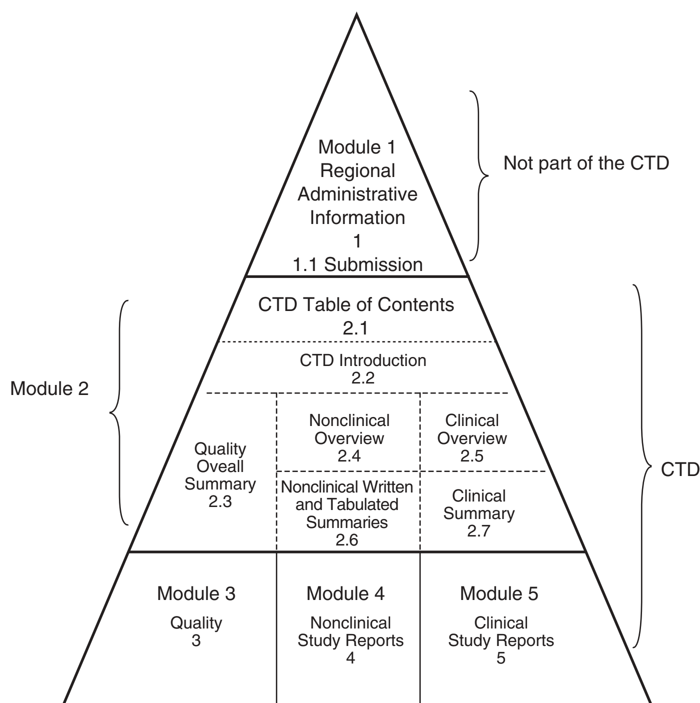

FIGURE 10 Schematic illustration of the ICH M4 common technical document (CTD); the contents of the Quality Overall Summary (2.3) and Quality (3) modules are most relative to PAT.

current definition, operation outside of the established design space would initiate a regulatory postapproval change process. Thus, complete and accurate communication of the knowledge supporting a company's design space is vital for a company to maximize productivity while maintaining regulatory compliance. Furthermore, with the new communication pathways in place, companies have incentive to pursue manufacturing studies beyond marketing approval to expand their design space or to update specifications and controls. In addition to the product under review, if appropriate, experiences gained from the development (and manufacture) of similar drug products may be included.

Q9: Quality Risk Management The second working group (ICH Q9 EWG) is trying to better define the principles by which risk management will be integrated into decisions by regulators and industry regarding quality, including cGMP compliance. In November 2005, the Q9 EWG released the "Step 4" version of the Q9 guideline which defines the two primary principles of quality risk management, provides a model for the quality risk management process (Figure 11), and describes the terminology and tools for risk assessment and management. In addition, the document includes a concise reference list for more detailed information on risk management methods, such as failure mode effect and criticality analysis (FMECA), which are important tools for prioritized implementation of PAT. While it is not intended to be a "how to" manual for risk management, the Q9 guideline is a valu-

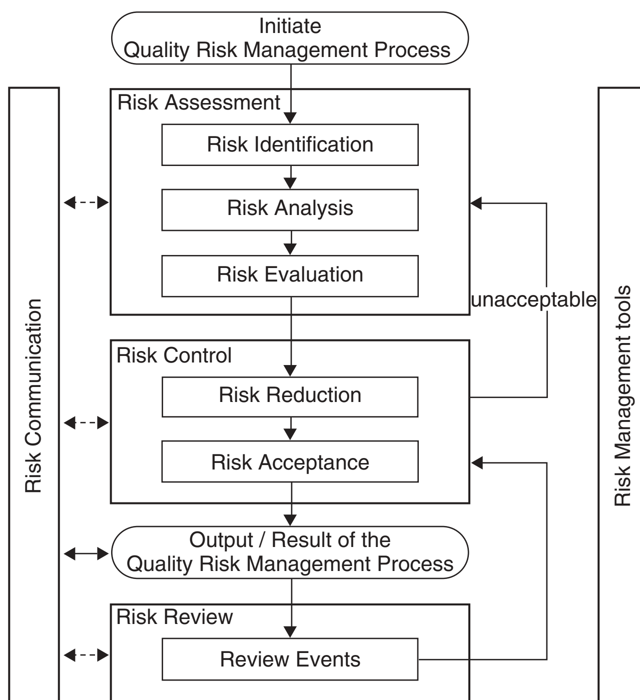

FIGURE 11 Schematic of quality risk management process described within ICH Q9.

able information source for companies seeking to incorporate quality risk management into their operations [62].

Q10: Pharmaceutical Quality Systems While the Step 2 document for the third tripartite guideline, Q10: Pharmaceutical Quality Systems, has not yet been released, the final concept paper has been available since 2005 [63]. Similar to the manner by which the FDA's quality systems approach guidance mapped the relationship between cGMPs and other industrial quality management systems, the Q10 guideline is anticipated to serve as a bridge between the approaches to quality systems taken by the different regional regulations, thereby helping to achieve global harmonization of quality systems. The guideline is expected to strengthen and complement issues covered in Q6A, Q8, and Q9 and will provide a foundation for a pharmaceutical quality system based on elements from the ISO 9001 and 9004 standards. The guideline is also expected to develop harmonized definitions for issues critical to PAT, including continuous improvement activities, data-gathering methods, and the approach to measurement system validation.

4.1.5 PAT EVOLUTION IN PHARMACEUTICAL MANUFACTURING

Though it may be tempting to characterize PAT as a revolutionary change in pharmaceutical manufacturing, history will likely show that the beginning of the twenty-first-century cGMPs initiative and the development of the PAT guidance mark the

beginning of a period of rapid evolution in pharmaceutical manufacturing which will extend far into the future. Even though the twenty-first-century cGMPs initiative is more extensive (with regard to changing the relationship between the FDA and the pharmaceutical industry), interest in the PAT guidance and the opportunities it presents for the industry were initially much greater. More recently, perhaps in parallel with some changes in leadership in the agency, there has been a palpable shift of emphasis toward QbD, which was barely mentioned in many of the twenty-first-century cGMPs documents. It is important to keep in mind that, just as most industries have seen a parade of "new" quality systems initiatives over the years since Shewhart's first methods were published, the principles upon which PAT and QbD are built, such as robust process design, quality monitoring, and effective controls, will persist regardless of the name of the initiative. Furthermore, as with PAT, QbD is not a new concept. Indeed, Dr. Genichi Taguchi, who has been credited by some as the father of QbD, began applying QbD in pharmaceutical manufacturing while working as a statistical consultant for Morinaga Pharmaceuticals Company of Japan from 1947–1949 [25].

The PAT guidance is unique when compared with typical FDA guidance documents in that it is not instructive or limiting per se; rather, the guidance describes the principles and tools upon which the PAT *framework* is built, with the goal of "highlighting opportunities and developing regulatory processes that encourage innovation." The FDA's goal in developing the PAT guidance was to eliminate the specter of regulatory uncertainty which has been identified as a major factor limiting innovation in pharmaceutical manufacturing. The guidance works with existing regulations and was designed to be consistent with the agency's twenty-first-century cGMPs initiative. Furthermore, the guidance emphasizes that the decision on the part of manufacturers to work with the agency to implement PAT is voluntary. Since the guidance is not prescriptive in nature, it neither describes "how to do PAT" nor identifies any particular practice or technology as "approved for PAT."

4.1.5.1 Process Understanding

The agency considers PAT to be a "system for designing, analyzing, and controlling manufacturing through timely measurements of critical quality and performance attributes of raw and in-process materials and processes, with the goal of ensuring final product quality." Based on this definition, it would be practical to consider PAT to be an expansion of PAC; PAT builds on the measurement and control aspects of PAC by incorporating additional emphasis on QbD and process understanding.

According to the PAT guidance, a process is generally considered well understood when:

1. All critical sources of variability are identified and explained.
2. Variability is managed by the process.
3. Product quality attributes can be accurately and reliably predicted over the design space established for materials used, process parameters, manufacturing, environmental, and other conditions.

Furthermore, according to the guidance, the ability to predict "reflects a high degree of process understanding."

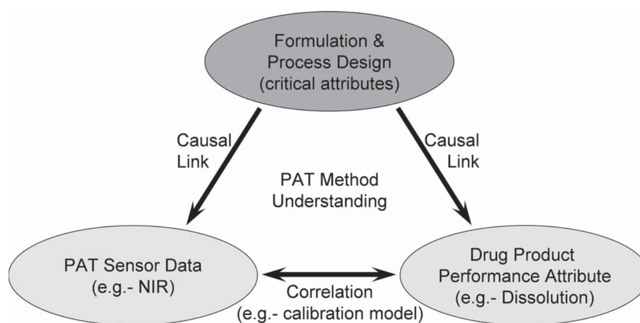

FIGURE 12 Illustration of aspects of method understanding which must be in place to justify product performance measurements using indirect and/or nondestructive analyses.

Possession of a predictive model (for product quality attributes) alone does not necessarily constitute process understanding, however. A relatively common example would be prediction of material or product performance characteristics using multivariate measurements, such as prediction of tablet dissolution rate using NIR spectroscopy. Multiple researchers have demonstrated that (in some cases) it is possible to predict drug release from tablets in vitro using nondestructive NIR spectra by generating a calibration model for dissolution rate. Without demonstrating at least mechanistic understanding of the physicochemical feature (correlated to dissolution rate) being detected by NIR, the calibration model would constitute nothing more than pattern recognition (Figure 12) [64]. While such a calibration may be useful, without greater insight as to the basis for correlation, it would not likely be a useful demonstration of process understanding.

Design Space and Quality by Design The concept of a multidimensional space of acceptable operating conditions, or design space, is perhaps one of the most important aspects of the twenty-first-century cGMPs which facilitates continuous improvement. In a PAT-enabled environment, the process design space must provide evidence of QbD [65] and should be the mathematical medium by which process understanding and real-time control decisions are communicated (Figure 13).

The current ICH Q8 definition of design space, unfortunately, offers little guidance with regard to the aspects of a process design space which are required for implementation. As a result, a variety of interpretations of what constitutes a suitable process design space have recently surfaced among industry participants. One of the most popular misconceptions is that an effective design space for a process or unit operation can be determined by the common trajectory of PAT measurements (i.e., "process signature") related to product batches known to have acceptable quality (i.e., "golden path"). While such data are useful for monitoring, they are nothing more than a modern version of "3-batch" process validation. Golden paths or process trajectories are not sufficient for control since 1) the path itself is not necessarily predictive and 2) such controls would imply that a process is limited by its historical path in the space of process parameters. Originally, the term process signature was defined as a multivariate process measurement, that is, NIR spectrum, which contained features useful for describing the impact of the process on the chemical and physical aspects of the processed material [38].

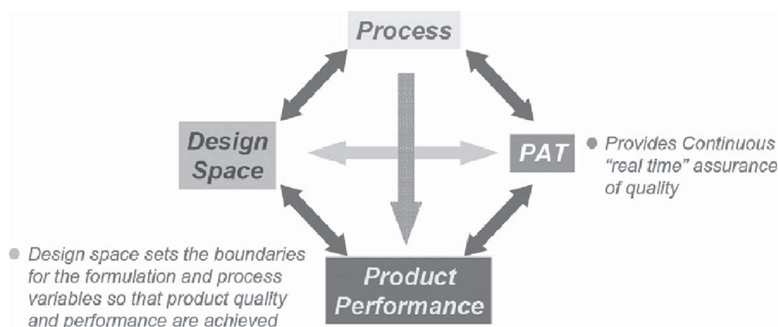

FIGURE 13 Interrelation between design space, PAT, and process control in a manufacturing system based on quality-by-design. (*Source*: R. C. Lyon, Process monitoring of pilot-scale pharmaceutical blends by near-infrared chemical imaging and spectroscopy, Eastern Analytical Symposium (EAS), Somerset, NJ, 2006.)

While it is perhaps too early to posit a conclusive standard for pharmaceutical process design space development, the following minimum criteria should be achieved for a process design space to be suitable for process control:

- The process design space should be expressed in the form of a mathematical model which *quantitatively* links *process capability*, quality of input materials, and process operating parameters.
- Relevant critical-to-quality product attributes should be considered by the design space model (e.g., content uniformity, bioavailability, stability).
- Borrowing from a famous quote by Albert Einstein, the (design space) model should be as complex as necessary (for accurate prediction), but no less.
- Product attributes that are superfluous or are not known to be critical to quality should not be considered by the design space model (there should not be a penalty for monitoring such parameters, however).
- In the same way that in vitro–in vivo correlation (IVIVC) is required to be granted a biowaiver for implementation of postapproval changes, the ability of the design space model to predict the quality of finished goods must be validated prior to implementation.
- If the accuracy of the design space model cannot be established a priori with statistical significance within portions of the parameter hyperspace, operation in such regimes should initiate supplementary quality assurance (inspection) activities until the design space model can be updated and revalidated.
- If unacceptable product quality is observed during operation within a region of the design space expected to yield acceptable quality, the design space should be considered unsuitable for process control (due to drift or the appearance of new factors in the parameter space) until the missing factor(s) can be identified and incorporated into the model and the model is revalidated.

If such a *model-based process design space* includes a sufficient portion of the factors affecting product quality variance, the process *control space* can be projected to define the bounds of normal operation. Based on this definition, the control

model algorithm for each operation in the manufacturing process would be generated from a subset of the control space spanned by the material qualities and processing parameters which impact that operation. Each unit operation control model seeks to adjust process parameters in a timely manner in response to changes in raw material (feedforward) or finished-product (feedback) quality. In other words, *control* the process and *validate* the controls.

The mathematical linkage of the design space, process, and control models enables continuous optimization of product quality by seeking the optimal point within the control space. As the level of process understanding increases or as processing conditions evolve, factors might be added or removed from the design space and the process and control models updated. Furthermore, by considering other factors such as yield, efficiency, or C/T as a function of the variables spanned by the process design space, the process might be co-optimized for quality and profitability.

It is likely that many pharmaceutical manufacturing operations are not understood in a way that product quality variance can be fully described in functional form (e.g., transfer functions); attaining such a level of manufacturing knowledge should be a goal for the industry. Using functional representations of process understanding as the basis set for a process design space, rather than historical performance, offers many operational advantages:

- *Efficient Process Development* While the current definition of design space does not preclude the incorporation of knowledge from other products and processes, model-based knowledge representation offers a more robust framework for incorporation of external or *a priori* information. Even though the level of quality expected by a particular combination of input and process parameters from another product is not likely to transfer to a new product or process (in absolute terms), the functional relationships which predict quality may be quite similar. Furthermore, model-based design space development enables direct incorporation of first principles and mechanistic knowledge, which might significantly reduce the complexity of experimental designs required for process development since significant terms may be identified in silico.
- *Quality by Design* The incorporation of functional relationships between inputs, parameters, and product quality (or efficiency), which inherently imply magnitude and directionality, enables the use of a process design space as a tool for multiobjective process optimization. Furthermore, the model-based representation of knowledge is compatible with concepts of risk management, enabling more flexible operation since the risk associated with extrapolation could be predicted.
- *Control System Development* Model-based design space development offers an ideal segue between process and control development. Quite literally, a model-based design space would provide the template for development of feedforward process control models. Moreover, development of a process design space using a model-based framework would facilitate control system validation and identification of science-based, in-process, and release specifications.

- *Scaling and Technology Transfer* Within the current system for process development, it is common to use designed experiments (i.e., DOE) where some input variables are product specific (e.g., excipient "grade") or process parameters are device dependent (e.g., chopper speed, damper angle). In a model-based paradigm, however, a process design space would ideally be generated using product- and device-independent units which have more basic physical meaning (e.g., modulus, viscosity, energy, or work). Designing and describing production processes in fundamental terms or, perhaps, standardized dimensionless units would facilitate scaling and transfer of design space and process control models to similar manufacturing processes that are based on the same physical operating principles.

Academic research is currently underway to further develop the model-based design space concept. Working within the limits of the current system, though, producers who are able to demonstrate process understanding or are willing to invest in a PAT system to facilitate their development of process understanding can use the tools and provisions of the framework to pursue innovation and continuous improvement with more efficient regulatory oversight (i.e., the ability to make changes without supplemental review). The PAT framework is described as consisting of two components: (1) a set of scientific principles and tools supporting innovation and (2) a strategy for regulatory implementation that will accommodate innovation. The following paragraphs will describe selected aspects of both components in detail.

4.1.5.2 PAT Principles and Tools

Central to the PAT framework is the acceptance that certain physical and mechanical attributes of pharmaceutical ingredients are not necessarily well understood and that even processes which have achieved significant process understanding are subject to a finite level of stochastic variation. Thus, the core of the PAT guidance is allocated to describing the principles and tools, such as process analyzers and risk analysis, which producers can employ to augment process understanding and mitigate latent risks to product quality.

PAT Tools The guidance describes four categories of PAT tools:

- Multivariate tools for design, data acquisition, and analysis
- Process analyzers
- Process control tools
- Continuous improvement and knowledge management tools

Since each of the four categories draws upon methods and technology which are already established in other fields such as PAC, the discussion of each category within the guidance is focused on aspects which are unique or significant to pharmaceutical manufacturing, such as *process signature* [2]. Furthermore, in keeping with the spirit of the framework as a catalyst for innovation, the agency made an effort to avoid mention of any particular tool or technology in the final version of

the PAT guidance. The PAT tools section of the guidance does, however, include cross-references to relevant portions of current regulations which should be considered by a manufacturer developing a PAT strategy or system.

Standards for Pharmaceutical Applications of PAT During the early stages of developing the PAT framework, the agency was aware that the lack of international standards was a significant impediment to regulatory coordination and implementation of PAT in the global pharmaceutical industry. In 2003, the FDA's PAT team worked with ASTM International to form Technical Committee E55 on Pharmaceutical Application of Process Analytical Technology. The E55 committee addresses issues related to process control, design, and performance as well as quality acceptance/assurance tests for the pharmaceutical manufacturing industry. Stakeholders in the committee include manufacturers of pharmaceuticals and pharmaceutical equipment, federal agencies, design professionals, professional societies, trade associations, financial organizations, and academia (www.ASTM.org).

As of mid-2006, there were three subcommittees of E55: PAT system management, PAT system implementation and practice, and PAT terminology. The PAT team has been represented on E55 committees with a goal to ensure that standards developed are aligned with the PAT guidance and acceptable to the FDA. To date, one active standard has been published, while 16 additional standards have been proposed. The ASTM International provides another venue for international cooperation (consistent with the twenty-first-century cGMPs initiative); the definitions of PAT (in the FDA guidance and ASTM E55) as well as other concepts are being incorporated into the ICH Q8 guidance.

Real-Time Release (RTR) The PAT guidance defines RTR as "the ability to evaluate and ensure the acceptable quality of in-process and/or final product based on process data." Whereas finished products are typically released for marketing only after sampling, inspection (i.e., laboratory-based QC testing), and review, implementation of an RTR system enables release of finished products concurrent with the completion of manufacturing operations. Practically speaking, RTR is one of the most significant, tangible benefits for producers who implement PAT, because it can facilitate dramatic reductions in process C/T.

Real-time release is considered by the guidance to be comparable to *alternative analytical procedures* for final product release and is defined within the guidance as an extension of parametric release. The defining characteristic of RTR is that it considers simultaneously the degree to which material attributes and process parameters are measured and controlled during manufacturing. It was not intended that RTR be implemented by simply installing a rapid measurement system at the end of a manufacturing process; such uses for PAT tools would be tantamount to inspection and would do nothing to improve quality management.

The guidance does suggest, however, that it may be feasible to implement RTR without finished-product quality monitoring by using "the combined process measurements and other test data gathered during the manufacturing process." Similar language is found in the USP general notices, where it is suggested that data derived from "[validation studies and] in-process controls may provide greater assurance that a batch meets a particular monograph requirement than analytical data derived from an examination of finished units drawn from that batch." It would not be

difficult to create a system more capable of detecting quality variation than current methods based on inspection. Recent statistical analyses [42] have demonstrated that, for determining batch quality, the traditional USP ⟨905⟩ method of content uniformity testing may indeed have little more statistical power than a coin toss until more than 5% of the product exceeds specification limits (corresponds to within-batch C_{pk} of approximately 0.65, only slightly worse than has been observed in a recent industry benchmarking study [28]).

On the other hand, deployment of an RTR system without finished-product monitoring would require the manufacturer to demonstrate a very high level of process understanding based on, for example, their development of a comprehensive design space and/or a well-validated process model. Even though it may be feasible to implement RTR without end-of-process monitoring, a well-designed PAT system will typically include some form of final product quality monitoring as a means for mitigating latent risk and creating strategic redundancy in process controls and as an additional tool to bolster process understanding.

4.1.5.3 Strategy for Implementation

One of the FDA's goals for the PAT guidance is to "tailor the Agency's usual regulatory scrutiny to meet the needs of PAT-based innovations that (1) improve the scientific basis for establishing regulatory specifications, (2) promote continuous improvement, and (3) improve manufacturing while maintaining or improving the current level of product quality." Recognizing that the achievement of this goal requires a unique interface between regulators and manufacturers seeking to implement PAT, a strategy for implementation based on the integrated systems approach was developed. An objective of the strategy for implementation is to facilitate clear, effective, and meaningful communication between the agency and industry, for example, in the form of meetings or informal communication.

In practice, the strategy breaks with traditional industry–FDA modes of communication; whenever PAT is concerned, it is anticipated that regulators will communicate directly with the pharmaceutical scientists and engineers involved with development and operation of the PAT system rather than indirectly via a department of regulatory affairs. The components of the agency's regulatory strategy include:

- A PAT team approach for CMC review and cGMP inspections
- Joint training and certification of PAT review, inspection, and compliance staff
- Scientific and technical support for the PAT review, inspection, and compliance staff
- Recommendations provided within the PAT guidance

PAT Team Approach FDA's assembly of the PAT team was one of the most significant incentives for the industry to pursue manufacturing innovation as described in the twenty-first-century cGMPs initiative and the PAT guidance. The PAT team was put in place to ensure that industrial PAT applications were handled with expediency and accuracy by scientists familiar with the most up-to-date PAT methods.

At one point the PAT team included more than 20 scientists, including investigators, compliance officers, reviewers, training coordinators, and a policy development team. More recently the agency has begun steps to "sunset" the PAT team, the duties of which will ultimately be handled by FDA staff trained in PAT systems. A comprehensive scientific training program was developed for the PAT team with guidance from the ACPS PAT subcommittee. Initial training began in January 2006, with plans for further training to be provided by faculty at Duquesne and Delaware Universities [47].

Research Data Provision In developing the PAT guidance, the FDA recognized that, even with the guidance in place, manufacturers seeking to evaluate the suitability or potential value of new technologies for process control may be hesitant, figuring that such data will be subject to cGMP inspection, thereby increasing their liability with respect to regulatory actions. To allay these fears, the agency included a statement which applies to investigational deployment of new technologies [2]:

> Data collected using an experimental tool should be considered research data. If research is conducted in a production facility, it should be under the facility's own quality system. . . . FDA does not intend to inspect research data collected on an existing product for the purpose of evaluating the suitability of an experimental process analyzer or other PAT tool. FDA's routine inspection of a firm's manufacturing process that incorporates a PAT tool for research purposes will be based on current regulatory standards (e.g., test results from currently approved or acceptable regulatory methods). Any FDA decision to inspect research data would be based on exceptional situations similar to those outlined in Compliance Policy Guide Sec. 130.300. Those data used to support validation or regulatory submissions will be subject to inspection in the usual manner.

4.1.6 PAT IMPLEMENTATION PROCESS

The PAT guidance identifies three possible plans for companies seeking to implement PAT:

- PAT can be implemented under the facility's own quality system; cGMP inspections by the PAT team or PAT-certified investigator can precede or follow PAT implementation.
- A changes being effected (CBE), CBE in 30 days (CBE-30), or prior approval (PAS) supplement can be submitted to the agency prior to implementation, and, if necessary, an inspection can be performed by a PAT team or PAT-certified investigator before implementation.
- A comparability protocol (CP) can be submitted to the agency outlining PAT research, validation, and implementation strategies and time lines. Following approval of this comparability protocol by the agency, one or a combination of the above regulatory pathways can be adopted for implementation.

Reflecting its nonprescriptive nature, the three implementation plans are essentially the only "how to" portions of the PAT guidance. This leaves industrial (and academic) scientists and engineers with the burden of determining how best to proceed

in the deployment of a PAT system. Despite the fact that some pioneering companies have been incorporating aspects of PAT in their operations since long before the start of the FDA's twenty-first-century cGMPs initiative, there continues to be significant diversity in their approaches to implementation. While perhaps the ambiguity (in how best to proceed) has slowed the uptake of PAT to some degree, in the long run, the latitude is preferable since the optimal path of implementation will likely be unique for most facilities.

With regard to drug manufacturers' implementation of PAT, a list of 10 questions has been presented which provides an initial checklist for companies seeking approval of their plans [10, 66]:

1. Is this a PAT system?
2. Does it have aspects of design, measurement, and manufacturing control?
3. Are PAT principles and tools used?
4. Which tools specifically are used for manufacturing control?
5. How are continuous improvement and knowledge management performed?
6. What risk-based approach has the company taken—assessment, prevention, and management?
7. How are the PAT systems integrated?
8. What kind of RTR is being proposed or used?
9. What regulatory process is being considered?
   a. Can the companies' quality systems manage the PAT change?
   b. Are the submission proposals appropriate and justified?
10. What are the critical aspects that will be evaluated during site visits/ inspections?

Drawing from aspects of the DMAIC model, as well as the risk-based orientation and quality systems approach espoused by the FDA's twenty-first-century cGMPs initiative, the Duquesne University Center for Pharmaceutical Technology (DCPT) has proposed a six-phase, iterative cycle for process improvement based on PAT (Figure 14). While there are certainly many acceptable variants of this strategy, some of which have begun to appear in conferences and the industrial literature, any successful PAT deployment, large or small, will most likely include some combination of these elements. In addition, each project phase will necessarily include one or more modules of training. Finally, while the project phases are presented as being discrete, most of the phases will overlap to some degree. In particular, consideration of the objectives for control, release strategies, and plans for continuous improvement should begin, along with management buy-in, early in the cycle.

4.1.6.1 Preparation

The preparation phase is arguably the most critical step in the path toward PAT implementation. Process analytical technology projects are inherently multidisciplinary, requiring acceptance and buy-in from corporate divisions which sometimes

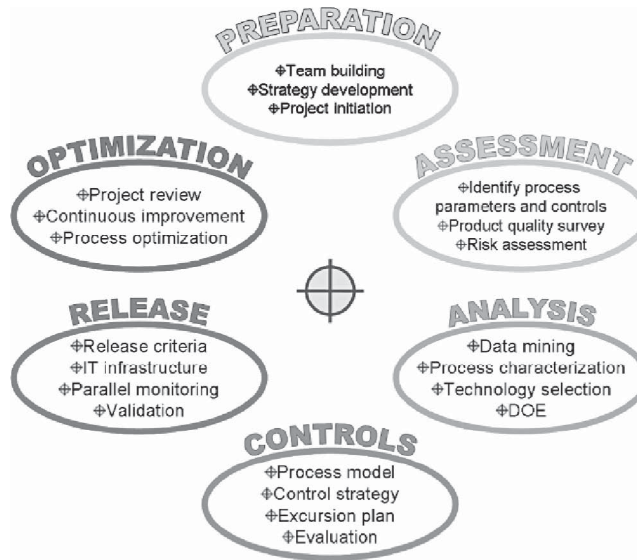

FIGURE 14 PAT implementation cycle with examples of associated activities for each phase.

operate with rather divergent goals and procedures. Most importantly, those who are seeking to initiate a PAT project will need to obtain management buy-in at a level high enough in the corporate structure to ensure sufficient resources will be available and that the company will be committed to positive change. During the preparation phase, a PAT team having a diverse background and critical skills should be assembled, and formal planning of the project should begin, including selection of the product and process to address. Ideally, dialogue with the FDA PAT team should begin early in the preparation phase.

4.1.6.2 Assessment

The PAT guidance clearly states that industrial implementations should be risk based. Soon after the PAT team and objective have been identified, the project should commence with a formal risk assessment. The risk assessment should be focused on identifying and characterizing the failure modes which present risks to product quality; the outcome of the risk assessment will provide a means prioritizing the allocation of PAT resources and a baseline for review of the effect of PAT in mitigating risks to quality.

4.1.6.3 Analyze

The “analyze” phase of the project consists of the activities which are typically associated with PAC, including identification and assessment of potential sensor technologies, method development, qualification, and validation. In addition, designed experiments (DOE) or data-mining exercises may be performed to

generate process understanding or to support PAT goals. Plans for the IT infrastructure, sampling protocols, and development of controls should also be considered.

4.1.6.4 Control

The implementation of controls begins as each new analytical method or technology is deployed. Controls may be as simple as automated termination of a unit operation upon reaching an endpoint. With greater process understanding, more complex controls can be deployed, including feedback (e.g., control of punch force during tablet compaction, control of temperature or airflow during fluid bed processing) or feedforward controls (e.g., adjustment of process parameters based on incoming raw-material quality). The development and implementation of controls should also consider operating procedures for adverse situation management and should initiate a reassessment of risk to determine the suitability of controls.

4.1.6.5 Release Philosophy

For PAT projects including implementation of RTR or some modification of a preexisting release mechanism for an approved process, additional method development and validation procedures will be required. The real-time release decision will typically be determined by a process model, which can be a mathematical equation or algorithm within the control system; furthermore, the IT system must accurately convey the release decision and supporting data to downstream operations (i.e., warehouse, logistics), upstream operations (i.e., production scheduling, accounting), or the facility information repository. The interwoven IT and scientific components require an integrated systems approach to development, validation, deployment, and operation. Finally, implementation of PAT systems enables redefinition of product quality acceptance criteria for release; the task of identifying robust release criteria suitable for large sample sizes, for example, continues to merit examination [43].

4.1.6.6 Optimization

The optimization phase of the project provides an opportunity to assess the performance of the PAT system relative to the goals of the project as well as the level of latent risk in the system. Ideally, with the PAT s2ystem in place, the level of process understanding will be improving as more data are collected for every batch. The added insight into the operation may yield new opportunities for improving quality or efficiency or for solving similar problems with another product. The key to success in the optimization stage is realizing that it is only the beginning of continuous improvement.

4.1.7 PERSPECTIVES ON THE IMPACT OF PAT

PAT and the twenty-first-century cGMPs initiative have clearly made an impact within the pharmaceutical and associated industries. Significant sums of capital are now flowing in new directions to meet the challenges and opportunities pre-

sented by the changes. Some people within the industry, however, question whether there will be much of a long-term impact, citing the litany of new eras in the industry (and their careers) that turned out to be more of the same. With just a bit of observation, though, it is not hard to see that it really is different this time.

The modern pharmaceutical manufacturing industry finds itself in a difficult situation that perhaps few anticipated just 10 or 15 years ago. The rate of new blockbuster drug approvals has continued to wane, while new drug therapies become inexorably more expensive to discover and develop. Despite the fact that the market for drug sales has never been larger, drug company profit margins are shrinking while consumers, feeling that pharmaceutical company profits are unjust, have reached new lows in their opinion of the industry. A recent survey by the Kaiser Family Foundation placed pharmaceutical companies just above oil and tobacco companies, and right below health management organizations (HMOs), in terms of public opinion [67]. Entities of significant magnitude in both the public and private sector are increasingly applying pressure to capture an even greater portion of the industry's compensation. Indeed, there is no shortage of industrial and financial publications which have chronicled the pharmaceutical industry's troubles [34, 45, 68, 69].

The pharmaceutical industry is fortunate, perhaps, to follow (rather than lead) most other major industries in adopting truly automated controls, process analytics, quality management, and lean manufacturing. The performance of pharmaceutical companies relative to the benchmarks for world-class manufacturers provides a roadmap for improvement. If the pharmaceutical industry, as a whole, were able to at least approach the benchmarks for world-class manufacturing performance (by implementing PAT), the savings returned to consumers and shareholders would be immense (Figure 15). Finally, the returns on investment in PAT are not limited to major producers. Estimates based on recent benchmarks suggest that, by successfully transforming operations through the deployment of PAT and lean, a typical small or mid-sized pharmaceutical manufacturer could improve operating margins by up to 600 basis points [44].

Forces which are out of the industry's control are providing more reasons than ever before to seek efficiency in pharmaceutical manufacturing, and the FDA is doing its part to clear the way. While the pharmaceutical industry has likely been unjustly cast as a culprit behind America's fiscal crisis in health care, the industry has ample opportunity to change for the benefit of patients as well as investors.

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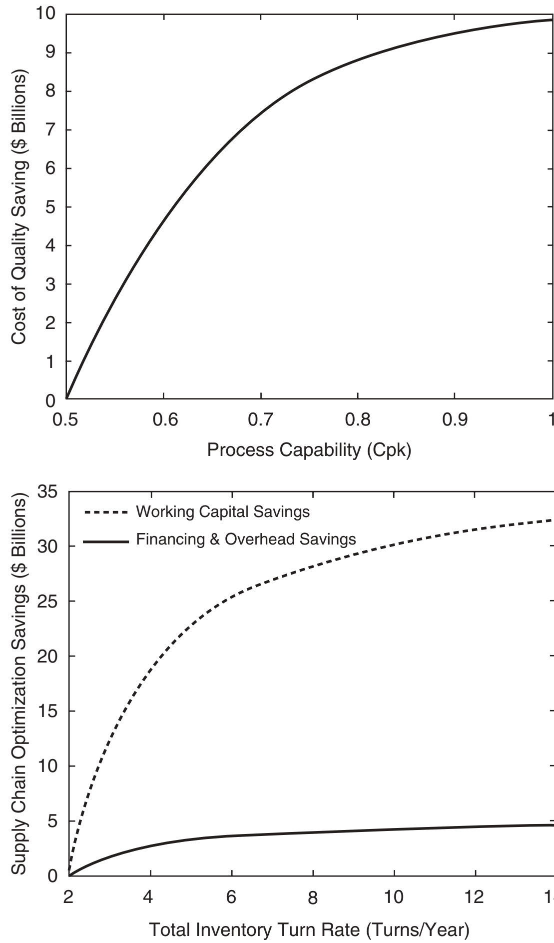

FIGURE 15 Potential financial returns from deployment of PAT and lean. The curves are calculated based on the aggregate COGS and inventories reported in the 2005 annual reports of the top 16 branded and generic pharmaceutical manufacturers (according to market capitalization). It is important to keep in mind that working capital savings are a one-time-only benefit, while cost of quality and inventory financing and overhead savings represent on-going returns on investment. Furthermore, while the curves may overestimate savings because of innacuracies in benchmark data or the limits on the opportunities for PAT implementation, they do not account for numerous other potential pathways for returns from PAT such as capacity increase, labor productivity enhancement, reduction of QC expense, or decreased time to market.

REFERENCES

1. U.S. Department of Health and Human Services (2004), Pharmaceutical CGMPs for the 21st century—A risk-based approach, Final report, Food and Drug Administration, Rockville, MD.
2. U.S. Department of Health and Human Services (2004), Guidance for industry: PAT—A framework for innovative pharmaceutical development, manufacturing, and quality assurance, Food and Drug Administration, Rockville, MD.
3. Clevett, K. J. (1986), *Process Analyzer Technology*, Wiley, New York.
4. Illman, D. L. (1986), CPAC: An industry—university cooperative research center for process analytical chemistry, *TrAC Trends Anal. Chem.*, 5, 164.
5. Callis, J. B., Illman, D. L., and Kowalski, B. R. (1987), Process analytical chemistry, *Anal. Chem.*, 59, 624A.
6. Balboni, M. L. (2003), Process analytical technology: concepts and principles, *Pharm. Technol*.
7. Koch, M. V. (2006), Optimizing the impact of developments in micro-instrumentation on process analytical technology: a consortium approach, *Anal. Bioanal. Chem.*, 384, 1049.
8. Küppers, S., and Haider, M. (2003), Process analytical chemistry—future trends in industry, *Anal. Bioanal. Chem.*, 376, 313.
9. Küppers, S., and Haider, M. (2006), Process analytical chemistry, *Anal. Bioanal. Chem.*, 384, 1034.
10. Hinz, D. C. (2006), Process analytical technologies in the pharmaceutical industry: the FDA's PAT initiative, *Anal. Bioanal. Chem.*, 384, 1036.
11. Beebe, K. R., et al., (1993), Process analytical chemistry, *Anal. Chem.*, 65, 199R.
12. Blaser, W. W., et al., (1995), Process analytical chemistry, *Anal. Chem.*, 67, 47R.
13. Workman, J. J., et al., (1999), Process analytical chemistry, *Anal. Chem.*, 71, 121R.
14. Workman, J. J., et al., (2001), Process analytical chemistry, *Anal. Chem.*, 73, 2705.
15. Workman, J. J., Koch, M. V., and Veltkamp, D. J. (2003), Process analytical chemistry, *Anal. Chem.*, 75, 2859.
16. Workman, J. J., Koch, M. V., and Veltkamp, D. J. (2005), Process analytical chemistry, *Anal. Chem.*, 77, 3789.
17. Gluckman, P., Roome, D. R., Deming, W. E., and Delavigne, K. (1993), *Everyday Heroes of the Quality Movement: From Taylor to Deming, the Journey to Higher Productivity*, 2nd ed., Dorset house, New York.
18. Shewhart, W. A. (1980), *Economic Control of Quality of Manufactured Product*, ASQC Quality Press, Milwaukee, WI.
19. Shewhart, W. A. (1986), in Deming W. E. ed., *Statistical Method from the Viewpoint of Quality Control*, Dover, New York.
20. Scherkenbach, W. W. (1991), *The Deming Route to Quality and Productivity: Road Maps and Roadblocks*, ASQC Quality Press, Milwaukee, WI.
21. Juran, J. M. (2003), *Architect of Quality: The Autobiography of Dr. Joseph M. Juran*, McGraw-Hill, New York.
22. Bhote, K. R. (2002), What is Six Sigma? *The Ultimate Six Sigma*. Vol 1. New York: AMACOM; 9–14.
23. Galvin, R.W. (2002), Forward. *The Ultimate Six Sigma*. Vol 1. New York: AMACOM; xxi–xxii.
24. Marash, S. A., Berman, P., and Flynn, M. (2004), *Fusion Management: Harnessing the Power of Six Sigma, Lean, ISO 9001:2000, Malcom Baldridge, TQM and Other Quality Breakthroughs of the Past Century*, QSU Pub., Fairfax, VA.

25. Ealey, L.A. (1988), *Quality by design. Taguchi Methods and U.S. industry*. Dearborn, MI: ASI Press.
26. Taguchi, G., Chowdhury, S., and Wu, Y. (2005), *Taguchi's quality engineering handbook*. Hoboken, NJ: John Wiley & Sons.
27. e-Handbook of Statistical Methods. *NIST/SEMATECH*. Available at: http://www.itl.nist.gov/div898/handbook/.
28. Macher, J., and Nickerson, J. (2006), Pharmaceutical manufacturing research project: Final benchmarking report, Georgetown University, McDonough School of Business and Washington University Olin School of Business, Washington DC.
29. Levinson, W. A. (2002), *Henry Ford's Lean Vision*, Productivity Press, New York.
30. Leiper, K. (2006), paper presented at the The Heidelberg PAT Conference, Heidelberg, Germany.
31. Lewis, N. A. (2006), A tracking tool for lean solid-dose manufacturing, *Pharm. Technol.* 30, 94–108.
32. Roumeliotis, G. (2006), Lean proves mean in drug manufacturing, available: in-PharmaTechnologist.com.
33. Gerecke, G., and Knight, T., Improving performance and reducing cycle time using flow path management: A case study, *Pharm. Eng.* 21.
34. Abboud, L., and Hensley, S. (2003), Factory shift: New prescription for drug makers: Update the plants, *Wall Street J.*, Sept. 3, p. 1.
35. Cooley, R. E., and Egan, J. C. (2004), The impact of process analytical technology (PAT) on pharmaceutical manufacturing, *Am. Pharm. Rev.*, 7, 62–68.
36. Cogdill, R. P. (2006), in Brittain, H. G., Ed., *Spectroscopy of Pharmaceutical Solids*, Taylor & Francis Group, New York, pp. 313–412.
37. Cogdill, R. P., et al., (2005), Process analytical technology case study, Part II: Development and validation of quantitative for tablet API content and hardness, *AAPS Pharm. Sci. Tech.*, 6, Article 38.
38. Cogdill, R. P., et al., (2005), Process analytical technology case study, Part I: Feasibility studies for quantitative NIR method development, *AAPS Pharm. Sci. Tech.*, 6, Article 37.
39. Cogdill, R. P., Anderson, C. A., and Drennen, J. K. (2005), Process analytical technology case study, Part III: Calibration monitoring and transfer, *AAPS Pharm. Sci. Tech.*, 6, Article 39.
40. Lorber, A. (1986), Error propagation and figures of merit for quantification by solving matrix equations, *Anal. Chem.*, 58, 1167.
41. Braga, J. W. B., and Poppi, R. J. (2004), Figures of merit for the determination of the polymorphic purity of carbamazepine by infrared spectroscopy and multivariate calibration, *J. Pharm. Sci.*, 93, 2124.
42. Lunney, P., and Drennen, J. K. I. (2005), A prevention based strategy for quality control using PAT, *NIR News*, 16, 7.
43. Sandell, D., Diener, M., Vukovinsky, K., Hofer, J., and Pazdan, J. (2006), Development of a content uniformity test suitable for large sample sizes, *Drug. Info. J.*, 40, 337.
44. Cogdill, R. P., Knight, T. P., Anderson, C. A., and Drennen, J. K. (2007), The financial returns on investments in process analytical technologies and lean manufacturing: Benchmarks and case study, *J. Pharm. Innov.*, 2(1–2), 38–50.
45. du Pre Gauntt, J. (2005), Quality manufacturing: A blockbuster opportunity for pharmaceuticals, Economist Intelligence Unit.
46. Steyer, R. (2005), Merck names clark CEO, available: TheStreet.com, accessed May 5, 2005.

47. Watts, D. C. (2006), PAT—An FDA paper presented at the The Heidelberg PAT Conference, Heidelberg, Germany.
48. Hussain, A. S. (2001), Emerging science issues in pharmaceutical manufacturing: Process analytical technologies, paper presented at the Science Board Presentations to FDA, Rockville, MD.
49. Chisholm, R. S. (2001), TQMS, statistically based in-process control with real time quality assurance, the AstraZeneca total quality management strategy, paper presented at the Science Board Presentations to FDA, Rockville, MD.
50. Raju, G. K. (2001), Pharmaceutical manufacturing: New technology opportunities, paper presented at the Science Board Presentations to FDA, Rockville, MD.
51. Scherzer, R. H. (2002), Quality by design: A challange to the pharma industry, paper presented at the Science Board Presentations to FDA, Rockville, MD.
52. U.S. Department of Health and Human Services (2004), Risk-based method for prioritizing cGMP inspections of pharmaceutical manufacturing sites—A pilot risk ranking model, Food and Drug Administration, Rockville, MD.
53. U.S. Department of Health and Human Services (2006), SMG 2020—FDA quality system framework for internal activities, Food and Drug Administration, Rockville, MD.
54. U.S. Department of Health and Human Services (2006), Guidance for industry: Quality systems approach to pharmaceutical cGMP regulations, Food and Drug Administration, Rockville, MD.
55. U.S. Department of Health and Human Services (2004), Guidance for industry: Sterile drug products produced by aseptic processing—Current good manufacturing practice, Food and Drug Administration, Rockville, MD.
56. U.S. Department of Health and Human Services (2003), Guidance for industry: Comparability protocols—Chemistry, manufacturing, and controls information, draft guidance, Food and Drug Administration, Rockville, MD.
57. U.S. Food and Drug Administration (FDA) (2004, Mar.), Sec. 490.100 Process validation requirements for drug products and active Pharmaceutical ingredients subject to pre-market approval (CPG 7132c.08), FDA, Rockville, MD.
58. International Conference on Harmonization (ICH) (2004), Q8: Pharmaceutical development, International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH, Geneva.
59. International Conference on Harmonization (ICH) (2004), M4: Organisation of the common technical document for the registration of pharmaceuticals for human use, International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH, Geneva.
60. U.S. Department of Health and Human Services (2006), Guidance for industry: Providing regulatory submissions in electronic format—Human pharmaceutical product applications and related submissions using the eCTD specifications, Food and Drug Administration, Rockville, MD.
61. Yu, L. X. (2006), Implementation of quality-by-design: Question-based review, paper presented at the Drug Information Association 42nd Annual Meeting, Philadelphia, PA.
62. International Conference on Harmonization (ICH) (2005), Q9: Quality risk management, International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH, Geneva.
63. International Conference on Harmonization (ICH) (2005), Q10: Pharmaceutical quality systems, final concept paper, International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH, Geneva.

64. Hussain, A. S. (2006), Quality by design and bioequivalence/bioavailability assessment, paper presented at the The Heidelberg PAT Conference 2006, Heidelberg, Germany.
65. Lyon, R. C., and Hammond, S. (2006), Process monitoring of pilot-scale pharmaceutical blends by near-infrared chemical imaging and spectroscopy, paper presented at the Eastern Analytical Symposium, Somerset, NJ.
66. D'Sa, A. (2005), Process analytical technology (PAT): regulatory process, review and inspection, paper presented at the 19th International Forum on Process Analytical Technology—IFPAC 2005, Arlington, VA.
67. Views on prescription drugs and the pharmaceutical industry, The Kaiser Family Foundation, 2005.
68. Arlington, S., Barnett, S., Hughes, S., Palo, J., and Shu, E. (2002), Pharma 2010: The threshold of innovation, IBM Business Consulting Services, Somers, NY.
69. Arlington, S., et al., (2005), The metamorphosis of manufacturing, IBM Business Consulting Services, Somers, NY.

4.2

PROCESS ANALYTICAL TECHNOLOGY

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4.2.1 BASIC CONCEPTS AND IMPACT

4.2.1.1 Definition

Process analytical technology (PAT) is one of the objectives contained in the *Initiative for Pharmaceutical cGMPs for the 21st Century* published by the Food and Drug Administration (FDA). In a few words and according to the FDA's guideline, PAT can be defined as a system for designing, analyzing, and controlling pharmaceutical manufacturing through the measurement of critical quality and performance parameters. The measurements performed on raw and in-process materials or process parameters are intended to enhance final product quality.

Process analytical technology encourages technological innovation, specifically the adoption of new analytical techniques by the pharmaceutical industry designed to improve the understanding and control of manufacturing processes. Both the FDA and industry experts expect benefits over conventional manufacturing practices: higher final product quality, increased production efficiency, decreased operating costs, better process capacity, and fewer rejects. Correspondingly, fundamental changes are also expected within the regulatory framework. The future of pharmaceutical production will require innovative technological approaches and more science-based processes. PAT will boost collaboration between research and development (R&D) and manufacturing departments inside companies and increase overall efficiency. Approvals and inspections will increasingly focus on scientific and engineering principles. As a result, regulators will set higher expectations for new products from the outset.

4.2.1.2 What Motivated PAT?

Preliminary discussions of PAT concepts between the FDA and certain pharmaceutical companies already active in this field date back to the late 1990s. In September

2004 the FDA released a document for the industry entitled "PAT Guidance for Industry: A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance." PAT is clearly anchored in FDA corporate culture.

Pharmaceutical companies are facing growing demands for increased productivity and reduced manufacturing costs. They also have to meet the evolving need for higher quality standards and higher drug expectations. At the same time the quest for new active substances remains a significant issue. Reducing the attrition rate among selected candidates will bring more new medicines onto the market. In terms of drug marketing, the goal is to improve formulations so as to offer patients innovative and more efficient solutions, and thus achieve commercial success or breakthrough. By prioritizing science-based design and introducing novel or improved process techniques, backed by the generation of increased critical data throughout a drug's life cycle, the aim of the emerging PAT strategy is to direct the drug industry toward these essential goals.

Because they have been used for many years, a variety of existing experimental methods and manufacturing processes are considered well established. They are trusted to generate few errors and make only modest contributions to process variation. Due to their longevity, they continue to be widely used in recent drug developments. Improvements in existing technologies are always possible and are constantly being made. However, this makes it difficult to consider or identify potential technological alternatives without critical review or a voluntary management decision to replace well-established techniques. The FDA noticed that nearly all recent drug developments lacked the possibility of enhancing and extending process capabilities toward newer or alternative technologies. More specifically, the FDA wanted to encourage drug manufacturers to achieve more innovation and improve risk management when releasing new medicines on the market.

4.2.1.3 Root-Cause Analysis and Process Control

When a quality problem arises in present-day production, it is increasingly difficult to identify the root cause. Thorough understanding of process and product performance often comes up against knowledge barriers, whether due to the escalating documentation burden, lack of time, or loss of expertise. The goal of PAT is to enhance process control and understanding so that procedures can be performed differently and more efficiently. The PAT initiative facilitates and encourages the introduction of innovative approaches. It makes it possible to consider shifting from validation to continuous verification. The next step is effective real-time release with continuous processing as an alternative to the conventional batch-after-batch production scheme.

4.2.1.4 When to Introduce PAT

Building quality into a pharmaceutical product has to be considered from the very beginning of the product's life. Essential preconditions are the equal involvement of—and seamless communication between—R&D and manufacturing. One purpose of PAT is to provide a motivating framework to bring quality into a product from the outset. It is thus essential for it to be involved in the R&D phase. If product quality requirements are understood and implemented from the beginning,

root-cause analysis of quality or process failure after scale-up to commercial manufacturing will be much easier. This is why PAT could play an even more important role in the design and analysis of manufacturing processes, enabling performance control to be based on timely measurement of well-described critical processing data.

Data processing needs should also be considered in the context of overall process analysis strategy to meet emerging requirements for the speed and volume of data collection. Real-time analysis supported by knowledge management requires collecting and gathering all production batch information, for example, by data warehousing. Thus, a PAT data management strategy based on online process analysis or data mining can be set up long before generating large sets of measurement data. Historical data analysis should aim to cover method development, method validation, and ongoing performance monitoring, as well as routine results for a given manufacturing process.

4.2.1.5 PAT Enhances Process Understanding

Process analytical technology can greatly enhance process understanding. In fact, introducing PAT can act as a key driver to better process knowledge. The expected steps in implementing the PAT approach are the collection of online, in-line, and at-line data (Figure 1) on critical attributes, extraction of information, and analysis of process status data, ending with closure of the loop by dynamic process control. Innovating during development, applying cutting-edge techniques, and process modeling whenever possible, all contribute to a more fundamental exploration of the science behind the process. It is important to realize that PAT is not only the straightforward introduction of additional analytical techniques into a process but also the development of methods to predict future behavior according to given settings of the critical parameters. That means being able to predict final product quality. For example, while implementing the process, it is important to explore all sources of component variation as well as their effect on the finished product in order to select which quality parameters (i.e., attributes) have to be measured for optimal and realistic process control.

Science, engineering, and control technologies can provide a very high level of process understanding and control capability. A process is well understood when all

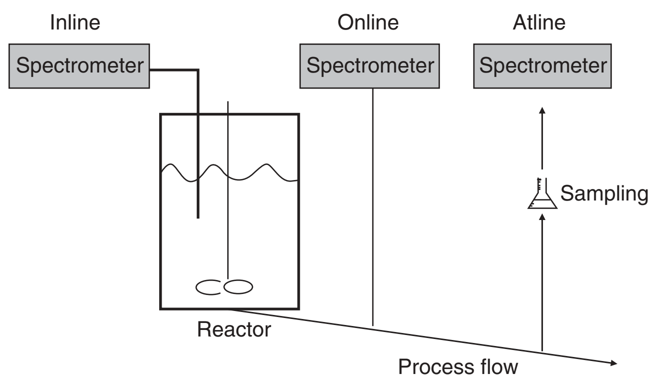

FIGURE 1 In-line, online, and at-line process measurements.

critical sources of variability are identified and explained. The process should be robust enough to manage this variability. It is also expected that critical quality attributes can be accurately and reliably predicted in an adequate design space when other unexpected variables are encountered (e.g., change of raw material supplier).

4.2.1.6 Changing Current Practice Using PAT

An approach integrating R&D and manufacturing will enhance process understanding and make acceptable risk management possible. By establishing transferable process models, it will be possible to develop and implement adequate measurement technologies that match process needs rather than vice versa. More efficient and cost-effective technology transfers will facilitate process knowledge, continuous process verification, and compliance, thereby enhancing final product quality. Better process understanding makes it possible to operate by continuous process verification instead of three-batch validation. Measurement technique selection and integration occur very early. Accumulated pertinent knowledge is readily available through data-mining techniques to confirm or control processing. A series of dynamic closed control/compliance loops at the process steps identified as critical will increase confidence in final product quality. In addition knowledge accumulated over time will provide a basis for immediate and rapid intervention in the event of deviation or failure.

A typical illustration of a PAT approach to quality improvement is the use of near-infrared spectroscopy (NIRS) to qualify excipients and active principles just before they enter the production process, for example, in dispensing. As discussed in the next part, near-infrared (NIR) spectra are informative about product structure and overall quality. Because with substances such as excipients the quality range was investigated at some time in the past and fixed into a calibration, NIR measurement can provide simultaneous nondestructive confirmation of the predominant physical and chemical parameters. This is an effective method of reducing uncertainties about possible causes of failure or poor quality during production. Each time a given excipient fails its quality requirements at the moment of use, immediate action can be taken. Control is possible before the risk of failure is increased. Such an approach is complementary to container-wise identification of materials on delivery to a warehouse.

4.2.1.7 Promoting Physical Pharmacy and Pharmaceutical Sciences

Process analytical technology supposes a more science-based approach to pharmaceutical processes. As a matter of fact, it underlines the observed weakness in formal knowledge of the physical phenomena behind pharmaceutical processes. The physics is less well understood than the chemistry. Conventional physics has moved increasingly into the field of activity of engineers and technologists. Formal approaches are lacking. As a consequence, much highly valuable knowledge of physical phenomena is dispersed across various disciplines. Expertise in physics is often purely technological rather than being formalized and integrated into a specific discipline.

Just as the boundaries of physics and chemistry once merged to create physical chemistry, there is an opportunity now for assembling complementary

scientific knowledge from various disciplines. It is a major challenge to improve understanding through in-depth investigation of the physical phenomena behind pharmaceutical processes. This objective motivates the enforcement of physical pharmacy to improve process understanding through a grounding in theoretical physics.

One major issue is the science and technology of solid particles and powders: characterization, size and shape analysis, processing understanding, and so forth. Others include particle formation and fluid–particle separation, mixture stability, and understanding and simulating the dynamics of powder mixtures. For example, the compaction state of powders and mixtures may change rapidly depending on storage time and conditions. Time to use is not always under control and unexpected changes may occur. Stirring a mixture of two free-flowing powders of different size may result in segregation rather than improved mixture quality. The flow properties of powders depend not only on intrinsic characteristics of the different materials, such as particle size distribution, particle shape, and surface properties, but also on external conditions, such as humidity or compaction status. Further areas of interest include liquid drops, emulsions and colloids, bubbles, and polymers, as well as surface properties, surface analysis, interfacial and electrostatic phenomena, surface reactivity, wet chemistry properties, and solubility.

4.2.1.8 Data Mining

Complex processes generate large volumes of data over time. As ever-increasing volumes are collected and stored, the gap between buried information and usable accessible knowledge can quickly expand if care is not taken. Data mining extracts new knowledge out of accumulated observations and thus provides a basis for decision making and action. How to turn understanding of buried knowledge to best use? How to extract operational feedback from preexisting, but latent, dormant empirical knowledge? Such questions precede any data-mining project.

As a multidisciplinary technique, data mining sits at the interface between statistics, mathematics, and computer science. It is a collection of methods for detecting regularities and patterns and for extracting knowledge from massive databases using conventional and advanced analytical tools. Another approach to data mining is to view it as the multivariate modeling of a real environment on the basis of multidimensional and accumulated historical data. Thus, data mining is similar to explorative data analysis. It is driven by the data itself. However, it must be considered as different from conventional statistics due to the huge volume of processed data, far above the megabyte scale. Beyond this critical database dimension, most conventional statistical packages exceed their operational limit. Data mining can also be performed without the help of professional statisticians. It runs according to semiautomatic procedures, which makes it widely attractive and more likely to be used in an industrial environment.

Such situations are characteristic of pharmaceutical processes which accumulate a variety of historical data without consideration of pertinence. Accumulation is systematic and exhaustive. However, cross-links between data sources or types may not be established, leading to irrelevant and undetected redundancies. Reliability of the collected data is not clearly established over time and variations may not be detected.

4.2.1.9 Data Warehousing

The 1990s saw the development of data warehouses. An ideal data warehouse is a collection of historical data varying with time, organized by topic, aggregated in a unique database, and stored in a way that facilitates decision making (Figure 2). Three main functions are required to manage data warehouses. First, the data must be collected or else accessed by an alternative method, for example, as preexisting databases or files. Second, the data warehouse requires management and control tools. Only then can the third function operate, namely data analysis for the purpose of decision making and new knowledge. Dedicated information management tools mediate all external, operational, and historical data to the warehouse. Decisional information management components are used to extract and visualize the data warehouse information. Online analysis processing (OLAP) consists of the real-time analysis and visualization of the historical data. Data mining involves the extraction of rules and models constructed from the collected data.

Online analysis processing mainly comprises the interactive exploration of multidimensional data sets, or data cubes, which are manipulated by operations from matrix algebra, for example, slice-and-dice, roll-up, and drill-down. Computing performance is related to data warehouse size and also data quality, for example, missing data, unsharpness, and redundancy. The multidimensionality issue is critical for extracting pertinent information and selecting the results to be stored and visualized.

The data-mining tools now incorporated in much commercial software are a set of techniques and algorithms for exploring large databases in order to extract semantic links pertinent to event explanation and new knowledge acquisition. The more general goal of data mining is to extract rules and models for understanding connections and assisting decision making. There are numerous fields of application: risk analysis, manufacturing trends, raw material management, maintenance, process validation, development, quality control, and so forth. The idea behind data mining consists in introducing or proposing rules associated with likelihood coefficients established from a large set of existing (i.e., historical) data. The techniques used

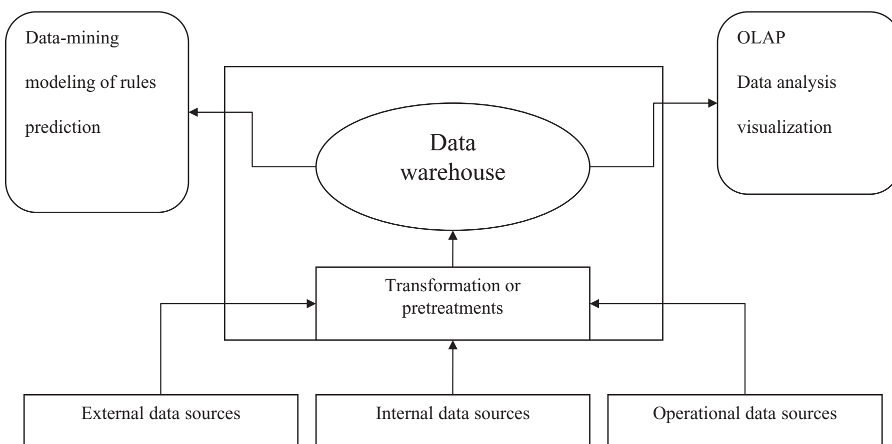

FIGURE 2 Schematic structure of a data warehouse.

are drawn from the fields of artificial intelligence and numerical and statistical data analysis, for example, functional modeling, learning machines, neuronal networks, Bayesian networks, support vector machines, modeling of associations, and explanatory rules, classifications, and segmentations. Their computing complexity derives from the dramatic up scaling from database to data warehouse level (from megabase to petabase, i.e., 10^6–10^{15}).

4.2.1.10 Data-Mining Methods for Pharmaceutical Processes

The data warehouse is a central repository of data accumulated over time from various origins: quality control, quality assurance, production, development, and the like. The accumulated data represent a potential gold mine, conferring competitive advantage by facilitating understanding of pharmaceutical process and optimizing it in the light of buried empirical knowledge.

Data mining is used to extract previously unexploited data and knowledge. Its potential for acquiring knowledge and generating explanatory rules can overcome the loss of data or underused accumulated data. There are two ways of proceeding. The first is proactive or directed, for example, hypothesis testing. Particular groupings or features are suspected, and verification or confirmation of identity is sought. The second is reactive or undirected, consisting of simple data exploration. Groupings are unknown, properties undetected or latent, and patterns unidentified. Alternative terms for these approaches are supervised and unsupervised learning, respectively. Top-down and bottom-up approaches complement one another. For example, the confirmatory tools of supervised learning can be used to verify and certify the quality of the discoveries obtained using the exploratory approach.

What can be obtained using data-mining tools? Here is a short list of achievable goals:

- Data characterization to extract or determine descriptors or indicators, for example, by generalizing, summarizing, or grouping
- Establishment of associative and explanatory rules
- Classification (supervised learning) of items or objects in classes according to a given probability
- Clustering of data items (unsupervised learning) in classes, after establishing class limits inductively from existing data sets
- Detection of similarities in time series
- Pattern recognition

Data from external and internal sources is integrated, aggregated, or associated in time series. Data items may contain errors or the data may be missing, unsharp, redundant, or contradictory. A language with operators and variables is required to establish models. Validity levels also have to be defined using suitable optimization and validation criteria. In addition, a search method is required to extract the data from the data warehouse and prepare it for analysis.

Data mining can, therefore, be considered as a three-step operation. Prior to any analysis, the collected data is preprocessed to integrate the warehouse, and some verification is performed to maintain the data level: for example, integration,

aggregation, or grouping of data from different internal and external sources. The data is then selected and data mining performed applying the appropriate algorithms or models. Results are visualized and interpreted for experts in the field.

4.2.1.11 Data-Mining Practice

Data mining is part of an action process known as a business intelligence chain (Figure 3). Data mining is a flexible solution to the recurrent problem of how to derive knowledge from data. The source for data mining is the existence of a large but buried data set. The corresponding data analysis is an intellectual method that applies only if integrated into the current operational process. Hypothesis testing, knowledge acquisition, and the generation of explanatory rules are directed by active collaboration between different process actors. Data mining is teamwork that requires expertise in various areas, such as information technology (IT), database management, and data analysis. However, the methods are available in commercial packages and may not require the expertise of traditional statisticians. It is the computer which is responsible for discovering patterns or identifying rules or features. In summary, data mining is a logical loop involving the following steps:

- Business understanding
- Precise setting of the data-mining project, for example:
  - Definition of realistic objectives
  - Field of treated data
  - Inventory of available or usable data
- Data preparation
  - Extraction from internal or external sources
  - Verification and correction
  - Pretreatment
- Warehouse construction
- Modeling, for example:
  - Description and visualization
  - Affinity grouping
  - Rules of association, explanatory rules
  - Clustering

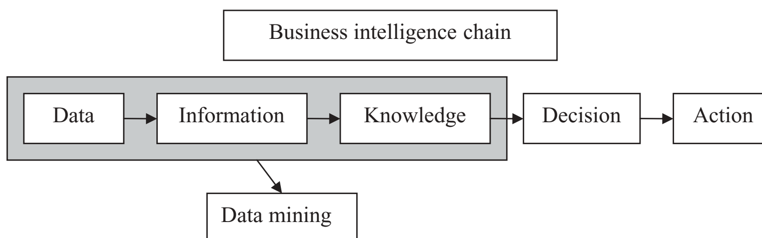

FIGURE 3 Place of data mining in the decision chain.

  - Classification
  - Estimation
  - Prediction
- Evaluation and comparison of models
- Documentation and presentation of results
- Deployment for action
- Back to business understanding.

4.2.1.12 Comments about Data Mining

Data mining provides an explanatory analysis from a confirmatory analysis. It is tempting to extract maximal value from available resources such as any kind of accumulated data. But maximal efficiency requires critical insight into the expertise actually buried in data collections or warehouses. The goal of data exploration is to access the buried data to acquire the knowledge that will make explanation, prediction, or estimation possible. That is why data mining requires team effort from data specialists, users, information technologists, and specialists in the relevant field (in this case, pharmaceutical process). It also requires senior management support throughout the organization. Mining is a matter of good practice according to established rules but also a challenge for innovative mathematical techniques. Not all patterns or rules found by data mining are interesting, although the results should remain logical and actionable by experts in the relevant field. Because the algorithms involved tend to be complex and the data volume is huge, software implementation together with the level of information technology are major considerations.

Data mining is driven by the accumulated data but always directed at solving a process, business, or research problem. The results are designed to make it easier to reach a diagnosis or make a decision. They are only likely to be useful in context: that is, they are not simply numbers and graphics but an aid to insight for experts in the relevant field. Also, no single mining technique is equally applicable. A range of different methods or algorithms should be considered, as no one particular technique will work equally well or outperform all other techniques on all problems. Nor will the value of an analytical technique exceed that of the data upon which it is based.

4.2.1.13 PAT Methods

Almost any existing analytical method can serve the objectives of PAT. Many online applications already exist. With newer techniques, like NIR imaging or matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, there are technological problems about performing online or inline analytics. Implementing a given analytical technique close to or during process does not always provide better process understanding. Attributes which are not informative should not be measured at all and are not worth the burden of complex process implementation.

Use of the various techniques listed in Table 1 depends on process requirements. The validity of a given technique or analytical application is challenged by every

TABLE 1 Analytical Methods for PAT

Method	Description	Online Application	Chemical Identification	Pharmaceutical Application Examples
Infrared, near-infrared, and Raman spectroscopy	Vibrational spectroscopy (discussed in this chapter)	×	×	Reaction monitoring Polymorphism Content determination Process monitoring (drying, granulation, blending)
Hyperspectral imaging	Vibrational spectroscopy coupled with a spatial analysis (cf. chemical imaging chapter)		×	Chemical compound distributions Counterfeit detection
UV–Vis spectroscopy	Photoelectron spectroscopy	×	×	Color measurement Dissolution testing Cleaning validation (ppm-level detection)
Terahertz spectroscopy	Far-infrared spectroscopy; 3D imaging		×	Polymorphism Coating integrity and thickness API distribution possible
Laser-induced breakdown spectroscopy	Plasma generated by a laser pulse and detection of the emitted light (destruction of sample)		×	Drug development Process troubleshooting
Laser diffraction	Interaction of a laser beam with particles and detection of the scattered light	×		Particle size determination
Effusivity	Combines thermal conductivity, density, and heat capacity	×		Mixing, blending, granulation monitoring
Acoustic methods	Active or passive	×		Solid, semisolid, and high viscose sample High shear granulation monitoring Crystallization monitoring

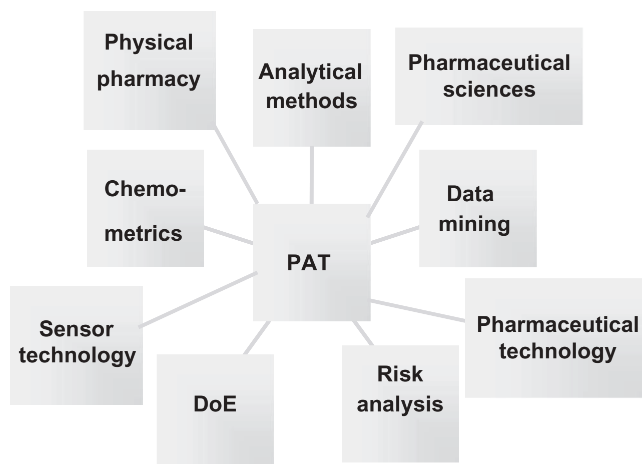

FIGURE 4 PAT constellation (DoE, design of experiments).

technological advance or new analytical technique. Innovation continuously drives optimization of overall process performance.

4.2.1.14 Conclusion

Process Analytical Technology can be viewed as a constellation placing greater or less emphasis on a given activity depending on the current problem or situation (Figure 4). There is no written rule or straightforward path to progress through PAT. Experience and expertise are necessary, together with a good knowledge of the pharmaceutical environment. Once a pharmaceutical company has decided to implement PAT, continuous management support for the development and maintenance of PAT-related activities is critical. It is a strategic and necessary step for the future success of PAT to encourage, stimulate, and initiate scientific collaboration and interaction as well as the relevant education and training. Better understanding and control of chemical and pharmaceutical processes are greatly needed, as well as the development of advanced measurement tools and data analysis methods.

A summary of PAT benefits follows:

- Immediate action if quality is not met
- Better process control and understanding
- Less uncontrolled variation and less production waste
- Better and more stable products
- Data collection and improved historical knowledge

Process analytical technology continuously improves product quality, extends the acquired knowledge base for new projects, and shortens time to market.

4.2.2 VIBRATIONAL SPECTROSCOPY

4.2.2.1 Introduction

Modern infrared (IR) spectroscopy is a versatile tool applied to the qualitative and quantitative determination of molecular species of all types. Its applications fall into three categories based on the spectral regions considered. Mid-IR (MIR) is by far the most widely used, with absorption, reflection, and emission spectra being employed for both qualitative and quantitative analysis. The NIR region is particularly used for routine quantitative determinations in complex samples, which is of interest in agriculture, food and feed, and, more recently, pharmaceutical industries. Determinations are usually based on diffuse reflectance measurements of untreated solid or liquid samples or, in some cases, on transmittance studies. Far-IR (FIR) is used primarily for absorption measurements of inorganic and metal-organic samples.

Within the electromagnetic spectrum (Figure 5), the IR region ranges from 12,800 to 10 cm^{-1} or from 0.78 to 1000 µm. The IR domain is conveniently subdivided into NIR, MIR, and FIR, respectively, with the following limits:

Near	0.78–2.5	4000–12,800
Mid	2.5–50	200–4000
Far	50–500	20–200

Methods and applications differ with the IR subregion considered. Academia and analytical chemists commonly consider MIR as the default region of interest. Current MIR instruments are completely different from traditional grating spectrophotometer technology. The generalization of Fourier transform (FT)–based spectrometers in the early 1980s lowered instrument prices and increased the number and types of MIR applications, in particular thanks to the use of interferometers in improving signal-to-noise ratios and detection limits. IR applications were originally limited to qualitative organic analysis. Almost from the outset, absorption MIR became a well-established application for structure elucidation. Organic chemists were trained in the visual and direct interpretation of MIR spectra. Nowadays mid-IR spectroscopy (MIRS) tends to be more viewed as a useful tool for the quantitative analysis of complex samples by absorption and emission spectrometry, which may require calibration and data pretreatment.

Near-IR measurements can be performed similarly to those using dedicated ultraviolet (UV) or visible spectrophotometers. Historically, the most important

0.1	0.4	0.8	2.5	50	500	3 000	µm
	UV	VIS	IR		Microwaves		
			Near	Middle	Far		
10^5	25 000	12 500	4 000	200	20	3.3	cm^{-1}

FIGURE 5 Limits and designation of the spectroscopic domains.

application was quantitative analysis in the food and feed industries. Only more recently have the chemical and pharmaceutical industries shown increasing interest in the NIR range. The major reason for the delay is in the type of information delivered. All observed bands result from overtones or combinations of overtones originating in the fundamental MIR region of the spectrum. Because the measurement method is nondestructive, samples are measured with little or no specific preparation. NIR spectra contain chemical and physical information on the sample. Direct interpretation is limited, if not impossible, meaning that multivariate data processing is routinely required to extract the relevant information. This led most analytical chemists to ignore the potential of NIR. Until the early 1990s, NIR spectrophotometers tended to be the dispersive type based on diffraction gratings. Subsequent technological advance has brought FT and diode array instruments. Filter instruments remain used for ultrarapid measurement of material composition in the food and feed industries.

Being at the edge of the IR region, FIR is believed to have less industrial potential. This is partly due to unresolved experimental and technological difficulties. FIR may provide relevant information, but at the cost of disproportionate effort. Routine use in the pharmaceutical environment is not anticipated in the near future, and for this reason we shall not discuss FIR further.

The most recent developments in IR/NIR technology include imaging large sample surfaces, nondestructive analysis of solids by attenuated total reflectance (ATR), and photoacoustic measurement. Instrument performance continues to increase, with particular respect to reliability and modularity. Spectrometer downsizing, speed of measurement, and mobility no longer represent critical challenges. However, what has really expanded the scope of MIR applications, and use of the full NIR region, has been the constant increase in computing power. The field of application of IR spectroscopy is moving toward the quantitative analysis of complex samples in various measurement modes. These types of samples are characteristic of the pharmaceutical industry. Noninvasive spectral sampling using light probes is at last making in situ analytics attractive, for example, for performing online real-time measurements.

Infrared microscopy was introduced in the early 1980s. Two microscopes, an ordinary optical microscope and an FT IR instrument with reflection optics, were combined. The optical microscope is used to visually locate the spot of interest. The spot is then irradiated with the IR or NIR beam. There are numerous applications for noninvasive measurement, including of contaminants, particles, imperfections, and for fiber identification. Chemical imaging systems (CIS) are a refinement of the technique. Spectra are collected from adjacent areas (pixels) on a larger surface. In practice, an imaging breakthrough became possible after moving away from pixel-after-pixel scanning. CIS flexibility and speed of acquisition improved with the introduction of new detectors, for example, focal plane array (FPA) detectors. Multiple IR/NIR spectra (up to many thousand) are scanned in a single step on the sample surface. With image analysis algorithms and fast computers, current NIR/IR imaging techniques hold fresh promise for resolving quality problems.

4.2.2.2 IR Spectroscopy Theory

In a typical IR absorption spectrum of an organic substance (Figure 6), the ordinate is transmittance and the abscissa is the wavenumber. A linear wavenumber scale is

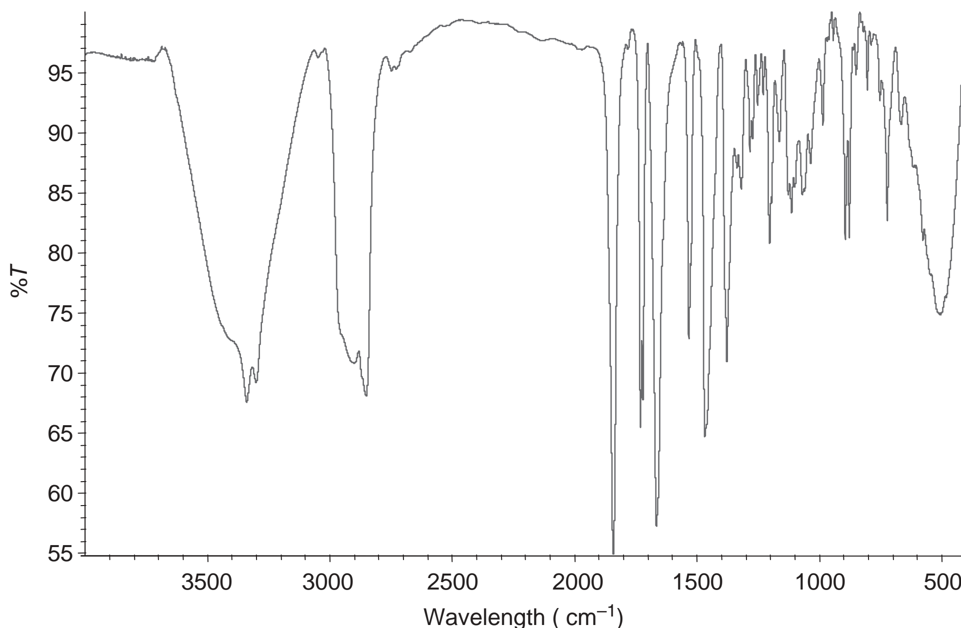

FIGURE 6 Typical example of an infrared absorption spectrum.

preferred because of the linear relationship between wavenumber and energy and frequency. The frequency of an absorbed radiation is the molecular vibrational frequency actually responsible for the observed absorption.

Infrared absorption, emission, or reflection for molecular species can be explained by assuming transitions from one rotational or vibrational energy state to another. IR radiation is not energetic enough to produce electronic transitions similar to those resulting from UV, visible (Vis), or X-ray radiation. Absorption of IR radiation is limited to molecular species with small energy differences between various vibrational and rotational states. In order to absorb IR radiation, a molecule must undergo a net change in dipole moment as a consequence of its vibrational or rotational motion. Under these circumstances an alternating electrical field interacts with the molecule and causes changes in the amplitude of one of its motions. The dipole moment is determined by the magnitude of the charge difference and the distance between the two charge centers. In addition, regular fluctuation in dipole moment occurs, and a field is established which interacts with the electrical field associated with the incident radiation. If the radiation frequency exactly matches a natural vibrational frequency of the molecule, a transfer of energy takes place that changes the amplitude of molecular vibrations and absorption of radiation results. Similarly, the rotation of asymmetric molecules around their centers of mass results in periodic dipole fluctuations which interact with radiation. Homonuclear species are not concerned and such compounds cannot absorb in the IR.

The amount of energy required to cause a change in energy level is approximately equivalent to radiation of $100\,cm^{-1}$ or less. The relative positions of atoms in a molecule fluctuate continuously, and multiple types of vibrations and rotations about the bonds in the molecule are possible. Exact analysis of all movements becomes

impossible for molecules comprising several atoms. Not only do larger molecules have more vibrating possibilities, but intercenter interactions occur that must be taken into account. Vibrations may be of the stretching and bending variety. Stretching vibration involves a continuous change in interatomic distance along the axis of the bond between the atoms. Bending vibration is characterized by a change in the angle between two bonds and comes in four types: scissoring, rocking, wagging, and twisting. All vibration types may be possible in a molecule containing more than two atoms. In addition, vibration interaction or coupling may occur if the vibrations involve bonds to a single central atom with a change in the characteristic of the vibrations concerned.

4.2.2.3 Mechanical Model of IR Vibration

Infrared spectra result from light absorption by organic molecules. The easiest way to describe vibrational spectroscopy from a theoretical perspective is to consider the isolated vibrations of a mechanical model called the harmonic oscillator. Atomic stretching vibration behavior can be approximated by a mechanical model consisting of two masses, m_1 and m_2, connected by an ideal spring. Displacement of one such mass along the spring axis results in harmonic motion. Many fundamental frequencies may be calculated by assuming that band energies arise from the vibration of the ideal diatomic harmonic oscillator (Figure 7), obeying Hooke's law, that is,

$$\upsilon = \frac{1}{2}\pi\sqrt{\frac{k}{u}}$$

where υ is the vibrational frequency, k the classical force constant, and $u = m_1 m_2/(m_1 + m_2)$, the reduced mass of the two atoms.

The model provides a good description of true diatomic molecules and is not far from the average value of two atoms stretching within a polyatomic molecule. The corresponding potential-energy curve is the typical parabola illustrated in Figure 8. This approximation gives the average vibration frequency of the bond. For example, the reduced masses for C—H, O—H, and N—H are 0.85, 0.89, and 0.87. These figures are similar, so the frequencies would be quite similar too. However, the electron-withdrawing and -donating properties of neighbors within molecules act

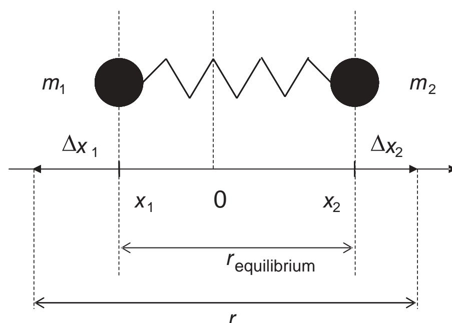

FIGURE 7 Ideal diatomic harmonic oscillator.

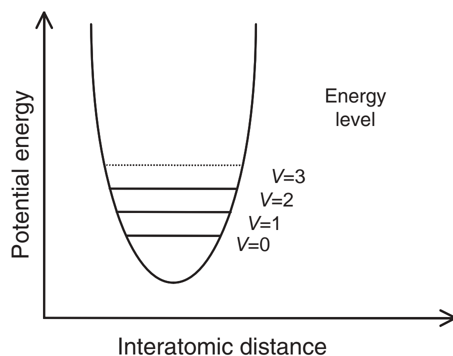

FIGURE 8 Energy diagram of the ideal diatomic oscillator.

on the observed band strength, length, and frequency. An average value is of little use in structural determinations and these differences cause a real spectrum to develop. The force constant k is a measure of the stiffness of the chemical bond and is the equivalent of the force constant of the spring in the harmonic model. The k values vary widely and cause energy differences which can both be calculated and utilized in spectral interpretation. It has been possible to evaluate some force constants for various types of chemical bonds by IR spectroscopy. Generally, k has been found to range between 3×10^2 N/m and 8×10^2 N/m for most single bonds (average: 5×10^2 N/m). Double and triple bonds are found to have k values two and three times this average, respectively. In practice, these average experimental values can be used to estimate the wavenumbers of fundamental absorption peaks, that is, peaks of the transition from the ground state to the first excited state, for a variety of bond types.

Classical mechanics does not apply to the atomic scale and does not take the quantized nature of molecular vibration energies into account. Thus, in contrast to ordinary mechanics where vibrators can assume any potential energy, quantum mechanical vibrators can only take on certain discrete energies. Transitions in vibrational energy levels can be brought about by radiation absorption, provided the energy of the radiation exactly matches the difference in energy levels between the vibrational quantum states and provided also that the vibration causes a fluctuation in dipole.

4.2.2.4 Quantum Mechanical Model

Unlike the classical spring model for molecular vibrations, there are not an infinite number of energy levels. Instead of a continuum of energies, there are discrete energy levels described by quantum theory. The time-independent Schrödinger equation is solved using the vibrational Hamiltonian for a diatomic molecule. Values for the ground state ($\upsilon = 0$) and succeeding excited states can be calculated by solving the equation (Figure 8). Absorption of a photon of the correct energy can cause the molecule to change between vibrational energy levels. At room temperature only the ground state has a significant population, and so transitions due to absorption at these temperatures occur from the ground state. Transitions between ground state to energy level 1 give the fundamental absorption if this leads to a

change in molecular dipole moment. Transitions between ground state and energy level 2 or above give overtones. Transitions between multiple states can occur and give rise to combination bands.

A simplified version of the energy levels may be written for the energy levels of a diatomic molecule:

$$E_\upsilon = \left(\upsilon + \frac{1}{2}\right)\frac{h}{2\pi}\sqrt{\frac{k}{u}} \qquad \upsilon = 0, 1, 2, \ldots$$

in which Hooke's law terms can be seen. Rewritten using the quantum term $h\bar{V} = (h/2\pi)\sqrt{k/u}$, the equation reduces to

$$E_\upsilon = \left(\upsilon + \frac{1}{2}\right)h\bar{V} \qquad \upsilon = 0, 1, 2, \ldots$$

In the case of polyatomic molecules, the energy levels become quite numerous. Ideally, one can treat such a molecule as a series of diatomic, independent, harmonic oscillators and the above equation can be generalized:

$$E(\upsilon_1, \upsilon_2, \upsilon_3, \ldots) = \sum_{i=1}^{3N-6}\left(\upsilon_i + \frac{1}{2}\right)h\bar{V}_i \qquad \upsilon_1, \upsilon_2, \upsilon_3, \ldots = 0, 1, 2, 3, \ldots$$

Any transition of an energy state from 0 to 1 in any one of the vibrational states (υ_1, υ_2, υ_3, …) is fundamental and allowed by selection rules. Where the transition is from the ground state to $\upsilon_i = 2, 3$, and so on and all others are zero, it is known as the first overtone, the second overtone, and so on. Transitions from the ground state to a state for which $\upsilon_i = 1$ and $\upsilon_j = 1$ simultaneously are combinations. Other combinations, such as $\upsilon_i = 1$, $\upsilon_j = 1$, $\upsilon_k = 1$, or $\upsilon_i = 2$, $\upsilon_j = 1$, and so forth are also possible. Typically, NIR spectra will contain these overtones and combinations derived from the fundamental vibrations which appear in the MIR. Overtones and combinations are not allowed, but appear as weak bands due to anharmonicity or Fermi resonance. As a rule, overtones occur at one-half and one-third of the fundamental absorption wavelength or 2 and 3 times the frequency. The majority of overtone peaks arise from the R—H stretching and bending modes because the dipole moment is high: O—H, C—H, S—H, and N—H are strong NIR absorbers and form most NIR bands. Since most absorption is repeated in the NIR range, this region is likely to be used to identify a molecule, as with MIR. As a consequence, IR bands are traditionally used to identify functional groups which have characteristic frequencies. NIR spectra are more overlapping, and, although bands can be identified, they cannot be placed in relation to the rest of the molecule. NIR spectra are, therefore, mainly used to confirm the identity of a material, as for true identification.

As given from the quantum mechanics equations, the energy for transition from energy levels 1 to 2 or 2 to 3 should be identical to that for transition from 0 to 1. Furthermore, quantum theory states that the only transitions that can take place are those for which, according to vibrational quantum theory, the vibrational quantum number changes by unity. This is the so-called selection rule.

So far we have illustrated the classic and quantum mechanical treatment of the harmonic oscillator. The potential energy of a vibrator changes periodically as the distance between the masses fluctuates. In terms of qualitative considerations, however, this description of molecular vibration appears imperfect. For example, as two atoms approach one another, Coulombic repulsion between the two nuclei adds to the bond force; thus, potential energy can be expected to increase more rapidly than predicted by harmonic approximation. At the other extreme of oscillation, a decrease in restoring force, and thus potential energy, occurs as interatomic distance approaches that at which the bonds dissociate.

In theory, the wave equations of quantum mechanics can be used to derive near-correct potential-energy curves for molecular vibrations. Unfortunately, the mathematical complexity of these equations precludes quantitative application to all but the very simplest of systems. Qualitatively, the curves must take the anharmonic form. Such curves depart from harmonic behavior by varying degrees, depending on the nature of the bond and the atom involved. However, the harmonic and anharmonic curves are almost identical at low potential energies, which accounts for the success of the approximate methods described.

Anharmonicity leads to deviations of two kinds. At higher quantum numbers, ΔE becomes smaller, and the selection rule is not rigorously followed; as a result, transitions of $\Delta \pm 2$ or ± 3 are observed. Such transformations are responsible for the appearance of overtone lines at frequencies approximately two or three times that of the fundamental line; the intensity of overtone absorption is frequently low, and the peaks may not be observed. Vibrational spectra are further complicated by the fact that two different vibrations in a molecule can interact to give absorption peaks with frequencies that are approximately the sums or differences of their fundamental frequencies. Again, the intensities of combination and difference peaks are generally low.

It is ordinarily possible to deduce the number and kinds of vibrations in simple diatomic and triatomic molecules and determine whether these vibrations contain several types of atoms as well as bonds; for these molecules, the multitude of possible vibrations gives rise to IR spectra that are difficult, if not impossible, to analyze. The number of possible vibrations in a polyatomic molecule can be calculated as follows. Three coordinates are needed to locate a point in space; fixing N points requires $3N$ coordinates. Each coordinate corresponds to one degree of freedom for one of the atoms in a polyatomic molecule; for this reason, a molecule containing N atoms is said to have $3N$ degrees of freedom. A molecule features three types of motion. First, the motion of the entire molecule through space; second, the rotational motion of the entire molecule around its center of gravity; and, third, the vibrations of each of its atoms relative to the other atoms. Since all atoms in the molecule move in concert through space, definition of translational motion requires three of the $3N$ degrees of freedom. Another 3 degrees of freedom are needed to describe the rotation of the molecule as a whole. The remaining $3N - 6$ degrees of freedom involve interatomic motion and hence represent the number of possible vibrations within the molecule. In a linear molecule 2 degrees of freedom suffice to describe rotational motion. Thus, the number of vibrations for a linear molecule is $3N - 5$. Each of the $3N - 6$ or $3N - 5$ vibrations is a normal mode. For each normal mode of vibration there is a potential energy relationship. In addition, to the extent that a vibration approximates harmonic behavior, the differences between the

energy levels of given vibrations are the same; that is, a single absorption should appear for each vibration in which there is a change in dipole.

However, fewer experimental peaks may be observed than would be expected from the theoretical number of normal modes. Fewer peaks can be found when the symmetry of the molecules is such that no change in dipole results from a particular vibration. The energies of two or more vibrations can be identical or nearly identical. In some cases absorption intensity is too low to be detected by ordinary means. It may also happen that the vibrational energy is in a wavelength region which is beyond the range of the instrument.

Conversely, more peaks may be found than expected from the number of normal modes. This is the typical situation that concerns the NIR domain. Overtone peaks at two or three times the frequency of a fundamental peak, or addition combination bands at approximately the sum or difference of two fundamental frequencies, are sometimes encountered. The energy of a vibration and thus the wavelength of its absorption peak may be influenced by, or coupled with, other vibrators in the molecule. A number of factors influence the extent of such coupling. Vibration coupling is a common phenomenon. As a result, the position of an absorption peak corresponding to a given organic functional group cannot always be specified exactly. While interaction effects may lead to uncertainties in the identification of functional groups contained in a compound, it is this very effect that provides the unique features of an IR absorption spectrum that are so important for the positive identification of a specific compound.

4.2.2.5 Anharmonicity

The ideal harmonic oscillator is a somewhat limited model. As the oscillating masses get very close, real compression forces—which are neglected in calculations—fight against the bulk of the spring. As the spring stretches, it eventually reaches a point where it loses its shape and fails to return to its original coil. This ideal case is shown in Figure 9. The barriers at either end of the cycle are approached in a smooth and orderly fashion. Likewise, in molecules, the respective electron clouds of the two bound atoms limit approach by the nuclei during the compression step, creating an energy barrier. At extension of the stretch, the bond eventually breaks when the vibrational energy level reaches the dissociation energy. The barrier at smaller dis-

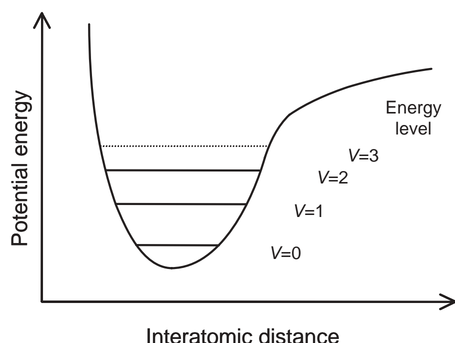

FIGURE 9 Energy diagram of the anharmonic diatomic oscillator.

tances increases at a rapid rate, while the barrier at the far end of the stretch slowly approaches zero (Figure 9). The shape of the potential energy curve is typical of an anharmonic oscillator.

Energy levels in the anharmonic oscillator are not equal, although they become slightly closer as energy increases. This phenomenon can be seen in the following equation:

$$E_\upsilon = \left(\upsilon + \frac{1}{2}\right) hW_e - \left(\upsilon + \frac{1}{2}\right)^2 W_e X_e + \text{higher terms}$$

where $W_e = \frac{1}{2}\pi(K_e/u)^{1/2}$ is the vibrational frequency, W_eX_e the anharmonicity constant, K_e the anharmonicity force constant, and u the reduced mass of the two atoms. In practice, anharmonicity is between 1 and 5%. Thus, the first overtone of a fundamental vibration set, for example, at 3500 nm would be

$$\upsilon = \frac{3500}{2} + (3500 \times [0.01, 0.02, \ldots])$$

Depending on structural or steric conditions, the number may range from 1785 to 1925 nm for this example. However, it would generally appear at 3500/2, plus a relatively small shift to a longer wavelength. As forbidden transitions, the overtones are between 10 and 1000 times weaker than the fundamental bands. Thus, a band arising from bending or rotating atoms would have to be in its third or fourth overtone to be seen in the NIR region of the spectrum. For example, a fundamental carbonyl stretching vibration at 1750 cm^{-1} or 5714 nm would have a first overtone at approximately 3000 nm, a weaker second overtone at 2100 nm, and a third very weak overtone at 1650 nm. The fourth overtone, at about 1370 nm, would be so weak as to be useless. These figures are based on an illustrative 5% anharmonicity constant.

The detailed examination of the spectra of simple molecules is a direct source to determine the characteristic NIR frequencies for selected vibration modes. For qualitative and quantitative analyses there is the requirement to interpret as much as possible the NIR spectrum. Although interpretation of spectra in a manner analogous to MIR is not conceivable, attempts exist to define and categorize observed NIR frequencies. Examples of reported frequencies for aliphatic hydrocarbons are given in the following list:

8547 cm^{-1}	C—H second overtone in CH═CH
8474 cm^{-1}	C—H group in cis olefins
7700–9000 cm^{-1}	C—H second overtone
8696 cm^{-1}	Second overtone of CH_2 antisymmetric stretching
8285 cm^{-1}	Second overtone of CH_2 symmetric stretching
1080–1140 cm^{-1}	Second overtone olefin
7692, 8237, 8576 cm^{-1}	C—H stretching second overtone in CH_2

4.2.2.6 Structure Elucidation Using MIRS

Mid-IR absorption and reflectance spectroscopy is typically used for determining the structure of organic and biochemical species. When used in conjunction with

other analytical methods, such as mass spectroscopy, nuclear magnetic resonance, and elemental analysis, IR spectroscopy usually achieves positive species identification. Spectra are obtained after sample preparation, usually involving dilution of the analyte. Sample handling is the difficult and time-consuming part of the analysis. Organic samples exhibit numerous IR absorption peaks used for qualitative structure confirmation. First, presumptive functional groups are identified by examining their frequency region from about 3600 to 1200 cm^{-1}. As mentioned earlier, the frequency at which an organic functional group absorbs radiation can be approximated from the atomic masses and bond forces between them. These group frequencies are not totally invariant because of interactions with other vibrations. However, such interaction effects are small, and a range of frequencies can be assigned within which it is highly probable that the absorption peak for a given functional group will be found. Group frequencies are listed in correlation charts, which serve as a starting point in the identification process.

Second, the spectrum of the unknown is compared with the spectra of reference compounds featuring all the functional groups found in the first step. The fingerprint region from 1200^{-1} to 600 cm^{-1} is extremely useful because small differences in structure and constitution produce significant changes in the appearance and distribution of absorption peaks in this region. Most single bonds give rise to absorption bands at these frequencies. Because their energies are about the same, strong interaction occurs between neighboring bonds. The absorption bands are thus composites of these various interactions and depend upon the overall skeletal structure of the molecule. Exact interpretation in this region is seldom possible because of spectral complexity. On the other hand, it is this complexity that leads to uniqueness and the consequent usefulness of the region in final identification. A close match between two spectra in the fingerprint region constitutes almost conclusive compound identification.

In employing group frequencies it is essential that the entire spectrum rather than a small isolated portion be considered and interrelated. Correlation charts serve only as a guide for further and more careful study. Catalogs of IR spectra that assist in qualitative identification by providing comparison and reference spectra for a large number of pure compounds are commercially available on electronic media. Optimized search systems for identifying compounds from IR spectral databases and algorithms for the matching step produce rapid and reliable potential hits.

4.2.2.7 Extending Use of MIRS

Organic and inorganic molecular species (except homonuclear molecules) absorb in the IR region. IR spectroscopy has the potential to determine the identity of an unusually large number of substances. Moreover, the uniqueness of a MIR spectrum confers a degree of specificity which is matched or exceeded by relatively few other analytical methods. This specificity has found particular applications for the development of quantitative IR absorption methods. However, these differ from quantitative UV/Vis techniques in their greater spectral complexity, narrower absorption bands, and the technical limitations of IR instruments. Quantitative determinations obtained from IR spectra are usually inferior in quality and robustness to those obtained with UV/Vis and NIR spectroscopy. In addition, univariate or linear cali-

bration curves require meticulous attention to numerous details. One cause of failure is the frequent nonadherence to Beer's law due to the inherent complexity of IR spectra, featuring overlapping absorption peaks or disturbance by stray radiation. Analytical uncertainties cannot be reduced to a level which is comparable to other methods, despite considerable effort or care.

Diffuse-reflectance MIRS has found a number of applications for dealing with hard-to-handle solid samples, such as polymer films, fibers, or solid dosage forms. Reflectance MIR spectra are not identical to the corresponding absorption spectra, but sufficiently close in general appearance to provide the same level of information. Reflectance spectra can be used for both qualitative and quantitative analysis. Basically, reflection of radiation may be of four types: specular, diffuse, internal, and attenuated total.

Specular reflection is encountered when the reflecting medium is a smooth polished surface. The angle of reflection is identical to the incident angle of the radiation beam. If the surface is IR absorbent, the relative intensity of reflection is less for wavelengths that are absorbed than for wavelengths that are not. Thus, the plot of reflectance R, defined as the fraction of reflected incident radiant energy versus the wavelength (or wavenumber) appears similar to a transmission spectrum for the sample.

Diffuse-reflectance spectra are obtained directly from powder samples after a minimum of preparation. In addition to the time saved, measurement is nondestructive, leaving the sample intact for further analysis. The widespread use of diffuse reflectance was only possible with the introduction of the FT technique. Reflected radiation from powders is too low to be measured at medium resolutions or inadequate signal-to-noise ratios. Diffuse reflectance (Figure 10) occurs when a beam of radiation strikes the surface of a finely divided powder. With this type of sample, specular reflection occurs at each plane surface. However, since there are many of these surfaces and they are randomly oriented, radiation is reflected in all directions. The intensity of the reflected radiation is independent of the viewing angle. If peak locations are identical in reflectance and transmittance spectra, relative peak heights differ considerably. For example, minor transmittance peaks generally appear larger in reflectance spectra.

Internal-reflection spectroscopy is used to obtain IR spectra of hard-to-handle or hard-to-prepare samples such as solids with limited solubility, films, pastes, adhesives, and powders. Reflection occurs when a beam of radiation passes from a denser to a less dense medium. The fraction of incident beam which is reflected increases as the angle of incidence becomes larger. Beyond a certain critical angle, reflection is complete. During the reflection process the beam penetrates a small distance into

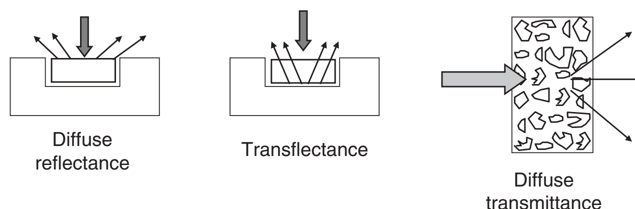

FIGURE 10 Diffuse reflectance, transflectance, and transmittance measurements.

the less dense medium before reflection occurs. The depth of penetration varies from a fraction of a wavelength up to several wavelengths and depends on the wavelength of incident radiation, the refraction indices of the two materials, and the angle of incident beam with respect to the interface.

Attenuated total reflection (ATR) is the most common reflectance measurement modality. ATR spectra cannot be compared to absorption spectra. While the same peaks are observed, their relative intensities differ considerably. The absorbances depend on the angle of incidence, not on sample thickness, since the radiation penetrates only a few micrometers into the sample. The major advantage of ATR spectroscopy is ease of use with a wide variety of solid samples. The spectra are readily obtainable with a minimum of preparation: Samples are simply pressed against the dense ATR crystal. Plastics, rubbers, packaging materials, pastes, powders, solids, and dosage forms such as tablets can all be handled directly in a similar way.

4.2.2.8 Raman Spectroscopy

When radiation passes through a transparent medium, a fraction of the beam scatters in all directions. A small fraction of the scattered radiation differs from the incident beam, showing shifts in wavelength determined by the chemical structure of the molecules in the medium. The same types of quantized vibrational changes associated with IR absorption occur, and the difference in wavelengths between incident and scattered radiations corresponds to wavelengths in the MIR. The Raman scattering spectrum and IR absorption spectrum for a given species are very similar. Figure 11 illustrates a typical Raman spectrum. IR is generally the method of choice, but in some cases Raman spectroscopy offers more information about certain types of organic compounds. For example, it is sensitive to conformational

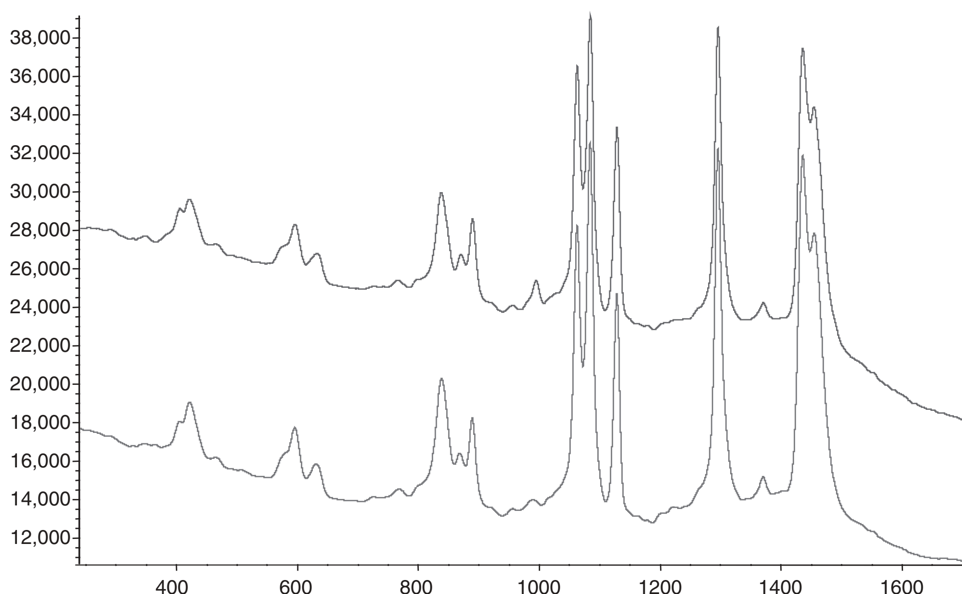

FIGURE 11 Example plot of two Raman spectra (two polymorphic forms of an excipient).

and environmental information. Peak overlap in compound mixtures is less likely, and quantitative determinations are easier. In particular, accurate quantitative determination can be performed on very small samples. Despite these advantages, Raman spectroscopy has not yet been exploited due to the rather high cost of the instruments.

There are differences between the kinds of groups that absorb in the IR and those that are Raman active. Parts of Raman and IR spectra are complementary, each being associated with a different set of vibrational modes within a molecule. Other vibrational modes may be both Raman and IR active. The intensity or power of a Raman peak depends in a complex way on the polarizability of the molecule, the intensity of the source, and the concentration of the active group, as well as other factors. Raman intensities are usually directly proportional to the concentration of the active species.

In Raman spectroscopy, the excitation radiation occurs at a wavelength distant from any absorption peaks of the analyte. The mechanism which leads to Raman spectra is different from that of MIR spectra, although dependent upon the same vibrational modes. IR absorption requires a change in dipole moment or its associated charge distribution. Only then can radiation of the same frequency interact with the molecule to promote an excited vibrational state. In contrast, scattering involves momentary distortion of the electron cloud distributed around a bond in a molecule, followed by reemission of the radiation as the bond returns to its normal state. In the distorted form the molecule is temporarily polarized. A dipole is momentarily induced which disappears upon relaxation and reemission. Thus, the Raman activity of a given vibrational mode may differ markedly from its IR activity. For example, a homonuclear molecule has no dipole moment either in equilibrium or when stretched, and IR absorption of radiation at the exciting frequency cannot occur. On the other hand, the polarizability of the bond between the two atoms of such a molecule varies periodically in phase with the stretching vibrations, reaching a maximum at the greatest separation and a minimum at the closest position. A Raman shift corresponding in frequency to that of the vibrational mode results. Raman shift magnitude is independent of excitation wavelength. Thus shift patterns are identical regardless of the laser used for excitation.

In MIR spectroscopy water always causes interference, which is not the case with Raman scattering. Thus, Raman spectra can be obtained directly from aqueous solutions. In addition, glass or quartz cells can be employed. The development of Raman spectroscopy was closely associated with the availability of readily usable laser beams. Raman spectra are obtained by irradiating the sample with a laser source of visible or NIR monochromatic radiation. During irradiation, the scattered radiation is acquired at some angle (e.g., 90°) with a suitable device. Raman lines are 0.001% or less intense than the source and more difficult to detect than IR spectra. Raman measurement can be restricted by fluorescence or impurities in the sample. This problem has been partly solved by the use of NIR laser sources, which operate at longer wavelengths. Much higher power can irradiate the sample without causing photodecomposition or simply heating. NIR lasers are not energetic enough to populate a significant number of fluorescence-producing excited electronic energy states in most molecules. Fluorescence is less intense or nearly nonexistent.

Scattered radiation is of three types: Stokes, anti-Stokes, and Rayleigh. The wavelength of Rayleigh scattering is identical to that of the excitation source and

significantly more intense than either of the other two types. By convention Raman spectra are plotted with the abscissa defined as the difference in wavenumbers between the observed radiation and that of the source. As anti-Stokes lines are less intense than the corresponding Stokes lines, only this part of the spectrum is used. However, when fluorescence occurs and interferes with the observations of the Stokes lines, the anti-Stokes part of the spectrum proves helpful, despite the lower intensities involved.

4.2.2.9 Introducing NIRS

Until recently the NIR region was not considered particularly useful for the spectroscopy of organic compounds. The combination and overtone bands of molecular vibrations occur in the relatively narrow region of 750–3000 nm compared to the fundamental bands occurring at 2800–50,000 nm. This was considered as a drawback. In addition it was observed that NIR bands severely overlapped, were difficult to resolve, and, once resolved, difficult to interpret. If samples were not dried prior to NIR analysis, the changes in hydrogen bonding due to the effects of sample temperature, ionic strength, and analyte concentration could also complicate interpretation of frequently overlapping NIR spectral bands. Changes in hydrogen bonding produce band shifts as well as flattening or broadening of band shapes. The overtone and combination molecular absorptions found within the NIR region are inherently much less intense than the fundamental IR absorptions. Thus, the changes in absorbance in the NIR region are quite small with respect to changes in concentration. The relatively small extinction coefficients due to combination and overtone NIR bands severely restrict the allowable noise levels and stability of any NIR instrument that is used for quantitative work. Sample presentation and the relatively straightforward reflectance measurement involved aspects which were not expected with traditional IR spectroscopy.

If analytical information is obtained with better resolution in the IR region, why should a chemist be interested in NIRS? Numerous difficulties inherent in the use of qualitative NIRS led to its rejection by analytical chemists. Karl Norris, an engineer at the U.S. Department of Agriculture, demonstrated the potential value of the NIR region for quantitative work by making measurements of agricultural products in the 1960s. The basic idea was to provide various research and production facilities with online NIR measurements of agricultural products. NIRS has now become widespread in the chemical and pharmaceutical industry, with the publication of multiple practical applications and a massively increased presence in specialized journals.

4.2.2.10 Benefits of NIRS

The NIR region is of great interest for pharmaceutical applications. NIRS is fast, nondestructive, and cost effective. Samples require no preparation and can be measured as such, intact and available for further analysis. NIRS can be performed in-, on-, and offline. Also, glass fiber optics can be used to perform remote analysis, thus bringing radiation directly to the sample. Many more advantages can be cited when considering the practical use of NIR in a pharmaceutical process, depending on the particular objective.

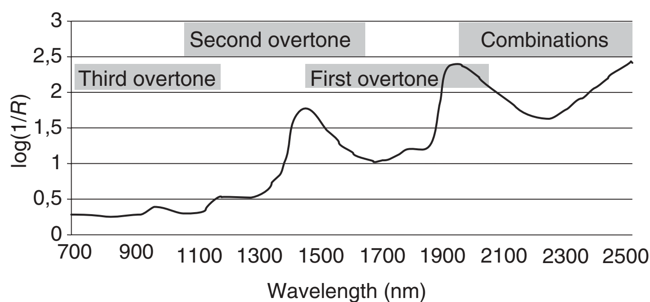

FIGURE 12 Typical NIR spectrum.

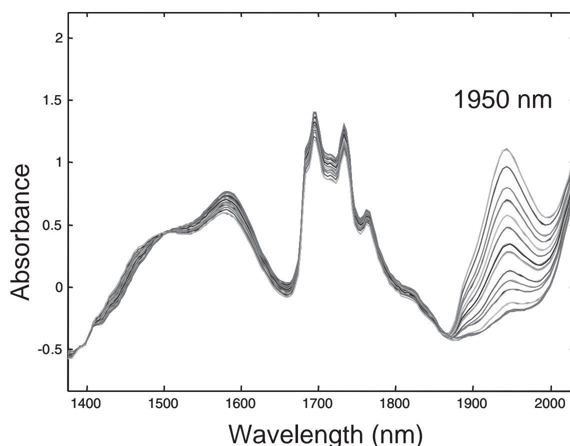

FIGURE 13 Collection of NIR spectra. The varying water band near 1950 nm can be clearly identified.

As already mentioned, absorption bands in this region are overtones or combination bands of fundamental stretching bands that occur in the 3000–1700 cm^{-1} region (Figure 12). The bonds involved are usually C—H, N—H, and O—H. Because the bands are overtones or combinations, their molar absorptivities are low and detection limits are around 0.1%.

Sample handling is simplified as glass can be used for windows, lenses, and any other optical components. In addition, the laser source is easily focused on small sample area. Very small samples can be investigated without time-consuming preparation. It is also possible for the source radiation to be transmitted through optical fibers. The fiber-optic probe can be in contact with the sample or immersed in it. The probe consists of input fibers surrounded by several collection fibers that transport the scattered radiation to the monochromator. This makes it possible to collect spectra directly under relatively adverse conditions.

In contrast to its MIR counterpart, an important application of NIRS is the routine quantitative determination of species, such as water, proteins, hydrocarbons, and fats, for example, in food or feed products, but also in the petroleum and chemical industries. Figure 13 illustrates a collection of spectra from a pharmaceutical product with varying water content. It shows that a quantitative application can be

developed once a given physical parameter varies, which can then be specifically modeled. The spread of the NIR technique in the pharmaceutical industry was encouraged by advances in computer technology and data handling. Both diffuse reflectance and transmittance measurements (Figure 10) are used, although diffuse reflectance much more widely because of its ease of use. Many spectrometers were specifically designed for the NIR range. Instrument variety is wide and much more varied than in the MIR range. The most sophisticated are dual-beam grating, diode-array, and FT instruments. Simpler filter instruments are still available and remain valuable tools. These instruments can basically be viewed as similar to those used for UV/Vis analysis. Tungsten–halogen lamps with quartz windows serve as sources. Detectors are usually lead sulfide (PbS) or arsine–gallium (AsGa) detectors and many instruments are designed to operate from 180 to 2500 nm.

The NIR spectra are less useful for identification and more useful for quantitative analysis of compounds containing functional groups made up of hydrogen bonded to carbon, nitrogen, or oxygen. Such compounds can often be determined with an accuracy and precision equivalent to UV/Vis spectroscopy, rather than with MIR spectroscopy. Many currently implemented applications concern the determination of water in variety of samples. Reflectance NIRS has become a reliable tool for the quantitative determination of constituents in solids. The first use of this fast technique was for the determination of protein, moisture, starch, oil, lipids, and cellulose in feed and food products. Sample handling, measurement procedure, and data treatment were established with these first applications. Typically in NIR the solid sample is irradiated with one or more narrow bands or the full range of radiation from 1 to 2.5 μm. Diffuse reflectance occurs in which the radiation penetrates the surface layer of the particles, excites the vibrational modes of the analyte molecule, and is then scattered in all directions. A reflectance spectrum is thus produced that is dependent upon the sample composition. In this case the ordinate is the logarithm of the reciprocal of reflectance R, $\log(1/R)$, where R is the ratio of the intensity of radiation reflected from the sample to reflectance from a standard reflector, such as finely ground barium sulfate or magnesium oxide. The typical reflectance band at 1940 nm is a water peak used for moisture determination, as can be seen in Figure 13.

Many diffuse-reflectance instruments are available. Some employ several interference filters to provide narrow bands of radiation. Others are equipped with grating monochromators. Ordinarily, calibration is often a stringent requirement as samples must be acquired of the material for analysis that contain the range of analyte concentrations likely to be encountered. It may be useful to grind solid samples to a reproducible particle size. Equations are developed and used for the analysis. Once method development has been completed and validated, solid samples can be analyzed in a few minutes. Accuracy and precision are reported to be of 1 to 2% relative.

In addition to the somewhat empirical and difficult development of NIR applications, thorough documentation must be produced. NIR methods have to comply with the current good manufacturing practice (cGMP) requirements used in the pharmaceutical industry. Various regulatory aspects have to be carefully considered. For example, NIR applications in classification, identification, or quantification require extensive model development and validation, a study of the risk impact of possible errors, a definition of model variables and measurement parameters, and

comprehensive data analysis. Further, clearly defined operating procedures and user training are required for routine analysis. General regulatory requirements also require valid documentation to have been maintained on the life cycle of the NIR model, spectrometer, computer, and the like.

4.2.2.11 Introducing MIR/NIR Chemical Imaging

Chemical compound homogeneity is an important issue for pharmaceutical solid forms. A classical spectrometer integrates the spatial information. In solid form analysis, use of a mean spectrum on a surface can be a drawback. For example, in the pharmaceutical industry it is important to map the distribution of active ingredients and excipients in a tablet so as to reveal physical interaction between the compounds and help to solve homogeneity issues. Spectroscopic imaging techniques that visualize chemical component distribution are thus of great interest to the pharmaceutical community.

Vibrational hyperspectral imaging is the most recent development to combine chemical information from spectroscopy with spatial information in the sample. In principle, hyperspectral images can be collected using single-point detectors, that is, classical scanning or mapping with microscopes. The commercialization of FPA detectors promoted imaging using a more rewarding analytical method. Array detectors with multiple detector elements measure all pixels on the mapped surface simultaneously, thus drastically reducing recording time, and provide a uniform background to improve the signal-to-noise ratio. A complete spectrum is acquired for each pixel, meaning that a hyperspectral image is in fact a data cube, that is, a three-dimensional (3D) matrix. Hyperspectral imaging provides spatial and spectral information as well as qualitative and quantitative information.

Imaging techniques are typically effective when applied to pharmaceutical tablets in order to explore qualitative aspects or visually address process issues: dissolution, polymorph distribution, moisture content determination, active pharmaceutical ingredient (API) localization and characterization, content uniformity, blending, and granulation. Conventional spectroscopy cannot provide this kind of information. Chemical compound identification or particle size determination can also be estimated by imaging. Selection of an imaging technique will depend on several criteria, such as spatial and spectral resolutions, time of measurement, and wavelength range.

The CISs are rapidly becoming more popular and reliable as their field of application broadens. This is mainly due to the production of surface images by multipoint scanning and mapping. Hyperspectral imaging has proven its potential for qualitative analysis of pharmaceutical products and can be used when spatial information becomes relevant for an analytical application. Even if online applications and regulatory method validation require further development, the power of CIS in quality control and PAT needs no further demonstration, whatever the wavelength domain or method of spectra collection.

4.2.2.12 Design of MIR Instruments

Different types of IR instruments are available: dispersive grating spectrophotometers, FT instruments, and nondispersive photometers. Until the 1980s, and the

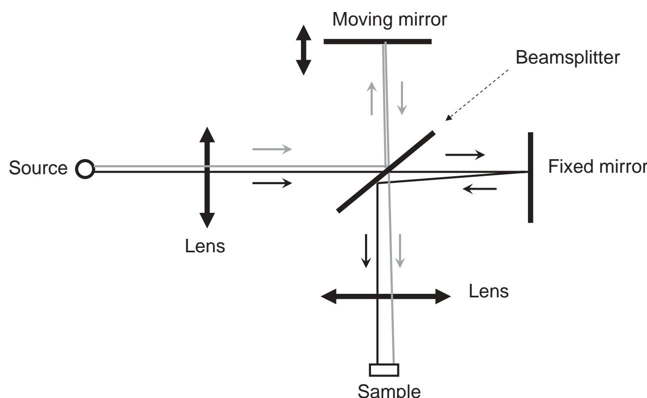

FIGURE 14 Michelson interferometer.

introduction of more reliable interferometers, the dispersive type was the most widely used. FT spectrophotometers are preferred for many applications because of their speed, reliability, and convenience. They have largely displaced other equipments in the analytical laboratories. Source radiation is split into two beams whose path lengths can be varied periodically to produce interference patterns. The reversed FT algorithm is then used for data recovery. Early FT spectrometers required frequent adjustments of the optical parts, but their unique characteristics of speed, high resolution, sensitivity, and wavelength precision and accuracy made them indispensable. Recent instrument design is much more reliable and easier to adjust. Typically, FT spectrometers are based on the Michelson interferometer (Figure 14), although other types of optical systems are also encountered. Laboratory FT spectrometers are usually proposed as single-beam instruments. For determining transmittance or absorbance spectra with this type of instrument a reference interferogram, air, for example, must first be measured and stored. The analyzed sample is then placed in the radiation path and the process repeated. Transmittance at various frequencies is obtained by computing the ratio of the sample and reference spectra.

Over most of the MIR spectral range, FT instruments appear to have better signal-to-noise ratios than do good-quality dispersive instruments by more than one order of magnitude. The enhanced signal-to-noise ratio can, of course, be traded for rapid scanning, producing spectra within a few seconds. Interferometric instruments also feature high resolutions (less than $0.1\,cm^{-1}$), high accuracy, and reproducible frequency determinations. FT instruments provide a much larger energy throughput (one or two orders of magnitude) than do dispersive instruments, which are limited in throughput by the necessity of narrow slit widths. However, this advantage is partially offset by the lower sensitivity of fast-response detectors required for interferometry.

To perform chemical imaging of sample surfaces, FT spectrometers can be coupled with a microscope or macrochamber with an FPA detector. CIS are available for Raman, NIR, and MIR spectroscopy. Figure 15 illustrates an optical arrangement for chemical imaging.

Instrument design depends on how measurements are performed (Figure 10). The ideal instrument has both transmittance and reflectance capabilities. For

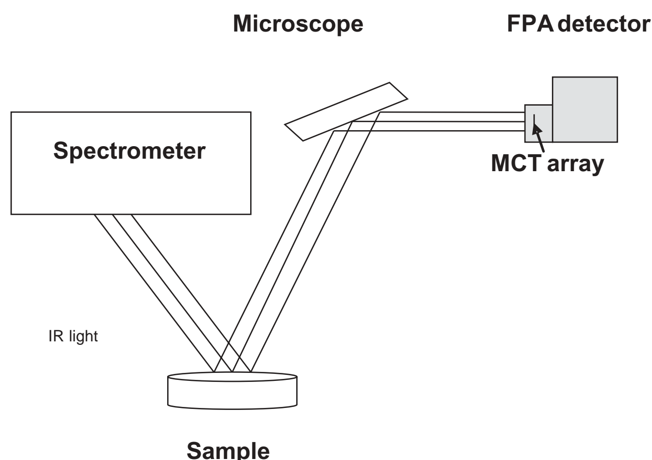

FIGURE 15 IR microscope coupled with a FPA detector.

example, reflectance measurements penetrate only 1–4 mm into the surface of solid samples. This shallow penetration of energy into a sample brings greater variation when measuring nonhomogeneous samples than transmittance techniques. In transmittance measurements, the entire path through the sample is integrated into the spectral measurement, thereby reducing error due to sample nonhomogeneity. Transmittance is suitable for measuring through compact samples, like intact tablets, but surface scattering induces a loss of transmitted energy with the net effect being a decrease in the signal-to-noise ratio. In some circumstances, particle size is so small that most of the energy striking the sample is scattered back. If the particle size is small enough, the instrument will not transmit enough energy through the sample for the detectors to record a signal. In transmittance, higher frequencies (800–1400 nm) are used to increase the depth of penetration into the sample. But higher frequency energy is more susceptible to surface scattering than lower frequency energy. Transmittance measurements must therefore be optimized based on the relationships between the frequencies used for measurement, surface scatter, and sample path length.

The first NIR spectrometers utilized a tilting filter concept. This concept was refined into a spinning system with a range of filters mounted in an encoder wheel for greater positioning accuracy (wavelength reproducibility) and greater reliability. The use of interference filters represents another type of instrument design, for which prespecified discrete interference filters are manufactured. The filters are mounted in a turret and rotated slowly to different positions during measurement scanning. These instruments are rugged, provided the calibrations are well established and specific to the analyte concerned. If the wrong interference filters are selected for the specific application, successful calibration is impossible. Systems currently exist configured from 6 to 44 discrete wavelength interference filters, in particular for routine application in the food and feed industries.

Dispersive, grating, scanning NIR instruments have been available since the late 1970s. These instruments varied in optical design, but all shared tungsten–halogen source lamps, a single monochromator with a holographic diffraction grating, and uncooled lead sulfide detectors. This design dates back to the early 1980s. Figure 16

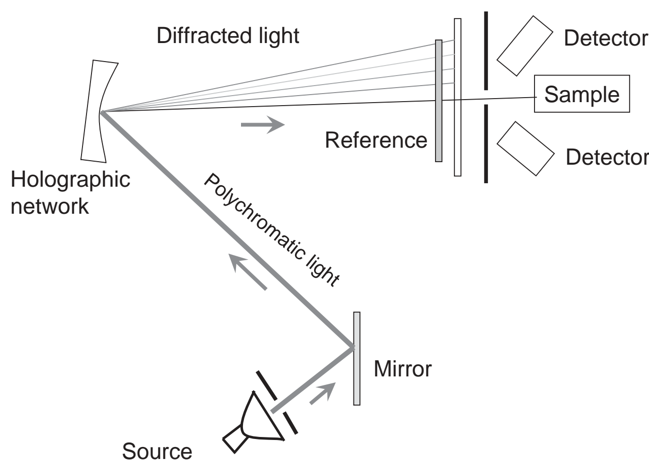

FIGURE 16 Predispersive grating spectrometer for diffuse reflectance.

shows a typical predispersive monochromator-based instrument where the light is dispersed before striking the sample, with a detector system for diffuse reflectance. The monochromatic light beam illuminates the sample at 0° (normal incidence), and the detectors collect the reflected light at 45°. Two to six detectors can be used, generally lead sulfide detectors for measurement in the 1100- to 2500-nm region. A computer is required for data processing, calibration, and storage. The spectrum is the difference between the raw reflectance measurement of the sample and the raw reflectance measurement of the reference material. Reflectance is converted to absorbance using the function absorbance = $-\log_{10}$ (reflectance), written as log $1/R$. Raw transmittance is converted to absorbance using the expression log $1/T$.

Postdispersive design is an optical arrangement allowing the transmission of more energy in either a single fiber-optic strand or fiber-optic bundle. Radiation is piped through the fiber-optic strand or bundle where it strikes the sample and returns to the dispersive element, a grating. After striking the grating, the light is separated into the various wavelengths before striking the detector(s).

The integrating sphere is still a common sample presentation geometry for NIR measurements. The use of integrating spheres dates back to the first commercial photometers. Their greatest advantage is that a detector placed at an exit port of the sphere is unlikely to lose energy. However, as detector technology improves, the advantages of integrating spheres for energy collection are no longer critical. Some special applications still require the use of spheres attached to fiber-optic bundles. The use of a sphere allows internal photometric referencing—a sort of double-beam instrument. The application of FT techniques to NIR dates back to the late 1980s. Transmittance/diffuse-reflectance FT-NIR instruments are widespread. As already mentioned, FT spectrometers differ from scanning spectrometers in that the recorded signal is an interferogram (details in the MIR section of this chapter). Other designs include diode array detectors (Figure 17) and NIR emitting diode sources. Acousto-optic tunable filters (AOTF) are devices based on diffraction. The NIR filter is in fact a transparent crystal in which an ultrasonic wave field is created. Thus, the selected wavelength is a function of the field intensity. AOTF scanning speed is

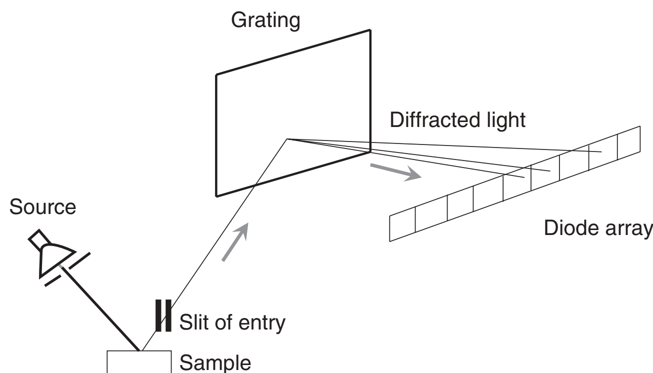

FIGURE 17 Diode-array spectrometer.

measured in microseconds. This kind of spectrometer is considered very fast but requires extra care to perform reliably in a pharmaceutical process environment. Other techniques are available, such as ultrafast-spinning interference filter wheels, interferometers with no moving parts, and tunable laser sources.

4.2.2.13 Conclusion

To summarize, instruments for vibrational spectroscopy can be categorized by the optimization of their optical design for a specific type of sample presentation and for the solution of a multiplicity of measurement problems. For development purposes, the instrument of choice is generally one with broad capabilities. Once an application has been clearly defined, an instrument suited to the specific sample presentation geometry or sample type will give the most reliable results. However, at this stage it is worth mentioning that due to the optical design and the mode of measurement, it can be very tricky to transfer calibrations or analytical methods from one instrument to another. For example, calibration transfer is a recurrent issue in NIRS and concerns all instrument designs. This is important if planning to perform measurements of a given sample attribute at different locations with different instruments, for example, laboratory versus process. The issue is more stringent with complex pharmaceutical samples where the signal from the analyte or property of interest strongly interacts with other parameters.

The choice of a given spectroscopy, that is, MIR, NIR, or Raman, has great importance for improving the monitoring and understanding of pharmaceutical processes. In Table 2 the major characteristics of use of MIR, NIR, and Raman spectroscopy are summarized. The most appropriate strategy is implemented according to the necessity and requirements of the manufacturing or analytical operations. In the selection of the spectroscopic technique, attention has to focus on criteria like chemical information content, location of the measurement, speed or real-time ability, robustness, ease of use, sample preparation, sample destruction, and the like. Multiconstituent analysis requires levels of accuracy which are comparable to those of the primary reference methods. Nowadays, a large number of technologies and numerous manufacturers exist. With this in mind, a given choice of equipment will probably match most significant criteria for the analytical problem of interest and

TABLE 2 Comprison of MIR/NIR Roman Spectroscopic Techniques

Spectroscopy	NIR	IR	Raman
Spectral range (cm^{-1})	12,000–4,000	4,000–400	4,000–50
Signal intensity	++	+++	+
Microscopic analysis	No	Yes	Yes
Fiber-optic interfacing	Yes	Yes (limited length)	Yes
Sampling through glass	Yes	No	Yes
Qualitative application	Yes	Yes	Yes
Quantitative application	Yes	Difficult	Yes
Instrument robustness	+++	+	++
Chemical Interpretation	Chemometrics	Direct	Direct

at the same time require to accept some inherent measurement limits or restrictions in use.

4.2.3 CHEMOMETRICS

4.2.3.1 Introduction

Chemometrics is a term which defines a discipline of chemistry involving mathematics and computer science in order to derive information from data of various type, origin, and complexity. Typical applications include relating the concentration of some analyte found in a sample to the sample's spectral data or identifying some physical or chemical characteristics of the sample. Although performing chemometrics without a computer is effectively impossible, the basic multivariate mathematic calculation has been known since the early twentieth century.

Near-IR spectroscopy is a typical application for chemometrics, for example, how to relate the NIR spectra, obtained at little expense, of the contents of a closed sterile vial with measurement of the concentration of a particular compound in the powdered mixture found by a reference method without opening the sample. To achieve this, the relationship between two data matrices has to be found and a quantitative calibration calculated. This task is a little different depending on whether the data has been generated using statistical experimental design (i.e., designed data) or has simply been collected, more or less at random, from a given population (i.e., nondesigned data). With designed data matrices, the variables are orthogonal by construction. Traditional statistical methods such as analysis of variance (ANOVA) and multiple linear regression (MLR) are well suited to find the regression model in orthogonal data tables. In nondesigned data matrices the variables are seldom orthogonal but are more or less collinear. MLR is likely to fail in these circumstances, and the use of projection techniques such as principal-component regression (PCR) or partial least squares (PLS) is recommended. A regression model can then be used to predict new, that is, unknown, samples. Prediction is a useful technique because it can be used in place of costly and time-consuming measurements.

Another example is the noninvasive identification of species from NIR absorbance spectra. Classification is simply a matter of finding out whether new samples

are similar to classes of samples that have been used to make the model. If a new sample fits a particular model well, it is said to be a member of that class. Many analytical tasks fall into this category. For example, raw materials like excipients may be sorted according to "good and bad quality," finished products classified into "grades A, B, C," and so on. Principal-component analysis (PCA) is a typical mathematical procedure for resolving such sets of data into orthogonal components whose linear combinations approximate the original data with a desired degree of accuracy. As successive components are calculated, each accounts for the maximum possible amount of residual variance in the set of data. In NIRS, the data usually consist of large sets of recorded spectra, and the number of components will be smaller than or equal to the number of known variables or the number of spectra.

4.2.3.2 From Univariate to Multivariate Regression

In spectroscopy the simplest method of quantitative calibration is based on a single independent variable, for example, wavelength, since a sample attribute such as analyte concentration is a linear function of absorbance at a given wavelength. Modeling of the concentration requires conventional least-squares fitting. A straight line is fitted through a set of data points to minimize the sum of squares of deviations between estimated and known data points. In this approach, a wavelength is selected when it shows a high degree of correlation between concentration and absorbance. Correlation is an indicator of goodness of fit between concentration and absorbance and of how well the calibration describes the data set. The linear relationship has a practical advantage in that it permits direct and visual estimation of goodness of fit, thus enhancing the analyst's trust in his data collection. Where pharmaceutical samples are concerned, the linear approach rapidly reaches its limits and another approach is required.

Multiple linear regression extends linear regression to one wavelength by least squares, with more than one wavelength selected to perform a calibration. The complexity of the test samples is again an issue with this approach. MLR requires independent variables for its ability to explain the data set. As pharmaceutical samples comprise a complex matrix in which species interact to various degrees in the NIR range, it is impossible to find appropriate wavelengths to select. Observed absorbance values are linked as they may describe related behaviors in the spectral data set. It is typical of NIR spectra of mixtures that collinearity is found among the wavelengths and MLR will never perform a usable linear calibration.

Partial least squares multivariate regression uses many wavelengths without the limitation due to collinearity. In fact, collinearity makes PLS the tool of choice when considering segments or entire NIR spectra. PLS treats constituent data and spectral data on the same basis, without statistical prerequisites of standard distribution or noncollinearity. Errors are assumed equally and in both data sets. Spectral and constituent data are modeled simultaneously according to an iterative algorithm. This training step is required for the model to learn to predict the sample attribute of interest. For each iteration a part of the spectral data and a part of the corresponding constituent data are combined until the data set is optimally described. The nonexplained part of the data set is made up of residuals, which are a measure of the quality of the modeling. The original data is combined in factors or principal components. Coefficients are calculated—loadings for spectral data and scores for

constituent data—to indicate the extent of original data involvement in the computation of each factor. Finally, the amount of variance modeled, that is, the explained part of the data, is maximized for each factor and the residuals are minimized.

The raw data is normally preprocessed to some extent before PLS calibration, as illustrated below. A critical step in PLS modeling is the selection of the number of factors. Selecting too few factors will provide an inadequate explanation of variability in the training data set, while too many factors will cause overfitting and instability in the resulting calibration. Optimal factor number is estimated during validation of the calibration, which is part of the PLS algorithm. Either an additional and independent data set is used (external validation in one computation step) or the training data set is split into subsets for continuous internal validation at each iterative step (cross-validation). In addition, the resulting PLS calibration must prove able to predict unknown samples. It is also good practice to challenge the resulting calibration in a way that enhances trust in future results. In no way are PLS calibrations black boxes despite the fact that visualization is far from evident when compared to basic linear regression.

4.2.3.3 Sample Quality and Data Error

In an ideal case, a given resulting calibration equation provides an explicit value for the concentration of an unknown analyte in terms solely of measurable absorbances. Only the concentrations of the desired analyte need be known during calibration. The values for other sample constituents need not be determined, although in order to develop a good calibration equation, variations of these values should also be evenly distributed. Indeed, in some cases, even the number of other compounds in the calibration samples may not be known. Using stepwise multiple regression, features in the spectra which correlate most closely with the analyte concentration are selected for a particular sample set. Once optimal calibrations are computed, the NIR instrument can be used to predict unknown samples for the determination of the quantity of desired analyte. Thus, regression analysis is a method to develop the relationship (i.e., regression calibration equation) between several spectral features and the constituent being investigated. For diffuse reflectance measurements, the effects of extraneous physical phenomena are superimposed on the absorbance readings and have to be taken into account. The equations can be formulated to account for these phenomena by implicitly including corrections for their effects in the calibration coefficients.

One problem that chemometrics cannot deal with is the appropriate selection or preparation of samples. Careful sample selection increases the likelihood of extracting useful information from spectral data. Whenever it is possible to actively experiment with selected variables or parameters, the quality of the results is increased. The critical part is deciding which variables can be changed and what limits to fix on their variation. Appreciation of which parameters may influence data depends mainly on the experience of NIR operators but is also a matter for specialists. Each problem will generate and implicate a specific set of more or less usable variables. The key to success lies in gaining experience in dealing with numerous and different problems. Experimental design could ideally help to generate the data indicating which parameters or design variables influence output or response variables.

Understanding the interactions between design variables while identifying the optimal conditions for extracting the relevant information should in principle minimize the number of experiments required, thus reducing cost. An experimental design program offers appropriate design methods and encourages good experimental practice by performing a small number of useful experiments that span the important variations. Experimental design will not be discussed here, but only the way in which data should be collected and organized in practice for multivariate regression. Typically, with spectral data, the problem is to find out which variables are really important for variation in the data matrix. The usual tasks when thinking about modeling a response variable are to find out which variables are necessary for describing the samples adequately, which samples are similar to each other, and whether the data set contains groups of samples. A good way of finding this information is by decomposing the spectral data matrix into a structured part and a noise part, typically by using PCA. Another problem is how to establish a regression model between a spectral data matrix and a response variable matrix.

Before performing multivariate data analysis, statistical analysis of sample response values or spectral data may help to check data quality. Descriptive statistics summarize the distribution of one or two variables at a time. They are not supposed to say much about the data structure, but they are useful for obtaining a quick look at each separate variable before starting an analysis. One-way statistics, that is, mean, standard deviation, variance, median, minimum, maximum, lower, and upper quartile, can be used to explore the data set and detect out-of-range values, abnormal spread, or asymmetry. These statistics reveal anything suspect in a data table and indicate whether a transformation might be useful. Two-way statistics, for example, correlation, show how variations in two variables may be linked in a data table. Checking these statistics is also useful to detect out-of-range values and outliers.

Experience in NIRS practice shows that the critical step(s) for successful implementation vary with each specific problem to solve. However, the following procedure is recommended when analyzing nondesigned data. First, investigate the origin and availability of the data. In formulating the study problem, define the precise objective of data collection and the expected analysis results. The data collection should span appropriate variation in the explored variable(s) or attribute(s). If the naturally available data do not span the expected variation, prepare or measure samples with corresponding experimental data. Raw spectra may have to be transformed and mathematical pretreatments performed. Calibrate and validate the model either using PCA or PLS. Explore and challenge the way the calibrations behave on real samples or data, for example, validate the method according to current pharmaceutical usage and requirements.

There are three types of data error: random error in the reference laboratory values, random error in the optical data, and systematic error in the relationship between the two. The proper approach to data error depends on whether the affected variables are reference values or spectroscopic data. Calibrations are usually performed empirically and are problem specific. In this situation, the question of data error becomes an important issue. However, it is difficult to decide if the spectroscopic error is greater than the reference laboratory method error, or vice versa. The noise of current NIR instrumentation is usually lower than almost anything else in the calibration. The total error of spectroscopic data includes

sample-induced errors which can be much greater than the instrument's noise level. Such errors include particle size effects, variations in the packing density of powders, effects of impurities, and effects due to changing physical characteristics of the sample (e.g., crystallinity). Practical experience shows that in many cases sample-induced error in the optical data remains small, prompting the empirical assumption that optical data error is always smaller than reference laboratory error.

4.2.3.4 Mathematical Preprocessing of Spectroscopic Data

As discussed above, the greatest source of error in NIR calibration is usually reference laboratory error, sample nonhomogeneity, and nonrepresentative sampling in the learning (training) set or calibration set population. Instrument quality and equation selection account for only a fraction of the variance or error attributable to NIR analytical technique in current routine application.

When radiation is reflected from solid matter surfaces, diffuse and specular reflected energies are superimposed. The intensity of diffusely reflected energy depends on the angles of incidence and observation but also on sample packing density, crystalline structure, refractive index, particle size distribution, and absorptive qualities. Thus, in practice, an ideal diffusely reflecting surface can only be approximated, even with the finest possible grinding of the samples. There are always coherently reflecting surface regions acting as elementary mirrors whose reflection obeys Fresnel's formulas. Radiation returning to the sample surface from the interior can be assumed to be largely isotropic, thus meeting the requirements of the Beer–Lambert law. It is assumed that radiant energy is continuously removed from the incident NIR beam and converted to thermal vibrational energy of atoms and molecules. Decrease in the intensity of the diffusely reflected light depends on the absorption coefficient of the sample. The absorption coefficient (K), when taken as the ratio K/S, where S is the scattering coefficient, is proportional to the quantity of absorbing material in the sample. In the Kubelka–Munk theory, reflectance (R) is related to absorption (K) and the scattering coefficient (S) by the equation

$$\frac{K}{S} = \frac{(1-R)^2}{2R} = F(R)$$

Diffuse reflectance R is a function of the ratio K/S and proportional to the addition of the absorbing species in the reflecting sample medium. In NIR practice, absolute reflectance R is replaced by the ratio of the intensity of radiation reflected from the sample and the intensity of that reflected from a reference material, that is, a ceramic disk. Thus, R depends on the analyte concentration. The assumption that the diffuse reflectance of an incident beam of radiation is directly proportional to the quantity of absorbing species interacting with the incident beam is based on these relationships. Like Beer's law, the Kubelka–Munk equation is limited to weak absorptions, such as those observed in the NIR range. However, in practice there is no need to assume a linear relationship between NIRS data and the constituent concentration, as data transformations or pretreatments are used to linearize the reflectance data. The most used linear transforms include log $1/R$ and Kubelka–Munk as mathemati-

cal pretreatments. To some extent PCR, PLS, and multilinear regression compensate for nonlinearity. Calibration equations can be developed which compensate to some extent for the nonlinear relationship between analyte concentrations and log $1/R$ or Kubelka–Munk transformed data. If a matrix absorbs at different wavelengths than the analyte, Kubelka-Munk can prove a useful linearization method for spectroscopic data. If the matrix absorbs at the same wavelength as the analyte, log $1/R$ will prove a better choice for relating reflectance to concentration. This transformation is basically well suited for diffuse reflectance NIR spectra of most mixtures with absorbing matrices. Plots of $F(R)$ versus concentration are less linear than plots of log $1/R$ versus concentration.

4.2.3.5 Preprocessing NIR Data

When generating calibration equations using samples of known composition, the independent variable is represented by the spectroscopic readings (i.e., log $1/R$) at specific wavelengths, while the concentration of the analyte of interest, for example, determined by traditional laboratory techniques, is the dependent variable.

Spectral raw data may have a distribution or shape that is not optimal for analysis. Background effects, baseline shifts, measurements in different conditions, different variances in interfering variables and the like can complicate information extraction using multivariate methods. It is important to minimize the noise introduced by such effects. Preprocessing operations include centering, weighting, and numerous mathematical transformations. Mean centering consists of subtracting the average spectra from each individual spectrum. This ensures that all results will be interpretable in terms of variation around the mean considered as the model center. This is useful with models in which a linear relationship between spectral data and response data is supposed to go through the origin. Depending on the kind of information to be extracted from the spectral data, weights based on the standard deviation (i.e., square root of the variance, which expresses the variance in the same unit as the original variable) may be used for scaling. This may be a typical pretreatment for PCA, PLS, or PCR calibrations which are projection methods based on finding directions of maximum variation, thus depending on the relative variance of the variables. A possible weighting option is the 1/SD standardization, which gives all variables the same variance, that is, 1. In this case all variables are given the same chance to influence estimation of the components. This is advisable if the variables are measured with different units, have different ranges, or are of different types. It is also possible to fix a constant weight for each variable manually. Weighting involves stretching and shrinking by measuring a position relative to the extremes in the actual spectral data table. By standardizing the spectra, variation in the data set is performed relative to the extremes in the data table. However, this procedure emphasizes the relative influence of unreliable or noisy attributes.

4.2.3.6 Mathematical Pretreatment and Transformation

A wide range of transformations can be applied to spectral data before they are analyzed. The main purpose of transformations is to make the latent variables better available for powerful analysis. One of the most widely used is logarithmic transformation, which is especially useful to make skewed variables more symmetrically

distributed. It is also indicated when the measurement error in a variable increases proportionally with the level of that variable. Taking the logarithm will achieve uniform precision over the whole range of variation. This particular application is also called variance stabilization. In case of limited asymmetries, a square root can be sufficient. Smoothing is relevant for variables which are themselves a function of some underlying variable, for instance, of time. It is also one of the first operations performed on recorded NIR spectra. It removes as much noise as possible without degrading important information content. Moving point average (MPA) is a classic smoothing method which replaces each observation with an average of the adjacent observations including it. The number of observations to average is a variable parameter. Polynomial smoothing, also called Savitzky–Golay smoothing, involves least-square fitting of a polynomial equation to a window of n sequentially selected spectral data points. If the polynomial order is less than the number of data points, the polynomial cannot pass through all selected data points, and the least-square fit gives a smoothed approximation to the original window. Normalization is a family of transformations which are computed sample wise. Here the purpose is to scale samples so as to improve specific properties. Mean normalization is the classic algorithm. It consists in dividing each row of a data matrix by its average, thus neutralizing the influence of possible hidden factors. It is equivalent to replacing the original variables by a profile centered on 1. Only the relative values of the variables are used to describe the sample, and the information carried by their absolute level is dropped. It is indicated in the specific case where all variables are measured in the same unit, and their values are assumed to be proportional to a factor which cannot be directly taken into account in the analysis. Maximum normalization is an alternative procedure which divides each row by its maximum absolute value instead of the average. The maximum value becomes +1 and the minimum value becomes –1. In range normalization, each row is divided by its range, that is, maximum value minus minimum value and the curve span becomes 1.

More specific transformations for spectroscopic data are the reflectance to absorbance, absorbance to reflectance, and reflectance to Kubelka–Munk transformations. Multiplicative scatter correction (MSC) is an additional transformation method used to compensate for additive and/or multiplicative effects in spectral data. MSC was originally designed to deal with multiplicative scattering alone. However, MSC successfully treats a number of similar effects such as path length problems, offset shifts, and interference. The idea behind MSC is to remove the two effects—amplification, which is multiplicative, and offset, which is additive—from the spectral data table to prevent them from dominating the table's information content. Derivation is typically relevant for spectral data that are a function of some underlying variable influencing absorbance at various wavelengths. Derivatives are also a simple but powerful technique for magnifying fine structure in raw spectra lacking structure, which is common in NIRS. By increasing the order of derivation, band structure resolution is increased. The inherent drawback is a decrease in the signal-to-noise ratio, which in the particular case of NIR spectra is not considered, since smoothing while performing a derivation may limit the effect of noise. On the other hand, it will tend to hide some weaker spectral features. The main advantage of the second derivative as usually performed is that band structure is maintained: Peak maxima still correspond while peak shape and resolution are improved. The Savitzky–Golay algorithm permits computation to higher order derivatives, includ-

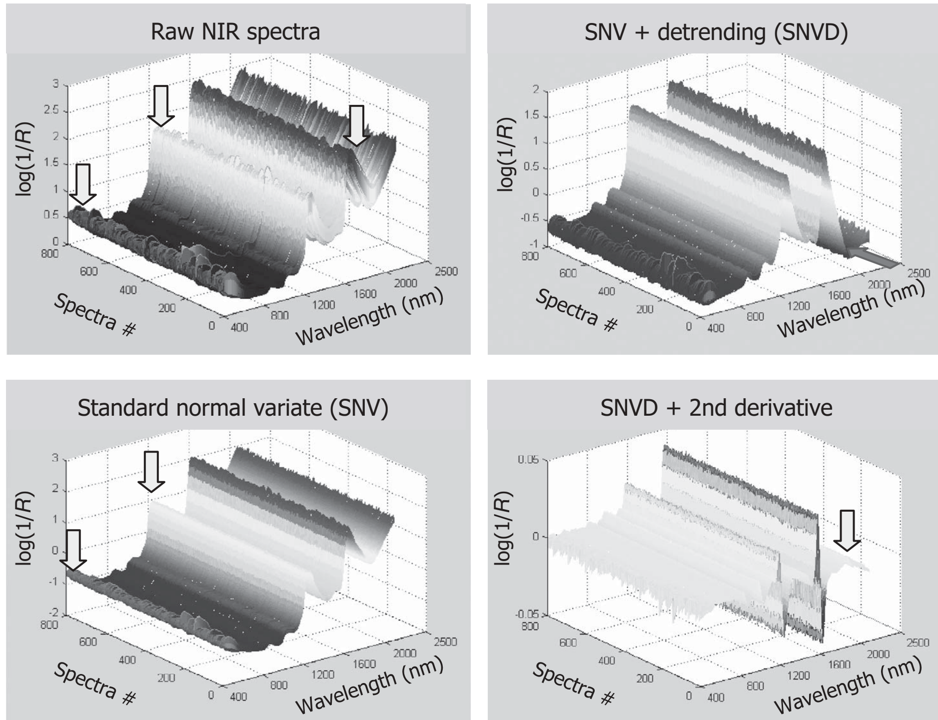

FIGURE 18 Examples of combined spectral preprocessing.

ing a smoothing factor which determines how many adjacent variables will be used to estimate the polynomial approximation for derivation. Norris derivation is an alternative algorithm for computing first derivatives only. A baseline correction algorithm is the standard normal variate (SNV) method, which does not affect overall spectra layout. Averaging samples in case of replicates or variables for variable reduction, for example, to reduce the number of latent variables, is a method of obtaining more stable and more readily interpretable results. Figure 18 illustrates the effect of selected combined transformations to a collection of spectral data.

4.2.3.7 Principal-Component Analysis

Large data tables contain an amount of information which is partly hidden because the data complexity prevents ready interpretation. This is typical of NIR spectra collections. PCA is a projection method used to visualize all the information contained in the data table. It can be used to show in what respect one sample differs from another, which variables contribute most to this difference, and whether these variables contribute in the same way and are correlated or independent of each other. It also reveals sample patterns or groupings. In addition, it quantifies the amount of useful information, as opposed to noise or meaningless variation, contained in the data table. Principal components are defined only for the data set from which they were computed. They may also hold for other data of identical type, but this is not guaranteed, and it is certainly not true for different types of data.

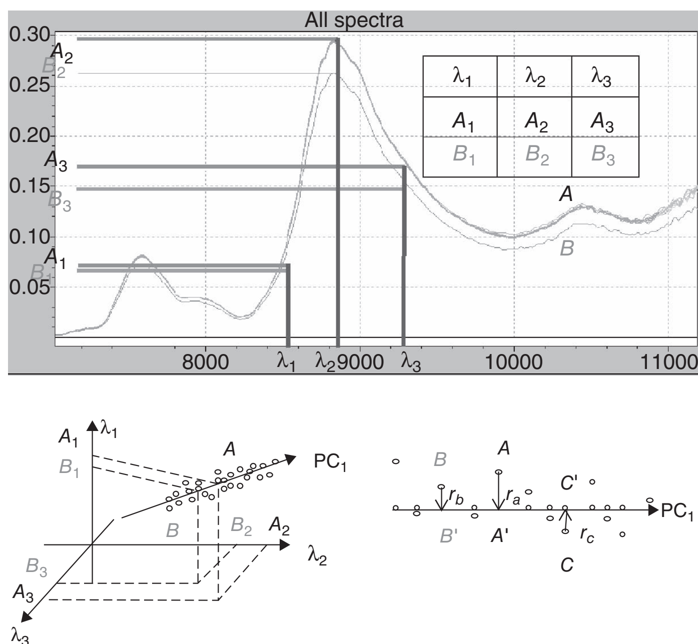

FIGURE 19 Geometric illustration of PCA. Each spectrum is plotted as a point, whose coordinates are the intensities collected at three different wavelengths. For a given collection of spectra, the first principal component is the direction in space which covers the greatest variation of the corresponding point set.

The PCA modeling forms the basis for several classification and regression methods. The underlying idea is to replace a complex multidimensional data set by a simpler version having fewer dimensions, but still fitting the original data closely enough to be considered a good approximation. Extracting information from a data table consists of exploring variations between samples, that is, finding out what makes a sample different from, or similar to, another. Two samples can be described as similar if they have close values for most variables. From a geometric perspective, in the case of close coordinates in the multidimensional space of variables, the two points are located in the same area. Likewise, two samples can be described as different if their values differ greatly with respect to at least some variables. The two points have different coordinates and are located far away from each other in the multidimensional space. An illustration of this geometrical concept is proposed in Figure 19.

The principle of PCA consists of finding the directions in space—known as principal components (PCs)—along which the data points are furthest apart. It requires linear combinations of the initial variables that contribute most to making the samples different from each other. PCs are computed iteratively, with the first PC carrying the most information, that is, the most explained variance, and the second PC carrying most of the residual information not taken into account by the previous PC, and so on. This process can go on until as many PCs have been computed as there are potential variables in the data table. At that point, all between-sample variation has been accounted for, and the PCs form a new set of axes having two

advantages over the original set. First, the PCs are orthogonal to each other. Second, they are ranked so that each carries more information than any of those following. It thus prioritizes their interpretation, starting with the first PCs. This method of generation ensures that the new set of axes is more suitable for interpreting the data structure. Usually only the first PCs contain pertinent information, with later PCs being more likely to describe noise. In practice, only the first PCs are examined rather than the whole raw data table: Not only is it less complex, but it also ensures that noise is not mistaken for information. If all PCs were retained, there would be no approximation at all and no gain in simplicity either. Deciding on the number of components to retain in a PCA model is a compromise between simplicity, completeness, and effectiveness. The PCA model is only an approximation of reality.

Each component of a PCA model is characterized by three complementary sets of attributes, that is, variances, loadings, and scores, respectively. The importance of a given PC is expressed by its variance. Loadings describe the relationships between variables, while scores describe sample properties. Variances are error measures which tell how much information is taken into account by successive PCs. The way they vary with the number of components can be studied to decide how complex the model should be. Residual variance designates the variation in the data that remains to be explained once the current PC has been taken into account, while explained variance, often measured as a percentage of total variance in the data, measures the proportion of variation in the data accounted for by the current PC. These variances can be considered either for a single variable or sample or for the whole data. They are computed as mean square variations, corrected for the remaining degrees of freedom.

Loadings describe the data structure in terms of variable correlations. Each variable has a loading on each PC. This reflects how much the variable contributed to that PC and how well that PC reflects the variation of the variable considered. In geometric terms, loading is the cosine of the angle between the variable and the current PC, with a value that ranges by definition between −1 and +1. The smaller the angle, the greater the link between variable and PC, and the greater the loading value. Variables with high loadings (i.e., close to +1 or −1) for a given PC contribute greatly to the meaning of that particular PC. Thus, in studying correlations between variables, the loadings indicate their respective angles in the multidimensional space. For instance, if two variables have high loadings along the same PC, their angle is small, which in turn means that the two variables are highly correlated. If both loadings have the same sign, the correlation is positive; when one variable increases, so does the other. If negative, the variables are anticorrelated.

Scores describe the data structure in terms of sample patterns and emphasize differences or similarities. Each sample has a score on each PC, which is the coordinate of the sample on the PC. Once the information carried by a PC has been interpreted with help of the loadings, the score of a sample along that PC can be used to characterize a given sample. It describes the major features of the sample, relative to the variables with high loadings on the same PC. Samples with close scores along the same PC are considered as similar because they have close values for the corresponding variables. Conversely, samples with greatly dissimilar scores differ greatly from each other with respect to those variables.

4.2.3.8 PCA Practice for NIRS

As already mentioned, any multivariate analysis should include some validation, that is, formal testing, to extrapolate the model to new but similar data. This requires two separate steps in the computation of each model component: calibration, which consists of finding the new components, and validation, which checks how well the computed components describe the new data. Each of these two steps needs its own set of samples: calibration samples or training samples, and validation samples or test samples. Computation of spectroscopic data PCs is based solely on optic data. There is no explicit or formal relationship between PCs and the composition of the samples in the sets from which the spectra were measured. In addition, PCs are considered superior to the original spectral data produced directly by the NIR instrument. Since the first few PCs are stripped of noise, they represent the real variation of the spectra, presumably caused by physical or chemical phenomena. For these reasons PCs are considered as latent variables as opposed to the direct variables actually measured.

A convenient analogy for understanding latent variables is reconstructing the spectrum of a mixture from the spectra of the pure chemicals contained in the mixture. The spectra of these pure chemicals would be the latent variables of the measured spectrum because they are not directly accessible in the spectrum of the mixture. However, PCs are not necessarily the spectra of the pure chemicals in the mixtures representing the samples. PCs represent whatever independent phenomena affect the spectra of the samples composing the calibration set. If one sample constituent varies entirely independently of everything else, and this constituent has a spectrum of its own, then one of the PCs will indeed represent the spectrum of that constituent. It is most unusual for any one constituent to vary in a manner that is exactly independent of any other. There is inevitably some correlation between the various constituents in a set of specimens, and any PC will represent the sum of the effects of these correlated constituents. Even if full independence is accomplished, there is dependence in that the sum of all constituents must equal 100%. Consequently, the PC representing that source of independent variability will look like the difference between the constituent of interest and all the other constituents in the samples. The spectrum of the constituent considered could be extracted mathematically, but the PCs will not look exactly like the spectrum of the pure constituent.

Once samples have been collected and the corresponding NIR spectra stored in suitable files, building and using a PCA model involves three steps: selecting the appropriate preprocessing procedure(s), running the PCA algorithm and diagnosing the model, and interpreting the loading and score plots. Once the model is built, it is important to assess its quality before using it for interpretation. There are two steps in diagnosing a PCA model. The variances must be checked to determine how many components the model should include and to estimate how much information the selected components take into account. It is important to verify the variances calculated during validation. Then it is advisable to look for outliers, that is, samples that do not fit into the general pattern.

Total explained variance measures how much of the original variation in the data is described by the model. It expresses the proportion of structure found in the data by the model. Total residual and explained variances show how well the model fits

the data. Models with small total residual variance (close to 0) or large total explained variance (close to 100%) explain most of the variation in the data. With simple models residual variance falls to zero with few components. If this is not the case, it means that there may be a large amount of noise in the data. Alternatively, it may also mean that the data structure is too complex to be accounted for by only few components. Variables with small residual variance and large explained variance for a particular component are well explained by the relevant model. Variables with large residual variance for all or the few first components have a small or moderate relationship with other variables. If some variables have much larger residual variance than other variables for all components or the first components, they may be excluded in a new calculation. This may produce a model that is easier to interpret. Calibration variance is based on fitting the calibration data to the model. Validation variance is computed by testing the model on data not used in building the model.

Outliers may sometimes account for large residual variance. An outlier is a sample which looks so different from the others that either it is not well described by the model or it influences the model too much. In practice, true spectral outliers are first considered to be samples whose spectral characteristics are not represented within the specified sample set. At least one of the model components may focus on trying to describe only this particular sample, even if this is irrelevant to the more important structure present in the other samples. In PCA, outliers can be detected by using different plots or analysis. For example, score plots show sample patterns according to one or two components. It is easy to identify a sample lying far away from the others. Such a sample is likely to be an outlier. Residuals measure how well samples or variables fit the model determined by the components. A sample with a high residual is poorly described by the model, which, however, fits the other samples quite well. Such a sample does not fit among the samples well described by the model and can be considered an outlier. Deciding to exclude or retain such spectral outliers solely on mathematical criteria is not necessarily correct. The outlier may be considered as not part of the group intended for use as a calibration set. However, such outliers may indicate additional characteristics not taken into account during initial sample selection.

4.2.3.9 Pattern Recognition

Pattern recognition can be classified according to the distinction between supervised and unsupervised techniques. Unsupervised methods such as cluster analysis classify data without calibration and are based solely on the collected sample data. Supervised classification uses the spectral data and some class membership information. Therefore, mathematical models are computed in a first step with a calibration set containing spectra and class information. This model is then applied to predict new sample classes. Feature extraction methods such as PCA or wavelet compression are often applied before cluster analysis. PCA is valuable for extracting features or visualizing the data set. Another benefit of PCA is to reduce the number of wavelengths. Many clustering algorithms are in use. Nonhierarchical methods include Gaussian mixture models, *K* means, and fuzzy *C* means, each of which can be subdivided into hard and soft clustering methods. For example, hard clustering by *K* means would declare one given item as one class membership, whereas soft

clustering by fuzzy *C* means would assign fractional membership degrees of each cluster.

Current methods for supervised pattern recognition are numerous. Typical linear methods are linear discriminant analysis (LDA) based on distance calculation, soft independent modeling of class analogy (SIMCA), which emphasizes similarities within a class, and PLS discriminant analysis (PLS-DA), which performs regression between spectra and class memberships. More advanced methods are based on nonlinear techniques, such as neural networks. Parametric versus nonparametric computations is a further distinction. In parametric techniques such as LDA, statistical parameters of normal sample distribution are used in the decision rules. Such restrictions do not influence nonparametric methods such as SIMCA, which perform more efficiently on NIR data collections.

4.2.3.10 SIMCA Classification

Classification is useful when the response considered is a category variable that can be interpreted in terms of several classes to which a sample may belong. The main goal of classification is to assign new samples reliably to preexisting classes, but classification results can also be used as a diagnostic tool to identify the most important variables to retain in the model or to find outliers. Applications include predicting whether a pharmaceutical product meets specified quality requirements, in which case the result is simply a binary response, or more generally testing or confirming the identity of a substance. PCA and discriminant analysis are techniques that have found extensive use in NIR analysis for that purpose. SIMCA, a multivariate technique optimized for NIR data analysis, combines PCA models for each defined class in the training set. The approach can be applied to a more general class of problems than simple classification, for example, identification. PCA is performed on a data set requiring qualitative analysis. After computing the PCs, scores are calculated and used to perform qualitative analysis by surrounding each region of multidimensional space containing each group's scores with a surface. Conceptually, this enclosing surface can be modeled as an ellipsoid and distances to these clouds are computed using Mahalanobis metrics based on the PC scores.

In practice, the optimal number of PCs should be chosen for each class model separately, according to a suitable validation scheme. Each model is checked for possible outliers and improved as much as possible, like any PCA model. Before using the models to predict class membership for new samples, the specificity of the models should be verified, that is, checked for class overlap and adequate interclass distance. Once each class has been modeled, and class overlap is not excessive, new samples can be fitted to each model. Unknown samples are then compared to the class models, and assigned to classes according to their similarity to the training samples. The modeling stage implies that enough samples have been identified as members of each class to allow reliable models to be built. Accurate sample description also requires a sufficient number of variables. Actual classification is based on statistical tests performed on Mahalanobis distances between sample and model. With each unknown sample, all variable values are computed using the model scores and loadings, before being compared to the actual values. The residuals are then combined into a measure of the object-to-model distance. The scores are also used to measure the sample's distance from the model center, known as leverage. Finally,

both object-to-model distance and leverage are taken into account to decide the classes to which the sample belongs. Any sample belonging to a class should have a small distance to the class model.

4.2.3.11 Regression

Historically, one motivation for performing NIRS was to develop fast and noninvasive quantitative analysis. To achieve this, it is not sufficient to extract PCs from the data. There must be a regression method relating these PCs to the constituent, analyte, or physical property for which the calibration is performed. Regression concerns all methods attempting to fit a model to the observed data. The fitted model may be used to describe the relationship between two groups of variables or to predict values of unknown samples. If X and Y are the two data matrices involved in regression, the purpose is to compute a $Y = f(X)$ model, which tries to explain, or predict, the variations in the Y variable(s) from those in the X variable(s). The link between X and Y is explored through a common set of samples from which both X and Y values have been collected and are clearly known. Building a regression model involves collecting variable values for selected samples and fitting a mathematical relationship to the corresponding spectral data. For example, spectroscopic measurements are performed on solutions with known concentrations of a given compound. Regression is used to relate the concentration to the spectrum. Once the regression model is build, the unknown concentration for new samples can be indirectly predicted using the spectroscopic measurements as predictors. The advantage appears obvious if one considers noninvasive and nondestructive measurement by NIR. If the concentration is difficult or expensive to measure directly, spectroscopic analysis offers an alternative and much cheaper method of determination. Thus the indication for using regression as a predictive tool is its potential for performing fast and low-cost measurement as a substitute for more expensive or time-consuming alternatives.

The data may require appropriate preprocessing before a regression can be built and used. The calibration step must be followed by a validation step, that is, the model must be checked for its efficiency in predicting independent data. Once the number of components has been selected based on the calibration and validation variances, the model is diagnosed by interpreting the loading and score plots (for PCR and PLS), the loading weight plots (for PLS), and B coefficients (PCR), and examined for the prediction of new data.

4.2.3.12 Multiple Linear Regression

Classic univariate regression uses a single predictor, which is usually insufficient to model a property in complex samples. Multivariate regression takes into account several predictive variables simultaneously for increased accuracy. The purpose of a multivariate regression model is to extract relevant information from the available data. Observed data usually contains some noise and may also include irrelevant information. Noise can be considered as random data variation due to experimental error. It may also represent observed variation due to factors not initially included in the model. Further, the measured data may carry irrelevant information that has little or nothing to do with the attribute modeled. For instance, NIR absorbance

spectra may contain information relative to solvents, processing path, instrument status, light probes, and the like, in addition to the analyte concentration for measurement. A good regression model should be able to pick out only the relevant information while leaving irrelevant variation aside.

Multiple linear regression is a method based on ordinary least-squares regression. It involves matrix inversion which leads rapidly to collinearity issues if the variables are not linearly independent. In MLR, all X variables participate in the model independently of each other, and their covariations are not taken into account. X variance is not meaningful in this context. Variable independence is also an essential precondition. Further, to perform the inversion, MLR requires more samples than predictors and no missing values in the data table. If the data table complies with these conditions, MLR will approximate the response values by linear combination of predictor values, yielding regression coefficients known as B coefficients. Other results are predicted Y values, residuals with error measures, and ANOVA. It is noteworthy that MLR is the only multivariate method for which formal statistical tests of significance for regression coefficients are available. To evaluate the goodness of the model, diagnostic tools are associated with the regression coefficients. The standard error is an estimate of the precision of a given coefficient. A Student's t value can be computed and compared to a reference t distribution, which in turn indicates a significance level or p value. It shows the probability of a t value equal to or larger than the observed value if the true value of the regression coefficient were 0. Predicted Y values are computed for each sample by applying the model equation with the estimated B coefficients to the observed X values. For each sample, the residual is the difference between the observed Y value and predicted Y value. The only relevant measure of how well the MLR model performs is provided by the Y variances. Residual Y variance is the variance of the Y residuals. It expresses how much variation remains in the observed response after the modeled part is removed. It is an overall measure of misfit, that is, the error made when fitted Y values are computed as a function of X values.

4.2.3.13 PCR and PLS Regression

Multivariate regression is better suited to fit a relationship between spectroscopic data and the variable to estimate. PC regression (PCR) is a two-step procedure which first decomposes the X matrix by PCA, then fits an MLR model, using the PCs instead of the raw data as predictors in the regression step. MLR and PCR model one Y variable at a time, while a PCR model using all PCs gives the same solution as MLR. Partial least squares or projection to latent structures (PLS) will model both X and Y matrices simultaneously to find the latent or hidden variables in X that will best predict the latent or hidden variables in Y. The difference between PCR and PLS lies in the algorithm. PLS components are similar to PCs and are also referred to as PCs. PLS1 deals with only one response variable at a time (like MLR and PCR). PLS2 handles several responses simultaneously. PCR and PLS are projection methods, like PCA. Model components are extracted in such a way that most information is carried by the first PC, then the second PC, and so on. At a certain point, the variation modeled by any new PC becomes mostly noise. Residual variances are used to determine the optimal number of PCs modeling useful information while avoiding overfitting. PLS uses both independent and dependent variables

to find the regression model. It switches iteratively between *X* and *Y*. PLS usually needs fewer PCs than PCR to reach the optimal solution because the focus is on the dependent variables. Results of PCR modeling are given as scores, loadings, predicted *Y* values, residuals, error measures, and *B* coefficients. Results of PLS modeling are given as *T* scores and *U* scores, *P* loadings and *Q* loadings, loading weights, predicted *Y* values, residuals, and error measures.

The PLS scores are interpreted in the same way as PCA scores since they are the sample coordinates along the model components. The additional feature in PLS is that two different sets of components are considered, summarizing variations in the *X* space or *Y* space. PLS loadings express the relatedness of each *X* and *Y* variable to the model component. *T* scores are the coordinates of data points located in the *X* space that describe the part of structure in *X* which is most predictive for *Y*. *U* scores summarize the part of structure in *Y* which is explained by *X* along a given model component. The relationship between *T* scores and *U* scores is a model of the relationship between *X* and *Y* along a specific component, and it can be visualized for diagnostic purposes. It follows that loadings will be interpreted differently in the *X* space and *Y* space. *P* loadings are similar to PCA loadings. They express how much each *X* variable contributes to a specific model component. Directions determined by the projections of *X* variables are used to interpret the meaning of the location of a projected data point on a *T* score plot, in terms of variations in *X*. *Q* loadings express the direct relationship between *Y* variables and *T* scores. Thus, the directions determined by the projections of *Y* variables by means of *Q* loadings can be used to interpret the meaning of the location of a projected data point on a *T* score plot in terms of sample variation in *Y*. When plotted on a single graph, *P* and *Q* loadings make it possible to interpret *T* scores by considering variations in both *X* and *Y*. In contrast to PCA loadings, PLS loadings are not normalized, so that *P* loadings and *Q* loadings do not share a common scale. Thus, only their directions can be interpreted, not their lengths. The residuals should be randomly distributed and free from systematic trends. The most useful residual plots are *Y* residuals versus predicted *Y*, and *Y* residuals versus scores plots.

Where there is more than one *Y* variable, PLS2 is designed for interpretation of all the variables simultaneously. It is often argued that PLS1 or PCR are better predictors; this is usually true if there are strong nonlinearities in the data, in which case modeling each *Y* variable separately according to its own nonlinear features might perform better than trying to build a common model for all *Y*'s. On the other hand, if the *Y* variables are somewhat noisy, but strongly correlated, PLS2 will model the whole information by excluding more noise.

As in PCA, outliers may influence modeling and should be detected. In regression, there are many ways a sample can be defined as an outlier. It may be an outlier according to *X* variables only or to *Y* variables only, or to both. It may also not be an outlier for either separate set of variables but become an outlier for (*X*, *Y*) regression.

4.2.3.14 Regression Practice in NIRS

Calibration is the fitting stage: The main data set, containing only the calibration samples set, is used to compute model parameters such as PCs, regression coefficients, and the like. The models must be validated to get an idea of how well a

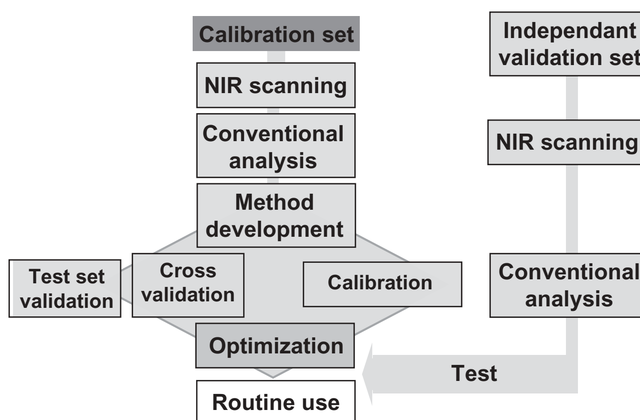

FIGURE 20 Typical working flowchart of the development of a PLS calibration with NIR spectra.

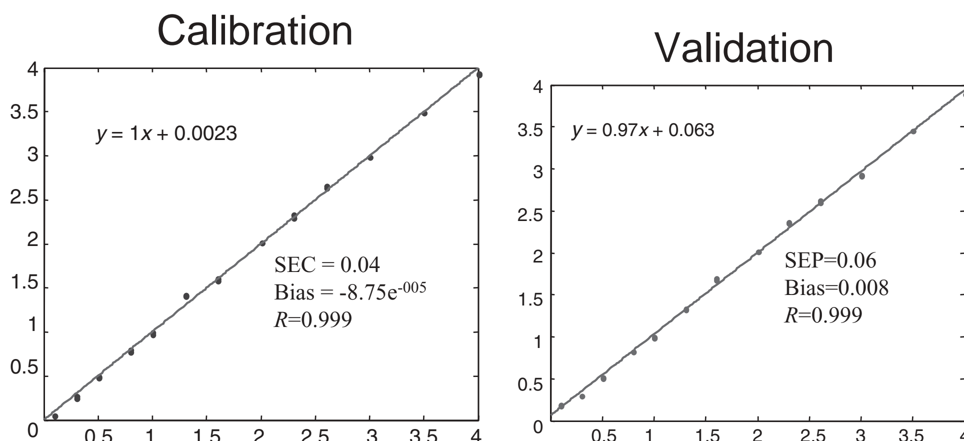

FIGURE 21 Typical output of a PLS calibration and validation.

regression model performs if used to predict new, unknown samples. A test set consisting of samples with known variable values is used. Only the *X* values are fed into the model, from which response values are predicted and compared to the known, true response values. The model is validated if prediction residuals are low. Validating a model means checking how well the model performs on real new data. As a regression model is usually made to perform predictions on future unknown samples, validation must estimate the uncertainty of prediction. If the uncertainty is reasonably low, the model can be considered usable. The steps required for complete modeling are illustrated in Figure 20. And in Figure 21 the end result obtained from a typical chemometric package performing calibration and validation is shown.

Independent (external) test-set validation and cross-validation are the most current methods of estimating prediction error. External test-set validation is based on testing the model on a subset of available samples, which will not be involved in

TABLE 3 Descriptors Used to Estimate Performance of Calibration

$$\sigma_y = \sqrt{\frac{\sum_{j=1}^{m}(y_j - \bar{y})^2}{m-1}} \qquad \mathrm{cov}(y', y) = \sqrt{\frac{\sum_{j=1}^{m}(y_j - \bar{y})(y'_j - \bar{y}')}{m-1}} \qquad R = \frac{\mathrm{cov}(y', y)}{\sigma_{y'}\sigma_y}$$

$$\mathrm{SEC} = \sqrt{\frac{\sum_{j=1}^{m}(y_j - y'_j)^2}{m-1-q}} \qquad \mathrm{SEP} = \sqrt{\frac{\sum_{j=1}^{n}(y_j - y'_j)^2}{n}} \qquad \mathrm{Bias} = \frac{\sum_{j=1}^{n}(y_j - y'_j)}{n}$$

where y_j = reference value for the sample j
y'_j = predicted value for the sample j
m = number of samples in calibration
n = number of samples in validation
q = PC number

computing the model components. For example, the global data table is split into two subsets. The calibration set contains all samples used to compute the model components, using both X and Y values. The test set contains all the remaining samples, for which X values are fed into the model once a new component has been computed. The predicted Y values are then compared to the observed Y values, yielding a prediction residual that can be used to compute a validation residual variance, or a measure of the uncertainty of future predictions, called root mean square error of prediction (RMSEP). This value is calculated for each modeled response and indicates the average uncertainty that can be expected when predicting Y values for new samples, expressed in the same units as the Y variables. Table 3 gives a formula for RMSEP. The results of future predictions can then be presented as the predicted values ±RMSEP. This measure is valid provided that the new samples are similar to those used for calibration. Otherwise, the prediction error might be much higher. No assumption about statistical error distribution is made for modeling. As a consequence, prediction error cannot be given as a proper statistical interval estimate (e.g., twice the standard deviation, etc.). RMSEP is a practical average prediction error and if both calibration and validation sample sets are representative of future samples to predict, it is a good error estimate.

With cross-validation, the same samples are used both for model estimation and testing. Cross-validation represents an alternative way of utilizing samples for validation if their number is small or moderate. The method consists in leaving out a few samples from the calibration data set and calibrating the model on the remaining data points. The left-out sample values are then predicted and the corresponding prediction residuals computed. The process is repeated with another subset of the calibration set, and so on until every object has been left out once. All prediction residuals are combined to compute the validation residual variance and RMSEP. Full cross-validation leaves out only one sample at a time. Segmented cross-validation leaves out a whole group of samples at a time. Segmented cross-validation is faster, but segment selection requires some care since it should feature unique information. For example, samples which can be considered as replicates of each other should not be present in different segments.

In the case of an independent test set the file should contain 20–40% of the full data. The calibration and test set must cover the same population of samples as

possible. Replicate measurements should never be present in both the calibration and test sets. This risk exists with a random selection which is proposed in current NIR software. If it is the simplest way to select a test set, it leaves the selection to the computer. Manual selection is recommended since it gives full control over the selection of a test set.

Multiple regression programs also calculate auxiliary statistics, designed to help decide how well the calibration fits the data, and how well it can be expected to predict future samples. For example, two of these statistics are the standard error of calibration (SEC) and the multiple correlation coefficient (R). The SEC (also called standard error of estimate, or residual standard deviation) and the multiple correlation coefficient indicate how well the calibration equation fits the data. Their formulas are given in Table 3.

The SEC has the same units as the dependent variables, and it reflects the differences between the instrumental value for the analyte of interest and the reference laboratory value. It expresses the modeling error and cannot be used to estimate future prediction errors. In the absence of instrumental error, SEC is only the measure of reference laboratory error. It is an indication of whether calculation using the calibration equation will be sufficiently accurate for the purposes for which it has been generated. In practice, SEC is less accurate than the reference laboratory, even in the absence of instrumental error, if the wavelength range used as independent variables in the calibration does not account for all interference in the samples or if other physical phenomena are present. The detection limit and sensitivity, or signal-to-noise, defines instrument performance for a specific NIR application. Detection limits can usually be approximated for any NIRS method as equal to three times the SEC for the specified application. Sensitivity for quantitative NIR methods can typically be evaluated as the slope of the calibration line for the concentration of the examined analyte (y axis), versus the change in optical response (x axis), between samples of varying concentration. Sensitivity, from a purely instrumental point of view, is expressed as a signal-to-noise or peak height ratio for a particular compound versus peak-to-peak noise at some absorbance (usually zero). However, in a practical sense, the above considerations do not really matter in NIR spectroscopy. This is due to the fact that the applications developed for practical NIR are mostly based on empirical calibration methods and that the calibration is specific to the problem of interest. It is a characteristic of calibration equations using multivariate mathematical techniques to compensate for the common variations found in noisy chemical samples and imperfect instrumental measurements. This is why properly calculated and validated NIR calibration models are robust and work extremely well.

The multiple correlation coefficient R is a dimensionless measure of how well the calibration fits the data. R can have values between −1 and +1, but in a calibration situation only positive values exist. A value close to zero indicates that the calibration fails to relate the spectra to the reference values. As the correlation coefficient increases, the spectra become better and better predictors of the reference values. Because the multiple correlation coefficient is dimensionless, it is a useful way of comparing data or results with different units, and that are difficult to compare in other ways. However, its value gives no indication of how well the calibration equation can be expected to perform on future samples.

4.2.3.15 Some Pitfalls

The first step before any multivariate data analysis is sampling. The selection or preparation of a set of calibration samples is a critical issue. For example, the analyst must collect or prepare samples which span the complete range of constituent concentrations as evenly distributed as possible. It is usual for random sample selection to cause the models to fit most closely to the mean concentration samples. Samples at high or low concentration levels will not influence the slope and intercept in the case of multivariate regression. Ideally, even concentration distribution will allow the model to minimize the residuals at the extremes and at the center with relatively equal weighting. Calibration sets must not only uniformly cover an entire constituent range, they must also be composed of a correctly distributed number of sample types. For example, ideal calibration sets are composed of more than 10–15 samples per analytical term. These samples ideally have widely varying composition evenly distributed across the calibration range. Last but not least, spectroscopic measurements should be performed under as identical conditions as possible between calibration samples and routine samples. A recurrent difficulty is the effect of moisture within a solid or powdered sample. The presence or absence of water in a sample influences the extent of hydrogen bonding, which affects both band position and width in the complete NIR domain. If a calibration model is developed from samples featuring a wide range of the component of interest but a small range in moisture, it will only be useful for samples with that narrow moisture range. Such a calibration may not be robust enough for routine application. To summarize, each calibration problem involves slightly different aspects.

Paying attention to finer details may be obvious, as unexpected error sources can affect the quality of computed models. Errors occur, for example, in sample preparation and measurement. Parameters of potential influence include temperature differences in samples or the instrument while recording the data, calibration sample instability, instrument noise and drift, changes in instrument wavelength setting, nonlinearity, stray light effects, particle size differences, concentration-dependent color differences, residual solvent interaction, and nonhomogeneous samples. The reference method may not measure the same components as the spectroscopic method. Controlling for these aspects may sound overly rigorous and may initially dampen enthusiasm for NIRS. However, multicomponent problems are inherently complex and require the management of several variables simultaneously in order to develop a usable calibration. The ultimate goal of successful calibration is to calculate a mathematical model with the calibration samples which is most sensitive to changes in the modeled parameter and least sensitive to all other noncalibration related factors, such as physical, chemical, and instrumental variables. Every case must be evaluated in terms of the chemical and physical data carried by the calibration samples and the information which the analyst wishes to obtain.

4.2.3.16 Example Analytical Applications of NIRS

Assuming NIR spectra have been recorded, once a set of pharmaceutical samples has been analyzed with high precision by some analytical reference method, the concentration of an analyte of interest clearly determined or the identity of a given

compound confirmed, the sample data can then be filed into a training set to generate a calibration for subsequent predictions. To form a usable training set, the samples should evenly span the concentration range for the analyte of interest or the expected variations in quality. An obvious pitfall is to develop calibrations that only use sample sets with uneven constituent distributions or too narrow variations of the attribute of interest. In that case the model will most closely fit the dominating samples in the calibration set. The calibration will be highly weighted to the mean value and behave poorly against variations in further samples. Conversely, an ideally evenly distributed calibration set will weight the calibration model equally across the entire concentration range. A properly developed calibration model of this kind will perform most accurately with samples at high and low concentrations.

Despite these sampling issues, which are important for NIR spectroscopists in the pharmaceutical industry, the potential of NIR applications remains intact. NIR is rapid and nondestructive, requiring little or no sample preparation. It can monitor concentrations of several chemical species and physical parameters simultaneously. Its speed and ease are a major bonus in many analytical or monitoring processes. All types of materials are concerned, from solid or liquid raw materials (e.g., excipients), active pharmaceutical ingredients (API), intermediate synthesis products, intermediate formulations in the form of powders, slurries, granulates, or pellets, up to final dosage forms such as capsules, tablets, or lyophilized substances. NIR measurements can be performed in close or direct contact with the sample, in both the laboratory or directly online or inline in the production plant, in order to obtain analytical information rapidly and save time. Transmittance measurements have become an alternative to the conventional reflectance spectroscopy of pharmaceuticals. The important difference is that transmittance NIR samples a volume, whereas reflectance NIR merely samples the surface of solid samples. This has the advantage of more representative values for less homogenous samples like tablets or capsules. On the other hand, more attention has to be paid to sample presentation with respect to stray light and light scattering.

Traditionally, pharmaceutical excipients have been characterized in the laboratory by viscosity, pH in dispersion/solution, water content, ash content, constituent amounts, particle size, and so on. In reality, there are also other subtle properties that are not covered by these parameters but that nevertheless affect the properties of a drug formulation. Many of these variations can be extracted from NIR reflectance spectra in addition to identity testing by other methods (Figure 22). The raw material spectra and results from other analytical methods can then be combined to predict the impact of raw material quality variations on final batch quality. This conformity test by NIR can be achieved by recording numerous raw material batches of known quality and calculating an envelope of acceptability around the mean spectrum. Conform raw material is only qualified when it lies within the threshold values of the envelope at each wavelength. This test would fail poor materials with high impurities and high water levels.

Most pharmaceutical production is performed in batches, both when synthesizing active compounds and manufacturing pharmaceutical formulations. NIR can measure the final state in batch production in terms of spectral similarity, using SIMCA, spectral correlation, or spectral distance. Individual batch development can also be monitored using PCA. Several parameters of the same product can be

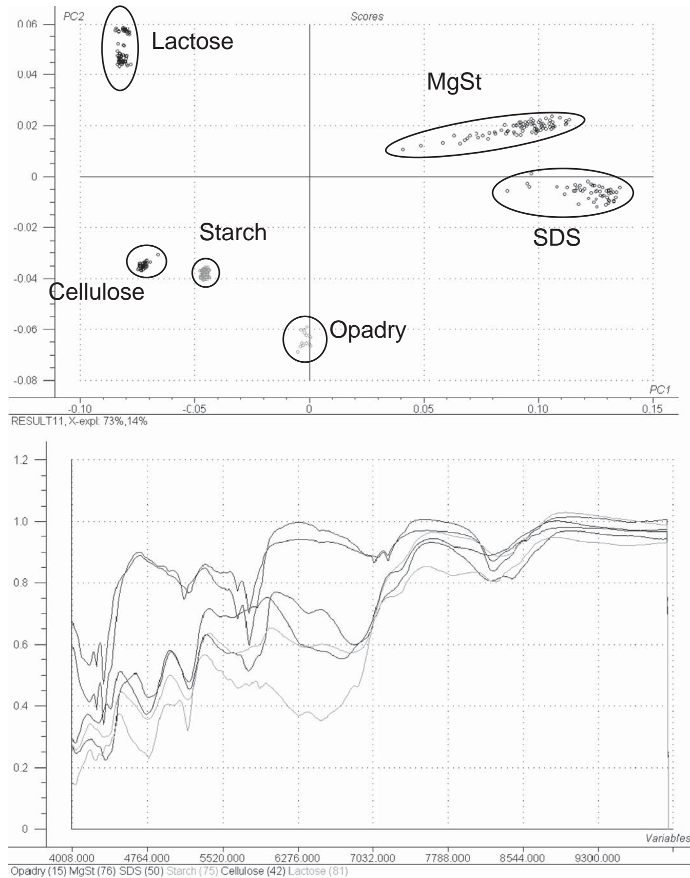

FIGURE 22 Excipient library with the result of a PCA analysis for identity testing.

determined quantitatively using PLS models. A current basis for comparison includes processing time as a *Y* variable in a PLS model based on several batches considered good for release. Limits for an accepted batch can then be calculated and compared with online NIR measurements during the batch process.

Drying of drug substances is an important step in the production of active materials. It is usually performed in an agitated vacuum dryer, carefully controlled to ensure a suitably dry product and to minimize the risk of overdrying which could damage the high cost material. Some drug substances are hydrates, which present special requirements for dryer control. If the waters of crystallization are removed, the batch may need reprocessing or may even be lost. The traditional means of monitoring and controlling drying operations is to use the slight changes in temperature within the dryer to estimate an endpoint and then to sample the volatile content for laboratory analysis. There is often no distinct temperature endpoint. Some products will also retain residual solvent within the crystals. Sampling vacuum dryers is difficult as the vacuum often has to be released, and the laboratory analysis can take several hours. The use of an NIR fiber-optic probe, inserted directly into the plant scale dryer, can provide real-time analysis of dryer contents and can thus

be considered as a valuable alternative. Differences in physical and chemical properties between polymorphs of an active compound (e.g., solubility, dissolution rate, chemical reactivity, resistance to degradation, bioavailability) are highly significant for the pharmaceutical industry. Effective methods are required not only to control the content in the active compound but also to detect and determine the undesirable form. NIRS can be used in some circumstances to confirm a low percentage of undesirable crystalline state in the amorphous polymorph of a compound. The underlying methods are both qualitative (e.g., classification) and quantitative. The simplicity, expeditiousness, and reliability of the NIR method make it a promising tool for controlling the polymorphic purity of the amorphous phase.

4.2.3.17 Conclusion

In many pharmaceutical companies, quality control departments already use NIRS to identify formulations. Figure 23 illustrates a PLS calibration for the active content determination in a low-dose tablet. Once identity testing is passed, it is straightforward to consider as a next step the determination of active content in intact tablets. Thus, qualitative and quantitative analysis can be performed by acquiring a single NIR spectrum per sample. Two analytical techniques are replaced by one—nondestructive—NIR measurement. For this purpose near-infrared spectroscopy is a fast and powerful alternative to traditional analysis, which only remains necessary as reference analytics.

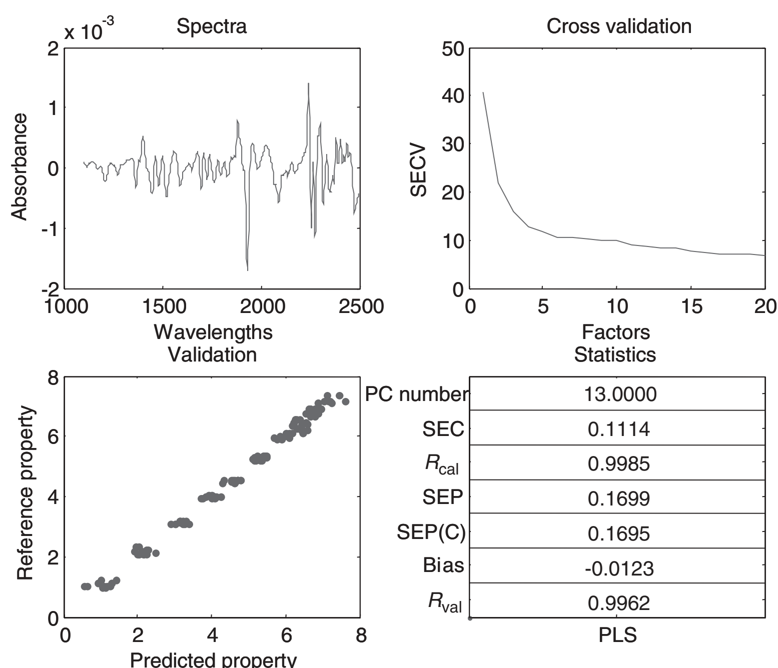

FIGURE 23 Quantification of the API content in a tablet and overview of the corresponding PLS regression.

The viability of analytical methods obtained by combining vibrational spectroscopy—mainly NIRS—and chemometrics, once validated, can be proved on numerous industrial examples. However, dominant application field of chemometrics is not the pharmaceutical industry, and most accumulated experience is found in the agricultural and food industries. Best practice in chemometrics is independent of the analytical problem or working field. To solve complex pharmaceutical problems by using a large band of spectroscopic methods and at the same time optimizing sample handling, multivariate data analysis algorithms are highly recommended. Thus, education of chemists, pharmacists, and analysts with regards to chemometrics remains a requirement to catch from the beginning the usefulness of multivariate data analysis applied to spectroscopic data.

BIBLIOGRAPHY

Section 4.2.1

Bakeev, K. A., Ed. (2005), *Process Analytical Technology*, Blackwell, London.

Giodici, P. (2003), *Applied Data Mining. Statistical Methods for Business and Industry*, Wiley, Hoboken, NJ.

Initiative for Pharmaceutical cGMPs for the 21st Century, FDA, Washington, DC.

Martin, A. (1993), *Physical Pharmacy*, 4th ed., Lippincot Williams & Wilkins. Philadelphia, PA.

Section 4.2.2

Colthup, N. B., Daly, L. H., and Wiberley, S. E. (1990), *Introduction to Infrared and Raman Spectroscopy*, 3rd ed., Academic.

The Proceedings of NIR-97, *J. Near-Infrared Spectros.*, 6 (1–4), NIR Publications, 1998.

Rouessac F., and Rouessac, A. (2000), *Chemical Analysis. Modern Instrumentation Methods and Techniques*, Wiley-VCH, New York.

Siesler, H. W., Ozaki, Y., Kawata, S., and Heise, H. M. Eds (2002), *Near-Infrared Spectroscopy. Principles, Instruments, Applications*, Wiley-VCH, New York.

Williams, P., and Norris, K. (2001), *Near-Infrared Technology in the Agriculture and Food Industries*, 2nd ed. American Association of Cereal Chemists, St Paul, MN.

Section 4.2.3

Brereton, R. G. (2003), *Chemometrics: Data Analysis for the Laboratory and Chemical Plant*, Wiley, Hoboken, NJ.

Kramer, R. (1998), *Chemometric Techniques for Quantitative Analysis*, Marcel Dekker, New York.

Manly, B. F. J. (2000), *Multivariate Statistical Methods. A Primer*, 2nd ed., Chapman & Hall/CRC, New York.

Mark, H. (1991), *Principles and Practice of Spectroscopic Calibration*, Wiley, New York.

Martens, H., and Martens, M. (2001), *Multivariate Analysis of Quality: An Introduction*, Wiley, New York.

Massart, D. L., Vendeginste, B. G. M., Buydens, L. M. C., De Jong, S., Lewi, P. J., and Smeyers-Verbeke, J. (1997/1998), *Handbook of Chemometrics and Qualimetrics, Parts A and B*, Elsevier, Amsterdam.

Naes, T., Isaksson, T., Fearn, T., and Davies, T. (2002), *A User-Friendly Guide to Multivariate Calibration and Classification*, NIR Publications, Chichester.

Peer Review Articles

Chalus, P., Roggo, Y., Walter, S., and Ulmschneider, M. (2005), Near-infrared determination of active substance content in intact low-dosage tablets, *Talanta*, 66, 1294–1302.

Inschneider, A. (2002), Validierung von chemometrischen Methoden am Beispiel multivariater Datenanalyse in der Nah-Infrarot Spektroskopie, in *Handbuch der Validierung*, Kromidas, S. Ed., Wiley-VCH, Weinheim, pp. 302–307.

Roggo, Y., Edmond, A., Chalus, P., and Ulmschneider, M. (2005a), Infrared imaging for qualitative analysis of pharmaceutical solid forms and trouble shooting, *Anal. Chim. Acta*, 535, 79–87.

Roggo, Y., Jent, N., Edmond, A., Chalus, P., and Ulmschneider, M. (2005b), Characterizing process effects on pharmaceutical solid forms using near-infrared spectroscopy and infrared imaging, *Eur. J. Pharm. Biopharm*., 61(1–2), 100–110.

Roggo, Y., Roeseler, C., and Ulmschneider, M. (2004), Near-infrared spectroscopy for qualitative comparison of pharmaceutical batches, *J. Pharm. Biomed. Anal*., 36, 777–786.

Russell, F., and Ulmschneider, M. (2004), Dissolution testing by means of NIR transmittance spectroscopy, *Am. Pharm. Rev*., July/August.

Sukowski, L., and Ulmschneider, M. (2005), In-line process analytical technology on qualitative NIR modelling: An innovative approach for the pharmaceutical quality control, *PharmInd*, 67(7), 830–835.

Ulmschneider, M., Barth, G., Reder, B., Vögel, A., and Schilling, D. (2000a), A transferable basic library for the identification of active substances using near-infrared spectroscopy, *Pharm. Ind*., 62(4), 301–304.

Ulmschneider, M., Barth, G., and Trenka, E. (2000b), Building transferable cluster calibrations for the identification of different solid excipients with near-infrared spectroscopy, *Pharm. Ind*., 62(5), 374–376.

Ulmschneider, M., and Pénigault, E. (2000a), Assessing the transfer of quantitative NIR-calibrations from a spectrometer to another one, *Analusis*, 28, 83–87.

Ulmschneider, M., and Pénigault, E. (2000b), Direct identification of key-intermediates in containers using Fourier-Transform near-infrared spectroscopy through the protective polyethylene primary packaging, *Analusis*, 28, 136–140.

Ulmschneider, M., and Pénigault, E. (2000c), Non-invasive confirmation of the identity of tablets by near-infrared spectroscopy, *Analusis*, 28, 336–346.

Ulmschneider, M., Wunenburger, A., and Pénigault, E. (1999), Using near-infrared spectroscopy for the non invasive identification of five pharmaceutical active substances in sealed vials, *Analusis*, 27, 854–856.

4.3

CHEMICAL IMAGING AND CHEMOMETRICS: USEFUL TOOLS FOR PROCESS ANALYTICAL TECHNOLOGY

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4.3.1 INTRODUCTION

Chemical compound homogeneity is an important issue for pharmaceutical solid forms. A classical spectrometer [1–3] integrates spatial information. However, use of a mean spectrum on a surface can be a drawback in solid-form analysis. For example, in the pharmaceutical industry, it is important to map the distribution of active ingredients and excipients in a tablet as this reveals physical interaction

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between components and helps to solve homogeneity issues—hence the increasing number of spectroscopic imaging studies on the visualization of chemical component homogeneity [4–9].

Vibrational hyperspectral imaging (chemical imaging) is a recent development that combines the chemical information from spectroscopy with spatial information. In principle, it is possible to collect hyperspectral images with single-point detectors, that is, classical mapping with microscopes. However, array detectors measure all pixels simultaneously, reduce recording time, provide uniform background, and improve the signal-to-noise ratio [4]. A complete spectrum is acquired for each pixel, meaning that a hyperspectral data set is in fact a data cube, that is, a three-dimensional (3D) matrix.

This chapter begins by defining hyperspectral imaging, then presents details of instrumentation and image formation, and concludes by describing some chemometric image analysis tools with the help of pharmaceutical examples.

4.3.2 DEFINITION OF HYPERSPECTRAL IMAGING

This chapter considers three types of digital image. The first is binary (or black and white), where the pixel value can be either 0 or 1. The second is monochromatic (e.g., gray scale), which can be presented as a two-dimensional (2D) $X \times Y$ array describing the distribution of light intensity, where X and Y are the numbers of digitization steps (i.e., pixels) along the two spatial directions. Each pixel has a value between 0 (black) and 255 (white). The third is color, which can be described in the red–green–blue (RGB) space, for example, with three planes (for red, green, and blue, respectively) as a 3D $X \times Y \times 3$ matrix. Each pixel has a value between 0 and 255 for red, green, and blue, generating 255^3 possible colors for such images (known as 24-bit images).

By analogy, a hyperspectral data set is defined by at least 50 planes; an absorbance map is acquired for each wavelength in the spectral range. If the wavelengths number less than 50, the term *multispectral imaging* is used.

With chemical imaging, a new type of data structure needs to be analyzed. Chemical imaging experiments yield a 3D $X \times Y \times \lambda$ matrix or data cube, where X and Y are the spatial dimensions and λ the spectral dimension. One spectrum per pixel is recorded and selection of a wavelength will show an absorbance picture of the sample [10] (Figure 1).

The data cube combines spectral and spatial information and therefore includes the requisite statistics for spectral classifications. However, new chemometric strategies have to be applied to interpret chemical imaging results.

4.3.3 HYPERSPECTRAL IMAGE ACQUISITION AND INSTRUMENTATION

4.3.3.1 Principles of Hyperspectral Image Acquisition

This section discusses three modes of acquisition: mapping, array detection, and fiber bundles.

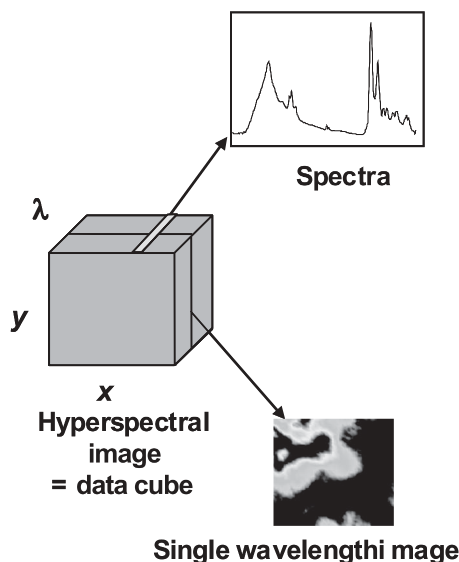

FIGURE 1 Data structure of chemical image.

Mapping Historically, mapping [11] was the first method used to acquire hyperspectral data cubes, in particular with Raman spectroscopy and infrared (IR) microscopy. The image is created pixel by pixel in a "step-and-acquire" mode: A spectrum is measured at one point of the sample, and then the sample moves to the next measurement position and another spectrum is acquired. The process is iterative for all positions in the area that define the image.

The drawback is the measurement time, which depends on the number of pixels. Spectrometer manufacturers therefore developed line mapping, in which samples are scanned line by line, thereby reducing the acquisition time. These devices were developed for Raman, IR, or near-IR (NIR) spectroscopy (with diode array detectors). However, due to the moving stage, this kind of imaging principle is only suitable for at-line applications.

Focal Plane Array Detectors Focal plane array (FPA) optical detectors are composed of several thousand individual detector elements forming a matrix of pixels. As their name indicates, they are placed in the spectrometer's focal plane. They can be manufactured to be sensitive to ultraviolet (UV), visible (Vis), NIR, or IR radiation. Recent developments in optics have produced cooled and uncooled FPAs with different numbers of pixels from 64×64 up to 1024×1024 and different spectral ranges of detection (from 1000 to 12,000 nm). The different types of FPA include those built with indium antimonide (InSb), platinum silicide (PtSi), indium gallium arsenide (InGaAs), and mercury cadmium telluride (HgCdTe) [12]. The mercury cadmium telluride (MCT) detector has become the dominant IR FPA through its entire range coverage.

Fiber Bundles Fiber bundles are used for Raman imaging. Several optical fibers are grouped together, each analyzing a specific sample area [13]. A 3D data cube is

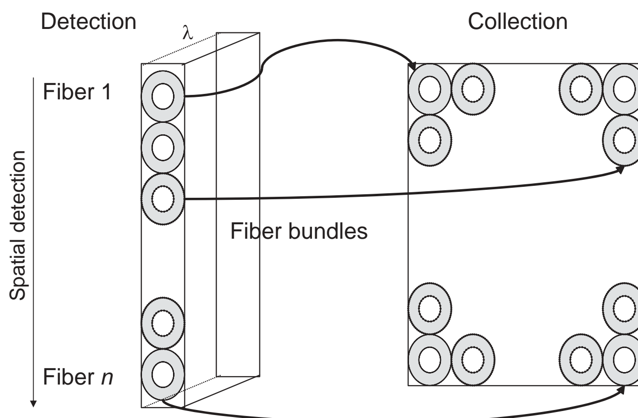

FIGURE 2 Principle of fiber bundles. (Adapted from A. D. Gift, J. Ma, K. S. Haber, B. L. McClain, and D. Ben-Amotz, *Journal of Raman Spectroscopy*, 30, 757–765, 1999.)

collected using a 2D charge-coupled device (CCD) detector. At the collection point the fibers are arranged in a circular pattern or square array in order to analyze defined surfaces on the samples (Figure 2). At the detection end, the fibers are aligned in order to detect the signal: The first CCD detector dimension is used for spatial information and the second for the spectral region. The technical difficulty is to assign the signal position on the detector end with the pixel position in the image. This technique's main advantage is fast image acquisition.

4.3.3.2 Spectroscopic Instrumentation

The imaging detectors, whether for point mapping, line scanning, or array detection, can be coupled with different types of spectrometers. Instrument types are classified by wavelength selection modality into imaging Fourier transform (FT) and tunable filter (TF) spectrometers, both of which are presented below, and dispersive spectrometers. FT imaging systems are classical laboratory instruments while TF spectrometers are compact and robust systems for chemical imaging.

FT Spectrometers FT spectrometers (Figure 3) differ from scanning spectrometers by the fact that the recorded signal is an interferogram [14] (see Chapter 6.2). They can be coupled to a microscope or macrochamber with an FPA detector. FT chemical imaging systems (CISs) are available for Raman, NIR, and IR spectroscopy. However, they can only be considered as research instruments. For example, most IR imaging systems are FT spectrometers coupled to microscopes. This type of spectrometer allows the acquisition of spectra in reflection, attenuated total reflection (ATR), or transmission mode.

TF Systems A TF is a device whose spectral transmission can be controlled by applying a voltage or acoustic signal. There are two main TF devices: acousto-optical TF (AOTF), based on diffraction, and liquid crystal TF (LCTF), based on birefringence. An AOTF is a transparent crystal in which an ultrasonic wave field is created,

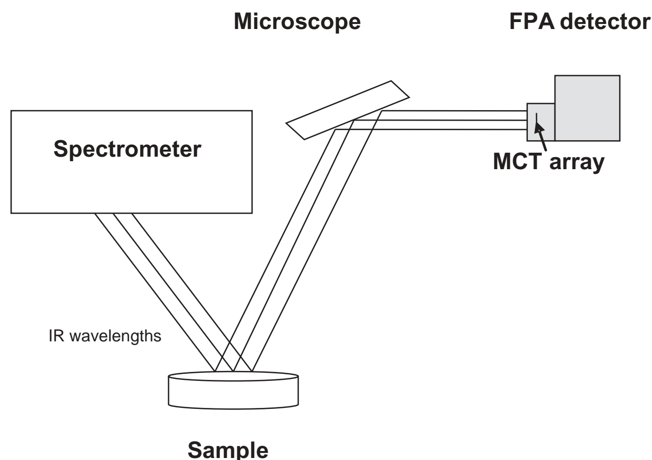

FIGURE 3 IR microscope linked to FPA detector.

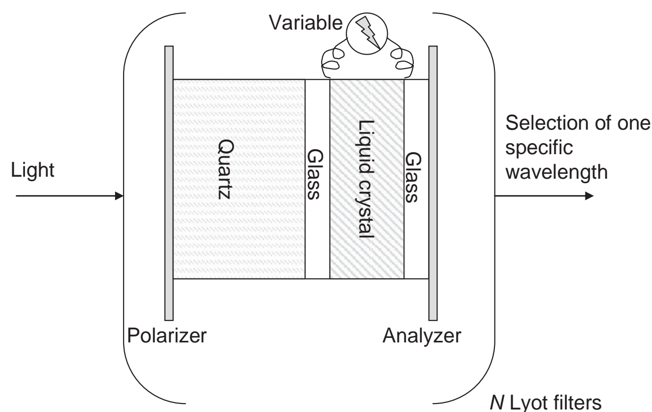

FIGURE 4 Lyot filter: LCTF element. (Adapted from N. Gat, *Proceedings SPIE*, 4056, 50–64, 2000.)

that is, the selected wavelength is a function of field intensity. An LCTF is built using several Lyot filters (Figure 4): The incoming light is first polarized for one Lyot filter element, then the birefringent crystal introduces a phase difference (δ) between the rays of light, and the light finally passes through the second "analyzer" polarizer, which selects the transmitted wavelength. The disadvantage of an LCTF compared to an AOTF is in speed: AOTFs scan in microseconds, LCTFs in milliseconds [15].

With TFs, samples can be scanned wavelength by wavelength. Single-wavelength images are then grouped into a data cube. Specific wavelengths (e.g., specific for particular chemical compounds) can be selected to reduce acquisition time. Commercial TF devices (Figure 5) are available for NIR and Raman imaging. TF imaging systems appear suitable for process analytical technology (PAT) applications in that they can be installed online due to their fast acquisition times and simple and robust mode of image acquisition.

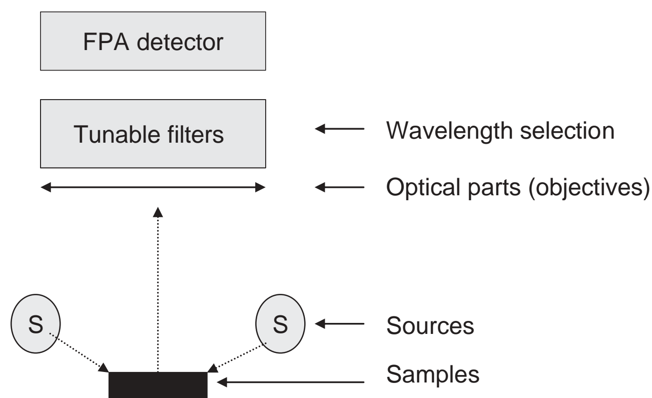

FIGURE 5 Acquisition by LCTF NIR imaging system. (Adapted from N. Lewis, J. Schoppelrei, E. Lee, and L. Kidder, in *Process Analytical Technology*, Editor K. A. Bakeev Blackwell, London., 2005, pp. 187–225.)

4.3.4 CHEMOMETRIC FOR IMAGING

Hyperspectral imaging systems generate a large amount of data which has to be processed in order to find the relevant information. Extraction methods must therefore be developed to display images of chemical compound homogeneity ("distribution maps"). The main steps in data cube analysis (Figure 6), presented in sequence below, begin with data preprocessing (to improve information quality), proceed with classification (to identify the main chemical compounds for each pixel and display the distribution maps), and terminate in the deployment of tools for distribution map analysis.

4.3.4.1 Data Preprocessing

Preprocessing enhances chemical information and removes noise and scattering effects [14]. Its specificity resides in the fact that data cubes can be preprocessed in both the wavelength and spatial dimensions.

Classical spectral preprocessing, described in Section 4.3.1, comprises normalization, smoothing, and baseline correction. Some CIS provide intensity as reflectance. The data are therefore converted to absorbance. Another type of spectral preprocessing can be added based on prior chemical knowledge: wavelength selection. The less informative spectral ranges are removed in order to reduce computation time and improve convergence of chemometric algorithms.

Image preprocessing techniques are also useful. Thus bad pixels (e.g., those without signals or outliers) can be removed and several data cubes grouped together for simultaneous analysis in order to simplify comparison. The hyperspectral image can also be masked to select only the regions of interest. Manuals describe other image preprocessing methods, such as spatial smoothing, contrast enhancement, deblurring, and filtering [16].

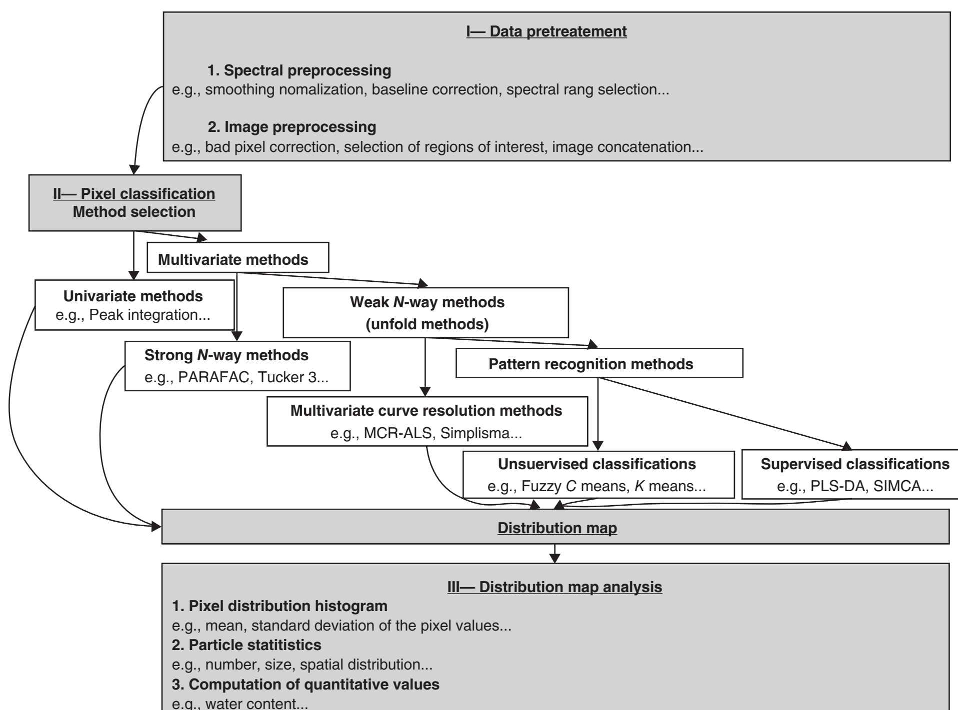

FIGURE 6 Image analysis workflow: processing chain.

4.3.4.2 Pixel Classification

After data preprocessing, classification is a critical step in distribution map extraction. Various algorithms can be applied. Although an exhaustive list is beyond the scope of this handbook, methods can be grouped to give an overview of data cube processing solutions (Figure 6). Chemometric techniques are grouped by dichotomy according the following criteria: univariate or multivariate analysis, strong or weak *N*-way methods, multivariate curve resolution or pattern recognition techniques, and supervised or unsupervised classifications.

Univariate and Multivariate Methods Univariate methods belong to classical spectroscopy: Depending on the chemical structure of the compound, a specific wavelength is selected to compute the distribution map. Peak height, area, and ratio between two peaks are used to display a false color picture in which the lowest value is displayed in black or blue and the highest in white or red. Univariate methods are the simplest ways of obtaining distribution maps. However, selection of compound-specific wavelengths can be difficult, especially when samples are complex mixtures. The advantage of hyperspectral imaging is that full spectra are available in the data cube. Drawing on all the information contained in the data sets rather than on a few wavelengths improves chemical map extraction. The techniques used in this case are termed multivariate. Multivariate image analysis (MIA) based on chemometrics generally improves distribution map extraction. Several types of MIA

methods are presented below: strong *N*-way, multivariate curve resolution, and pattern recognition methods.

Multivariate Image Analysis: Strong and Weak Multiway Methods Strong and weak *N*-way methods analyze 3D and 2D matrices, respectively. Hyperspectral data cube structure is described using chemometric vocabulary [17]. A two-way matrix, such as a classical NIR spectroscopy data set, has two modes: O object (matrix lines) and V variables (matrix columns). Hyperspectral data cubes possess two object modes and one variable mode and can be written as an OOV data array because of their two spatial directions.

Strong Multiway Methods Strong multiway methods analyze data cubes directly, without any matrix rearrangement, whereas weak multiway methods require a prior unfolding step. Examples of strong multiway techniques used for hyperspectral imaging analysis include parallel factor analysis (PARAFAC) and Tucker 3 [three-way principal-component analysis (PCA)] [18]. Their main advantage is that they take into account the correlations between image pixels in the OO modes: Unfold methods do not use pixel spatial proximity, resulting in neglect of some data cube information.

Even if strong *N*-way methods are used to reduce image noise, compress data, and improve data cube visualization, weak multiway methods are more often used as they facilitate classification using classical single-point spectra.

Classical chemometric methods, that is, the classification and regression presented in Section 4.3.1, are also applied to hyperspectral images. However, $X \times Y \times \lambda$ matrices have to be unfolded into $(X \times Y) \times \lambda$ matrices before processing. In other words, the three-way OOV array is unfolded into a classical two-way OV matrix.

Weak Multiway Methods Figure 7 shows the three steps in weak N-way analysis: Unfold the data cube, perform the selected chemometric methods, and refold the matrix in order to display distribution maps. Weak *N*-way analysis comprises two main variants:

Multivariate curve resolution (MCR) has been in common use for 30 years in the analytical chemistry community [high-performance liquid chromatography (HPLC), FTIR, UV, NIR, and Raman]. It refers to self-modeling mixture analysis. Of the various methods, simple-to-use interactive self-modeling mixture analysis (SIMPLISMA) [19] and MCR alternating least squares (MCR-ALS) [20] appear the most successful with hyperspectral imaging. Their aim is to extract the chemical compound natures (so-called pure spectra) and concentration profiles from multicomponent systems. Their main advantage is that they are calibration free, that is, no prior knowledge is required.

Pattern recognition can be classified according to several parameters. Below we discuss only the supervised/unsupervised dichotomy because it represents two different ways of analyzing hyperspectral data cubes. Unsupervised methods (cluster analysis) classify image pixels without calibration and with spectra only, in contrast to supervised classifications. Feature extraction methods [21] such as PCA or wavelet compression are often applied before cluster analysis.

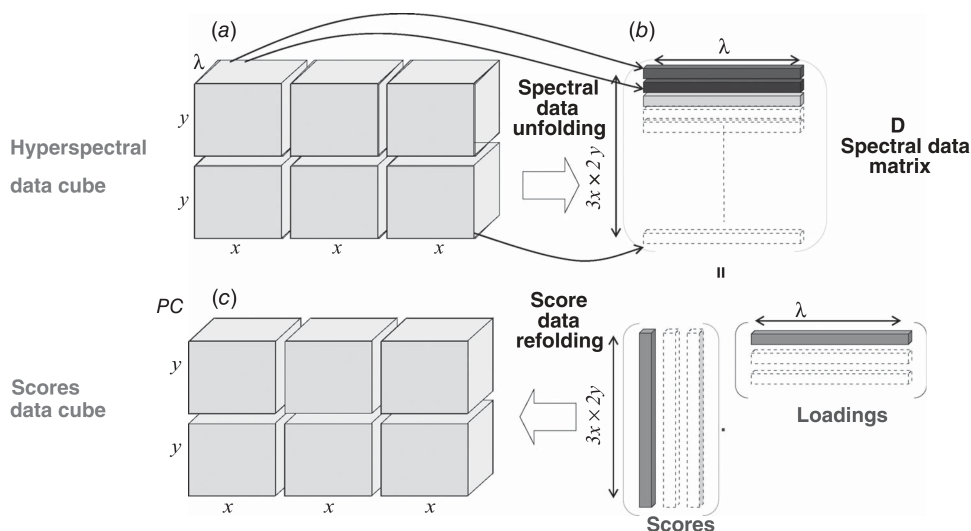

FIGURE 7 Application of weak *N*-way methods: (*a*) preprocessing; (*b*) unfold matrix analysis; (*c*) refolding. Multivariate curve resolution and pattern recognition techniques.

PCA is used to extract features and visualize data. The most important PCA application is the reduction in the number of wavelengths.

Examples of nonhierarchical clustering [22] methods include Gaussian mixture models, *K* means, and fuzzy *C* means. They can be subdivided into hard and soft clustering methods. Hard classification methods such as *K* means assign pixels to membership of only one cluster whereas soft classifications such as fuzzy *C* means assign degrees of fractional membership in each cluster.

Supervised classifications use spectral and class membership information [23]. Mathematical models are first computed with a calibration set containing spectral and class information. This model is then applied to predict new sample classes. Supervised pattern recognition algorithms fall into three main categories of contrasting pairs. The first comprises methods based on discrimination [e.g., linear discriminant analysis (LDA)] and those emphasizing similarity within a class [e.g., soft independent modeling of class analogy (SIMCA)]. The second comprises linear and nonlinear methods such as neural methods. The third comprises parametric and nonparametric computations. In parametric techniques such as LDA, the decision rules use statistical parameters of normal sample distribution. In summary, classical supervised classification methods are distance-based LDA, SIMCA, and PLS discriminant analysis (PLS-DA), which performs regression between spectra and class memberships.

Reference spectra choice is critical when applying supervised pattern recognition methods. The first solution is to use pure compound spectra as references. The drawback is that mixture spectra in data cubes often differ from the reference spectra. Applying the model may therefore give wrong results. The second solution, suitable in a few studies, is to select image pixels where only one compound is present in order to obtain the calibration sets.

4.3.5 PRACTICAL AT-LINE AND OFFLINE CHEMICAL IMAGING

Having reviewed the methods, we will now illustrate their application to pharmaceutical situations, with particular respect to univariate methods, PCA, and supervised classification (PLS-DA).

4.3.5.1 Practical Tools for Distribution Map Analysis

Image Comparison A solution for distribution map analysis is to compare images. References such as pure compounds or original samples can be included in the image. For example, references and samples can be measured simultaneously if the field of view is large enough; otherwise two data cubes can be concatenated, that is, grouped together. After the distribution map has been extracted, it can be readily interpreted simply by image comparison. In the example shown in Figure 8, the aim was to detect counterfeits. The original samples were compared with the suspected counterfeit. After PCA extraction, the differences between the two groups were clearly detected. The counterfeits had no active pharmaceutical ingredient (API) and the excipients were not identical. A self-calibrating comparison was then performed with NIR imaging for fast counterfeit detection.

Pixel Distribution and Particle Size Determination Quantitative parameters can also be computed to analyze a distribution map. The first tool (Figure 9) is to display the pixel histogram and calculate classical statistics such as mean, standard

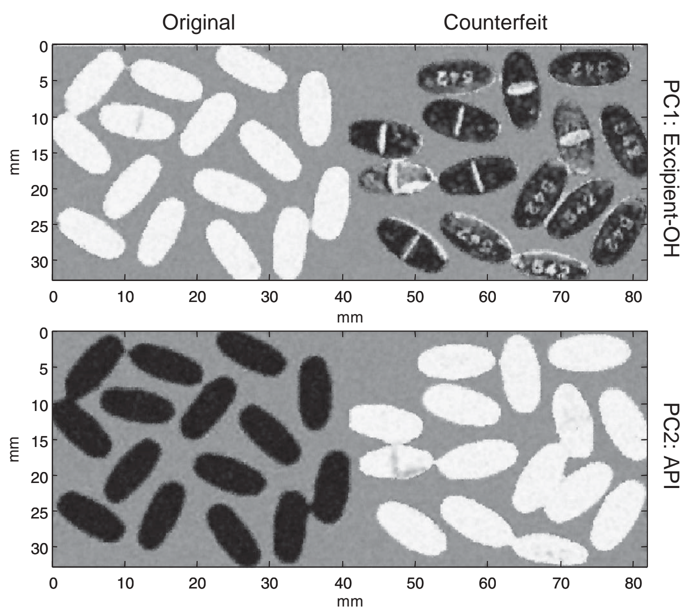

FIGURE 8 Self-calibrating image comparison for counterfeit identification: score images. White: higher score. Black: lower score.

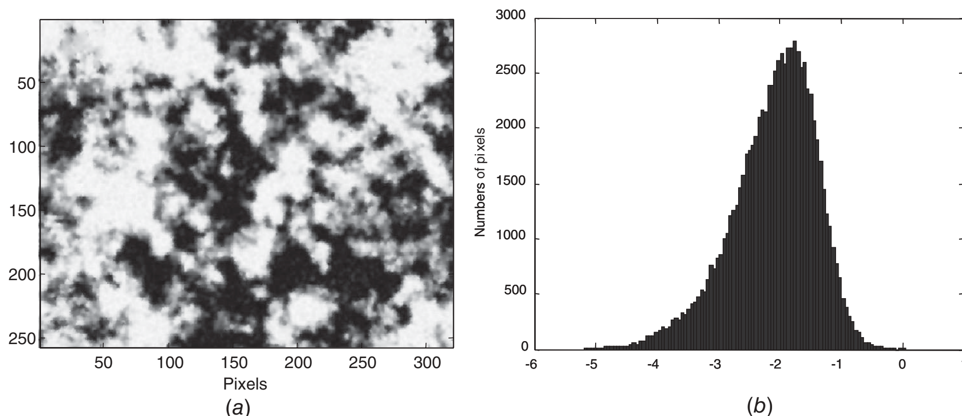

FIGURE 9 Image and associated pixel histogram (white: higher absorbance, black: lower absorbance).

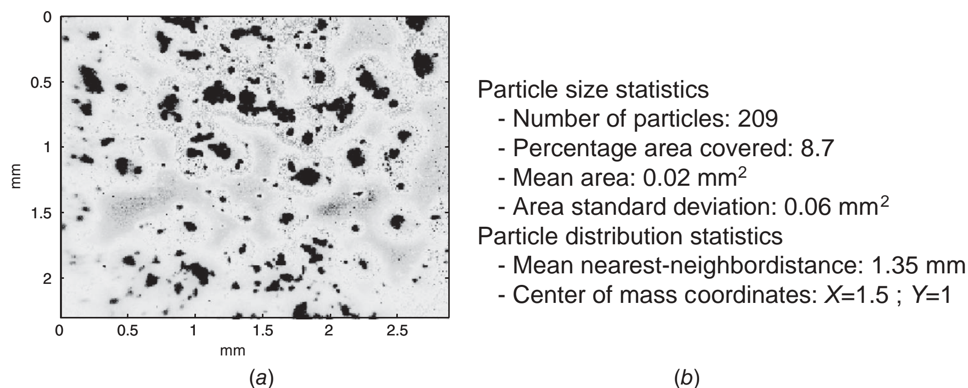

FIGURE 10 Particle size computation with imaging techniques: (*a*) powder NIR image (particles are in black); (*b*) statistics.

deviation, and the parameters of normal distribution, that is, skew and kurtosis. For example, a higher mean may be explained by a higher content of a chemical compound, and a lower standard deviation may be synonymous with greater homogeneity. The second tool is to compute particle size and obtain information about spatial particle distribution (Figure 10). Several quantitative parameters are obtained, such as particle number, particle size, and percentage area covered by the particles. Thus the particle distribution statistics provide a measure of sample homogeneity.

4.3.5.2 Wavelength Selection and Chemical Interpretation

The common aim of the Raman and NIR spectroscopy examples below was to display the API and excipient localization on the tablet surface in order to assess distribution homogeneity and characterize the solid-state properties of the API.

Example 1 API Mapping and Raman Spectroscopy

Method

A Raman microscope (Renishaw, 785-nm laser, spectral range 800–100 cm^{-1}) with a line-mapping detector (21 pixels/line) was used to analyze a solid dosage form. The image size was 105 × 88 pixels, that is, 325 µm × 270 µm, and acquisition time was about 40 min. Spectra were smoothed and normalized. Peak heights were determined for the three main compounds—API, lactose, and cellulose (Figure 11)—in order to create distribution maps.

Results and Discussion

Raman bands are narrow and specific peak selection is simple. One of the simplest classification methods, that is, univariate peak height, could thus be successfully applied. The drawbacks of Raman spectroscopy are long acquisition time and the effect of line scanning in the images (Figure 11). Another issue is fluorescence. Some chemical compounds fluoresce (e.g., cellulose), obscuring the relevant chemical signal and making the distribution map more difficult to extract.

Thus Raman imaging is a useful tool for detecting small API particles on the surface of pharmaceutical solid forms. It may even be the most suitable chemical imaging technique for API mapping due to its low spatial resolution (up to 0.5 µm/pixel) and the polymorphism of the spectral information.

Example 2 Tablet Reconstruction by NIR Imaging

Method

The tablet was cut lengthwise using a trimmer to leave a plane surface with the tablet coating removed (Figure 12). Sample and references were analyzed using a chemical imaging NIR spectrometer (Sapphire, Malvern) with the following acquisition parameters: detector size 320 × 256 array, spectral range 1100–2450 nm, and spatial resolution 40 µm/pixel. Acquisition time was about 5 min.

Results and Discussion

After the second derivative, wavelengths could be selected giving contrast images and displaying the localization of mannitol, API, and crospovidone (Figures 13 and 14) with the NIR images obtained at specific wavelengths. In this example, chemometrics can help define concentration maps by being able to use the information present in the whole spectra and not only at specific wavelengths. For example, the MCR-ALS technique was able to extract five compounds whereas wavelength selection could only display three components due to peak overlapping in the NIR range. However, simpler methods are also fast and easy to use. Because the aim was to localize the API, the wavelength selection method provided the expected results.

Images were displayed at specific wavelengths (peak-height method). The single-wavelength images were then binarized, in an operation similar to the transformation of a gray-scale image into a black-and-white image. A color image resulted (Figure 14). The red channel was associated with the API, the blue channel with mannitol, and the green channel with crospovidone. Tablet reconstruction was then possible. This highlights the main advantage of the spectroscopic technique: the large area of analysis, meaning that the images are more representative of the sample.

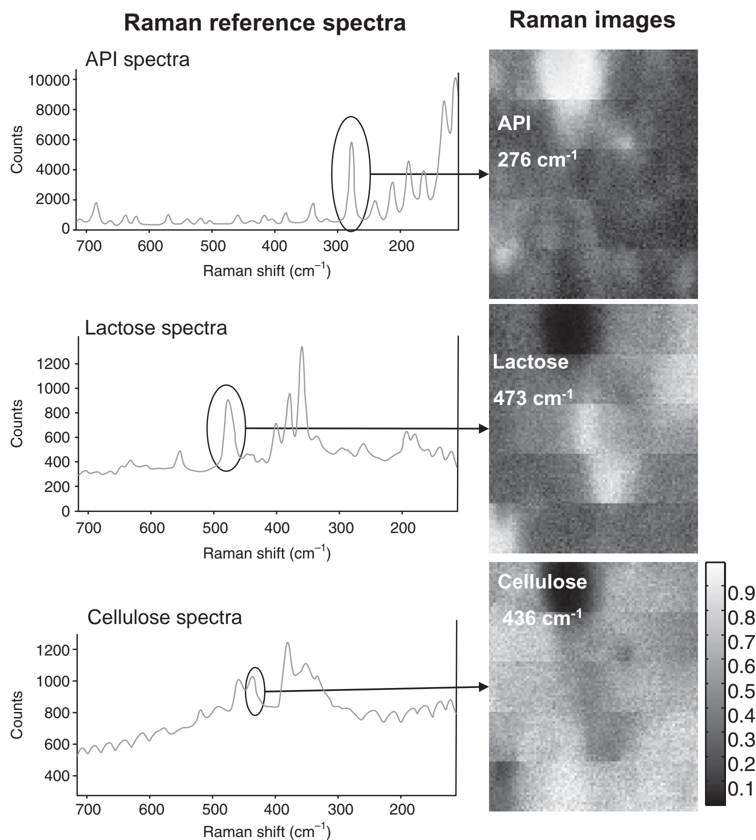

FIGURE 11 Raman images at specific wavelengths and reference spectra (image size 325 μm × 270 μm; white: higher absorbance, black: lower absorbance).

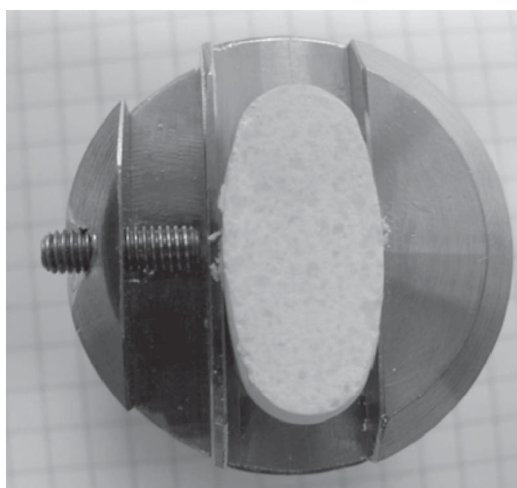

FIGURE 12 Sample and cutting holder.

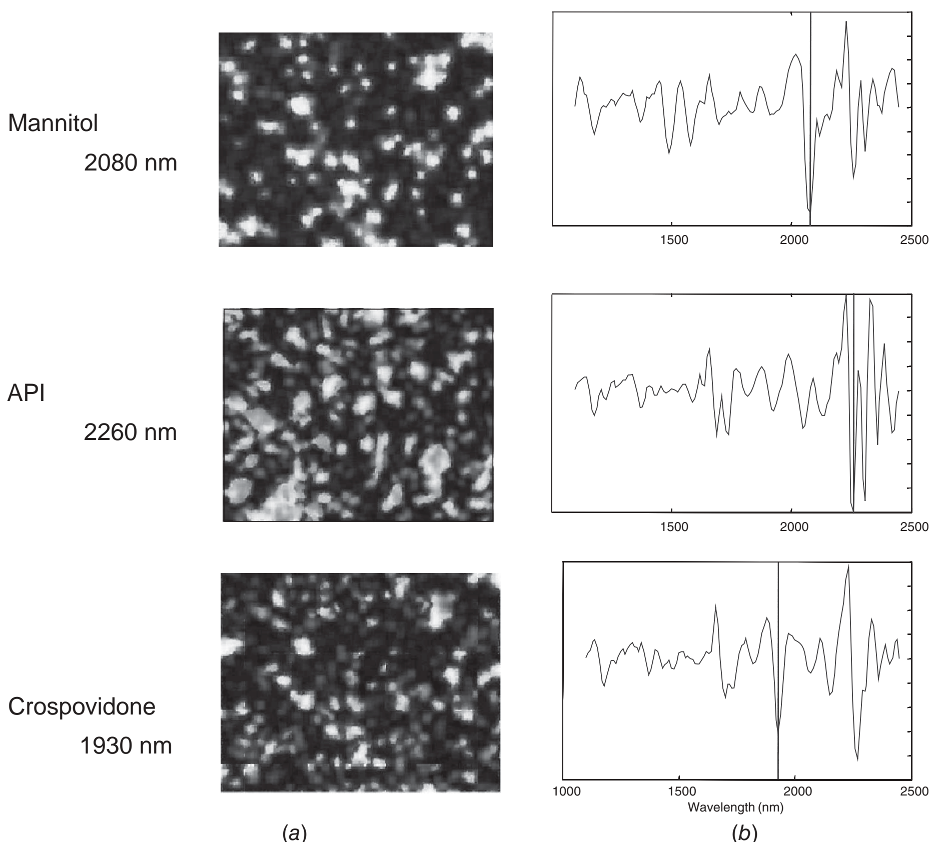

FIGURE 13 Images at component-specific wavelengths and second derivative spectra of high-absorption pixel (image size: 1.02 cm × 1.3 cm; white: higher absorbance, black: lower absorbance).

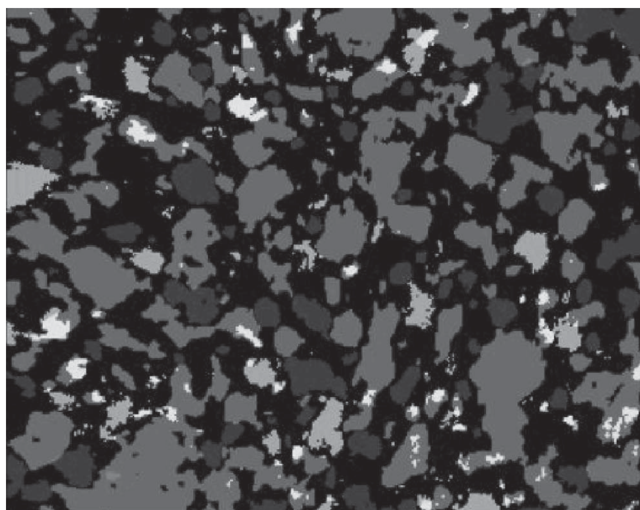

FIGURE 14 Tablet reconstruction. Gray (normally red): API. Dark gray (normally blue): mannitol. Light gray (normally green): crospovidone. Black: other. Image size 1.02 cm × 1.3 cm.

4.3.5.3 Unsupervised Classification for Process Troubleshooting

The aim of the example was to compare tablets with respect to "good" and "bad" dissolution properties using IR imaging and to identify the root causes of bad dissolution [24].

Methodology Six samples were analyzed: three passed the dissolution test (good samples) and three failed (bad samples). Several ingredients were used as references: Avicel (cellulose), the API, magnesium stearate, and poloxamer. Dissolution was tested after NIR imaging measurement to obtain the wet chemical references.

An Equinox 55 spectrometer coupled to a Hyperion 3000 microscope equipped with a 64 × 64 MCT FPA detector (Bruker, Ettlingen, Germany; www.brukeroptics.com) was used to acquire IR spectra between 3900 and 900 cm^{-1} at 16 cm^{-1} resolution (i.e., 376 data points) under N_2 purge. The pixel-grouping binning function was applied to improve the signal, and a 16 × 16 pixel image was finally acquired. The number of scans was 20 and the area of analysis per FPA measurement was 270 μm × 270 μm. Two FPA images were recorded per tablet, that is, a 32 × 16 pixel image per tablet. The three data cubes of the good samples were concatenated in the Y dimension to obtain a 32 × 48 image. The same 32 × 48 image was produced for the bad samples. The two sets of good and bad samples were then concatenated in the X dimension to obtain a 64 × 48 image. The final data cube was a 64 × 48 × 376 matrix.

Results and Discussion In the PCA results (Figures 15 and 16), loadings were interpreted using raw material reference spectra. The first loading was attributed to poloxamer, the second to magnesium stearate (Figure 16), the third to the API, and the fourth to Avicel. However, the other ingredients could also have contributed to the loadings because PCA loadings are not pure component spectra. PCA extracts orthogonal signals, which is not the case for reference spectra. In particular, the PC3 loading attributed to the API could have been contaminated by other components, especially magnesium stearate. This confirms the drawback of feature extraction methods.

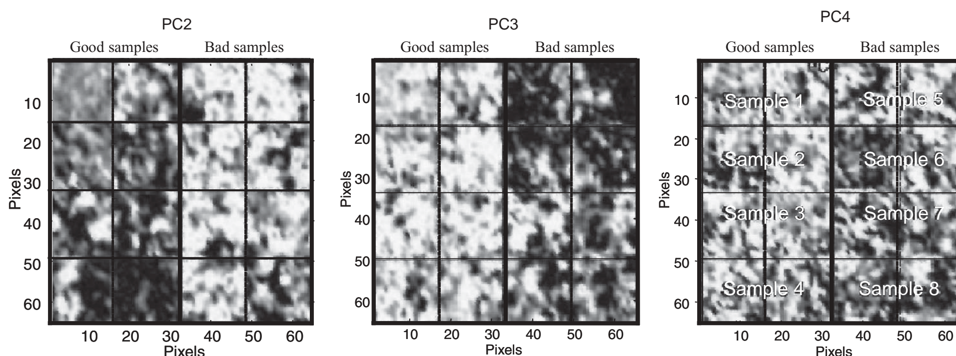

FIGURE 15 PCA score images: PC2: magnesium stearate; PC3: API; PC4: Avicel; PC1: poloxamer, not discriminant and not displayed (white: higher absorbance, black: lower absorbance).

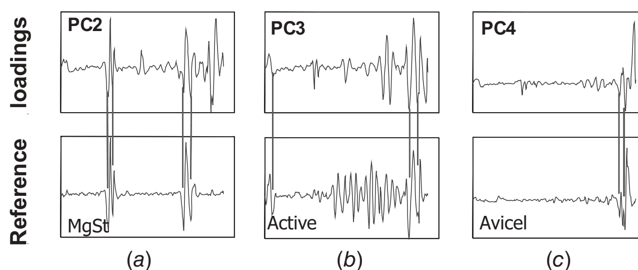

FIGURE 16 PCA loadings and comparison with reference spectra: (*a*) PC2, magnesium stearate; (*b*) PC3, active ingredient; (*c*) PC4, Avicel.

This PCA image study showed the differences between the two data sets, that is, it separated the good and bad dissolution samples. The main differences were due to the distribution of magnesium stearate and the API. No differences were observed in the spatial distribution of poloxamer or Avicel. Magnesium stearate is hydrophobic, thus protecting the tablet core from moisture and hence slowing dissolution. When a sample had more active ingredient on the surface, the dissolution properties were increased.

Peak height is a classical method of interpreting IR spectra. Its main advantage is in the selection of specific wavelengths. The disadvantage is that the bands often overlap and finding a specific band becomes problematic. PCA solves the problem of wavelength selection. Its main advantage is that it reduces the number of variables, that is, the number of images to analyze. The disadvantage lies in the interpretation of the loadings, which differ from pure substance spectra. Interpretation can be complicated by the fact that several chemical species may contribute to one loading.

4.3.5.4 Supervised Classification, NIR Imaging, and Process Development

The aim of this study was to use NIR imaging to solve granulation issues in new formulation development. Undesired powder agglomerations developed during the granulation step (Figure 17). Imaging was applied to characterize the agglomeration structure.

Method The sample contained starch, API, Avicel, crospovidone, and sodium lauryl sulfate. Sample and references were analyzed in triplicate by NIR imaging (Spectral Dimensions, 20 coadds, spectral range 1100–2450 nm). Full image size was 320×256 pixels or 4.1×3.3 mm. The NIR images were interpreted, and sample raw materials mapped, using PLS classification with five loadings (based on the reference spectra for starch, API, Avicel, crospovidone, and sodium lauryl sulfate).

Results and Discussion The PLS model identified all five chemical species. PLS multivariate analysis showed (Figure 18) that the core contained Avicel and API and that the periphery contained starch and crospovidone. The solution to the granulation issue was to add a premixing step to avoid agglomeration. NIR imaging proved useful for improving process understanding.

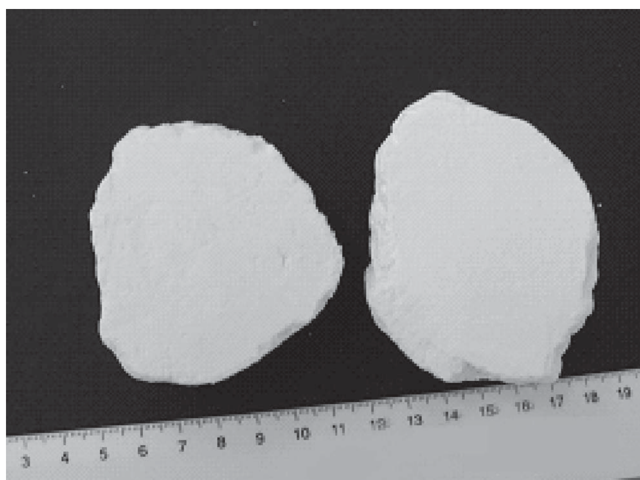

FIGURE 17 Powder agglomeration: visible picture.

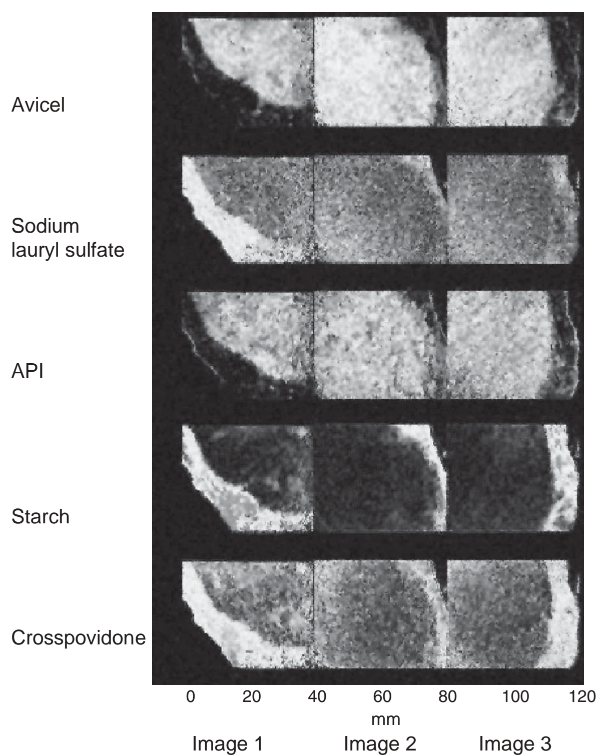

FIGURE 18 PLS classification image (three replicates and five chemical compounds). Simulation of granulation issues.

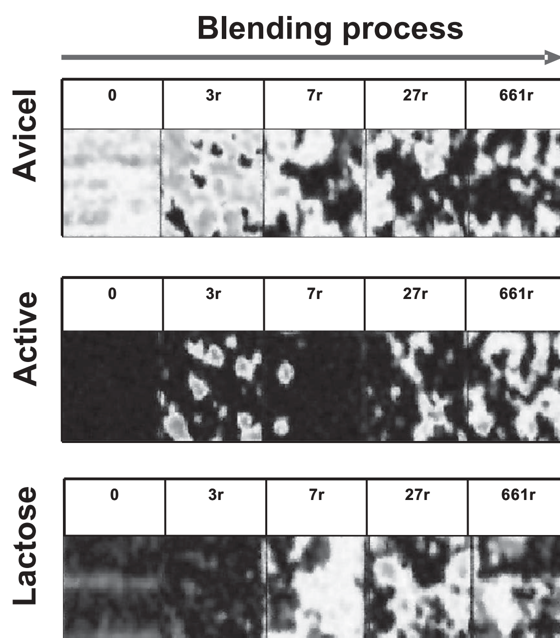

FIGURE 19 IR imaging for blend monitoring (r = blender rotation; white: higher absorbance, black: lower absorbance).

The advantage of supervised classification is that it avoids a wavelength selection step or the interpretation of PCA loading. It also rapidly extracts several chemical components. These methods provide accurate results provided the sample spectra are similar to the reference spectra. In our case the powder agglomeration was heterogeneous and the layers had a high content of excipient, making it possible to apply supervised classification.

4.3.5.5 Further Developments: Online Analysis by Hyperspectral Imaging

The two main online applications of hyperspectral imaging are blending endpoint determination (Figure 19) and capsule control (Figure 20). Other applications may be possible, such as content uniformity, determination of dissolution properties, and water quantification, but are not described in this chapter.

In blending, the main imaging advantage over single-point spectroscopy is the ability to analyze a larger area. We studied blends of three compounds (Figure 19), selecting single wavelengths for Avicel, API, and lactose. Only Avicel was present in the sample at the beginning of the experiment. Avicel and API were detected after three blender rotations; the two maps were complementary. All compounds in the mixture were homogeneous after 27 rotations. Timed distribution map analysis is thus a blending monitoring tool. Our example shows the IR images. However, NIR imaging has proved highly successful in the literature [25].

In capsule control, NIR radiation penetrates the shell, enabling the filling to be checked. The capsules in our example (Figure 20) were blue and opaque. However,

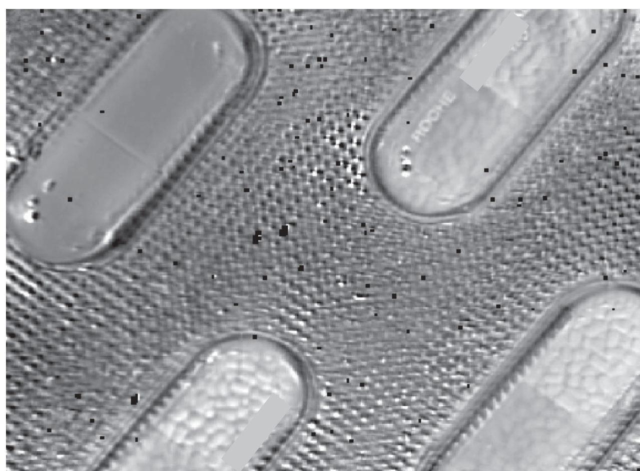

FIGURE 20 Capsule control by NIR imaging (image size 33 mm × 41 mm). Simulation of empty capsule in blister.

TABLE 1 Instrumentation Comparison

Parameters	FT System	Dispersive	Liquid Crystal Tunable Filter
Spectroscopy	NIR, IR, Raman	Raman, NIR	Raman, NIR
Image acquisition	Mapping, FPA detector	Mapping	FPA detector
Measurement mode	Reflexion, transmission, ATR	Reflexion, transmission	Reflexion
Image size	Microscopic, macroscopic	Microscopic	Microscopic, macroscopic
Acquisition time	Slow	Slow	Fast

NIR imaging was able to detect empty capsules and monitor fillings, including pellet appearance.

4.3.6 CONCLUSIONS

Hyperspectral imaging provides information that is spatial and spectral and both qualitative and quantitative. It can map chemical compound distribution and determine particle size. Such information cannot be obtained using classical spectroscopy. We have applied the method to the solution of quality control and process problems affecting pharmaceutical tablets: dissolution, polymorph distribution, moisture content determination, API localization and characterization, content uniformity, blending, and granulation. The choice of imaging technique is based on several criteria: spatial and spectral resolution, measurement time, and wavelength range. Tables 1 and 2 summarize the main advantages and disadvantages of the different methods.

TABLE 2 Comparison of Chemical Imaging Methods

Parameters	Raman Imaging	Infrared Imaging	Near-Infrared Imaging
Instrumentation robustness	LCTF: +++ other: +	+	+++
Spectral information specificity	+++	+++	++
Constituant map extration	Easy	Easy	Need chemometrics
Pharmaceutical application examples	Polymorph screening, small API particle detection	Unknown particle identification	Tablet reconstruction, blend homogeneity, process troubleshooting, counterfeit identification

Spectral imaging is a complex and multidisciplinary field. The introduction of new FPAs is making it increasingly powerful and attractive. It has proven potential in qualitative pharmaceutical analysis and can be used when spatial information becomes relevant for an analytical application. Even if online applications and regulatory method validation require further study, the potential contribution of imaging to quality control and PAT needs no further demonstration.

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REFERENCES

1. Roggo, Y., Roeseler, C., and Ulmschneider, M. (2004), Near infrared spectroscopy for qualitative comparison of pharmaceutical batches, *J. Pharm. Biomed. Anal.*, 36, 777–786.
2. Chalus, P., Roggo, Y., Walter, S., and Ulmschneider, M. (2005), Near-infrared determination of active substance content in intact low-dosage tablets, *Talanta*, 66, 1294–1302.
3. Roggo, Y., Duponchel, L., and Huvenne, J.-P. (2003), Comparison of supervised pattern recognition methods with McNemar's statistical test: Application to qualitative analysis of sugar beet by near-infrared spectroscopy, *Anal. Chim. Acta*, 477, 187–200.
4. El-Hagrasy, A. S., Morris, H. R., D'Amico, F., Lodder R. A., and Drennen, J. K. (2001), Near-infrared spectroscopy and imaging for the monitoring of powder blend homogeneity, *J. Pharm. Sci.*, 90, 1298–1307.
5. Beleites, C., Steiner, G., Sowa, M. G., Baumgartner, R., Sobottka, S., Schackert, G., and Salzer, R. (2005), Classification of human gliomas by infrared imaging spectroscopy and chemometric image processing, *Vibrat. Spectrosc.*, 38, 143–149.

6. Chalmers, J. M., Everall, N. J., Schaeberle, M. D., Levin, I. W., Neil Lewis, E., Kidder, L. H., Wilson, J., and Crocombe, R. (2002), FT-IR imaging of polymers: An industrial appraisal, *Vibrat. Spectrosc.*, 30, 43–52.
7. Juan, A. D., Tauler, R., Dyson, R., Marcolli, C., Rault, M., and Maeder, M. (2004), Spectroscopic imaging and chemometrics: A powerful combination for global and local sample analysis, *TrAC Trends Anal. Chem.*, 23, 70–79.
8. Lewis, E. N., Schoppelrei, J., and Lee, E. (2004), Near-infrared chemical imaging and the PAT initiative, *Spectroscopy*, 19, 26–36.
9. Lewis, E. N., Lee, E., and Kidder, L. H. (2004), Combining imaging and spectroscopy: Solving problems with near infrared chemical imaging, *Microscopy Today*, 12, 8–12.
10. Lewis, N., Schoppelrei, J., Lee, E., and Kidder, L. (2005), Near-infrared chemical imaging as a process analytical tool, in Bakeev, K. A., Ed., *Process Analytical Technology*, Blackwell, London, pp. 187–225.
11. Lewi, P. J. (2005), Spectral mapping, a personal and historical account of an adventure in multivariate data analysis, *Chemomet. Intell. Lab. Syst.*, 77, 215–223.
12. Tran, C. D. (2003), Infrared multispectral imaging: Principles and instrumentation, *Appl. Spectrosc. Rev.*, 38, 133–153.
13. Gift, A. D., Ma, J., Haber, K. S., McClain, B. L., and Ben-Amotz, D. (1999), Near-infrared Raman imaging microscope based on fiber-bundle image compression, *J. Raman Spectrosc.*, 30, 757–765.
14. Burns, D. A., Ciurczak, E. W. (2001), *Handbook of Near-Infrared Analysis*, 2nd ed., rev. and expanded, CRC Press, New York.
15. Gat, N. (2000), Imaging spectroscopy using tunable filters: A review, *Proc. SPIE*, 4056, 50–64.
16. Russ, J. (2002), *The Image Processing Handbook*, 4th ed., CRC Press, London.
17. Huang, J., Wium, H., Qvist, K. B., and Esbensen, K. H. (2003), Multi-way methods in image analysis—Relationships and applications, *Chemomet. Intell. Lab. Syst.*, 66, 141–158.
18. Bro, R. (2003), Multivariate calibration: What is in chemometrics for the analytical chemist? *Anal. Chim. Acta*, 500, 185–194.
19. De Braekeleer, K., and Massart, D. L. (1997), Evaluation of the orthogonal projection approach (OPA) and the SIMPLISMA approach on the Windig standard spectral data sets, *Chemomet. Intell. Lab. Syst.*, 39, 127–141.
20. Tauler, R. (1995), Multivariate curve resolution applied to second order data, *Chemomet. Intell. Lab. Syst.*, 30, 133–146.
21. Naes, T., and Martens, H. (1991), *Multivariate Calibration*, Wiley, New York.
22. Massart, D. L., Vandeginste, B. G. M., Buydens, L. M. C., Jong, S. D., Lewi, P. J., and Smeyers-Verbeke, J. (1997), *Handbook of Chemometrics and Qualimetrics: Part A, Data Handling in Science and Technology, 20A*, Elsevier, Amsterdam.
23. Massart, D. L., Vandeginste, B. G. M., Deming, S. M., Michotte, Y., and Kaufman, L. (2003), *Chemometrics: A Textbook, Data Handling in Science and Technology*, 2, Elsevier, Amsterdam.
24. Roggo, Y., Edmond, A., Chalus, P., and Ulmschneider, M. (2005), Infrared hyperspectral imaging for qualitative analysis of pharmaceutical solid forms, *Anal. Chim. Acta*, 535, 79–87.
25. Lyon, R. C., Lester, D. S., Lewis, E. N., Lee, E., Yu, L. X., Jefferson, E. H., and Hussain, A. S. (2002), Near-infrared spectral imaging for quality assurance of pharmaceutical products: Analysis of tablets to assess powder blend homogeneity, *AAPS PharmSciTech* [Electronic Resource], 3, E17.

SECTION 5

PERSONNEL

5.1

PERSONNEL TRAINING IN PHARMACEUTICAL MANUFACTURING

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5.1.1 OVERVIEW

5.1.1.1 Background

By law and by ethical commitment, companies that manufacture pharmaceutical products must make sure that what they produce is safe and effective. Ensuring that pharmaceutical manufacturing personnel possess the competencies necessary to perform their jobs correctly and efficiently is critical to a safe and successful

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manufacturing process. Developing required skills, providing discrete knowledge, and instilling an ethical and responsible approach to work are critical to training in an environment centered on good manufacturing practices.

This chapter includes the following types of pharmaceutical manufacturing organizations:

- Active pharmaceutical ingredient (API) or bulk manufacturers
- Biotechnology manufacturers
- Traditional manufacturers, solid and liquid dose
- Vaccine manufacturers

This chapter reviews the requirements for training personnel in a pharmaceutical manufacturing environment; it then focuses on how to develop and implement a training strategy that ensures pharmaceutical manufacturers are in compliance with the mandated *Code of Federal Regulations* (CFR), the current Food and Drug Administration (FDA) quality systems draft guidance document and good training practices (GTPs).

5.1.1.2 Training Requirements

The training requirements for personnel working in a pharmaceutical manufacturing environment are specified in (21 CFR) 211.25 [1]:

> (a) Each person engaged in the manufacture, processing, packing or holding of a drug product shall have education, training, and experience, or any combination thereof, to enable that person to perform the assigned functions. Training shall be in the particular operations that the employee performs and in current good manufacturing practices (including the current good manufacturing practice regulations in this chapter and written procedures required by these regulations) as they relate to the employee's functions. Training in current good manufacturing practice shall be conducted by qualified individuals on a continuing basis and with sufficient frequency to assure that employees remain familiar with cGMP requirements applicable to them.
>
> (b) Each person responsible for supervising the manufacture, processing, packing, or holding of a drug product shall have the education, training, and experience, or any combination thereof, to perform assigned functions in such a manner as to provide assurance that the drug product has the safety, identity, strength, quality, and purity that it purports or is represented to possess.
>
> (c) There shall be an adequate number of qualified personnel to perform and supervise the manufacture, processing, packing, or holding of each drug product.

In summary, the *Code of Federal Regulations* states that pharmaceutical manufacturing personnel must be trained as follows:

1. To do their specific jobs
2. In current good manufacturing practices (cGMPs)
3. On written procedures (required in 21 CFR 211:80)

The regulation also goes on to specify that training must fulfill the following conditions:

1. Be provided by qualified individuals
2. Be conducted on a frequent, continuing basis

Finally, the code states that manufacturing supervisors must be trained in the same manner as their employees.

In essence, the code specifies that pharmaceutical manufacturing companies must train their employees using qualified trainers; it also states that supervisors must have the same training as "qualified personnel." What the regulations do not say is *how to* conduct this training.

According to John W. Levchuck, then of the FDA, "The FDA has not published a guideline establishing acceptable procedures for personnel training, nor is a guideline being planned. Neither has the FDA specified strict training requirements" [2]. Levchuck's statement may be on its way to being updated. The latest pharmaceutical manufacturing training direction comes from the FDA's draft guidance document "Guidance for Industry: Quality Systems Approach to Pharmaceutical Current Good Manufacturing Practice Regulations" [3]. Published in 2004 this guidance document states in its develop personnel section:

> Under a quality system, senior management is expected to support a problem-solving and communicative organizational culture. Managers are expected to encourage communication by creating an environment that values employee suggestions and acts on suggestions for improvement. Management is also expected to develop cross-cutting groups to share ideas to improve procedures and processes.
>
> In the quality system, it is recommended that personnel be qualified to do the operations that are assigned to them in accordance with the nature of, and potential risk to quality presented by, their operational activities. Under a quality system, managers are expected to define appropriate qualifications for each position to help ensure individuals are assigned appropriate responsibilities. Personnel should also understand the impact of their activities on the product and the customer (this quality systems parameter is also found in the CGMP regulations, which identify specific qualifications, i.e., education, training, and experience or any combination thereof; see 21 CFR 211.25(a) & (b)). Under a quality system, continued training is critical to ensure that the employees remain proficient in their operational functions and in their understanding of CGMP regulations. Typical quality systems training would address the policies, processes, procedures, and written instructions related to operational activities, the product/service, the quality system, and the desired work culture (e.g., team building, communication, change, behavior).
>
> Under a quality system (and the CGMP regulations), training is expected to focus on both the employees' specific job functions and the related CGMP regulatory requirements.
>
> Under a quality system, managers are expected to establish training programs that include the following:
>
> - Evaluation of training needs
> - Provision of training to satisfy these needs
> - Evaluation of effectiveness of training
> - Documentation of training and/or re-training

> When operating in a robust quality system environment, it is important that supervisory managers ensure that skills gained from training be incorporated into day-to-day performance.

The quality systems draft guidance document reinforces the training requirements contained in 21 CFR 211.25 and goes on to add several key items. These include an evaluation of training needs, an evaluation of training effectiveness, and a documentation requirement. Once again, however, the document does not describe how training is to be designed, conducted, or evaluated. In addition, the guidance document does not provide details on the specific training information to be collected, how it should be stored, or how long the documentation records should be retained.

Managers have started to view training as a business-critical component in achieving improved performance and compliance with regulations, but at the same time they may have questions about how to direct training efforts. In the absence of firm guidelines for training, many in the industry have interpreted FDA commentary and audit results as supporting a competency-based approach to training, with validated and reliable training programs that produce measurable performance outcomes. Work responsibilities and tasks are specified in standard operating procedures, guidelines, batch records, employee directives, and protocol. Training must ensure that all employees know of the existence of all these documents, how to access them, and how they are used to direct work. Further, "qualified personnel" must demonstrate that they have read and understand these documents and can perform work as directed by them.

5.1.1.3 Good Training Practices and Pharmaceutical Manufacturing

Good training practices and a competency-based approach to training may first have been introduced to the general manufacturing industry in 1961 with the publication of a booklet by Robert Mager [4]. In this work, Mager tells educators and trainers how to follow a systematic approach to develop training materials and programs so students and trainees know what competencies they should possess after training. The idea of developing systematic, competency-based training in the pharmaceutical industry has its roots in an article published in 1982 by Ronald Tetzlaff [5], then of the FDA, which builds on the work done by Mager and others in the training field by explaining how a systematic approach to training program design is the best way to build effective, consistent training for employees in pharmaceutical manufacturing.

5.1.1.4 What Is Competency-Based Training?

Competency-based training is training aimed at ensuring that certain competencies (skills and knowledge) are achieved. Competency-based training measures trainees' mastery of materials through a test or skill demonstration or both to ensure that trainees have acquired competencies. For example, a competency-based program for tablet operators may be designed to ensure that operators can follow all steps in equipment start-up, operation, shut-down, and troubleshooting. The test to ensure that trainees acquired thoses competencies during training might include labeling

a diagram of the equipment and performing a demonstration of how to start up, operate, shut down, and troubleshoot the equipment.

5.1.1.5 Why Is Competency-Based Training So Important?

Competency-based training is important because it fulfills FDA training guidelines while at the same time meeting business needs. Businesses need a trained, competent workforce capable of manufacturing products effectively and efficiently. How effectively does pharmaceutical manufacturing respond to the challenge? According to one report, "A lack of trained and experienced technical and production staff will have an impact on more than half of the world's biopharmaceutical developers and contract manufacturers in the next five years, and impact their ability to meet demand, according to a survey of 100 international biopharmaceutical manufacturers and contract manufacturing organizations" [6].

From a compliance perspective, interest in training is also starting to rise: "The U.S. FDA is paying more attention to drug manufacturers' training programs under its quality system approach to inspections. While personnel training is not the hottest compliance topic in pharmaceutical GMP literature, it is a frequent U.S. FDA investigator observation, is appearing on a growing percentage of warning letters, and has been involved in some of the highest profile FDA regulator actions in recent years" [7]. So competency-based training makes sense from both business needs and compliance requirements. A well-thought-out, efficiently executed training plan can help ensure pharmaceutical companies not only meet the intent of regulations but also show a return on money invested in training.

5.1.2 DEVELOPING A TRAINING PLAN: STRATEGY TO ENSURE TRAINING COMPLIANCE IN A PHARMACEUTICAL MANUFACTURING FACILITY*

Ensuring training compliance within a pharmaceutical manufacturing facility requires the facility to establish and implement a comprehensive training plan. Set up and executed properly, this plan will encompass and meet all of the training requirements of:

- 21 CFR 210.25
- "Guidance for Industry: Quality Systems Approach to Pharmaceutical Current Good Manufacturing Practice Regulations"
- Good training practices

Managers in many organizations have an idea of what they want their training to look like, but few have a well-defined, written training plan to help them get there. A training plan is a roadmap that guides and integrates the training process throughout the facility. This plan helps ensure a facility accomplishes its goals consistently, according to its standards of quality, production, cost, and safety. The plan should

*The material in this section was originally published in 1999 in the *PDA Journal* [8]. The information is updated and expanded and examples of the material described are presented.

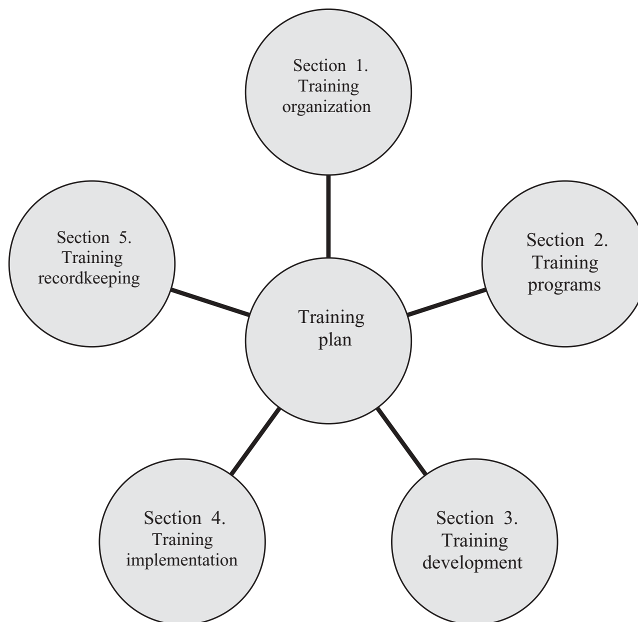

FIGURE 1 Training plan schematic.

carry the full weight and authority of other corporate directives with the highest levels of management support for training as an essential component in the manufacture of safe and effective health care products.

Why develop a training plan rather than a standard operating procedure (SOP) that covers training? Some managers believe that such a procedure is "required" [9]. Remember, though, that developing a training SOP—or any SOP—mandates training on that SOP. Ideally, the training plan should be more comprehensive than what is normally captured in an SOP. The training plan is a document that guides the development of training programs and materials and the way those programs and materials are implemented. A training plan includes training policy and goes beyond a procedure. And because it is not a procedure, it need not be trained.

A training plan might look like the schematic in Figure 1. The plan has five sections.

Note: The facility should have a master training plan that covers the entire site. Each department should develop its own training plan, including the training needed to qualify personnel for all job functions. The departmental plan should provide a schedule of training and retraining to ensure the availability of a sufficient number of qualified personnel to perform all job functions at all times.

A checklist for ensuring all components of a training plan are identified is shown in Figure 2.

Section 1 Training Organization

This section of the training plan calls for the development of a training philosophy and mission statement. These provide the overall foundation for a strong training

1. Training organization
- ☐ 1.1. Develop a training philosophy
- ☐ 1.2. Develop a training mission statement
- ☐ 1.3. Develop a training organization
- ☐ 1.4. Develop a training support network

2. Training programs
- ☐ 2.1. Identify all training programs/personnel to be trained
- ☐ 2.2. Establish personal qualification pathways
- ☐ 2.3. Training program materials

3. Training development
- ☐ 3.1. Establish a training program design model
- ☐ 3.2. Ensure development of valid training programs
- ☐ 3.3. Establish a change control methodology

4. Training implementation
- ☐ 4.1. Develop an implementation schedule
- ☐ 4.2. Establish a training failure response process

5. Training recordkeeping
- ☐ 5.1. Establish a recordkeeping system
- ☐ 5.2. Training record requirements

FIGURE 2 Training plan checklist.

organization. In addition, a training organization must be established with persons designated to support the training effort throughout the facility.

1.1 Develop a Training Philosophy Once the decision is made to develop a training plan, those involved need to formulate a training philosophy. Most organizations have developed philosophies around production, quality, cost, safety, and other aspects of their operation. Ask an employee what the company's philosophy is on meeting production quotas, and he or she can probably sum it up in a few words. But how many organizations have developed a well-articulated training philosophy? The answer is very few.

A training philosophy that acknowledges training as a critical component in achieving corporate business objectives is a key element in establishing the credibility of training in an organization. A training philosophy represents an organization's commitment to training. It tells employees, suppliers, customers, and other stakeholders the organization is committed to ensuring its people have the skills and knowledge needed to compete in an increasingly competitive environment. A training philosophy should acknowledge that training is conducted not only to meet regulatory requirements but also because properly trained personnel contribute to the quality of the operation and financial success of the company. Figure 3 provides an example of a training philosophy.

1.2 Develop a Training Mission Statement The training philosophy provides the basis for the training mission statement. The mission statement identifies the overall goals of organizational training and emphasizes the importance of establishing personnel standards of performance. It also positions training as critical to quality

We are committed to a strong training organization as supported by our mission statement. Our training philosophy includes the following elements:

- Commitment to preparing all employees to do their jobs competently and efficiently to produce products that consistently meet or exceed customer requirements for safety and efficacy
- Commitment to continuous training of all employees throughout the organization so they may reach their full human potential

FIGURE 3 Sample training philosophy.

Our training mission statement is based on our training philosophy. We will…

- Support all corporate objectives in the business plan by providing excellent training programs to all employees
- Evaluate the efficacy of those programs on an ongoing basis
- Grow and develop our employees in all areas of their lives, including interpersonal and technical areas
- Commit to support the organization as a top-tier growth company that can adapt to change in an ongoing regulated environment
- Provide ongoing training to ensure all personnel are in compliance with corporate and regulatory guidelines
- Provide resources for employee growth while meeting the organization's quality, productivity, safety, and financial goals

FIGURE 4 Sample training mission statement.

outcomes. Representatives from every department should participate in drafting a company training mission statement to ensure it reflects their interests. A sample training mission statement is presented in Figure 4.

Once the training philosophy and mission statement are developed, they need to be communicated to all employees to solicit feedback and support. The communication should focus on the direction the company is taking and the role managers will play in ensuring training is carried out effectively in the organization. A clearly worded philosophy and mission statement set the groundwork for building a plan that integrates training into all areas of personnel development.

1.3 Develop a Training Organization If it has not already been set up, a training organization needs to be established by the corporate or facility management team.

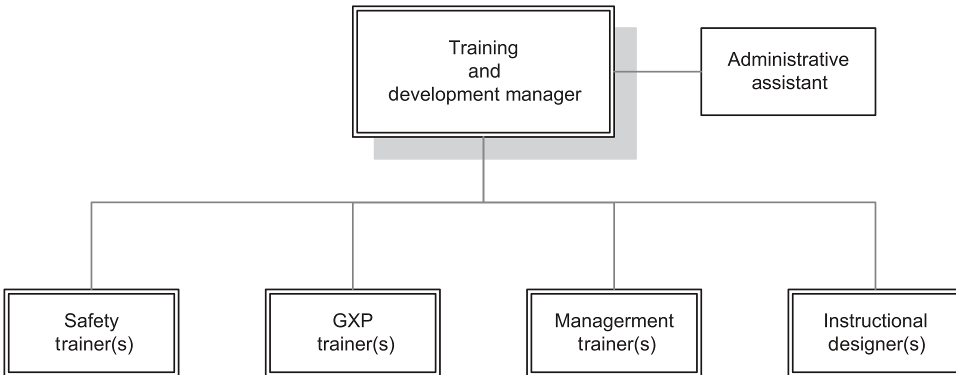

FIGURE 5 Sample training department structure.

Our experience has shown that training is most effective when the training organization or department is a facility function—on the same level as other major departments within the manufacturing facility. A typical organization chart for the training department within a pharmaceutical manufacturing facility might look like the one in Figure 5.

Roles and responsibilities should be defined in the plan as follows. Depending upon the size of the facility, some of the roles may be combined.

Training and Development Manager Since the goal of training is to ensure that personnel are capable of performing their jobs to produce consistent, quality products, the training function must support all other functions and be supported by them. The training and development manager must have lines of communication with all other functions, including quality, safety, production, information systems, and laboratory. Although many organizations agree on this in principle, the practice is likely to be different.

The training and development manager should direct, monitor, and build support for the training effort throughout the organization. The position should meet regularly with site management to review the training plan of action and the progress to date and ensure the training effort supports business and compliance goals. He or she also directs training coordinators and assists in communicating and developing training plans and outcome assessments for the departments to ensure they are implemented effectively.

This function should extend to departmental training to ensure that systems exist in each department to meet specific training requirements. The training and development manager should develop guidelines to be followed by each department for complying with the corporate training policy. These guidelines will identify the training coordinators, instructional designers, departmental trainers, and subject matter experts (SMEs), as well as persons responsible for scheduling and recording participation in training.

The training and development manager should be responsible for at least the following:

- Developing, maintaining, and administering the facility training plan
- Identifying training needs for each department to support site business objectives and compliance efforts
- Developing training plans and schedules to meet organizational training needs
- Reviewing training plans and schedules to ensure support for training from all departments
- Establishing metrics to measure training effectiveness
- Directing and monitoring training coordinators in executing training plans

Safety Trainer(s) Safety trainers are those individuals that provide safety training to the manufacturing employees. The safety trainers are responsible for at least the following:

- Working with instructional designers to develop safety training programs
- Presenting required safety training to appropriate departments, such as:
  - Lockout/tagout
  - Confined space entry
  - Hearing protection
  - Respiratory protection
  - Bloodborne pathogens
- Identifying ongoing safety training needs

Management Trainer(s) Management trainers are individuals that provide "soft skill" training programs to the other employees at the pharmaceutical manufacturing site. The management trainers are responsible for at least the following:

- Working with instructional designers to develop management development programs
- Presenting management development programs, such as:
  - Leadership
  - Problem solving/decision making
  - Coaching and counseling
  - Time management

GXP Trainer(s) GXP trainers are primarily responsible for presenting training programs on current regulations within the pharmaceutical manufacturing industry. The GXP trainers are responsible for at least the following:

- Working with instructional designers to develop GXP training programs
- Presenting initial GXP training during new employee orientation
- Presenting ongoing GXP topics in:
  - Good manufacturing practices
  - Good documentation practices
  - Good laboratory practices

Instructional Designers The instructional designers develop training programs as directed by the training coordinators. Instructional designers serve as a liaison between the training coordinators, training and development manager, departmental supervisors, and SMEs. Instructional designers are responsible for at least the following:

- Identifying appropriate SMEs for training projects, in coordination with department training coordinators
- Developing competency-based training programs according to established principles of instructional systems design
- Developing evaluations and assessment measures
- Developing train-the-trainer programs for department trainers

1.4 Develop a Training Support Network To function properly, a training department needs to establish a network for training support within each functional department of the manufacturing facility. Within each functional department, three roles should be identified to help support the training effort. Sometimes these roles can be performed by one person; sometimes the roles are performed by individuals within the department. Typically, the roles are not full-time positions. Figure 6 provides a list of departments and the training support roles necessary for each department; you may want to add to the list. Then, each support role is described.

Training Coordinators Training coordinators are the liaison between functional departments and the training department. They are responsible for communicating with departmental training needs to the training and development manager. Training coordinators also serve as the liaison between departmental SMEs, departmental trainers, and instructional designers. They work to identify training needs and develop training programs that support the plans set out by the training and development manager with site management approval. They are also responsible for building support within the departments for executing training plans. Training

Department	Training Coordinator(s)	Subject Matter Experts	Departmental Trainers
• Administration and finance			
• Buildings and grounds			
• Facilities and engineering			
• Human resources			
• Information systems			
• Manufacturing			
• Regulatory compliance			
• Research and development			
• Safety and security			
• Shipping and receiving			

FIGURE 6 Departments/training support roles.

coordinators may serve more than one department within a facility. Training coordinators are responsible for at least the following:

- Consulting with department management to identify training needs
- Communicating departmental training needs and progress of training to the training and development manager
- Developing departmental training plans and specifying training programs as directed by the training and development manager and department management
- Directing instructional designers in developing training programs, evaluations, and assessments

Subject Matter Experts Subject matter experts are individuals who possess a broad base of knowledge about the area where they work. These individuals are typically employees who have been with the company and department a significant amount of time—typically five years or more. Subject matter experts are responsible for at least the following:

- Working with training coordinators to identify departmental training needs
- Working with instructional designers to design and develop training programs
- Working with departmental trainers to implement training programs

Departmental Trainers The departmental trainers should be selected for their skill and interest in training and ability to perform their jobs. Some SMEs may become departmental trainers—but only with the stipulation that they want to learn how to become a trainer. Departmental trainers are responsible for at least the following:

- Working with instructional designers to design and develop training programs
- Training new hires and incumbents on job-specific skills and regulatory issues

In order to meet the 21 CFR 211.25 requirements, departmental trainers must complete a train-the-trainer program. An outline of topics to be covered in a typical train-the-trainer program is shown in Figure 7.

Section 2 Training Programs

This section includes a listing of all personnel to be trained as well as a listing of suggested training programs that need to be provided at the manufacturing facility. In addition, employee qualification pathways are discussed and illustrated.

2.1 Identify All Training Programs/Personnel to be Trained Figure 8 provides a sample list of personnel who need to be trained and the training programs they need to work effectively and efficiently at a pharmaceutical manufacturing facility; you will want to add to the list.

1. Introductions
2. Program objectives
3. Trainer responsibilities
4. Training process: trainee characteristics
5. Training process: adult learning theory
6. Training process: what makes successful training?
7. What makes a successful trainer?
8. Training techniques
9. Plan
10. Prepare
11. Present
12. Feedback and coaching
13. Using an OJT checklist
14. Exercise 1— OJT with SOP
15. Exercise 2— OJT checklist

Review and wrap up

FIGURE 7 Sample train-the-trainer program outline.

2.2 Establish Personal Qualification Pathways Good business practice, as well as CFR 21 211.25, mandates that each employee possess the education, training, and experience to enable him or her to perform assigned functions in a safe and effective manner. The training plan must contain a policy allowing personnel to demonstrate their qualifications. This should cover training and supervisory personnel as well as those directly involved with operations.

A qualification pathway should include all of the training programs necessary to qualify employees in job functions. A sample personal qualification pathway for a compression technician is shown in Figure 9.

2.3 Training Program Materials While there are basically three methods for delivering training available—classroom led, self-instruction, or a combination of the two—training materials should include:

1. A method of imparting knowledge or desired attitudes. This type of training typically occurs in a classroom-led or self-instructional system (paper or computer based).
2. A method of assessing if that knowledge or attitude transfer took place, that is some form of assessment.

Management Training Programs	
Training Programs	**Personnel to be Trained**
Leadership	Supervisors and above
Coaching and counseling	Supervisors and above
Problem solving/decision making	Supervisors and above
Coaching and counseling	Supervisors and above

Specialty Training Programs	
Training Programs	**Personnel to be Trained**
Bloodborne pathogens	Appropriate personnel
Conducting effective investigations	Appropriate personnel
Drug-labeling regulations	Appropriate personnel
Electronic signatures and batch records	Appropriate personnel
FDA inspections	Appropriate personnel
Hazcom	Appropriate personnel
SOP writing	Appropriate personnel
Surveillance monitoring	Appropriate personnel
Working with contract research organizations	Appropriate personnel
Validation concepts	Appropriate personnel

FIGURE 8 Training programs/personnel to be trained.

If the training requires employees to be competent in a particular task or skill, then these materials must also be developed:

3. A structured checklist that allows different trainers to demonstrate the same way of doing a particular task to each trainee.
4. An evaluation checklist that can be used by a trainer (it is suggested that the trainer who provided the initial training not do the competency evaluation) to determine if a trainee is competent in performing the assigned skill.

Frequency of Training In general, providing refresher or retraining on a routine basis is not necessary. Some training programs are mandated to be repeated, or refreshed, usually by federal, state, or local government, that is, some safety training.

Department: Manufacturing		Job title: Compression operator

Job Task	Job Duties	Training Start Date	Training Completion Date
Completing documentation	Master batch record (MBR)		
Performing cleaning procedures	Minor equipment cleaning		
	Major cleaning of detachable parts		
	Major cleaning of work centers		
Operating equipment—basic	Fette		
	Killian		
	Courtoy		
Operating equipment—advanced	Compression theory		
operating support equipment	Use and cleaning of friability tester		
	Use and cleaning of hardness tester		
	Use and cleaning of micrometer		
	Use and cleaning of metal detectors		
	Use of weigh checkers		
Transporting materials	Transferring sacks to pallets		
	Use and handling of totes		
	Staging totes/sacks for coating		

FIGURE 9 Sample personal qualification pathway.

Other training programs may be mandated to be repeated by a company—such as sexual harassment. Retraining may be necessary when a person has been absent from a job for a period of time due to illness, pregnancy, other job assignment, and so on. In that case, the company may require retraining after a certain period of missed job function. In addition, retraining or training needs may be indicated when situations such as the following occur:

- Out-of-specification product (quality issues)
- Excessive waste
- Decrease in production

Periodic audits of work performance may identify training needs as well. However, our experience has been that retraining is typically identified through situations similar to those mentioned above.

Section 3 Training Development

Employees need job-specific training in environment, SOPs, safety, GMP regulations and awareness, and technical skills. The training plan should ensure a systematic approach to develop and implement competency-based training for all programs and materials.

3.1 Establish a Training Program Design Model Effective competency-based training is the result of applying a systematic process to training program design. This process involves following certain well-defined steps to develop a training program that meets both the trainee's and the organization's needs. The most reliable method of training and qualifying people in the safe and effective performance of work is using an instructional system design model. One instructional systems

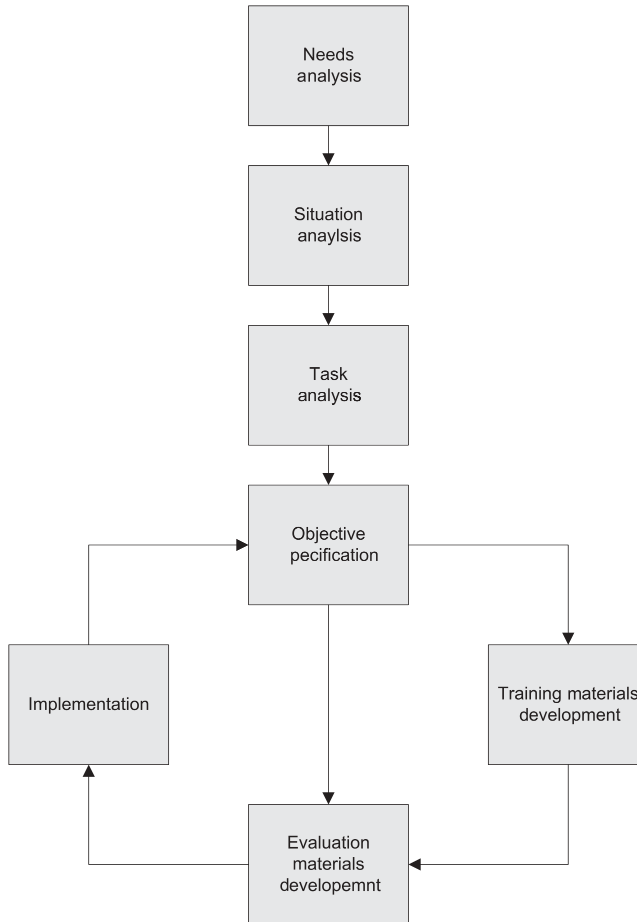

FIGURE 10 Training program design model.

design model, the training program design model (TPDM) developed by Gallup and Griffin, is shown in Figure 10.

3.2 Ensure Development of Valid Training Programs Validity may be defined as something that accomplishes that which it purports to accomplish. A training program is considered valid when it accurately imparts the competencies required to do a job. How can a training program be validated? The way to ensure program validation is to follow a training program design module and the following process:

1. Conduct a task analysis that identifies discrete bits of knowledge and specific job competencies; have SMEs sign off on completed task analyses indicating that all discrete bits of knowledge and specific tasks required to complete a job have been identified.
2. Write measurable performance objectives that state exactly what a trainee is to know or do after training has been completed; have SMEs sign off on com-

pleted performance objectives indicating that they agree that the level of stated performance is acceptable.

3. Develop assessment instruments that match up with the performance objectives; have SMEs sign off on assessments indicating they agree that the instruments adequately measure trainee competency.

Assess trainee's knowledge and skills using the approved assessment instruments; ensure that assessments are conducted in the same manner for all employee groups.

3.3 Develop a Change Control Policy and Procedure A change control policy helps ensure personnel are prepared to carry out new and revised policies and procedures effectively. An effective change control policy should include directions for regular reviews and revision of training materials and programs as well as scheduling training for new and incumbent employees on all pertinent SOPs. The plan must also include a procedure for responding to developments in operations, processes, and documentation.

Section 4 Training Implementation

4.1 Develop an Implementation Schedule The plan must include a schedule to ensure that training begins at initial employment and is ongoing. The schedule should include time frames for completing job qualification training and address training required for changes in process and new or increased performance expectations. Developing a training implementation schedule can be accomplished by adding to the matrix developed in Section 2, "Identify All Training programs/Personnel to be Trained." This revised matrix is shown in Figure 11.

4.2 Establish a Training Failure Response Process The training plan should describe a process for dealing with personnel who do not pass qualifying evaluations. It should list steps for isolating the cause of the failure and differentiate between discrepancies in training program design and inappropriate candidates for the job. It should include publishing the protocol for dealing with test failure. A typical failure response for a task-based process is shown in Figure 8. After a person completes a training program covering the knowledge to perform a specific task, an assessment is given. If that assessment is passed at a predetermined score, the person goes on to complete the skill-based portion of the training. Once the skill portion is completed, the person is observed as they perform the skill. If they perform it correctly, they are certified or qualified to perform the specific process operation. If they do not pass the skill demonstration check, they must repeat the training again. The number of times they are allowed to repeat the training and assessment should be part of the training failure response process. Figure 12 illustrates a basic failure response process.

Section 5 Training Recordkeeping

The FDA does not specify training documentation/recordkeeping requirements in the *Code of Federal Regulations*, but it has made them an industry requirement by virtue of precedent-setting industry demands.

Specialty Training Programs		
Training Programs	**Personnel to be Trained**	**Training Scheduled**
Bloodborne pathogens	Appropriate personnel	As needed
Conducting effective investigations	Appropriate personnel	As needed
Drug-labeling regulations	Appropriate personnel	As needed
Electronic signatures and batch records	Appropriate personnel	As needed
FDA inspections	Appropriate personnel	As needed
Hazcom	Appropriate personnel	As needed
SOP writing	Appropriate personnel	As needed
Surveillance monitoring	Appropriate personnel	As needed
Working with contract research organizations	Appropriate personnel	As needed
Validation concepts	Appropriate personnel	As needed

FIGURE 11 Sample implementation schedule.

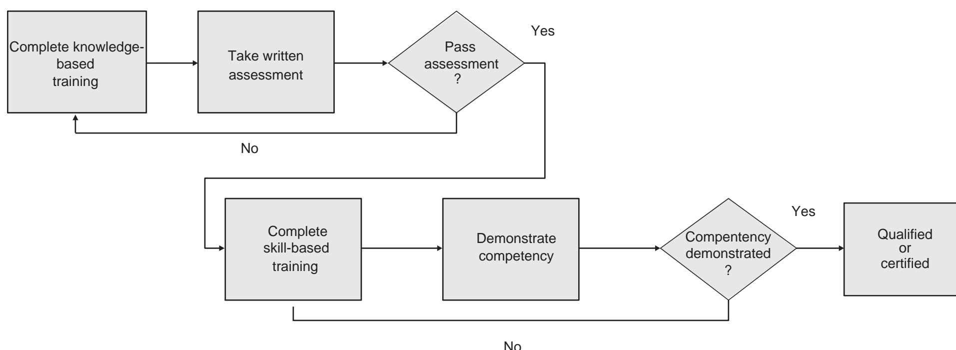

FIGURE 12 Basic failure response process.

Discussions with training managers at pharmaceutical manufacturing companies audited by the FDA suggest that it is through documentation/recordkeeping that the FDA "backs into" an audit of a company's training. This typically happens in one of three ways:

1. Auditors ask to see a SOP and observe an employee perform the procedure. Depending on the employee's performance, the auditors may ask to review the documents or records of the observed employee's training.

2. Through investigation of production records, auditors spot deviations or out-of-specification issues. This in turn may lead to an examination of an operator's work and qualifications for the job. The auditors may ask how or what additional training was conducted and request the records that prove training was provided.
3. Auditors identify processes or work practices that appear to be performed incorrectly during a routine tour of a manufacturing facility. The auditors may ask to see the training materials or training records associated with individuals working in the area.

Further, training managers note that if the training department can produce training documentation quickly and in an organized fashion, auditors are more likely to view the overall training effort as effective. At the same time, when solid training documentation cannot be produced quickly, a more in-depth review of the training system may occur [10]. As a result of these requirements to produce training records, it is critical that an efficient training recordkeeping system be in place at the pharmaceutical manufacturing facility.

5.1 Establishing a Recordkeeping System Any documentation system may meet the requirements of "produce … quickly and present in an organized fashion."

Three types of methods are commonly used for training documentation/recordkeeping in the pharmaceutical manufacturing industry: paper systems, electronic systems with paper backup, and stand-alone electronic systems. Some pharmaceutical companies use a paper-based documentation system. Electronic systems with paper backup, also known as learning management systems (LMSs), range from elaborate programs such as IsoTrain, SAP, Plateau, and Registrar to departmental databases created in Microsoft Access. Paper backup typically includes at least sign-in sheets and sometimes evaluation instruments. An LMS is essentially a database made up of multiple tables to store discrete units of information. These might include employee information, course information, evaluation data, and training programs or materials. These tables can be merged and queried to produce specific reports. Report capabilities allow users to compare, analyze, question, and project needs.

5.2 Training Record Requirements The following basic information should be included in any training recordkeeping system:

- Employee name
- Employee identification
- Personal qualification pathway, including training programs that need to be completed and targeted completion dates
- Results of training programs completed

In addition, the training program materials, including trainee guides and assessment instruments, should be readily accessible—either electronically or in a paper-based filing system.

REFERENCES

1. U.S. Food and Drug Administration (FDA) (2003), Current good manufacturing practice for finished pharmaceuticals, *Code of Federal Regulations*, Title 21, Part 211.25, FDA, Rockuille, MD.
2. Levchuck, J. W. (1991), Training for GMPs, *J. Parenter. Sci. Technol.*, 45(6), 270–275.
3. U.S. Food and Drug Administration (FDA) (2004), Guidance for industry: Quality systems approach to pharmaceutical good manufacturing practice regulations, FDA, Rockuille, MD.
4. Mager, R. (1962), *Preparing Instructional Objectives*, Center for Effective Performance, Atlanta, GA.
5. Tetzlaff, R. (1982), A systematic approach to GMP training, *Pharm. Technol.*, 6(11), 42–51.
6. Langer, E. S. (2004), Training day for International Biopharma Manufacturers, *Contract-Pharma*, 6(2), 46.
7. Morris, W. (2006), Personnel training: A growing compliance concern, *PDA Newslett.*, XLII(4), 1–38.
8. Gallup, D. A., Beauchemin, K. A., and Gillis, M. (1999), A comprehensive approach to compliance training in a pharmaceutical manufacturing facility, *PDA J. S. Technol.*, 53(4), 163–167.
9. Vesper, J. L. (2000), Defining your GMP training program with a training procedure, *BioPharm.*, 13(7), 28–32.
10. Gallup, D. A., Beauchemin, K. A., Gillis, M., Altopedi, D. and Manor, J. (2003), Selecting a training documentation/recordkeeping system in a pharmaceutical manufacturing environment, *PDA J. Sci. Technol.*, 57(1), 49–55.

SECTION 6

CONTAMINATION AND CONTAMINATION CONTROL

6.1

ORIGIN OF CONTAMINATION

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6.1.1 INTRODUCTION

There are many ways to approach the issue of contamination. Although the origin of contaminants in pharmaceutical products can easily be identified, definitions on this theme can be looked at from different angles.

In a first approach, the definition of contamination in pharmaceutical products can be referred to in terms of related substances and process contaminants: While *related substances* are structurally related to the drug substance, *process contaminants* are introduced during manufacturing or handling procedures. These two categories include all types of contaminants but do not define them.

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The U.S. Pharmacopeia and National Formulary (USP-NF 27) [1] presents definitions of terms related to contaminants in *impurities in official articles* <1086> and *ordinary impurities* <466>. In these monographs, impurities are classified into inorganic, organic, biochemical, isomeric, and polymeric, and definitions are given in terms of foreign substances, toxic impurities, concomitant components, signal impurities, and ordinary impurities. According to these definitions, *foreign substances* are not the consequence of the synthesis or preparation of the drug, yet they are introduced by contamination or adulteration. *Concomitant components* are characteristic of bulk chemicals and are not considered impurities in the pharmacopeial sense. Ordinary impurities are species in bulk chemicals that are innocuous and do not present significant undesirable biological activity in the given amounts.

Different from foreign impurities, which theoretically are not present and, therefore, are not included when monograph tests and assays are selected, *toxic impurities* and *signal impurities* may arise from the synthesis, preparation, or degradation of compendial articles and, differently from *ordinary impurities*, may present undesirable biological activity, even as minor components. In this context, the following topics should be taken into account:

- Source of a drug substance: natural, synthetic, biotechnological
- Ratio impurity/drug substance and its toxicological effects
- Pharmacology of the impurity

Other factors that should also be taken into account when limits for impurity levels in bulk substances are to be set are:

- Route of administration
- Dose
- Target population
- Duration of the therapy

Not included in *process contaminants*, but equally an external source, are those impurities arising during packaging, during drug reconstitution or admixing, and also during the administration to the patient. Very important under this category is the particulate matter in intravenous formulations, mainly infusion solutions due to the large volume administered.

Figure 1 presents an overview of impurity sources, both endogenous and exogenous, considering the steps at which these can occur. It also summarizes how "the origin of contamination" will be addressed in this chapter. It is divided into endogenous and exogenous contaminants. The first part, endogenous contaminants, is devoted to water since it is the main raw material, if not in the drug form at least in any step of its preparation. Following water, the contamination arising with raw materials will be treated, considering the presence of concomitants (natural impurities of raw materials) and by-products, synthesized along with the drug. Also important while dealing with endogenous contaminants are those formed through inadvertent drug decomposition. Exogenous source of contaminants—aid materials used during drug or formulation preparation, containers, and delivery systems—will also be considered.

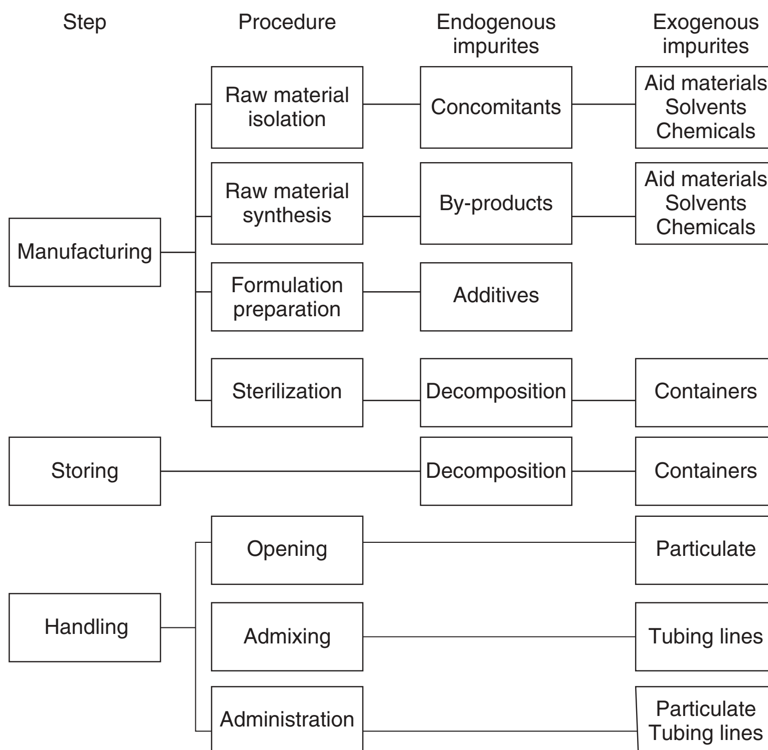


FIGURE 1 Schematic representation of an overview on impurity sources.

6.1.2 ENDOGENOUS CONTAMINANTS

Endogenous contaminants can already be present as concomitants in raw substances, whether formed during drug synthesis as a by-product or arising from reactions among formulation constituents. The generation of contaminants in formulations after processing and packaging is generally promoted by external agents such as light, ultraviolet (UV) radiation, heat, or air, causing unexpected reactions between constituents.

Pharmacopeial compendia establish a couple of tests that should be carried out with raw material batches to determine their level of impurities. Concomitants usually detected are heavy metals, chloride, or sulfated ash. The presence of by-products varies from raw material to raw material. Examples can be both innocuous substances such as monohydrogen phosphate, which is an impurity in dihydrogen phosphate salts and glycine, which is an impurity in alanine, and toxic substances, such as the classical enantiomer S of thalidomide.

6.1.2.1 Raw Materials

Raw materials employed in the pharmaceutical industry may have two different origins. They are either naturally occurring substances or synthesized drugs. Among the natural products are active ingredients from plant extracts or animals, chemicals,

and biotechnological products. As vast as the variety of different raw materials is the array of impurities. This section will deal with the impurities already present in raw materials as concomitants or those arising during drug synthesis. Concomitants include the usual impurities considered by pharmacopeial compendia, such as heavy metals and arsenic, for which limits are prescribed in all monographs. By-products arising during drug synthesis may or may not be a problem when present as contaminants.

Water Water is the primary raw material in pharmaceutical formulations. It is the most used vehicle since it is the major component of the human body. For many products, it is the main component, and, even in those containing non-water-soluble substances, water must be present. Depending on the product and the form of administration, lipophilic drugs are prepared as water–oil emulsions.

The amount and level of contaminants or impurities in water for pharmaceutical purpose depend on its use. Since water is used in all industries and scientific work, international and national standard authorities have established water quality parameters for all types of applications. Health-related water standards are given by organizations such as the World Health Organization (WHO) [2], the Environmental Protection Agency (EPA) [3], and the American Society for Testing and Materials Standards (ASTM) [4] in the United States and by pharmacopeial compendia when the aim is specifically related to water for pharmaceutical products for human and veterinary consumption.

Standards for water quality are similar among pharmacopeias (USP [1], BP [5], DAB [6], Ph. Eur. [7], IP [8]), with only a few minor differences in the allowed levels for chemical contaminants. Pharmacopeias classify water in three categories: *purified water*, *highly purified water*, and *water for injection.* Whatever the process for water purification, the raw material is always drinking water. Drinking water parameters, given by governmental regulatory agencies, include a large number of chemicals. They comprise not only naturally occurring substances but also a series of chemicals that may be present from anthropogenic activities in natural waters such as benzene, ethylene diamine tetraacetic acid (EDTA), and cadmium. Table 1 shows the guidelines for drinking water quality adopted by WHO [2].

The water purification process adopted by pharmaceutical industries must be able to furnish water with the quality parameters presented in Table 2.

The ability to achieve a guideline value depends on a number of factors, including:

- Concentration of the chemical in the raw water
- Nature of the raw water
- Treatment processes

Although there are no levels for them, pesticides, for example, should not be present in water for pharmaceutical purposes.

There are treatments available for the pharmaceutical industry for purifying water, which should be chosen according to the degree of purity necessary. They are listed in Table 3 according to their degree of complexity: the higher the ranking, the more complex the process.

TABLE 1 WHO Guideline Values for Chemicals in Drinking Water and EPA National Drinking Water Standards [2, 3]

Contaminant	Guideline Value (mg/L)
Primary Standards	
Acrylamide	0.0005
Alachlor	0.002
Antimony	0.006
Arsenic	0.010
Asbestos (fibers > 10 μm)	7 million fibers per liter
Atrazine	0.003
Barium	2
Benzene	0.005
Benzo(*a*)pyrene (PAHs)	0.0002
Beryllium	0.004
Boron	0.5
Bromate	0.010
Cadmium	0.005
Carbofuran	0.04
Carbon tetrachloride	0.005
Chloramines (as Cl_2)	4.0
Chlordane	0.002
Chlorine (as Cl_2)	4.0
Chlorine dioxide (as ClO_2)	0.8
Chlorite	1.0
Chlorobenzene	0.1
Chromium (total)	0.1
Copper	1.3
Cyanide (as free cyanide)	0.2
2,4-DB	0.07
Dalapon	0.2
1,2-Dibromo-3chloropropane (DBCP)	0.0002
o-Dichlorobenzene	0.6
p-Dichlorobenzene	0.075
1,2-Dichloroethane	0.005
1,1-Dichloroethylene	0.007
Cis-1,2-Dichloroethylene	0.07
Trans-1,2-Dichloroethylene	0.1
Dichloromethane	0.005
1,2-Dichloropropane	0.005
Di(2-ethylhexyl) adipate	0.4
Di(2-ethylhexyl) phthalate	0.006
Dinoseb	0.007
Dioxin (2,3,7,8-TCDD)	0.00000003
Diquat	0.02
EDTA	0.6
Endothall	0.1
Endrin	0.002
Epichlorohydrin	0.0004
Ethylbenzene	0.7
Ethylene dibromide	0.00005

TABLE 1 ***Continued***

Contaminant	Guideline Value (mg/L)
Fluoride	4.0
Formaldehyde	0.9
Glyphosate	0.7
Haloacetic acids (HAA5)	0.060
Heptachlor	0.004
Heptachlor epoxide	0.0002
Hexachlorobenzene	0.001
Hexachlorocyclopentadiene	0.05
Lead	0.015
Lindane	0.0002
Mercury (inorganic)	0.002
Methoxychlor	0.04
Nickel	0.02
Nitrate (measured as nitrogen)	10
Nitrite (measured as nitrogen)	1
Oxamyl (Vydate)	0.2
Pentachlorophenol	0.001
Picloran	0.5
Polychlorinated biphenyls (PCBs)	0.0005
Selenium	0.05
Simazine	0.004
Styrene	0.1
Tetrachloroethylene	0.005
Thallium	0.002
Toluene	1
Total trihalomethanes (TTHMs)	1
Toxaphene	0.003
2,4,5-TP (Silvex)	0.05
1,2,4-Trichlorobenzene	0.07
1,1,1-Trichloroethane	0.2
1,1,2-Trichloroethane	0.005
Trichloroethylene	0.005
Uranium	0.030
Vinyl chloride	0.002
Xylenes (total)	10
Secondary Standards	
Aluminum	0.05 to 0.2
Chloride	250
Copper	1.0
Fluoride	2.0
Foaming agents	0.5
Iron	0.3
Manganese	0.05
Silver	0.10
Sulfate	250
Total dissolved solids	500
Zinc	5

TABLE 2 Water Quality for Pharmaceutical Purposes (Pharmacopeial Standards)

	Water Grade		
Parameter	Purified Water	Highly Purified Water	Water for Injection
Conductivity (at 20°C) $\mu S\,cm^{-1}$	4.3	1.1	1.1
Total organic carbon (TOC) (mg/L)	0.5	0.5	0.5
Nitrates (ppm)	0.2	0.2	0.2
Aluminum (µg/L)	10	10	10
Heavy metals (ppm)[a]	0.1	0.1	0.1
Chloride	Pass/fail	—	0.5 ppm
Sulfate	Pass/fail	—	Pass/fail
Ammonium (ppm)	0.2	—	0.2
Calcium/magnesium	Pass/fail	—	Pass/fail
Residue evaporation mg/100 mL	1	—	0.4 (volume ≤ 10 mL) 0.3 (volume > 10 mL)

[a]Measured as lead.
— = not informed.

TABLE 3 Ranking of Complexity of Water Treatment Processes for Chemicals

Ranking	Process
1	Distillation
2	Ion exchange
3	Reverse osmosis
4	Membrane filtration

Table 4 presents the quality parameters of water obtained by the purification processes listed in Table 3.

The effectiveness of each treatment in removing the contaminants listed in Tables 1 and 2 are given in Table 5 [2].

Even when water complies with quality parameters as a raw material, it can present some impurities after being turned into a pharmaceutical product. Table 6 presents the level of some contaminants found in water for injection (WFI). Since the raw material should have passed in the quality test, contaminants either were below the allowed concentration level or were introduced after packaging. Contaminants introduced after packaging most likely originate from the packaging materials. Section 6.1.3.2 discusses containers as sources of contamination.

In summary, water can be a source of contaminants. If the raw material (drinking water) complies with the quality parameters established by authorities, contaminants still present can be eliminated by usual water purification processes available to the pharmaceutical industry. While distillation and reverse osmosis provide water with the quality specifications for *purified water* and *highly purified water*, *WFI* is generally obtained by membrane filtration (associated with another purification process) not only because of chemical contamination but mainly because of sterility requirements.

TABLE 4 Quality Parameters of Water after Different Purification Treatments

Purification Process	Conductivity (at 20°C) µS cm^{-1}	Total Organic Carbon (mg/L)	Nitrates (ppm)	Aluminum (µg/L)	Heavy Metals (ppm)[a]	Ammonium (ppm)	Calcium/ Magnesium (mg/L)	Residue Evap. mg/100 mL (as silicate)
None (tap water example)	240	10	<10	<200	1	1	35	1
Distillation (single)	10.2	0.03	—	—	0.5–1	0.01	1–3	0.5–1
Distillation (double)	2.1	0.06	—	—	0.1–0.8	0.01	0.3–0.1	0.1–0.7
Ion exchange	2–30	—	—	—	<0.01	—	—	1
Reverse osmosis	10–25	0.03	—	—	<0.04	0.4	1.6	0.1
Membran filtration	0.056	0.01	—	—	<0.01	<0.01	—	<0.01

[a]Measured as lead — not informed.

TABLE 5 Effectiveness of Each Treatment in Removing the Contaminants

	Treatment			
Contaminants	Distillation (%)	Ion Exchange	Reverse Osmosis (%)	Membran Filtration (%)
Ions	>70	>80%	Monovalent > 95 Polyvalent > 97	>80
Organic compounds	>80	Not efficient	>99	>80
Particles	>80	Not efficient	>99	>99

Source: From refs. 2 and 9.

TABLE 6 Contaminants Found in Water for Injection

Parameter	Sample	Content (μg/L)	Reference
Aluminum	Sterile water, Abbott	<5	10
	Sterile water, McGaw	<5	10
	Sterile water, Travenol	<5	10
	Aqua ad injectabilia, Braun, 50 mL	1	11
Arsenic	Water for injection, EMS	39.3	12
	Water for injection, Geyer	30.9	12
Zinc	Sterile water	13.9	13
Silicate	Sterile water, Geyer	280 ± 13	14

TABLE 7 Main Inorganic Impurities Listed in Pharmacopeial Compendia and Their Limits

Impurity	Limit	Impurity	Limit
Sulfated ash	0.01–1%	Heavy metals	1–50 ppm
Chloride	10–500 ppm	Arsenic	1–4 ppm
Sulfate	50–400 ppm	Aluminum	0.2–1 ppm
	0.1–0.6%	Cadmium	5–10 ppm
Fluoride	3 ppm	Chromium	0.05–10 ppm
Bromide	50 ppm	Copper	0.1 ppm
Oxalate	100–350 ppm	Iron	2–100 ppm
Phosphate	25–400 ppm	Lead	0.1–50 ppm
Sulfite	15 ppm	Nickel	0.2–1 ppm
Ammonium	200 ppm	Silver	250 ppm
		Zinc	10–30 ppm

Note: Observed: Some impurities are related to certain products only. For example, the limit for silver is in cysplatin and sulfite in sugars.

Concomitants Concomitants can be considered impurities present in naturally occurring, nonsynthesized raw materials. They may either present toxic effects, as with arsenic, or be as harmless as chloride ions. An overview of usual concomitants and their limits cited in pharmacopeial compendia are listed in Table 7.

The presence of concomitants in pharmaceutical products, although inevitable, may not exactly be a problem. The presence of magnesium in calcium salts is very

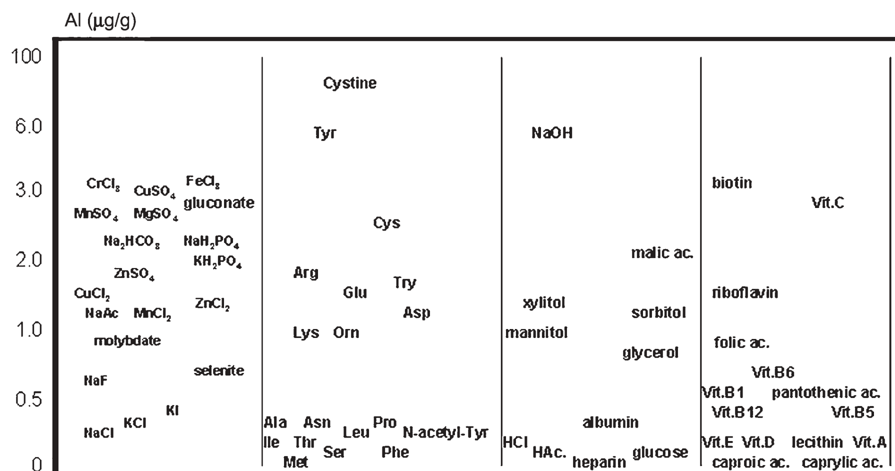


FIGURE 2 Aluminium present as impurity in substances used in parenteral nutrition [15].

common due to the similarity between these cations, both alkaline earth metals with a very similar chemical behavior. The consequence is that most raw materials containing calcium also contain small amounts of magnesium. The same occurs between sodium and potassium, so that the limit for potassium as an impurity in sodium chloride (according to the BP monograph) is 550 ppm or 0.55 mg potassium per gram sodium salt.

If species such as sodium, potassium, chloride, and sulfate are tolerable in a fairly large range, other toxic species such as arsenic and lead have narrower limits. For arsenic and lead, the limits in pharmacopeial compendia are generally set between 1 and 10 ppm; in fact, these limits are high for such harmful species.

Studies showing the presence of impurities in raw materials are not very frequent, mainly because the quality of raw materials is certified by guarantee bulletins, and products are supposed to be within the quality attested by the certificate.

Two studies with raw materials used to prepare parenteral formulations were carried out to show their content of aluminum and arsenic [15, 16]. It is possible to see in Figures 2 and 3 that aluminum and arsenic were present in all investigated raw materials. There were also different levels of contamination among the substances. While salts such as NaCl and KCl presented low aluminum contaminations, phosphates, gluconate, and also citric acid were relatively contaminated. The authors attributed this difference to the affinity of aluminum to the latter substances. Arsenic showed a more uniform distribution of contamination. With the exception of the amino acid tyrosine, the arsenic level in all substances was below 1 μg/g, not exceeding the limits prescribed by pharmacopeias.

Since analyses to check the presence of impurities in pharmaceutical products are generally carried out with finished products, it is not possible to attribute the presence of contaminants to the raw materials. Unless the raw material itself is checked, many other sources can aggregate impurities to the final product.

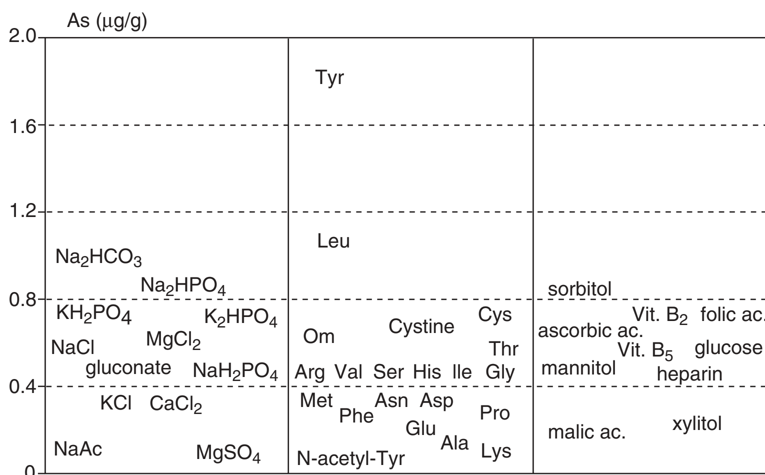


FIGURE 3 Arsenic level in substances used in formulations for parenteral nutrition [16].

By-products By-products are perhaps the most difficult impurities to summarize since each drug has its own by-products that may appear as impurities. They are synthesized along with active ingredients and are generally difficult to separate, owing to their similarity to the drug of interest. Most are isomeric species, differing from each other by the presence of only a small group or just by the position of a hydrogen atom. Even more difficult to separate and, therefore, purify are chiral isomers. Modern drugs that contain only one chiral isomer as the active ingredient are yet more difficult. Pharmacopeias usually present in the monographs the by-products that can exist as impurity of an active ingredient.

6.1.2.2 Additives

Additives are all formulation constituents other than the active ingredient. Although additives could be classified into excipients and vehicles (excipients for solid preparations and vehicles for liquid ones), there are several other agents used in pharmaceutical formulations with specific functions such as preservatives, sweeteners, coatings, colorants, antioxidants, surfactants, emulsifying agents, and flavors. Since they comprise a vast amount of products, this section will deal with additives for compounding pharmaceutical products for internal use only [17, 18].

Table 8 lists additives and the possible impurities they might present. The limits for these impurities were taken from manufacturers' specification bulletins. To compound Table 8, products of pharmacopeial grade (USP, BP, Ph. Eur., DAB) were selected from qualified suppliers (Merck, Aldrich, Sigma, Fluka, EMS, Riedel deHaën).

6.1.2.3 Decomposition of Formulation Constituents

The most relevant endogenous contamination arising from the decomposition of formulation constituents is the generation of peroxides in vitamins, amino acids, and lipid emulsions by action of light and air, that is oxygen.

TABLE 8 Additives for Pharmaceutical Formulations and Impurities and Their Limits According to Manufacturers (Merck, Aldrich, Sigma, Fluka, EMS, Riedel deHaën)

Additive	Impurity	Limit
	Solvents	
Ethanol	Acetone and isopropyl alcohol	≤0.01%
	Acetaldehyde and acetal	≤0.001%
	Benzene	≤0.0002%
	Methanol	≤0.01%
	Total of other impurities	≤0.03%
Glycerol	Chloride	≤0.0010%
	Sulfate	≤0.0010%
	Halogen compounds (as Cl)	≤0.0030%
	Heavy metals	≤0.0005%
	Arsenic	≤0.0001%
	Calcium	≤0.0001%
	Cadmium	≤0.0001%
	Mercury	≤0.0001%
	Ammonium	≤0.0005%
	Lead	≤0.0002%
	1,2,4-Butantriol	≤0.2%
	Residual solvents, class 3[a]	≤0.5%
	Aldehydes	≤10 ppm
	Sulfated ash	≤0.01%
Polyethylene glycol	Dioxane	10 ppm max
	Ethylene glycol and diethylene glycol	0.4% max
	Ethylene glycol (class 2)	620 ppm max
	Ethylene oxide	1 ppm max
	Formaldehyde (HCHO)	30 ppm max
	Heavy metals	20 ppm max
	Sulfated ash	0.2% max
	Preservatives	
Benzyl alcohol	Peroxide value[a]	≤5
	Benzaldehye	≤0.15%
	Cyclohexylmethanol	≤0.10%
	Benzene	≤0.0002%
	Chlorobenzene	≤0.01%
	Toluene	≤0.01%
Benzoic acid	Sulfate	≤0.02%
	Heavy metals	≤0.001%
	Arsenic	≤0.0001%
	Cadmium	≤0.0010%
	Copper	≤0.0010%
	Mercury	≤0.0001%
	Lead	≤0.0005%
	Zinc	≤0.0010%
	Halogen compounds (as Cl)	≤0.01%
	Toluene	≤890 ppm

TABLE 8 ***Continued***

Additive	Impurity	Limit
Chlorobutanol	Chloride	≤0.01%
	Chloroform	≤60 ppm
	Residual solvents, class 3[a]	≤0.5%
	Sulfated ash	≤0.1%
Methylparaben	Heavy metals	≤0.001%
	Arsenic	≤0.0003%
	Cadmium	≤0.001%
	Copper	≤0.001%
	Mercury	≤0.0001%
	Lead	≤0.0005%
	Zinc	≤0.001%
	Methanol	≤0.3%
	Sulfated ash	≤0.05%
Methylparaben sodium salt	Chloride	≤0.03%
	Sulfate	≤0.03%
	Heavy metals	≤0.001%
	Arsenic	≤0.0003%
	Cadmium	≤0.001%
	Copper	≤0.001%
	Mercury	≤0.0001%
	Lead	≤0.0005%
	Zinc	≤0.001%
Propylparaben sodium salt	Chloride	≤0.03%
	Sulfate	≤0.03%
	Heavy metals	≤0.001%
	Arsenic	≤0.0003%
	Cadmium	≤0.001%
	Copper	≤0.001%
	Mercury	≤0.0001%
	Lead	≤0.0005%
	Zinc	≤0.001%
	1-Propyl alcohol	≤0.5%
	Sulfated ash	34–36%
Propylparaben	Heavy metals	≤0.001%
	Arsenic	≤0.0003%
	Cadmium	≤0.001%
	Copper	≤0.001%
	Mercury	≤0.0001%
	Lead	≤0.0005%
	Zinc	≤0.001%
	Residual solvents, class 3[a]	≤0.5%
	Sulfated ash	≤0.05%
Potassium sorbate	Arsenic	≤0.0003%
	Cadmium	≤0.001%
	Copper	≤0.001%
	Mercury	≤0.0001%
	Lead	≤0.0002%
	Zinc	≤0.001%
	Aldehydes (as acetaldehyde)	≤0.15%
	Residual solvents, class 3[a]	<0.5%

TABLE 8 *Continued*

Additive	Impurity	Limit
Sodium benzoate	Chloride	≤0.02%
	Sulfate	≤0.01%
	Total chlorine	≤0.03%
	Heavy metals	≤0.001%
	Arsenic	≤0.0001%
	Cadmium	≤0.001%
	Copper	≤0.001%
	Mercury	≤0.0001%
	Lead	≤0.0002%
	Zinc	≤0.001%
Sorbic acid	Heavy metals	≤0.0010%
	Arsenic	≤0.0003%
	Cadmium	≤0.001%
	Copper	≤0.001%
	Mercury	≤0.0001%
	Lead	≤0.0002%
	Zinc	≤0.001%
	Aldehydes (as acetaldehyde)	≤0.15%
	Residual solvents, class 3[a]	≤0.5%
	Sulfated ash	≤0.2%
	Antioxidants	
Ascorbic acid	Heavy metals	≤0.001%
	Ign. residue	≤0.05% (as SO_4)
	Chloride	≤50 mg/kg
	Sulfate	≤20 mg/kg
	Copper	≤5 mg/kg
	Iron	≤2 mg/kg
L(+)-Ascorbyl palmitate	Heavy metals	≤0.001%
	Arsenic	≤0.0003%
	Copper	≤0.0025%
	Lead	≤0.001%
	Zinc	≤0.0025%
	Sulfated ash	≤0.1%
	Residual solvents, class 3[a]	≤0.5%
Butyl hydroxyanisole	Arsenic	0.0003% max
	Heavy metals	0.001% max
	Lead	0.0005% max
	Mercury	0.0001% max
	3-*tert*-Butyl-4-methoxyphenol	10% max
	Hydroquinone	0.2% max
	Sulfated ash	0.01% max
Butyl hydroxytoluene	Arsenic	0.0003% max
	Heavy metals	0.001% max
	Lead	0.0005% max
	Mercury	0.0001% max
	Residual solvents, class 2 (MeOH)[a]	0.2% max
	Sulfated ash	0.002% max

TABLE 8 ***Continued***

Additive	Impurity	Limit
Formaldehyde sulfoxylate sodium	Iron	0.0025% max
	Sodium sulfite	5.0% max
	Residual solvents, class 2 (MeOH)[a]	0.3% max
Phosphoric acid	Volatile acids (as CH_3COOH)	≤0.001%
	Chloride	≤0.0005%
	Fluoride	≤0.0010%
	Nitrate	≤0.0003%
	Phosphite and hypophosphite (as H_3PO_3)	≤0.02%
	Sulfate	≤0.005%
	Heavy metals	≤0.001%
	Arsenic	≤0.0002%
	Cadmium	≤0.00010%
	Copper	≤0.002%
	Iron	≤0.005%
	Mercury	≤0.0001%
	Potassium	≤0.005%
	Sodium	≤0.03%
	Lead	≤0.0010%
	Zinc	≤0.002%
Sodium bisulfite	Arsenic	0.001% max
	Heavy metals	0.003% max
	Iron	0.005% max
Sodium metabisulfite	Chloride	≤0.01%
	Heavy metals	≤0.001%
	Thiosulfate	≤0.02%
	Arsenic	≤0.0002%
	Iron	≤0.001%
	Mercury	≤0.0001%
	Lead	≤0.0005%
	Selenium	≤0.0006%
Sodium thiosulfate	Sulfate and sulfite (as SO_4)	≤0.2%
	Heavy metals	≤0.001%
Tocopherol	Heavy metals	≤0.001%
	Arsenic	≤0.0003%
	Copper	≤0.0025%
	Mercury	≤0.0001%
	Lead	≤0.0005%
	Zinc	≤0.0025%
	Methanol	≤3000 ppm
	Pyridine	≤200 ppm
	Toluene	≤890 ppm
	Sulfated ash	≤0.1%
	Vehicles	
Cellulose powder	Ether-soluble substances	≤0.15%
	Water-soluble substances	≤1.0%
	Heavy metals	≤0.001%
	Sulfated ash	≤0.3%

TABLE 8 *Continued*

Additive	Impurity	Limit
Gelatine	Sulfur dioxide (SO_2)	≤0.004%
	Heavy metals	≤0.001%
	Arsenic	≤0.00008%
	Chromium	≤0.001%
	Iron	≤0.003%
	Zinc	≤0.003%
	Peroxide (as H_2O_2)	≤0.001%
	Ash	≤2.0%
Lactose	Heavy metals	≤0.0005%
	Arsenic	≤0.0001%
	Copper	≤0.0025%
	Lead	≤0.00005%
	Zinc	≤0.0025%
	Sulfated ash	≤0.1%
Starch	Reducing matter (as maltose)	max 0.7%
	Sulfated ash	max 0.4%
Sorbitol	Chloride	≤0.002%
	Sulfate	≤0.006%
	Heavy metals	≤0.0005%
	Arsenic	≤0.00013%
	Nickel	≤0.0001%
	Lead	≤0.00005%
	Related substances (mannitol)	≤2.0%
	Reducing sugars after hydrolysis/ total sugar (as glucose)	≤0.5%
	Reducing sugars (as glucose)	≤0.11%
	Sulfated ash	≤0.02%
Sucrose	Chloride	≤0.0035%
	Sulfate	≤0.005%
	Sulfite (as SO_2)	≤0.0010%
	Heavy metals	≤0.0005%
	Arsenic	≤0.0001%
	Lead	≤0.00005%
	Sulfated ash	≤0.02%
	Residual solvents, class 3[a]	≤0.5%
Talc	Heavy metals	≤0.004%
	Aluminum	≤2.0%
	Arsenic	≤0.0003%
	Calcium	≤0.9%
	Iron	≤0.25%
	Lead	≤0.0005%
	Asbestos (according to Ph. Eur.)	Not detectable
Zinc oxide	Chloride	≤0.005%
	Sulfate	≤0.02%
	Arsenic	≤0.0005%
	Cadmium	≤0.001%
	Iron	≤0.001%
	Lead	≤0.005%

TABLE 8 *Continued*

Additive	Impurity	Limit
	Chelating Agents	
EDTA	Heavy metals	≤0.001%
	Calcium	≤0.001%
	Iron	≤0.001%
	Magnesium	≤5 ppm
	Nitrilotriacetic acid	≤0.1%
	Sulfated ash	≤0.1%
	Buffers	
Boric Acid	Sulfate	≤0.04%
	Heavy metals	≤0.0015%
Disodium hydrogen phosphate	Chloride	≤0.02%
	Fluoride	≤0.001%
	Sulfate	≤0.05%
	Heavy metals	≤0.001%
	Arsenic	≤0.0002%
	Cadmium	≤0.0001%
	Iron	≤0.002%
	Mercury	≤0.0001%
	Lead	≤0.0004%
Sodium dihydrogen phosphate	Chloride	≤0.005%
	Fluoride	≤0.001%
	Hydrogen phosphate (HPO_4)	≤0.5%
	Sulfate	≤0.01%
	Heavy metals	≤0.0005%
	Arsenic	≤0.0002%
	Cadmium	≤0.0001%
	Iron	≤0.001%
	Lead	≤0.0004%
	Mercury	≤0.0001%

[a]Residual solvents see Section 9.1.3.1; peroxide value: mmol peroxide/L.

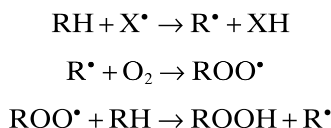
Lipid emulsions are essential components of parenteral nutrition. However, due to the amount of polyunsaturated fatty acids (PUFA), it is possible that chemical degradation occurs, forming hydroperoxides.

The exposure of lipid emulsions to room light and spotlight simulating phototherapy conditions in a neonatal intensive care unit for 24 h increases the level of hydroperoxides in these formulations by up to 60 times [19]. Lipid admixtures stored in ethyl vinyl acetate (EVA) bags presented a significant formation of lipid hydroperoxide. After one month under stressful conditions (gas-permeable container, 40°C), the peroxide value (PV = millimoles of peroxide per liter) was 450 times higher compared with controls (nitrogen-overlaid glass bottles) [20]. The results presented in Table 10 show that in contrast to lipids stored in closed glass containers gassed with nitrogen, significant and time-dependent peroxide formation occurred in all-in-one (AIO) plastic bags. Moreover, the lower PV of the lipid stored in V90 bags (polypropylene–polyamide 7 : 3, double layered) compared with EVA bags

(single layered) is indicative of the decreased oxygen permeability of the double-layered material and therefore the stabilization of the PV, although the increase of the PV occurred slowly in Intralipid samples stored in V90 bags.

Differences were found in the PV of Intralipid (LCT = long-chain triglyceride) and Lipofundin (MCT = medium-chain triglyceride) samples. Initial PV in Intralipid (0.02 mmol/L) was lower than in Lipofundin (0.10 mmol/L), whereas in the latter the formation rate of PV was slower over time. This behavior is due to the double PUFA content of Intralipid.

Polyunsaturated fatty acids are oxidized by enzymatic and nonenzymatic pathways. Nonenzymatic oxidation is a free-radical mediated peroxidation. It is a chain reaction providing a continuous supply of free radicals that initiate further peroxidation. The whole process can be depicted as follows [21]:



$$RH + X^{\bullet} \rightarrow R^{\bullet} + XH$$
$$R^{\bullet} + O_2 \rightarrow ROO^{\bullet}$$
$$ROO^{\bullet} + RH \rightarrow ROOH + R^{\bullet}$$

The reaction is initiated by any existing free radical ($X^{\bullet}$), by light, or by metal ions that react with lipids (RH) generating peroxy radicals ($ROO^{\bullet}$) and finally lipid hydroperoxides (ROOH). Besides hydroperoxides, malondialdehyde (MDA), ethane, and pentane can also be formed if PUFA with three or more double bonds and if ω-3 and ω-6 PUFA are present, respectively [14]. The formation of pentane (2 µmol/L) and MDA (10 µmol/L), along with hydroperoxides was observed in a lipid emulsion (Intralipid 10%) [22]. Malondialdehyde was also found in parenteral nutrition admixtures for newborn infants, in 12 samples analyzed, MDA levels varied from 1632 to 14,679 nmol/L [23]. Pironi and colleagues [24] also found MDA in fat emulsions. They compared three 20% lipid emulsions containing different amounts of PUFA and α-tocopherol, 24 hours after admixing them to make AIO solutions. They concluded that lipid peroxide generation is directly related to PUFA content and inversely related to the α-tocopherol/PUFA ratio of the emulsion. Moreover, MDA was also formed in these samples, presenting an increased concentration in the soybean-oil-containing emulsions when compared with the olive oil emulsion. Table 10 shows the PV and MDA measured in these samples.

Peroxide formation has also been attributed to a prooxidant action of vitamin E on PUFA, as vitamin E is another component present in lipid emulsions [19, 25]. Lipid oxidation occurring despite high concentrations of vitamin E (tocopherol) may appear surprising since the latter is generally regarded as the most efficient chain-breaking, lipid-soluble antioxidant [26]. Studies have revealed, however, that for vitamin E to be an efficient antioxidant in an isolated lipoprotein emulsion, it requires a suitable “co-antioxidant,” such as vitamin C [27, 28]. The co-antioxidant eliminates the vitamin E radical formed in the interaction of the vitamin with initiating radical oxidants, which can have an adverse effect by promoting lipid peroxidation.

Through the measurement of triglyceride hydroperoxides in Intralipid samples stored in glass and plastic syringes exposed to direct light or after being wrapped with aluminum foil, it was possible to demonstrate that Intralipid is highly oxidizable

TABLE 9 Peroxidation of Intralipid 20% and Lipofundin MCT 20% during Storage: Influence of Container Material, Light Exposure, and Temperature

Exposure, Condition, Time (day)	Nutrimix 2/3 Bag (EVA) 20–27°C Daylight[a]	Nutrimix 2/3 Bag (EVA) 20–27°C Light Protetion	3-chamber Bag (V 90) 20–27°C Daylight[a]	Control sample Closed Glass Bottle 20–27°C Daylight[a]
Intralipid 20%				
1				0.02 ± 0.006[c]
5	0.47 ± 0.016[b]	0.06 ± 0.004[b,c]	0.33 ± 0.011	
8				0.02 ± 0.005
9	0.84 ± 0.010	0.05 ± 0.007	0.50 ± 0.017	
14	1.46 ± 0.020	0.17 ± 0.002	0.60 ± 0.014	
15				0.02 ± 0.001
19	1.87 ± 0.027	0.27 ± 0.017	0.76 ± 0.018	
22	2.48 ± 0.038	0.40 ± 0.013	0.92 ± 0.025	
28				0.02 ± 0.005
29	2.93 ± 0.052	0.52 ± 0.023	1.24 ± 0.032	
		Lipofundin MCT 20%		
1				0.11 ± 0.006[c]
5	0.57 ± 0.018[b]	0.19 ± 0.017[d,e]	0.64 ± 0.014	
8				0.10 ± 0.018
9	0.88 ± 0.024	0.35 ± 0.002	0.69 ± 0.010	
14	1.31 ± 0.048	0.54 ± 0.025	0.64 ± 0.021	
15				0.08 ± 0.003
19	1.59 ± 0.064	0.61 ± 0.018	0.67 ± 0.036	
22	1.99 ± 0.076	0.84 ± 0.007	0.70 ± 0.054	
28				0.10 ± 0.032
29	2.48 ± 0.040	0.99 ± 0.044	0.69 ± 0.032	

Source: Form ref. 20.

[a,b] $P < 0.001$ (Nutrimix 2/3 with light protection).

[c,d] $P < 0.002$ (Nutrimix 2/3 with light protection versus control samples).

TABLE 10 Lipid Peroxide (LPX) and MDA Concentration in Lipid Emulsions

	LPX (μM/L)				MDA (μM/L)			
Emulsions	Bottles (n = 6)	No fat PN (n = 6)	AIO–T0 (n = 6)	AIO–T24 (n = 6)	Bottles (n = 6)	No fat PN (n = 6)	AIO–T0 (n = 6)	AIO–T24 (n = 6)
Soybean oil	28.8 ± 29.9	<2	<2	11.3 ± 15.0	8.8 ± 3.1	4.9 ± 0.8	5.0 ± 0.3	6.9 ± 1.0
Soybean oil + MCT	1.7 ± 0.5	<2	<2	5.2 ± 8.8	3.7 ± 0.4	3.4 ± 0.4	4.1 ± 0.3	7.8 ± 1.8
Olive oil	4.1 ± 2.8	<2	<2	5.5 ± 6.4	0.7 ± 0.1	5.4 ± 0.3	5.3 ± 0.3	3.3 ± 0.4

Source: From ref. 24.

Note: Values are mean ± standard deviation of six bags (one sample per bag, analyzed in triplicate).
AIO, all-in-one mixture immediately after the addition of lipid emulsion; AIO–T24, all in one mixture 24 h after the addition of lipid emulsion.

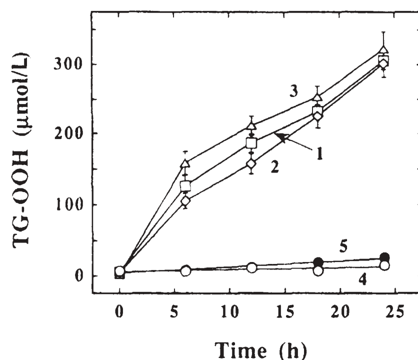


FIGURE 4 Kinetics of accumulation of triglyceride hydroperoxide in Intralipid exposed to phototherapy light under conditions that mimic the situation in an NICU (neonatal intensive care unit). A solution of 20% Intralipid was dispensed into a glass reservoir containing plastic tubing (line 3), with aluminum foil (line 4), or a plastic reservoir after supplementation with 1 mmol/L sodium ascorbate (line 5) and exposed to phototherapy light for up to 24 h. For all samples the light fluxes measured were 11.7–25.9 μW/cm^2 per nanometer for 24 h. At the time points indicated, aliquots were withdrawn, extracted, and their hexane extracts analyzed for the presence of triglyceride hydroperoxides and α-tocopherol as described in the methods section [19].

under routine clinical conditions, in spite of its vitamin E content (≅60 μmol/L γ-tocopherol and 20 μmol/L each of the α and δ isomers).

The problem is particularly evident for neonates undergoing phototherapy. Besides the fact that lipids are used extensively to supply premature babies with calories, bags and extension sets are exposed for longer periods of time to the intense radiation of spotlights due to the low infusion rates of neonatal solutions. Figure 4 depicts the evolution of triglyceride hydroperoxide formation in a 20% Intralipid emulsion under different conditions of light exposure. It shows that lipid oxidation occurs despite the high concentration of vitamin E present in the samples. Decomposition can be prevented by wrapping containers and tubing sets in aluminum foil or by adding ascorbate to the infusate.

Lipid peroxides are also able to react with other components of parenteral nutrition admixtures (trace elements), causing a drop in pH with the subsequent potential for physical–chemical instability [29]. Table 11 shows the peroxide value and the pH drop in a pure lipid emulsion and a lipid-containing AlO admixture stored in EVA bags under different conditions of temperature and light exposure in the presence and absence of trace elements.

Peroxide formation has also been observed in multivitamin solutions for parenteral nutrition. Lavoie and co-workers [30] have studied the action of light, air, and composition on the stability of multivitamin formulations, and also total parenteral nutrition (TPN) admixtures containing and not containing vitamins and fatty acids. They analyzed the generation of peroxide in multivitamin solutions and in TPN for adults and neonates. The analysis of multivitamin solutions for enteral use revealed the presence of peroxides at the initial opening of the bottle. The levels were higher in Poly-Vi-Sol (vitamin A, Vitamin D, and vitamin C, vitamin B_1, riboflavin, and

TABLE 11 Peroxide Value and pH Drop in Pure Lipid Emulsion and AIO Admixture Stored in EVA Bags under Different Conditions of Temperature and Light Exposition in Presence and Absence of Trace Elements

Sample	Conditions	Parameters	Without Trace Elements	With Trace Elements
AIO	2–8°C	PV (mmol/L)	0.04	0.19
	Light protected 29 days	pH drop	0.01	0.02
AIO	20–30°C	PV (mmol/L)	0.52	1.92
	Light exposed 29 days	pH drop	0.03	0.11
Intralipid 20%	40°C	PV (mmol/L)	2.77	18.04
	Light protected 14 days	pH drop	0.77	1.54

Note: PV: peroxide value.

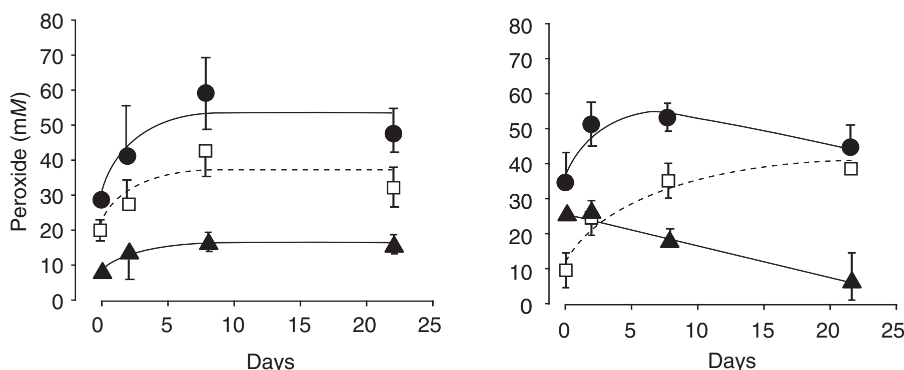


FIGURE 5 Total peroxide (circles), catalase-resistant peroxides (squares), and H_2O_2 as the difference (triangles) were measured in three lots of oral multivitamins without riboflavin (Tri-Vi-Sol) and three lots of oral multivitamins with 0.6 mg of riboflavin (Poli-Vi-Sol) by time after the initial opening of the bottle. Compared with the preparation without riboflavin, the level of H_2O_2 was initially higher ($P < 0.01$) in (Poli-Vi-Sol), and that level dropped over time ($P < 0.05$). In both multivitamin preparations, the levels of catalase-resistant and total peroxide rose ($P < 0.05$) until day 8. Data represent the mean ± standard error of the mean. Variations too small relative to the symbol are not shown [30].

niacinamide) than in Tri-Vi-Sol (vitamin A, vitamin D, and vitamin C) (Fig. 5) [30].

The authors attributed the difference in the peroxide content of the preparations to riboflavin. This vitamin catalyses the photoinduced reaction between ascorbate and oxygen, which leads to hydrogen peroxide generation. The drop in hydrogen peroxide concentration over time observed with Poly-Vi-Sol is explained by the transformation of hydrogen peroxide into a more reactive species, which in turn could react with other components of the formulation. In contrast, the increase in catalase-resistant peroxides in the Tri-Vi-Sol preparation suggests a greater contribution of air in the peroxide generation. In comparison to the formulation for parenteral use, the enteral multivitamin presented levels of 100 times higher, where catalase-resistant peroxides represented the bulk of peroxides. To study the effect of air and light on the generation of peroxides in multivitamins for TPN, the authors

limited the experiment to fat-free TPN since lipids are also able to generate peroxides [31]. In neonate solutions, the peroxide concentration ranged from 190 to 300 μmol/L in sets unprotected from light in contrast to the values for sets protected from light, which ranged from 60 to 130 μmol/L. In the adult formulation, peroxide reached less than one-tenth of the concentration measured in the neonatal preparation (Fig. 6). The authors attributed the difference to the nutrient composition, as the adult formulation was four times lower in vitamins and higher in final amino acid and glucose concentrations. Amino acids and glucose contributed to a decrease in the concentration of peroxides [32].

It is possible to see in Figure 6 that protecting solutions from light and air decreases peroxide generation in these formulations. However, because of the high peroxide concentration in neonatal solutions, protection from air during the infusion is not efficient.

The action of vitamins in the presence of lipids was also investigated by these researchers [33]. The generation of peroxide was compared between TPN preparations left at dark and at daylight for 6 h. Four different formulations containing and not containing vitamins and lipids were included in the experiment. The results, presented in Figure 7 allowed for the conclusion that lipids had a significant but minor additive effect compared with vitamins in generating peroxides. Contamination of TPN by air during compounding accounted for the photoinduced generation of peroxide in TPN solutions. It was, however, more effective to protect the solution from light exposure than to avoid contact with oxygen.

Since ascorbate reduces photooxidation of lipid emulsions and multivitamin preparations (see Figure 4) [19], Lavoie et al. [34] studied the formation of oxidative by-products of vitamin C in multivitamins exposed to light. They found that the loss of ascorbic acid in photoexposed multivitamin preparations was associated with the generation of products other than dehydroascorbate and 2,3-diketogulonic acid, which are the usual products of vitamin C oxidation. The authors showed that hydrogen peroxide at concentrations found in TPN solutions induced the transformation of dehydroascorbate into new, biologically active compounds that had the potential to affect lipid metabolism. They believe that these species have peroxide and aldehyde functions [35].

Since air (oxygen) is one of the factors responsible for peroxide generation in lipid emulsions and vitamin solutions, Balet et al. [36] compared multilayered versus single-layered (EVA) bags in terms of oxidation of parenteral nutrition solutions. They measured PV, α-tocopherol, and ascorbic acid at the moment of admixing and after 6 and 14 days in 24 parenteral solutions. Admixtures in multilayered bags showed less oxidation than in EVA bags; no important difference was observed in α-tocopherol content, but just after 6 days storage, ascorbic acid and dehydroascorbic acid disappeared in the EVA bags.

Sodium metabisulfite is an antioxidant agent widely used in pharmaceutical preparations to reduce or prevent oxidation. There are some studies, however, that have shown that metabisulfite, under specific conditions, may have indirect oxidant properties. Baker et al. [37] demonstrated that sulfite propofol emulsion, but not EDTA propofol emulsion, underwent chemical changes during a simulated intravenous infusion. Compounds were identified as propofol oxidation products. The increase of propofol oxidation products demonstrated that sulfite from metabisulfite created a strong oxidant environment when air was introduced. Lavoie et al. [38]

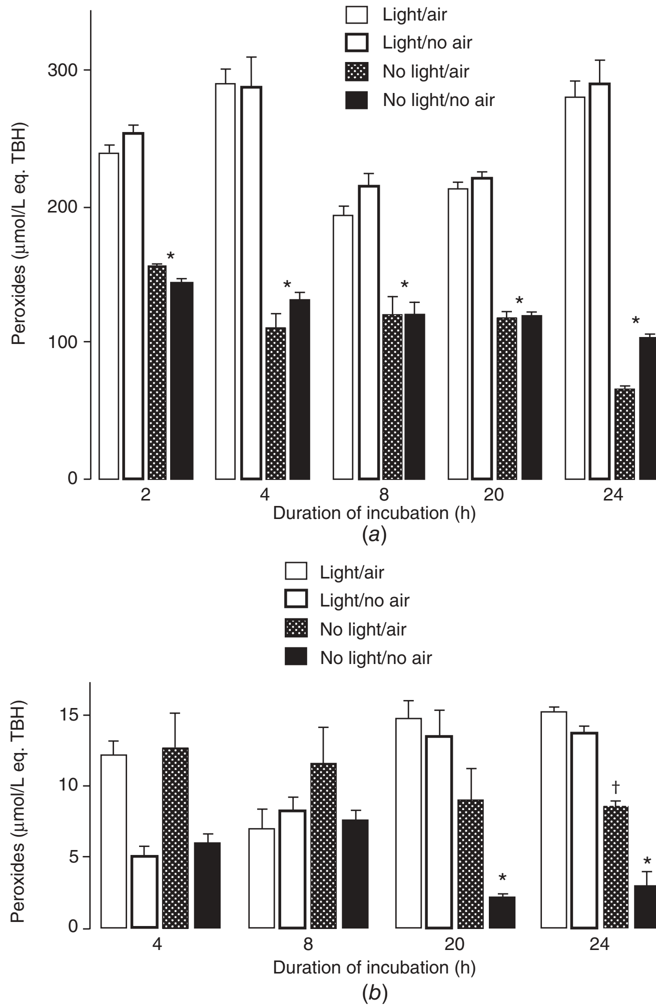


FIGURE 6 (*a*) Peroxide concentration in a neonatal fat-free solution of parenteral nutrition run through four different infusion sets: with or without air inlet, protected and unprotected from light (lux). Photoprotection was associated with a significantly lower peroxide content (indicated by *; $P < 0.05$). Protection from light resulted in a greater prevention against the generation of peroxides than did protection from air contamination. (*b*) Peroxide concentration in an adult solution of fat-free parenteral nutrition run through four different infusion sets: with or without air inlet, protected and unprotected from light (lux). The concentration of peroxides is 10-fold lower than in the neonatal solution. Protection from light and air was not associated with an overall effect on peroxide generation. Protection from light and air resulted in a significant decrease in peroxide generation at 20 and 24 h of infusion (indicated by *; $P < 0.05$), whereas photoprotection alone resulted in a significant difference at 24 h (indicated by +; $P < 0.05$). Results are expressed as mean ± standard error of the mean (SEM); $n = 3$. TBH = *tert*-butylhydroperoxide [31].

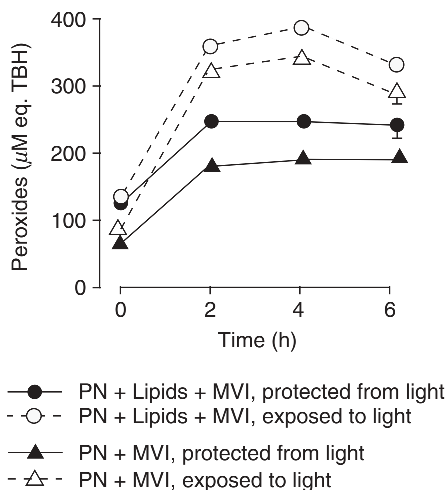


FIGURE 7 Influence of a lipid emulsion and daylight on peroxide levels in freshly prepared solutions of parenteral nutrition containing multivitamins (PN + MVI and PN + Lipid + MVI). (PN = parenteral nutrition; MVI = multi vitamin preparation.) The data represent the mean ± SEM, $n = 3$; the variations are not depicted because of their small size relative to the symbols. The peroxide content rose significantly over time ($P < 0.001$), and exposure to daylight had a significant effect on peroxide generation ($P < 0.001$) [33].

showed that sulfite has been able to cause the oxidation of lipids. The reaction of sulfite with oxygen may lead to several sulfite-derived oxidant species [39].

Besides the oxidation to sulfate ($SO_3^{2-} \rightarrow SO_4^{2-} + 2e^-$), sulfite may undergo a one-electron oxidation leading to the formation of the reactive sulfite radical ($SO_3^{2-} \rightarrow SO_3^{\bullet-} + e^-$), which reacts with oxygen forming two strong oxidant species: sulfite peroxyl ($SO_3^{\bullet-} + O_2 \rightarrow SO_3OO^{\bullet-}$) and sulfate radical ($SO_3^{\bullet-} + O_2 \rightarrow SO_4^{\bullet-}$). These species and the sulfite radical itself react as oxidants, turning sodium metabisulfite into a prooxidant agent, depending on the circumstances.

Peroxidation and free-radical formation should be considered as important aspects of pharmaceutical stability and quality of parenteral nutriton and intravenous drugs. Peroxidation and free-radical formation depend on environmental factors, such as storage conditions and container material, but are also influenced by formulation components or additives such as tocopherols and metabisulfite. Since the generation of these harmful species occurs generally at the time of use, manufacturing quality controls fail in demonstrating their existence.

6.1.3 EXOGENOUS IMPURITIES

6.1.3.1 Residual Solvents

Residual solvents are organic volatile chemicals that remain in active substances, excipients, and other pharmaceutical products after processing. In spite of their toxic properties, solvents play an important role in the production of pharmaceuticals, during the synthesis, separation, or purification, and their use cannot be avoided. Solvents in this category do not include those used as excipients.

TABLE 12 General Characteristics of Residual Solvents

Class	Action	Characteristics
1	Solvents to be avoided	Strongly suspected human carcinogens and environmental hazards
2	Solvents to be limited	Nongenotoxic animal carcinogens or possible causative agents of other irreversible toxicity such as neurotoxicity or teratogenicity
3	Solvents with low toxic potential	No health-based exposure limit is needed; solvents with permitted daily exposure of 50 mg or more per day

Source: From ref. 40.

Residual solvents are organic volatile chemicals that were not completely removed by practical manufacturing techniques and may, therefore, be contaminants in pharmaceutical products.

The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) [40] has adopted impurities guidelines for residual solvents that prescribe limits for the amount of residual solvents allowed.

Residual solvents are divided into three classes. Class 1 solvents are those known to cause toxic effects and should be avoided in the production of active substances and excipients. Class 2 solvents present less severe toxicity than class 1, and class 3 solvents have such low toxic potential that exposure limits are not necessary. Table 12 presents the general characteristics of the solvents included in each class, and Table 13 lists the solvents and their concentration limit in pharmaceutical products.

6.1.3.2 Containers

The primary function of packaging is to provide adequate protection. Pharmacopeial compendia have established requirements for containers based on drug form characteristics. Thus, while for capsules and tablets the requirements are generally related to the design of the container (e.g., tight, well closed), for injectables, and ophthalmic, and inhalation products, materials of construction are also addressed, as compatibility is a very important issue for this kind of dosage. Considering the purpose of this chapter, only those packaging materials whose interaction with the formulation is a factor will be focused on here.

Packaging components for pharmaceuticals are basically made of glass and polymeric materials such as plastics and rubbers.[1] In spite of this simple classification, glass, plastic, and rubber are not elementary materials but rather complex mixtures.

The evaluation of the chemical stability of a packaging component depends on the likelihood of packaging component–dosage form interaction and is usually

[1]Although metallic packaging is also used for pharmaceutical products, its use is restricted to blisters' supports, which have no contact with the formulation, and tubes for ointments.

TABLE 13 Solvents Included in Each Class and Their Concentration Limit in Pharmaceutical Products

Solvent	Concentration Limit (ppm)	Solvent	Concentration Limit (ppm)
Class 1		*Class 3*	
	2	Acetic acid	—
Benzene	4	Acetone	—
1,2-Dichloroethane	5	Anisole	—
1,1-Dichloroethane	8	1-Butanol	—
1,1,1- Trichloroethane	1500	2-Butanol	—
		Butyl acetate	—
Class 2		*tert*-Butyl methyl ether	—
		Cumene	—
Acetonitrile	410	Dimethyl sulfoxide	—
Chlorobenzene	360	Ethanol	—
Chloroform	60	Ethyl acetate	—
Cyclohexane	3880	Ethyl ather	—
1,2-Dichloroethene	1870	Ethyl formate	—
Dichloromethane	600	Formic acid	—
1,2-Dimethoxyethane	100	Heptane	—
N,*N*-Dimethylacetamide	1090	Isobutyl acetate	—
N,*N*-Dimethylformamide	880	Isopropyl acetate	—
1,4-Dioxan	380	Methyl acetate	—
2-Ethoxyethanol	160	3-Methyl-1-Butanol	—
Ethylene glycol	620	Methyl ethyl ketone	—
Formamide	220	2-Methyl-1-propanol	—
Hexane	290	Pentane	—
Methanol	3000	1-Pentanol	—
2-Methoxyethanol	50	1-Propanol	—
Methyl butyl ketone	50	2-Propanol	—
Methylcyclohexane	1180	Propyl acetate	—
N-methylpyrrolidone	4840	Tetrahydrofuran	—
Nitromethane	50		
Pyridine	200		
Sulfolane	160		
Tetralin	100		
Toluene	890		
1,1,2-Trichloroethene	80		
Xylene	2170		

Source: From ref. 40.
— = not limited.

carried out by exposing a sample of the packaging component to an appropriate solvent at elevated temperatures. The resulting extract should be analyzed for extractables. Thus, for glass samples the released alkalinity should be evaluated, whereas for plastics and rubbers the amount of extractables, as well as their nature, are to be determined. The elevated temperature has the purpose of increasing the extraction rate and simulating in a short period of time a longer exposure time at room temperature. The solvent used for the extraction test should present the same

TABLE 14 Glass Classification According to Pharmacopeial Prescription and Their Pharmaceutical Usages

Glass Type	General Description	Uses
I	Highly resistant borosilicate glass	Parenteral preparations
II	Treated soda–lime glass	Acidic or neutral parenteral preparations
III	Soda–lime glass	Not for parenteral preparations
NP	Soda–lime glass	Oral or topic use

TABLE 15 Typical Composition of Glass Containers According to the Pharmacopeial Classification

Component	Type I, Borosilicate (%)	Types II, III, IV, Soda–Lime (%)
SiO_2	70	73
B_2O_3	10	—
Na_2O	9	14
Al_2O_3	6	2
BaO	2	—
K_2O	1	—
CaO	1	7
MgO	0.5	4
ZnO	0.5	—

Source: From Corning, Life Science Catalogue: Technical information Description of Glasses Used in Corning Labware.

propensity to interact with the packaging material as the dosage form. Although it is desirable that the dosage form itself be used in the test, pharmacopeial compendia prescribe tests with standard solvents such as purified water, drug vehicle, and isopropyl alcohol.

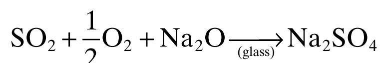
Even when impurities and degradation profiles of a drug substance have been established and containers comply with guidelines, some unexpected drug–container interactions can occur during the sterilization procedure or shelf life.

This section includes the most important container materials: glass, plastic, and elastomers and addresses the contamination issues for injectable formulations.

Glass Containers Glass containers for packaging pharmaceutical preparations should meet stability specifications, which are related to the presence of certain components in the structure of the glass and to the formulation type and use. According to pharmacopeial prescriptions, four different types of glass may be used for storing pharmaceutical preparations. Table 14 presents the classification of glass containers in accordance with the product's nature and pharmaceutical usage.

Though mostly made of silica, the different glass types are obtained by adding or subtracting certain glass components. Table 15 lists the principal components of pharmaceutical glasses, based on the criteria presented in Table 14. Type I glass must have at least 10% boron oxide and a higher concentration of aluminum oxide than the ordinary soda–lime glass to improve its resistance. The reduced amount of sodium oxide lessens the solubility of type I glass in water. Type II glass is made of

common soda–lime glass after a dealkalinization process on the inside surface at the time of manufacture. In this process, known as sulfur treatment, sulfur dioxide is introduced as newly formed bottles pass from the forming machine, and it reacts at the surface of the glass to form sodium sulfate, according to the equation:



$$SO_2 + \frac{1}{2}O_2 + Na_2O \xrightarrow[\text{(glass)}]{} Na_2SO_4$$

The container is delivered with a haze of sodium sulfate, which should be rinsed away before filling.

Stability test for glass containers prescribed by pharmacopeial compendia is limited to the action of water on the glass surface to measure the alkalinity released (water attack). After autoclaving the container filled with water at 121°C for 60 min, 100 mL of the resulting extract is titrated with 0.02 *N* H_2SO_4. The volume of the acidic titrant should not be greater than a specific value based on the container's capacity. Briefly, types I and II glass containers with a capacity of less than 10 mL should consume less than 2.0 mL titrant, whereas for type III glass containers the limit is 20 mL. For containers with a capacity of between 20 and 500 mL, the titrant consumption should be from 0.8 to 0.2 mL for types I and II glass and from 8.0 to 2.2 mL for type III glass. For non-parenteral (NP) glass, no limit of titrant is established.

The released alkalinity is, however, not enough to show the actual stability of a glass container. Glass is not as inert as it appears, and water is not the only substance that attacks the glass surface. In spite of this, the only test prescribed at USP for toxic impurities in glass is arsenic (<661> containers, USP 27); type I glass containers should be assayed according to the water attack procedure and present not more than 0.1 ppm As. Though not listed as a glass constituent, As may be used as a fining agent in the glass industry (see below) and, therefore, may be present in the glass structure.

Studies have shown that even type I glasses are not completely inert. Bohrer et al. [41] showed that the hydrolytic resistance of glass containers is diminished in the presence of some substances commonly present in infusion solutions. Table 16 presents the released alkalinity and also the concentration of some glass constituents (silicate, sodium, boron, aluminum) in the aqueous extract obtained after the hydrolytic resistance test.[2] These data show that, despite complying with pharmacopeial prescription, glass can release its constituents by action of species in the solutions and even in pure water. Basic solutions of bicarbonate and gluconate presented the highest level of glass constituents extracted, confirming the potential of basic solutions to attack and dissolve the glass network. Glucose and citric acid interacted with the glass surface, selectively extracting not only Al but also Cu and Pb (traces also present in the glass). This specific action of citrate and glucose could be related to their metal-complexing ability.

The presence of arsenic as a contaminant in pharmaceutical products is a pharmacopeial concern. Practically all monographs present limits for arsenic, ranging from 0.1 to 3 ppm. Bohrer et al. have shown that arsenic is a ubiquitous contaminant

[2]The hydrolytic resistance test described in this chapter is the "powdered glass test" and not the "water attack test" as described above; see Appendix <661>, USP 27 [1].

TABLE 16 Volume H_2SO_4 Solution Consumed in Titration of Solutions before and after Heating in Contact with Glass Mass for 30 min at 121°C and Species Extracted from Glass during the USP Powdered Glass Test Carried out with Clear Ampoules in Presence of Different Solutions

	Volume 0.02 *N* H_2SO_4 (mL)		Species Concentration (mg/L) ± SD ($n = 3$)					
Sample	Before Heating	After Heating	Na	Silicate	Borate	Al	Cu	Pb
NaCl	[a]	0.56 ± 0.01	—	8.30 ± 2.01	2.22 ± 0.11	0.15 ± 0.01	0.09 ± 0.02	0.03 ± 0.01
KCl	[a]	0.69 ± 0.01	14.42 ± 1.51	9.96 ± 1.71	1.58 ± 0.03	0.19 ± 0.01	0.14 ± 0.08	0.04 ± 0.02
$CaCl_2$	[a]	0.45 ± 0.02	13.41 ± 1.33	7.31 ± 2.08	2.00 ± 0.11	0.18 ± 0.01	0.06 ± 0.01	0.02 ± 0.01
$MgCl_2$	[a]	0.13 ± 0.01	15.25 ± 1.64	6.54 ± 0.53	3.25 ± 0.21	0.16 ± 0.01	0.09 ± 0.03	0.02 ± 0.01
Sodium gluconate	2.68 ± 0.22	1.83 ± 0.05	—	246.0 ± 18.7	1.91 ± 0.09	2.04 ± 0.02	0.49 ± 0.07	0.05 ± 0.01
Sodium hydrogen phosphate	[a]	10.22 ± 0.91	—	10.21 ± 3.41	2.66 ± 0.09	4.93 ± 0.12	0.05 ± 0.01	0.05 ± 0.02
Potassium hydrogen phosphate	[a]	8.78 ± 0.95	18.38 ± 2.34	45.32 ± 3.76	1.73 ± 0.06	4.30 ± 0.07	0.13 ± 0.04	0.04 ± 0.02
Sodium bicarbonate	24.3 ± 0.41	12.68 ± 1.03	—	271.7 ± 23.2	5.98 ± 0.14	5.22 ± 0.24	0.88 ± 0.09	0.06 ± 0.03
Citric acid	[a]	1.92 ± 0.04	19.82 ± 1.90	16.46 ± 0.87	1.44 ± 0.10	6.36 ± 0.53	0.49 ± 0.03	0.10 ± 0.05
Glucose	[a]	1.08 ± 0.05	13.86 ± 1.22	11.10 ± 0.88	3.18 ± 0.17	6.34 ± 0.33	0.28 ± 0.01	0.15 ± 0.03

Note: Concentration of the solutions: 0.01 mol/L [41].

[a] pH ≤ 7.

in raw materials [16] (see discussion of water in Section 9.1.2.1), but its presence in final products occurs in concentrations much higher than in the corresponding raw substances [12]. According to the authors, the main source of arsenic in injectable formulations is glass containers [16]. As mentioned above, glass can contain arsenic because arsenic oxide(III) is a fining agent to improve glass transparency [42], a feature especially important for solutions for intravenous administration that must be subjected to visual inspection of the content before use [43]. Arsenic oxide(III) is supposed to react with potassium nitrate in the glass melt to release oxygen and nitrogen oxides. These gases form large bubbles that rapidly rise to the surface, stirring the bath and sweeping small bubbles formed by the decomposition of batch materials. Table 17 shows the arsenic levels measured by these authors in solutions for parenteral nutrition. Among these products, sodium bicarbonate and calcium gluconate are the most contaminated, presenting levels even higher than the limit of 0.1 mg/L for arsenic in infusion solutions.

Other elements such as chromium, barium, and zinc have also been found in solutions for parenteral nutrition, and, though not stated in the studies, the origin of these elements is probably the glass packaging. Since glass is an inorganic material, inorganic species may arise as contaminants from glass containers. Exceptions could be Zn and Ba, which, besides being present in type I glasses, are components of plastic additives as well. Table 18 summarizes inorganic elements as contaminants in different infusion solutions and their respective concentrations.

Data on Al are being presented separately because of its special behavior and toxicity. Since the discovery in 1976 by Alfrey and co-workers [48] that Al can accumulate in patients with reduced renal function causing toxic manifestations including neurological diseases, much has been done to reveal and reduce aluminum contamination sources. Nowadays, Al is also considered toxic for patients with normal renal function who receive parenteral nutrition, mainly preterm infants [49]. Recognized sources of Al include water used for dialysis, solutions for total parenteral nutrition, and oral aluminum-containing compounds. Though additives and raw materials are sources as well, the most important source of Al in solutions for parenteral nutrition is the storage of parenteral preparations in glass containers. Figure 8 shows the Al level in different kinds of solutions stored in plastic and glass containers for a period of three months. While not more than 50 μg/L Al was leached from polyethylene (PE) containers, over 3000 μg/L Al was found in solutions stored in glass containers, confirming that glass is a valid and continual source of Al.

The same authors also showed that though glass is the source, Al leaching depends on the nature of the substance in contact with its surface [51, 52]. In an experiment with glass and an ion exchanger containing Al attached, complexing agents and also amino acids were able to extract Al from both sources, but following an extraction yield related to the affinity of the ligand for the metal. The results of this interaction, measured over 2 months, can be seen in Figure 9. Based in this conclusion, the notable contamination by Al in gluconate and phosphate solutions can be attributed to the affinity of these species with Al. They are able to selectively withdraw Al from the glass network when in contact with the glass containers (see Table 20).

Figure 10 shows the result of one year of storage of different amino acids, components of parenteral solutions in type I glass containers. Not all the solutions were contaminated by Al, but only those whose amino acids presented affinity with this element.

TABLE 17 Arsenic Species Present as Contaminant in Commercial Parenteral Solutions[a]

Product	Total As (μg/L)	As(V) (μg/L ± RSD)	As(III) (μg/L ± RSD)
KCl 19.1% (10)	41.3	41.3 ± 1.6	n.d.
KCl 10% (4)	23.6	23.6 ± 0.8	n.d.
NaCl 20% (10)	43.8	40.9 ± 5.7	2.9 ± 0.2
NaCl 20% (4)	15.9	15.9 ± 3.2	n.d.
Sodium acetate 2 meq/mL (1)	46.1	41.8 ± 2.0	4.3 ± 0.6
Sodium phosphate 0.5 mol/L (10)	36.7	36.7 ± 2.5	n.d.
Sodium bicarbonate 8.4% (5)	248.6	227.8 ± 0.5	20.8 ± 2.0
Sodium bicarbonate 8.4% (9)	198.3	147.2 ± 1.2	51.1 ± 0.8
Calcium gluconate 10% (11)	73.1	46.8 ± 4.1	26.3 ± 2.3
Calcium gluconate 10% (9)	92.7	72.8 ± 0.5	19.9 ± 1.9
Calcium gluconate 10% (4)	239.6	176 ± 7.2	63.6 ± 2.0
Magnesium sulfate 50% (11)	15.7	15.7 ± 0.2	5.7 ± 0.2
Magnesium sulfate 50% (10)	33.5	12.2 ± 1.2	21.3 ± 1.8
Magnesium sulfate 50% (9)	53.8	39.9 ± 5.6	13.9 ± 3.0
Glucose 25% (6)	21.8	16.5 ± 2.6	5.3 ± 0.6
Glucose 25% (6)	18.6	14.0 + 2.2	4.6 ± 0.7
Vitamins (Tiaminose[b])	103.7	86.1 ± 0.9	17.6 ± 3.2
Vitamins (Dextrovitase[c])	10.2	10.2 ± 0.2	3.8 ± 0.1
Vitamins (Frutovena[d])	61.7	21.5 ± 1.7	40.2 ± 6.7
Amino acids 10% (3)	2.5	2.5 ± 0.1	n.d.
Amino acids 10% (1)	15.4	15.4 ± 2.3	n.d.
Amino acids 10% (2)	41.0	41.0 ± 0.9	n.d.
Amino acids 8% (2)	21.1	16.7 ± 1.2	4.4 ± 0.4
Amino acids 8% (2)	94.7	87.9 ± 5.7	6.8 ± 1.0
Lipid emulsion 10% (2)	0.9	0.9 ± 0.3	n.d.
Lipid emulsion 20% (2)	1.7	1.7 ± 0.6	n.d.
Heparin 5000 UI/mL (7)	56.7	17.2 ± 2.8	39.5 ± 2.3
Heparin 5000 UI/mL (8)	79.4	79.4 ± 4.9	23.3 ± 1.2

[a]Number in parentheses refer to product brands: (1) B. Braun, (2) Fresenius, (3) Baxter, (4) Halex Istar, (5) JP, (6) Merck, (7) EMS, (8) Elkins Sinn, (9)Ariston, (10) Geyer, (11) Hipolabor. RSD, relative standard deviation; n.d., not detected (below LOD).
[b]Glucose 3 g, ascorbic acid 0.25 g, thiamin chloridrate 0.015 g in 10 mL.
[c]Glucose 2 g, ascorbic acid 2 g, pyridoxine chloridrate 20 mg, nicotinamide 30 mg, riboflavin in 20 mL.
[d]Frutose 5 g, ascorbic acid 1 g, pyridoxine chloridrate 20 mg, sodium pantothenate 10 mg, riboflavin 4 mg in 20 mL.
Source: Form ref. 16.

Table 20 presents the Al level in parenteral formulations, collected by different authors in different countries. Not all of them mention glass packaging as responsible for Al contamination, but it seems to have been the major source. These data also confirm that the nature of the formulation components plays a key role in selective Al leaching.

These results show that significant variations in the contaminant and level of contamination depend on the formulation constituents, brand, and mostly the nature of the packaging material.

TABLE 18 Metallic Contaminants Found in Commercial Solutions for Parenteral Nutrition

Sample	Element Concentration (μg/L)											Ref.
	Zn	Cr	Fe	Pb	As	Mn	Ba	Sn	Ge	Cu	Cd	
TPN bag	9.1	—	—	—	—	—	—	—	—	—	—	13
Amino acids	60–4,70	n.d.	—	—	—	—	—	—	—	—	—	44
L-Cysteine HCl	32,000–86,000	110–230	—	—	—	—	—	—	—	—	—	44
NaCl, KCl, Na acetate	350–560	20–230	—	—	—	—	—	—	—	—	—	44
Ca gluconate	280–2,380	—	—	—	—	—	—	—	—	—	—	44
Phosphate	910–2,330	390–440	—	—	—	—	—	—	—	—	—	44
Ca gluconate	47–244	—	237–655	—	—	—	—	—	—	—	—	45
TPN	233–703	—	84	—	—	—	—	—	—	—	—	45
Standard adult TPN formula, Baxter	—	86.8	—	0.6	288.0	309.9	11.0	0.5	5.5	—	—	46
Standard adult TPN formula, Abbott	—	25.8	—	1.1	65.0	109.9	16.0	0.4	5.5	—	—	46
Standard adult TPN renal formula, Abbott	—	99.8	—	0.6	298.0	259.9	28.0	2.2	28.9	—	—	46
Standard adult TPN formula	—	139.8	—	0.4	65.9	299.9	22.0	1.4	15.9	—	—	46
Standard adult TPN formula, Baxter	—	15.8	—	0.7	61.0	47.9	73.0	0.6	9.2	—	—	46
Standard adult amino acid solution, Fresenius	131.2	—	—	4.99	—	—	—	—	—	2.96	0.35	47
Standard adult amino acid solution, B. Braun	88.92	—	—	16.80	—	—	—	—	—	6.66	4.37	47
Standard adult amino acid solution, Baxter	1.39	—	—	4.39	—	—	—	—	—	40.81	0.71	47

Note: n.d. = not detected, — = not measured.

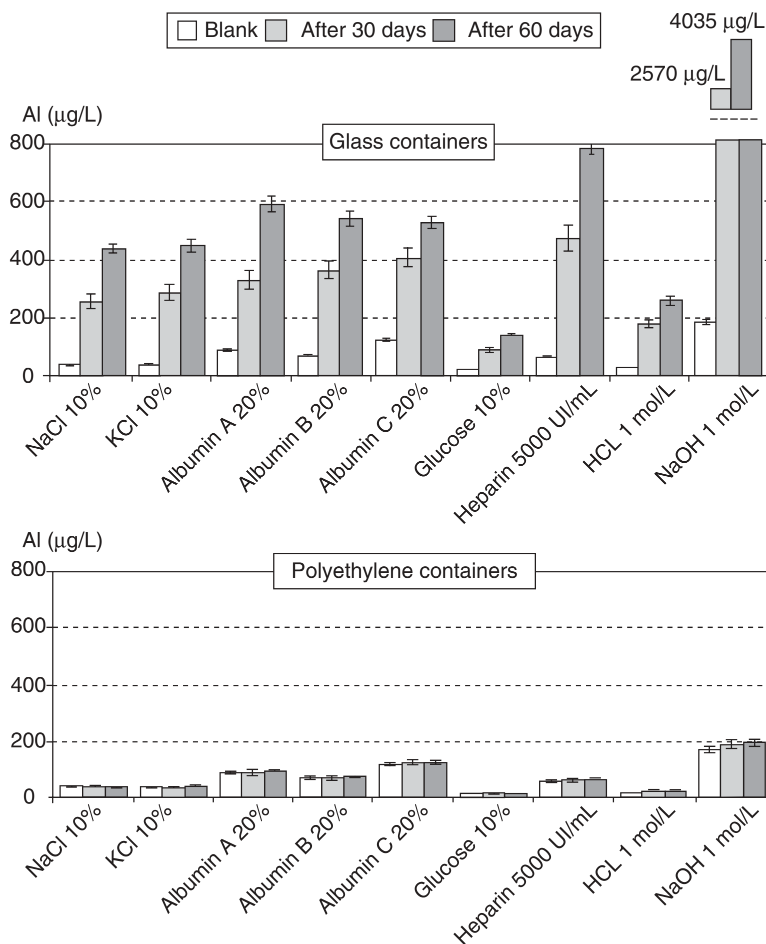


FIGURE 8 Aluminum extracted from type II glass containers and from polyethylene containers by action of NaCl, KCl, albumin, glucose, heparin, HCl, and NaOH solutions after 30 and 60 days storage at room temperature. The three different albumins are: A, bovine (Merck); B, bovine (Reagen), and C, egg (Sigma) [50].

Plastic Containers Plastics are organic and polymeric in nature. A polymer is a large molecule built up by the repetition of small and simple chemical units. The repeated unit of the polymer is usually equivalent or nearly equivalent to the monomer or the starting material from which the polymer is formed. The structural units of the polymers most used to manufacture plastic containers, along with their uses for pharmaceutical purposes, are shown in Table 21.

Modern polymer technology is founded on catalysis, and catalytic methods are extensively used in the production of plastics. Catalysts, since they only catalyse reactions, do not count as polymer constituents but may be present as impurities in the polymeric material. Table 22 lists the usual catalysts used for the polymerization of the polymers mentioned above, which can be found as contaminants in formulations stored in plastic containers.

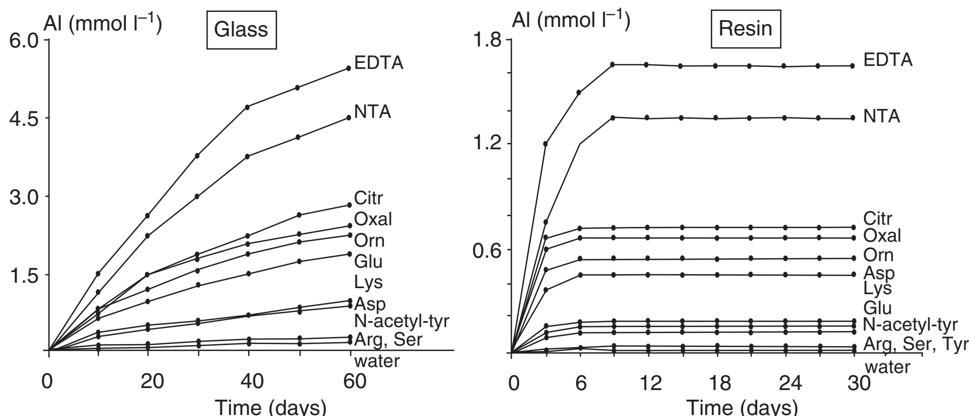


FIGURE 9 Aluminum extracted from glass particles (18 mesh) and Al-form exchanger (18 mesh) as function of time by action of some amino acids and complexing agents. Concentration of ligands: 0.05 mol/L [51].

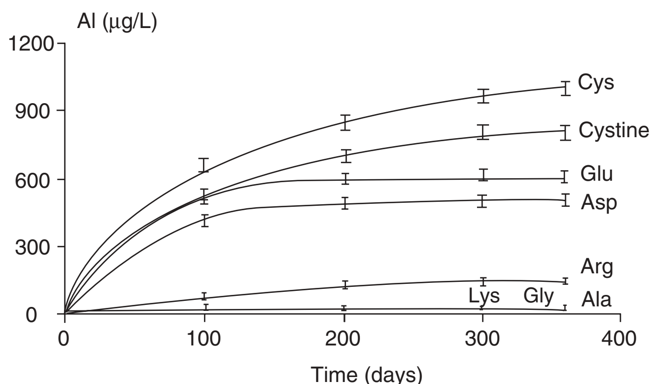


FIGURE 10 Aluminum leached from glass containers by amino acids as a function of time at room temperature. Amino acid concentration: 0.028 mol/L [53].

Other contaminants that can originate from plastic containers are the additives necessary to turn the raw polymer into adequate containers. While PE may be used without any additive, the other plastics are virtually useless alone but are converted into highly serviceable products by combining them with other substances or materials. The additives most commonly found in plastics used for pharmaceutical products are antioxidants, heat stabilizers, lubricants, plasticizers, fillers, and colorants. These additives can be in liquid, solid, or fine particle forms and are used in amounts varying from less than 1% to more than 50% of the plastic mass. The additives necessary for each of the selected types of polymers are described in Table 23.

Additives authorized for use in plastics for pharmaceuticals, along with their limits in the polymer mass (according to BP and Ph. Eur.), are summarized in Table 24.

Since additives, together with the polymers, provide a large variety of substances, and the leachability of such components cannot be predicted a priori,

TABLE 19 Aluminum Present as Contaminant in Commercial Parenteral Solutions, and Al Present in Container Materials

Product	Al Solution ± SD (μg/L)	Container	Al Container (%)
NaCl 20%	149 ± 10	Glass ampoule	1.43
	13 ± 4	Polyethylene	0.04
KCl 10%	68 ± 6	Glass ampoule	1.25
	23 ± 5	Polyethylene	0.06
Magnesium sulfate 50%	560 ± 85	Glass ampoule	1.25
	380 ± 288	Glass ampoule	1.43
Sodium acetate 2 meq/mL	45 ± 7	Glass ampoule	2.14
	17 ± 8	Polyethylene	0.05
Potassium phosphate 2 meq/mL	988 ± 76	Glass ampoule	1.98
	1325 ± 142	Glass ampoule	2.45
Sodium phosphate 0.5 *M*	933 ± 88	Glass ampoule	1.65
	879 ± 203	Glass ampoule	2.05
Calcium gluconate 10%	5621 ± 1165	Glass ampoule	1.51
	5960 ± 62	Glass ampoule	2.21
Sodium bicarbonate 8.4%	833 ± 141	Glass bottle	0.99
	922 ± 102	Glass bottle	1.03
Oligoelements[a]	1129 ± 33	Glass ampoule	2.14
Oligoelements[b]	1854 ± 744	Glass ampoule	2.21
Amino acids 10%	164 ± 6	Glass bottle	0.82
		Rubber closure	3.91
Amino acids 10%	116 ± 30	Glass bottle	0.76
		Rubber closure	4.23
Amino acids 10%	93 ± 23	Glass bottle	0.84
		Rubber closure	5.34
Amino acids 10%	65 ± 13	Glass bottle	0.89
		Rubber closure	5.70
Amino acids 10%	23 ± 8	Plastic bag	0.01
Glucose 50%	13 ± 1	Polyethylene	0.04
	293 ± 14	Glass ampoule	1.15
Glucose 25%	9 ± 3	Polyethylene	0.08
	370 ± 23	Glass ampoule	1.87
Albumin 20%	644 ± 58	Glass flask	0.67
		Rubber closure	4.06
	149 ± 23	Glass flask	0.66
		Rubber closure	3.99
Heparin 5000 UI/mL	732 ± 23	Glass ampoule	2.88
	738 ± 54	Glass ampoule	3.03

Note: All solutions were within their guaranteed period of shelf life.
Source: From ref. 52.
Suppliers: Abbott, Ariston, Aster, Baxter, Behring, B.Braun, Darrow, Fresenius, Fujisawa, Gayer, Halex Istar, Hypofarma, Santisa, Roche, Zenalb.

[a]Composition: 22.0 mg $ZnSO_4$, 6.3 mg $CuSO_4$, 2.46 mg $MnSO_4$, 102.5 mcg $CrCl_3$ per ampoule.
[b]Composition: 8.8 mg $ZnSO_4$, 1.60 mg $CuSO_4$, 123.04 mcg $MnSO_4$ 20.50 mcg $CrCl_3$ per ampoule.

TABLE 20 Aluminium Present in Commercial Solutions for Parenteral Administration

Product	Specification	Brand	Al concentration μg/L	Reference
Electrolytes				
NaCl	10%	Abbott	3	10
	10%	Commercial Polfa	14	54
	5.85%	Braun	1	11
	1 M	Kabi	22	11
	10%	Abbott	43 ± 8*	15
	10%	Elkins Sinn	78 ± 7*	15
KCl	15.0%	Commercial Polfa	18	54
	—	Abbott	4 (5–11)	10
	—	Elkins Sinn	3	10
	7.45%	Braun	<0.6	11
	7.45%	Kabi	12	11
	7.45%	Braun	33 ± 5*	15
	10%	Abbott	97 ± 8*	15
	10%	Aster	23 ± 5*	15
Ca chloride	—	Elkins Sinn	15 (12–19)	10
	—	Abbott	5	10
	0.5 M	Boehringer	27	11
	1 N	Kabi	224	11
Na acetate	2 meq/ml	Braun	17 ± 8*	15
K acetate	—	Abbott	<5	10
Mg sulphate	50%	Abbott	5	10
	50%	Braun	606	11
	—	Geyer	560 ± 85*	15
	—	Ariston	380 ± 288*	15
Na phosphate	—	Invenex	2236 (2026–2370)	10
	0.5 M	Abbott	933 ± 88*	15
	0.5 M	Geyer	430 ± 177*	15
K phosphate	—	Invenex	92	10
	—	Abbott	2189 (2069–2301)	10
	—	Braun	188	11
	—	Kabi	2826	11
	2 meq/l	Fresenius	1021 ± 188*	15
	2 meq/l	Braun	988 ± 76*	15
	2 meq/l	Aster	332 ± 27*	15
Ca gluconate	10%	Elkins Sinn	3973 (1095–5565)	10
	10%	Lyphomed	2245 (2000–2586)	10
	10%	Braun	4734	11
	10%	Fresenius	6549	11
	10%	Pharma Hameln	4421	11
	10%	Commercial Polfa	4400	54
	10%	Elkins Sinn	3987 ± 993*	15
	10%	Braun	4530 ± 1072*	15
	10%	Ariston	5960 ± 62*	15
	10%	Halex Istar	6781 ± 1842*	15

TABLE 20 ***Continued***

Product	Specification	Brand	Al concentration μg/L	Reference
Trace elements (TE)				
Zinc chloride	—	Abbott	99 (81–123)	10
TE	Tracitrans	Fresenius	994	11
Pediatric TE	Ped-el	Pharmacia	3000	54
Pediatric TE	Peditrace	Pharmacia	130	54
Pediatric TE	Ped-element	Darrow	1423 ± 68*	15
TE	Ad-element	Darrow	3574 ± 237*	15
TE	Tracitrans	Fresenius	5712 ± 988*	15
Amino acids				
	Freamine 8.5%	McGaw	12 (5–24)	10
	Travasol 10%	Travenol	7 (6–8)	10
	HepatAmine 8%	McGaw	22	10
	Aminoplasmal 10%	Braun	55	11
	Aminopaed 10%	Kabi	38	11
	Aminosteril 8%	Fresenius	17	11
	Primene 10%	Clintec	120	54
	Aminomel 12.5%	Clintec	121	54
	Vaminolact 6.5%	Pharmacia	30	54
	Aminoplasmal L10	Braun	160 ± 48*	15
	Pediamino 10%	Braun	116 ± 30*	15
	Aminoped 10%	Fresenius	195 ± 27*	15
	Nefroamino AEH	Braun	272 ± 66*	15
	Primène 10%	Baxter	65 ± 13*	15
Carbohydrates				
	Glucosteril 70%	Fresenius	9	11
	Glucose 40%	Fresenius	20	11
	Glucose 50%	Schiwa/Hormonchemie	18	11
	Glucose 20%	Schiwa/Hormonchemie	3	11
	Glucose 10%	Schiwa/Hormonchemie	<0.6	11
	Dextrose (10, 20, 50%)	Abbott	<5	10
	Dextrose (10, 50%)	McGaw	<5	10
	Dextrose (5, 10, 50%)	Travenol	<5	10
	Glucose 20%	Commercial Polfa	16	54
	Glucose 25%	Darrow	9 ± 3*	15
		Equiplex	9 ± 2*	15
	Glucose 50%	Fresenius	15 ± 3*	15
		Darrow	17 ± 3*	15
		J.P. Ind. Farm	8 ± 2*	15
		B. Braum Lab.	13 ± 1*	15
		Ariston	11 ± 4*	15

TABLE 20 *Continued*

Product	Specification	Brand	Al concentration µg/L	Reference
Lipids				
	Intralipid 10%	Kabi Vitrum	<5	10
	Intralipid 20%	Kabi Vitrum	<5	10
	Intralipid 10%	Pfrimmer Kabi	5	11
	Intralipid 20%	Pfrimmer Kabi	7	11
	Lipofundin 20%	Braun	35	11
	Lipofundin 20%	Braun	14	11
	Intralipid 20%	Pharmacia	30	54
	Lipofundin 20%	Braun	180	54
	Lipofundin 10%	Braun	29 ± 6*	15
	Lipofundin 20%	Braun	34 ± 7*	15
Vitamins				
	Vitamin C 500 mg	EMS	3443 ± 233*	15
	Vitamin B12 1mg	Bunker	92 ± 23*	15
	B complex	Hipolabor	1089 ± 127*	15
	B complex	Ariston	709 ± 65*	15
	Multivitamin MVI	ICN	588 ± 63*	15
Others				
	Albumin 20%	—	190.4	55
	Albumin 20%	Behring	235 ± 12*	15
	Heparin 1000 U/mL	—	211.7	55
	Heparin 5000 U/mL	Fujisawa Inc.	732 ± 23*	15
	Heparin 5000 U/mL	Cristália	72 ± 6*	15

—: not informed.
*mean value ± standard deviation of three samples of the same lot.

pharmacopeial compendia have determined procedures to investigate biological and physical–chemical properties of plastics. In general, tests do not include the measurement of the polymeric material or the additives themselves but the biological reactivity (toxicity screening) of their extracts. Extracts are generally obtained by autoclaving the plastic container filled with water at 121°C for 30 min or at 100°C for 2 h. Only containers for parenteral and ophthalmic preparations have a more controlled regulation since their risk of toxicity is more significant, and therefore assays for plastic additives are foreseen in some pharmacopeial compendia [5].

Regulatory guidelines for plastics for pharmaceutical containers also set limits for impurities other than additives. These are inorganic species (metallic and nonmetallic) that can also be present in plastic containers, and their determination serves as a quality criterion for the plastic material. They may or may not be related

TABLE 21 Polymeric Materials, Their Structural Units, and Uses in Pharmaceutical Products

Polymer	Monomers	Uses in Biotechnological Products
Polyethylene (PE)	Ethylene	Dry dosage forms Noninjectable aqueous solutions Intravenous aqueous infusions
Polypropylene (PP)	Propylene	Dry dosage forms Noninjectable aqueous solutions Intravenous aqueous infusions
Polyvinyl chloride (PVC)	Vinyl chloride	Dry dosage forms Noninjectable aqueous solutions Intravenous aqueous infusions Blood and blood components Tubing for blood
Polyethylene vinyl acetate (EVA)	Ethylene and vinyl acetate	Intravenous aqueous infusions Tubing for parenteral nutrition preparations
Polyethylene terephthalate (PET)	Terephthalic acid or dimethyl terephthalate and ethylene glycol	Dry oral dosage forms Liquid oral dosage forms

Source: Data from refs. 5 (BP) and 56 (FDA).

TABLE 22 Catalysts Used for Polymerization Reaction of Plastics Materials Listed in Table 21

Polymer	Catalysts
Polyethylene (PE)	$TiCl_4 + Al(C_2H_5)_3$; CrO_3/SiO_2, MoO_3/Al_2O_3
Polypropylene (PP)	α-$TiCl_3 + Al(C_2H_5)_3$
Polyvinyl chloride (PVC)	—
Polyethylene vinyl acetate (EVA)	—
Polyethylene terephthalate (PET)	—

Source: From ref. 57.

TABLE 23 Overview on Additives Commonly Used in Plastic Manufacturing

	Additives					
Polymer	Antioxidant	Heat Stabilizer	Lubricant	Plasticizer	Filler	Colorant
PE	×	—	×	—	×	×
PP	×	—	×	—	×	—
PVC	—	×	×	×	×	×
EVA	×	—	×	—	×	×
PET	—	—	×	—	×	×

Source: From ref. 58.

TABLE 24 Acceptable Additives Allowed in Plastics for Pharmaceuticals According to the BP and Ph. Eur. and Their Limits

Additive Number	Additive Name	Polymer	Limit (%)
	Plasticizer		
1	Di(2-ethylhexyl)phthalate	PVC	40
2	Zinc octanoate	PVC	1
3	*N′N′*-diacylethylenediamines	PVC	1
4	Epoxidized soya oil	PVC	10
5	Epoxidized linseed oil	PVC	10
	Antioxidants		
7	Butylhydroxytoluene	PE, PP, EVA	0.125
8	Ethylene bis[3,3-bis[3-(1,1-dimethylethyl) -4-hydroxyphenyl]butanoate]	PE, PP	0.3
9	Pentaerythrityl tetrakis[3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propionate]	PE, PP EVA	0.3 0.2
10	2,2′,2″,6,6′,6′-hexa-*tert*-butyl-4,4′,4″ [(2,4,6-trimethyl-1,3,5-benzenetriyl) -trismethylene]triphenol	PE, PP EVA	0.3 0.2
11	Octadecyl 3-(3,5-di-*tert*-butyl-4 -hydroxyphenyl)propionate	PE, PP EVA	0.3 0.2
12	tri(2,4-di-*tert*-Butylphenyl) phosphite	PE, PP EVA	0.3 0.2
13	1,3,5-tris(3,5-di-*tert*-Butyl-4-hydroxybenzyl) -*s*-triazine-2,4,6(1*H*,3*H*,5*H*)-trione	PE, PP	0.3
14	2,2′-bis(octadecyloxy)-5,5′-spirobi [1,3,2-dioxaphosphinane]	PE, PP	0.3
15	Dioctadecyl disulfide	PE, PP	0.3
16	Didodecyl 3,3′-thiodipropionate	PE, PP	0.3
17	Dioctadecyl 3,3′-thiodipropionate	PE, PP	0.3
18[a]	2,4-bis(1,1-Dimethylethyl)phenyl biphenyl-4,4′-diyldiphosphonite 2,4-bis(1,1-Dimethylethyl)phenyl biphenyl-3,4′-diyldiphosphonite 2,4-bis(1,1-Dimethylethyl)phenyl biphenyl-3,3′-diyldiphosphonite 2,4-bis(1,1-Dimethylethyl)phenyl biphenyl-4-ylphosphonite 2,4-bis(1,1-Dimethylethyl)phenyl phosphite 2,4-bis(1,1-Dimethylethyl)phenyl 4′-[bis[2,4-bis (1,1-Dimethylethyl)phenoxy]phosphanyl] biphenyl-4-ylphosphonate 2,4-bis(1,1-Dimethylethyl)phenol	PE, PP	0.1
	Lubricants and Fillers		
19	Stearic acid		
20	Oleamide	EVA	0.5
21	Erucamide	EVA	0.5
22[a]	Copolymer of dimethyl butanedioate and 1-(2-hydroxyethyl)-2,2,6, 6-tetramethylpiperidin-4-ol	PE, PP	0.3

TABLE 24 ***Continued***

Additive Number	Additive Name	Polymer	Limit (%)
23	Hydrotalcite	PE, PP	0.5
24	Alkanamides	PE, PP	0.5
25	Alkenamides	PE, PP	0.5
26	Sodium silico-aluminate	PE, PP	0.5
27	Silica	PE, PP	0.5
28	Sodium benzoate	PE, PP	0.5
29	Fatty acid esters	PE, PP	0.5
30	Trisodium phosphate	PE, PP	0.5
31	Liquid paraffin	PE, PP	0.5
32	Zinc oxide	PE, PP	0.5
33	Magnesium oxide	PE, PP	0.5
34	Calcium stearate	PE, PP	0.5
35	Zinc stearate	PE, PP	0.5
36	Talc	PP	0.5
	Colorants		
37	Titanium dioxide	PE, PP	4
38	Ultramarine blue	PVC	n.i.

Note: PE and PP for parenteral and ophthalmic preparations may contain at most three oxidants; n.i.: not informed.

[a]Only for nonparenteral preparations.

to any of the additives listed in Table 24. For example, the investigation of aluminum in PE and polypropylene (PP) is related to the use of aluminum-based catalysts for the attainment of the polymers; barium as a contaminant in a plastic container is indicative of the presence of a barium soap stabilizer, possibly used in plastic compounding. The tests, including chemicals such as sulfated ash residue and heavy metals, are listed in Table 25 with the respective limits for each species in the different plastic materials.

While not mandatory from regulatory guidelines, much research has been carried out to investigate the extractability of plastic additives in contact with a variety of pharmaceutical formulations, mainly those for parenteral use. The research concentrates on the extractability of plasticizer phthalates, mainly di-2-ethylhexylphthalate (DEHP) from polyvinyl chloride (PVC) into the blood, blood components, and infusion solutions. The purpose for these studies lies in its, up to now, controversial hazardous effects on humans. The amount of additive necessary to turn rigid PVC into a flexible material (40% m/m) and the absence of chemical bonds between the polymer and the plasticizer make it a potentially extractable species.

Limits for DEHP in formulations stored in PVC bags are not set in pharmacopeial compendia. The BP prescribes a standard test whose results should show that the amount of DEHP extracted by action of ether on PVC bags does not exceed 40% of the polymer mass. This test is valid for PVC for all uses: Dry dosage forms, noninjectable aqueous solutions, intravenous aqueous infusions, blood and blood components, and tubing used in sets for blood and blood components. On the other

TABLE 25 Inorganic Impurities and Their Limits in Extracts of Plastic Containers for Pharmaceuticals

Impurity Number	Impurity	Polymer	Use	Limit
1	Heavy metals	PE, PP	Injectable, ophthalmic	2.5 ppm
		PVC	Aqueous parenteral, blood, tubing	50 ppm
2	Sulfated ash	PE, PP	Injectable, ophthalmic	0.2%, 1%
		EVA	Parenteral container and tubing	1.2%
		PET	Oral forms	0.5%
3	Aluminum	PE, PP	Injectable, ophthalmic	1 ppm
		PET	Oral forms	1 ppm
4	Chromium	PE, PP	Injectable, ophthalmic	0.05 ppm
5	Titanium	PE, PP	Injectable, ophthalmic	1 ppm
		PET	Oral forms	1 ppm
6	Vanadium	PE, PP	injectable, ophthalmic	0.1 ppm
	Zinc	PE, PP	Injectable, ophthalmic	1 ppm
		PVC	Aqueous parenteral, blood	0.2%
		PET	Oral forms	1 ppm
7	Zirconium	PE	Injectable, ophthalmic	0.1 ppm
8	Barium	PVC	Aqueous parenteral, blood, tubing	5 ppm
		PET	Oral forms	1 ppm
9	Cadmium	PVC	Aqueous parenteral blood, tubing	0.6 ppm
10	Calcium	PVC	Aqueous parenteral, blood	0.07%
11	Tin	PVC	Aqueous parenteral, blood, tubing	20 ppm
12	Ammonium	PVC	Blood	2 ppm
13	Chloride	PVC	Blood	0.4 ppm
14	Antimonium	PET	Oral forms	1 ppm
15	Cobalt	PET	Oral forms	1 ppm
16	Germanium	PET	Oral forms	1 ppm
17	Manganese	PET	Oral forms	1 ppm

Source: Adapted from Ph. Eur [ref. 7].

hand, the Food and Drug Administration (FDA) launched a nonregulatory publication entitled "Safety Assessment of Di-2-ethylhexylphthalate (DEHP) Released from PVC Medical Devices" [59], where concerns about its toxicity are discussed in detail. This publication intends to provide risk managers with the information necessary to make decisions about the safety of DEHP exposure from medical devices. Conclusions of the study were reached by calculating the dose of DEHP received by patients undergoing different medical procedures. For the pharmaceutical industry, what is truly relevant are concerns about the presence of DEHP in intravenous (IV) infusion fluids and drugs and in parenteral nutrition because of their storage in plastic bags and the delivery of these preparations through PVC tubing sets. The study concluded that there is little to no risk posed by patient exposure to the amount of DEHP released from PVC IV bags following the infusion of crystalloid fluids (e.g., normal saline, Ringer's lactate). There is a small risk, however,

TABLE 26 Concentration of DEHP in Infusion Solutions stored in Plastic containers

Sample	Volume (mL)	Time of storage	DEHP (μg/L)	Ref.
0.9% saline	1000	5 months	7	60
		14 months	24	
0.9% saline	500	0.5 month	13	60
		3 months	<4	
0.9% saline	100	6 months	8	60
0.9% saline	1000	—	8	60
5% glucose	1000	5 months	4	60
		12 months	4	
5% glucose	500	0.5 month	34	60
		3 months	7	
ACO bag	100	12 months	7	61
Travenol bag	100	12 months	5	61
Multivitamins		48 h (45 °C)	21	62
TPN—3.85% lipid	2200–	24 h	330	63
TPN—3.85% lipid	2200–	1 week (4 °C)	450	63
TPN—2.50% lipid	2200–	24 h	230	63
TPN—2.50% lipid	2200–	1 week (4 °C)	260	63
TPN—1.85% lipid	650	24 h	300	63
TPN—1.85% lipid	650–	1 week (4 °C)	360	63
TPN—1.00% lipid	800–	24 h	240	63
TPN—1.00% lipid	800–	1 week (4 °C)	270	63
5% glucose–5% paclitaxel		1 h	6,600	64
5% glucose–5% paclitaxel		2 h	18,500	64
5% glucose–10% paclitaxel		1 h	29,400	64
5% glucose–10% paclitaxel		2 h	56,600	64

posed by the exposure to the amount of DEHP released from PVC bags used to store and administer drugs that require a pharmaceutical vehicle for solubilization. The dose of DEHP received by adult patients receiving TPN is estimated to be less than the tolerable intake (TI),[3] suggesting that there is little need for concern about DEHP-mediated effects in these patients. The dose of DEHP received by neonates undergoing TPN is uncertain. Depending on the data used to derive the TI/dose ratio, neonates may be at an increased risk of DEHP-mediated adverse effects.

In fact, studies have shown that, depending on the constituents in the formulation, the extractable DEHP can be much higher than the expected. Our knowledge of the nature of the polymer and its additives, as well as of standard migration, is not great enough to predict container–formulation interactions. Migration is a unique phenomenon, and predictions based only on the structure and physical or chemical properties may not be effective for solving interaction problems. Table 26 shows the level of DEHP found in different kinds of parenteral formulations stored in PVC bags. It is clear that the amount of DEHP leached into the formulation depends on the nature of its constituents. While in saline solutions the amount did not exceed

[3]The tolerable intake values for DEHP are outlined in the International Organization for Standardization ISO/DIS 10993-17 standard, "Method for the Establishment of Allowable Limits for Leachable Substances."

24 μg/L in 14 months, in oleaginous vehicles, it reached over 300 μg/L in 2 h, which corroborates with FDA studies. Due to the lipophilic character of DEHP, its migration process from the packaging into hydrophilic preparations is limited to a random dissolution on the contacting surface. On the other hand, in lipophilic preparations such as lipid emulsions, DEHP is encouraged to leave the polymer surface through a dissolution process into the lipophilic milieu.

There are drug products whose interaction with PVC bags and infusion sets are so high that they must include labeling precautions for use with PVC containers. These drugs include antineoplastics such as paclitaxel, docetaxel, tacrolimus, and teniposide, and others such as ciprofloxacin, cefoperazone sodium, fluconazole, metronidazole HCl, cimetidine, and propofol [64, 65].

Although differences in the methods of the studies shown in Table 26 (time of contact, volume of solution, temperature, etc.) do not allow for direct comparisons, the data presented suggest that the leachability of DEHP is highly dependent on the nature of the pharmaceutical formulation. The question of migration of DEHP into solutions is relevant not only to lipophilic vehicles but to drugs such as paclitaxel and etoposide, which are able to extract much more DEHP than simple lipophilic lipidic emulsions do.

Attempts to substitute PVC containers have shown, however, that non-PVC containers may also contain plasticizer components resulting in the release of some DEHP into the preparation. A study conducted by Sautou-Miranda and co-workers showed that [65] leaches rapidly from coextruded and triple-layered IV tubing into etoposide infusion solution (see details of the results under Section 9.1.3.3).

Although most studies focus on DEHP, it is not the only additive that can be leached from plastic materials. Scalia et al. [66] investigated the migration of antioxidants from polyolefinic plastics into oleaginous vehicles. The study concentrated on two antioxidants, Irganox 1010 and Irgafos 168, used in PE and PP polymers.[4] The study was carried out by dipping plastic sheets containing 0.15% of antioxidants into a mixture of five oils (caprylic, capric triglyceride, cyclomethicone, dicaprylyl ether, isohexadecane, and $C_{12–15}$ alkyl benzoate) commonly used as pharmaceutical vehicles, and storing the oil mixture in bottles manufactured with the polymers. The results showed that the amount of antioxidant leached into the oleaginous mixture varied remarkably in relation to the polymeric material, and decreases in the order EP > RACO > PP > high-density polyethylene (HDPE) (RACO and EP are ethylene–propylene copolymers). Table 27 shows the percentage of antioxidants left in the polyolefin bottles filled with the mixture and stored at 25°C for 1 year. The authors concluded that the migration of both antioxidants is related to the polymer crystallinity and structure: The higher the crystallinity, the lower the additive release. They suggested that PP and HDPE are satisfactory for the manufacture of oil.

Elastomeric Closures Closures for pharmaceutical products are generally made of polymeric materials, which may be of either a synthetic or natural origin. While brittle closures such as screw caps are made of conventional thermoplastics with a single composition, elastomeric closures are made of complex mixtures of many

[4]Pentaerythrityl tetrakis(3,5-di-*tert*-butyl-4-hydroxyphenyl)propionate (Irganox 1010) and tris(2,4-di-*tert*-butylphenyl)phosphite (Irgafos 168), additives 9 and 12 in Table 25.

TABLE 27 Percentage of Antioxidants Left in Polyolefin Bottles Filled with Mixture and Stored at 25 °C for 1 year

	Antioxidant Left (%)	
Polyolefin Bottles	Irganox 1010	Irgafos 168
HDPE	92.7 ± 5.7	47.8 ± 5.2
PP	76.3 ± 7.1	47.0 ± 4.1
RACO	57.9 ± 6.3	36.4 ± 4.7
EP	0	0

Source: From ref. 66.
EP: ethylene-propulene amorphous copolymer blend; RACO: ethylene-*co*-propylene random copolymer.

ingredients. Because elastomers or rubbers can be molded into an almost limitless variety of permanent shapes and are easily penetrated by needles and reseal after needle withdrawal, they are primary closures for parenteral vials and for preparations intended for repeated use.

The polymeric materials usually used to manufacture rigid closures are practically the same as those seen under plastic containers (Section 6.1.3.2). The same impurities are therefore to be expected in these packaging components. On the other hand, though made of polymeric materials, elastomeric closures present a different structure. In the manufacture of rubber, elastomer, the chief component, is combined with other chemicals to produce a material with specific properties that meet target needs, such as its above-mentioned ability to reseal on repeated use. Table 28 lists the common elastomers used in the pharmaceutical industry and their monomeric structures.

The substances listed in Table 28 correspond to the basic structure of elastomeric closures. The other components in rubber formulations are curing or vulcanizing agents, accelerators, activators, antidegradants, plasticizers, fillers, and pigments. The most common additives used to compound rubber for the pharmaceutical industry are listed in Table 29. The amount of each component may vary from rubber to rubber, and, depending on the component, the amount can reach more than 50% of the total mass of a formulation. While accelerators are used in amounts of around 1%, fillers may make up more than 50% of the formulation mass.

Table 30 shows four typical pharmaceutical rubber formulations based on natural, halobutyl, ethylenepropylenediene (EPDM), and silicone elastomers.

The presence of such a variety of components makes elastomeric closures a potential source of contaminants. Polymeric materials present a reasonable inertness, however, the possibility of the other components reaching the drug formulation should be considered. Moreover, these ingredients, though intended for pharmaceutical purposes and therefore meeting pharmacopeial requirements, may still present their own impurities as contaminants. Carbon black is usually an impure material, and it can contain polynuclear aromatic hydrocarbons [68], which can be extracted into the packaged drug. Clays contain metallic impurities that can also be extracted into the drug formulation or even react with a drug constituent.

Pharmacopeial monographs do not set limits for additives, as they do for plastic polymers, and rubber closure (BP, Appendix XIX, USP ⟨381⟩) tests are limited to

TABLE 28 Common Elastomers Used in the Pharmaceutical Industry

Common Name	Chemical Name	Structure
Butyl rubber	Poly(isobutylene-isoprene)	$\left[-\!\left(CH_2 - C(CH_3)_2 \right)_{50}\!- CH_2 - C(CH_3) = CH - CH_2 - \right]_n$
Halobutyl rubber	Halogenated poly(isobutylene-isoprene)	$\left[-\!\left(CH_2 - C(CH_3)_2 \right)_{65}\!- CH - C(CH_3) - CH(X) - CH_2 - \right]_n$ X = Cl or Br
Ethylene–propylene rubber	Poly(ethylene-propylene)	$\left[-\!\left(CH_2 - CH_2 \right)_{3}\!- CH_2 - CH(CH_3) - \right]_n$

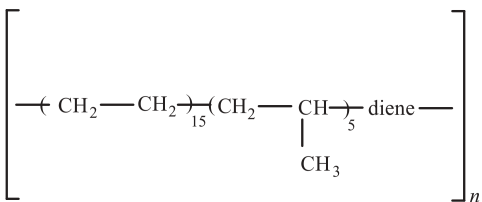
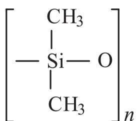
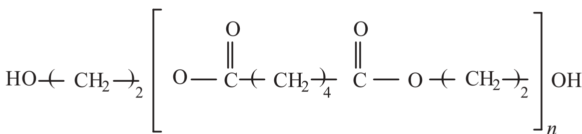
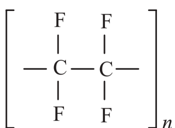
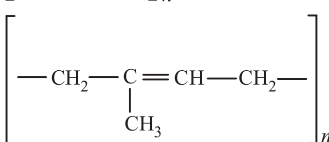
Ethylene–propylene–diene rubber	Poly(ethylene-propylene-diene)	
Silicone rubber	Polydimethylsiloxane	
Urethane rubber	Adipic acid–ethylene glycol polyester	
Fluoroelastomers	Poly(tetrafluoroethylene)	
Natural rubber	*cis*-1,4-Polyisoprene	

TABLE 28 ***Continued***

Common Name	Chemical Name	Structure
Polyisoprene rubber	*cis*-1,4-Polyisoprene	$\left[-CH_2-C(CH_3)=CH-CH_2-\right]_n$
Neoprene rubber	Polychloroprene	$\left[-CH_2-C(Cl)=CH-CH_2-\right]_n$
Styrene butadiene rubber	Poly(butadiene-styrene)	$\left[(CH_2-CH=CH-CH_2)_4-CH_2-CH(C_6H_5)-\right]_n$
Nitrile rubber	Poly(butadiene-acrylonitrile)	$\left[(CH_2-CH=CH-CH_2)_5(CH_2-CH(CN))_2\right]_n$
Polybutadiene	Polybutadiene	$\left[-CH_2-CH=CH-CH_2-\right]_n$

Source: From ref. 67.

TABLE 29 Ingredients Other Than Elastomers Used to Compound Rubbers for Pharmaceutical Industry

Curing Agents	Accelerators	Activators	Antidegradants	Plasticizers	Fillers	Pigments
Sulfur	Hexamethylene tetramine	Zinc Oxide	Amines	Paraffinic waxes	Carbon Black	Carbon black
Sulfur-containing chemicals	Ditiocarbamates	Stearic acid	Hindered phenols	Silicon oil	Alumnum silicates (clays)	Titanium dioxide
Peroxides	Sulfonamides		Waxes	Paraffinic oils	Magnesium silicate (talc)	Iron oxide
Cadmium oxide	Thiuram			Naphthenic oils	Barium sulfate	Chromium oxide
Magnesium oxides	Thiazole			Organic phosphates	Zinc oxide	Organic dyes
Zinc oxide					Silica	

Source: From ref. 67.

TABLE 30 Composition of Four Typical Rubbers Used as Closures for Pharmaceutical Formulations Based on Natural, Halobutyl, EPDM, and Silicone Elastomers

	Rubber Type			
Ingredient	Red Rubber	Gray Rubber	Gray Rubber	Black Rubber
Elastomer	Natural rubber	Halobutyl rubber	Dimethylpolysiloxane polymer	EPDM
Filler	Alumnum silicate	Alumnum silicate	Silica	Carbon black
Plasticizer	Paraffinic oil	Naphthenic oil	—	Naphthenic oil
Pigment	Iron oxide	Titanium dioxide Carbon black	Carbon black	—
Activator	Zinc oxide Stearic acid	—	—	Zinc oxide Stearic acid
Accelerator	Thiuram Thiazole	Thiuram	—	Thiuram Zinc dithiocarbamate
Antidegradant	Butylated hydroxytoluene	Butylated hydroxytoluene	—	—
Curing agent	Sulfur	Zinc oxide	2,4-Dichlorobenzoyl peroxide	Sulfur

Source: From ref. 67.

showing the content of sulfur, sulfated ash, volatile sulfides, and extractable zinc, ammonium, and heavy metals.

Extractability tests prescribed by other regulatory agencies [FDA, Parenteral Drug Association (PDA)] for closures for drug packaging [69, 70] are also limited to the amount of extractable residues or tests to evaluate the in vivo reaction of the extractable residue when the material fails in the in vitro tests.

The tests, however, are neither qualitative (in the sense of showing which substances can be extracted) nor specifically quantitative since they are conceived to show only the total amount of extractable as a residue. USP ⟨381⟩ "Elastomeric Closures for Injection," for example, recommends the calculation of the weight of the residue after evaporating the solvent (purified water, drug vehicle, or isopropyl alcohol) used for extraction. Tests in vivo are recommended only when the material does not meet the requirements of the in vitro tests.

These tests, however, are unable to show further contamination in the final product since the extractability also depends on the interaction between the container and the formulation constituents. In spite of possible interactions, very little is known about the leachability of closure constituents through the direct contact of closures with formulations.

Mennermaa and colleagues [71] determined the composition of three different types of rubber stoppers used to seal parenteral solutions. After immersing the stoppers in 0.9% NaCl solution and autoclaving at 121°C for 15 min, the analysis of the aqueous extract was carried out by proton-induced X-ray emission. Table 31 presents the elemental composition of the rubber stoppers investigated in micrograms/gram dry weight. The concentration of zinc varied from sample to sample by a factor of 40,000 (5–20,000 ppm). Titanium, Fe, and Br were present in all stoppers, and even Pb (2 ppm) was found in one extract.

TABLE 31 Elemental Composition of Rubber Stoppers Based on Element Concentration in Aqueous Extract

Element	Amount (μg/g)		
	Sample 1, Old Formulation of Bromolutyl Siliconized Rubber	Sample 2, New Formulation of Bromolutyl Siliconized Rubber	Sample 3, New Formulation of Chlorolutyl Siliconized Rubber
Ti	500	1,600	200
Fe	7,500	8,000	4,500
Cu	10	10	—
Zn	50	5	20,000
Br	100	7,500	50
Pb	2	—	—

Source: From ref. 71.

TABLE 32 Nature of Compounds Identified by GC-MS in Headspace and Soxhlet Extracts of Two Rubbers under Study

	Compound Identified in Extracts Obtained with	
	Headspace	Soxhlet
Alkanes	C_5 and higher	C_9–C_{30}
Oligomers	T_{max} (elution) = 235°C	T_{max} (elution) = 280°C
Aromatics	92 < MW < 132	168 < MW < 182
Fatty acids	No	228 < MW < 352
Esters	No	Yes
2,6-di-*tert*-Butyl-*p*-cresol	Yes	Yes
2,6-di-*tert*-Butyl-*p*-benzoquinone	Yes	Yes
Ketone	MW = 198	MW = 198

Source: From ref. 72.
Note: MW, molecular weight.

Schoenmakers et al. [72] analyzed two representative commercial rubbers by gas chromatography–mass spectrometry (GC–MS) and detected more than 100 different compounds. The rubbers, mixtures of isobutylene and isoprene, were analyzed after being cryogenically grinded and submitted to two different extraction procedures: a Sohxlet extraction with a series of solvents and a static-headspace extraction, which entailed placing the sample in a 20-mL sealed vial in an oven at 110°C for 5, 20, or 50 min. Although these are not the conditions to which pharmaceutical products are submitted, the results may give an idea of which compounds could be expected from these materials. Residual monomers, isobutylene in the dimeric or tetrameric form, and compounds derived from the scission of the polymeric chain were found in the extracts. Table 32 presents an overview of the nature of the compounds identified in the headspace and Soxhlet extracts of the polymers. While the liquid-phase extraction was able to extract less volatile compounds, the headspace technique was able to show the presence of compounds with low molecular mass

TABLE 33 Accumulation of Extractable from Synthetic Polyisoprene Rubber after Autoclaving for 1h

Compound	Concentration in Solution (mg/L)
Aniline	1.64
Diphenylguanidine	11.76
Dedenzylamine	2.12
Triisopropanolamine	3.73

Source: From ref. 73.

in the extract. A complete screening of the extracted substances can be seen in Figure 11, where the graphics depict the abundance of the different classes of compounds detected in the extracts of different solvents for both rubbers. Not only oligomers, but also phthalates, phenols, and acidic compounds, were found in the extracts, mainly from rubber P1.

Jenke [73] studied the extractability of aniline, diphenylguanidine, dedenzylamine, and triisopropanolamine from a synthetic polyisoprene rubber similar to the material used in pharmaceutical applications. Rubber samples were autoclaved (121°C) in contact with water or NaCl 0.9% solution for 1h. Table 33 presents the concentration of each compound in solution after the extraction procedure using 2g rubber material. Extraction profiles ranged between 1.64 and 3.73mg/L, with the exception of diphenylguanidine, whose extraction yield reached 11.76mg/L.

6.1.3.3 Delivery Systems

Pharmaceutical formulations also have direct contact with plastic materials through delivery sets, transfer tubing, and devices, as well as during the phases of their production, via gaskets, filters, and transportation.

Even though there is no specific pharmacopeial prescription for testing these materials, they should present the same profile stipulated for container and closures. In the case of tubing lines, which are also made of elastomeric materials, an extractability test for elastomeric closures is recommended, mainly for those made of PVC.

It is more difficult to generalize about the leachability of plastic tubing materials than it is for plastic containers or closures themselves since it depends not only on the nature of the plastic material but also on the flow rate, temperature, solvent, and time of contact with the tubing.

Studies conducted by different authors on the release of chemical substances from medical devices, mainly those used for infusing solutions, show that these are potential sources of contamination for pharmaceutical formulations. One of the most studied is diethylhexyl phthalate, the same plasticizer found in PVC infusion bags to give flexibility. The same concerns about the use of PVC bags for the storage of lipids or lipophilic formulations are valid for tubing.

Table 34 presents the amount of DEHP leached from typical PVC infusion lines from a delivery set of 2.25m kept at 27°C. A sample volume from 8 to 140mL was perfused in 24h through the lines and collected for analysis. It was possible to see that while amino acids did not promote the migration of DEHP from PVC tubing,

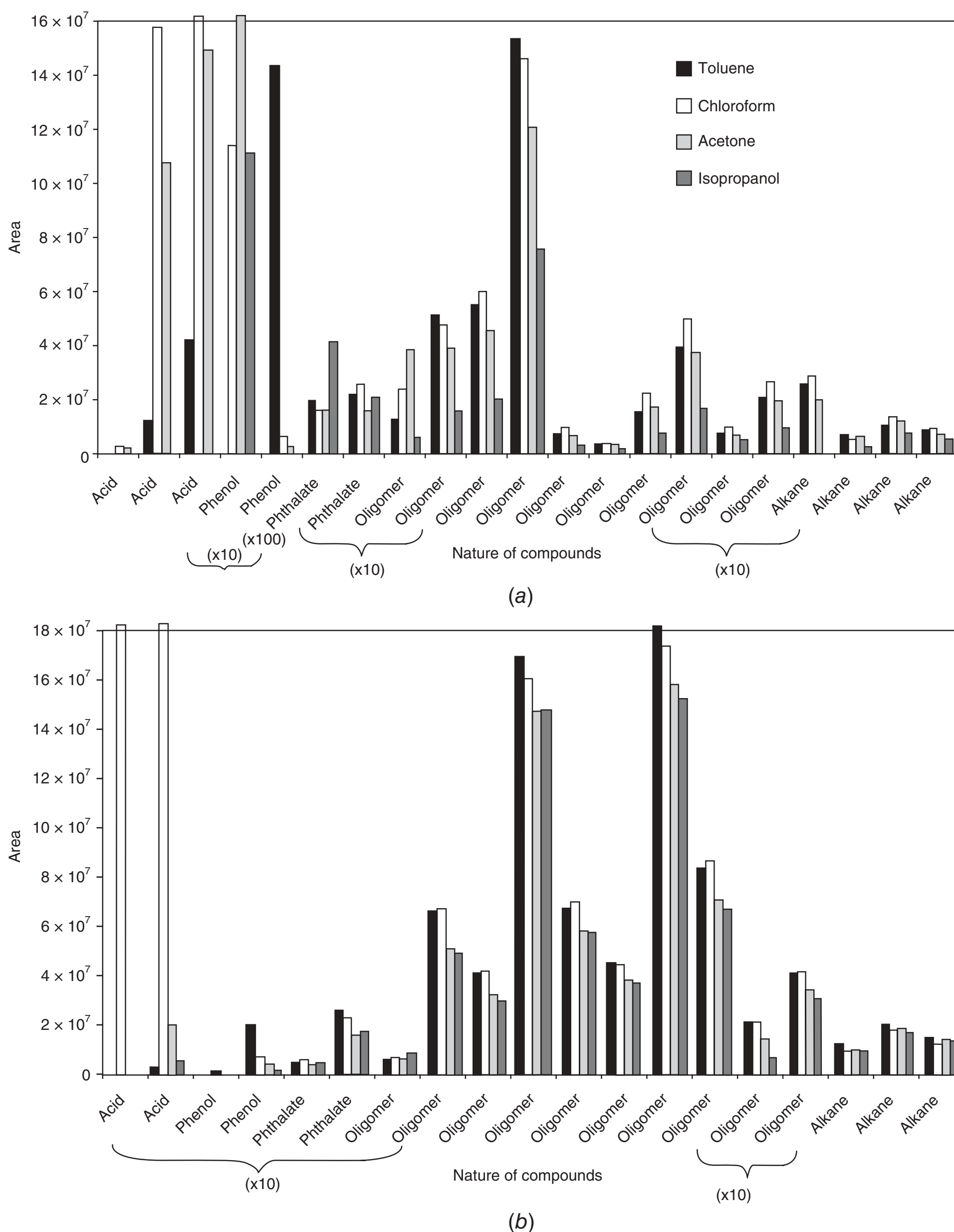


FIGURE 11 Influence of the solvent used for the Soxhlet extractions of two different commercial rubber closures (P1) and (P2) on the area of the chromatographic peaks of different classes of compounds detected in GC–MS [72].

the lipophilic lipid emulsion and propofol promoted the extraction of large amounts of DEHP into the preparations.

An investigation of DEHP extractability from PVC tubing into a large variety of pharmaceutical formulations used for different purposes was carried out by

TABLE 34 DEHP Loaded in Infusion Solutions after Exposure to PVC Infusion Lines

Sample	Volume (mL)	Number of Samples	DEHP (μg/mL)	Range DEHP (μg/mL)
Amino acid–glucose	140	06	0.31 ± 0.56	0.0–1.05
Lipid emulsion	24	10	422.78 ± 47.39	329.15–490.40
Midazolam	24	03	0.90 ± 0.38	0.55–1.30
Propofol infusion	10	10	654.87 ± 96.49	423.85–736.10
Fentanyl	28.8	10	3.63 ± 0.92	1.95–5.05
Imipenem	8	03	0	–0.10–0.05

Source: From ref. 74.

Haishima and colleagues [75]. The authors divided the IV drugs into five groups according to the properties of their active ingredients and additives. Group 1 included drugs that were practically insoluble or insoluble in water and contained additives such as surfactants, oils, glycerine, ethanol, or benzyl alcohol. Group 2 included drugs equally insoluble in water but soluble in acidic or basic solutions. Formulations slightly soluble in water were placed in group 3, and formulations very soluble or freely soluble in water were classified in groups 4 and 5. The difference between group 4 and 5 was the presence of additives suspected to induce DEHP migration in group 4. Tests were carried out by placing the drug formulations into PVC tubing of 10 cm in length with 2.13 mm inner diameter for 1 h at room temperature under shaking. As shown in Table 35, products included in group 1 promoted the release of large amounts of DEHP, with the exception of insulin and dinoprost, probably due to the absence of oleaginous excipients. No significant DEHP release was observed by most of the other drugs assigned to groups 2 through 5, with the exception of human serum albumin and antithrombin III (both group 4) and phenytoin solution (group 2), probably due to the presence of propylene glycol and ethanol in the formulation. These results confirm the affinity of DEHP to lipophilic media.

Due to their utilization modus, many studies conducted with tubing materials are essentially kinetic, where tubing length, time of contact, flow rate, and temperature are important parameters.

Kambia et al. [76] studied the kinetics of DEHP migration into TPN solutions from PVC tubing. They quantified the amount of DEHP leached into two types of emulsions 24 h after preparation and storage at 4 °C. The authors concluded that the extraction depended on the lipid content of the formulations and the flow rates. Figure 12 shows the DEHP concentration time course obtained from bags and outlet tubing during 10–11 h simulated infusion of TPN after 24-h storage at 4°C.

To overcome the problem of DEHP leaching into parenteral infusions containing lipophilic components, a triple-layered tubing material has been used. This type of tubing is made of a PVC outer layer, while its innermost layer, which comes into contact with the drug solution, is made of inert PE. In spite of this arrangement, it has been shown that, depending on the preparation, DEHP from the PVC outer layer may be released in the infusion solution.

Sautou-Miranda et al. [77] showed that DEHP is rapidly leached from PVC, coextruded, and also triple-layered IV tubing by action of an etoposide infusion

TABLE 35 DEHP Released from PVC Tubing into Intravenous Drug Preparations after 1h Contact at Room Temperature

Active Ingredient	Concentration	Additives	DEHP (µg/L)	SD (µg/L)
		Group 1		
Cyclosporin	500 µg/mL	Polyoxythene castor oil, ethanol	27,363.9	384.8
Tacrolymus hydrate	10 µg/mL	Absolute ethanol, HCO-60	4,091.9	31.9
Propofol	10 mg/mL	Soybean oil, concentrated glycerin, egg yolk lecithin, edetate	19,451.2	852.5
Flurbiprofen axetil	10 mg/mL	Soybean oil, egg yolk lecithin, concentrated glycerin	17,838.5	821.6
Vitamins—fat soluble	Whole amounts of Sorbita was mixed with PN-Twin no. 2 (2.2 L)	Sodium citrate, sodium pyrosulfite, sodium thioglycollate, HCO-60, benzyl alcohol, polysorbate 80	1,157.1	5.1
Menatetrenone	5 mg/mL	Aminoethylsulfonic acid, sesame oil, soybean lecithin, D-sorbitol, concentrated glycerin	8,457.5	62.9
Insulin human	40 units/mL	Concentrated glycerin, *m*-cresol	281.6	6.0
Dinoprost	2 mg/L	—	185.8	17.3
Miconazole	1 mg/L	HCO-60	30,098.3	423.3
Diazepam	5 mg/L	Propylene glycol, ethanol, benzyl alcohol, sodium benzoate, benzoic acid	2,008.8	257.6
Prednisolone sodium succinate	10 mg/L	Sodium carbonate, sodium hydrogen phosphate, sodium dihydrogen phosphate	915.6	182.3

TABLE 35 ***Continued***

Active Ingredient	Concentration	Additives	DEHP (μg/L)	SD (μg/L)
Prednisolone sodium succinate	1 mg/L	Sodium carbonate, sodium hydrogen phosphate, sodium dihydrogen phosphate	407.1	2.4
		Group 2		
Famotidine	20 mg/L	L-Aspartic acid, D-manitol	166.0	0.9
Droperidol	2.5 mg/mL	*p*-Oxymethyl benzoate, *p*-oxypropyl benzoate	171.0	0.6
Droperidol	50 μg/mL	*p*-Oxymethyl benzoate, *p*-oxypropyl benzoate	167.4	24.6
Sivelestat sodium hydrate	1 mg/L	D-Mannitol, sodium hydroxide, propylene glycol, ethanol	885.7	10.6
Phenytoin	50 mg/L			
Methotrexate	0.2 mg/mL	Sodium chloride, sodium hydroxide	372.8	6.8
Haloperidol	5 mg/mL	Glucose, lactic acid, sodium hydroxide	50.6	2.5
Epinephrine	0.25 mg/mL	Chorobutanol, sodium hydrogen sulfite, hydrochloric acid, sodium chloride	290.3	24.6
		Group 3		
Methilergometrine maleate	0.2 mg/mL	—	462.7	4.2
Vecuronium bromide	2 mg/mL	D-Mannitol	192.7	1.5
Panipenem betamipron	5 mg/mL	—	237.0	1.2
Mynocycline hydrochloride	1 mg/mL	—	150.0	8.9
Nicardipine hydrochloride	0.1 mg/mL	D-Sorbitol	211.6	24.0
Bromhexine hydrochloride	2 mg/mL	Glucose	174.9	23.7
Ceftazidime	10 mg/mL	Sodium carbonate	301.0	0.5
Fluconazole	1 mg/mL	—	210.5	0.2
Aspoxicillin	50 mg/mL	Sodium chloride	296.7	2.6
Carbazochrome sodium sulfonate	0.05 mg/mL	Sodium hydrogen sulfite, D-sorbitol, propylene glycol	246.1	3.0

		Group 4		
Oxytocin	0.01 units/mL	Chlorobutanol	423.1	0.8
Hydroxyzine hydrochloride	0.05 mg/mL	Benzyl alcohol	430.8	144.4
Ranitidine hydrochloride	0.1 mg/mL	Phenol	197.9	29.5
Human immunoglobulin G	50 mg/mL	D-Sorbitol	243.9	14.3
Panthenol	250 mg/mL	Benzyl alcohol	412.1	18.2
Human serum albumin	250 mg/mL	Sodium *N*-acetyl tryptophan, sodium caprylate, sodium hydrogen carbonate	10,080.8	84.1
Human antithrombin III	25 units/mL	Sodium chloride, sodium citrate, D-mannitol	2,008.2	21.8
Nitroglycerin	0.5 mg/mL	D-Mannitol	267.6	8.9
Sulpyrine	2.5 mg/mL	Benzyl alcohol	302.8	3.8
Erythromycin lactobionate	2.5 mg/mL	Benzyl alcohol	92.2	0.7
Clindamycin phosphate	3 mg/mL	Benzyl alcohol	274.9	4.0
		Group 5		
Imipenem cilastatin sodium	5 mg/mL	Sodium hydrogen carbonate	205.1	1.6
5% glucose	50 mg/mL	—	284.6	4.8
Ferric oxide, saccharated	0.4 mg/mL	—	244.5	5.5
Maltose, sodium chloride, magnesium chloride, potassium dihydrogenphosphate, sodium acetate	—	—	262.8	5.0
Atropine sulfate	0.5 mg/mL	—	200.7	5.1
Ampicillin sodium	10 mg/mL	—	262.3	6.8
Aminophyline	0.5 mg/mL	Ethylenediamine	301.1	4.0
Fosfomycin sodium	20 mg/mL	Glucose solution	289.6	6.7
Calcium gluconate	85 mg/mL	—	179.4	4.3
Cefazolun sodium hydrate	10 mg/mL	—	215.1	0.9
Amino acids, electrolytes	—	Sodium hydrogen sulfite	328.5	5.0
Suxamethonium chloride	2 mg/mL	—	228.6	2.1
Ioversol	320 mg/mL	—	404.0	79.5
l-Isoprenaline hydrochloride	1 μg/mL	Sodium hydrogen sulfite, L-cysteine hydrochloride	326.3	8.6

Source: From ref. 75.

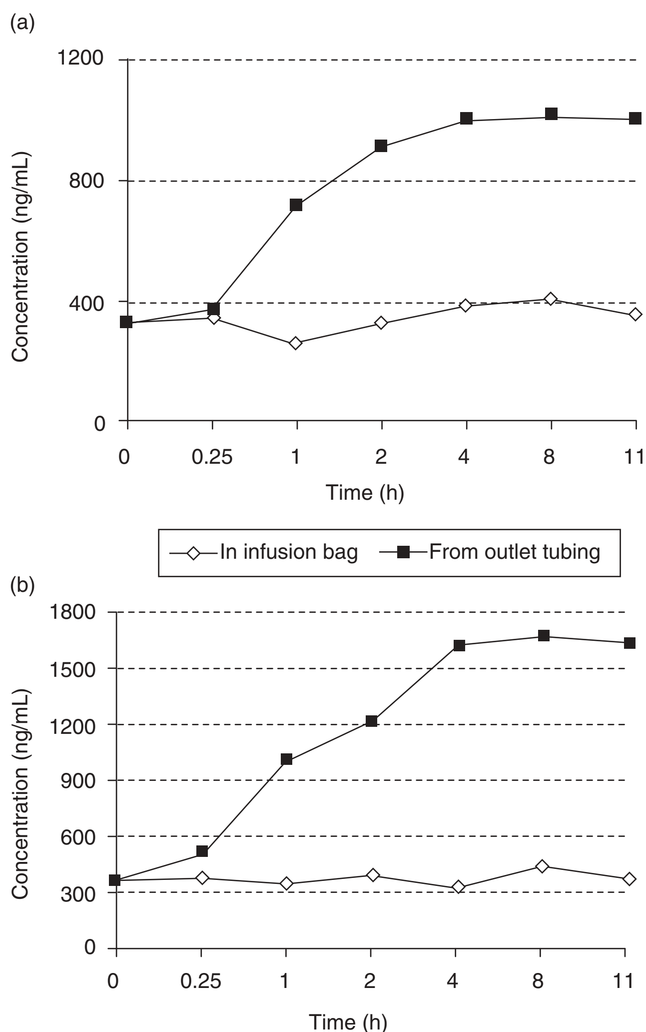


FIGURE 12 Comparative kinetics of DEHP leachability during simulated infusion of TPN [76]. (a) Kinetics of DEHP leachability during simulated infusion of TPN 24 hours after reconstitution of the preparation (n = 2 bag). Formula 1: infusion volume of 2200 mL (flow rate 177 mL/h, lipid concentration 3.85%). (b) Kinetics of DEHP leachability during simulated infusion of TPN 24 hours after reconstitution of the preparation (n = 2 bag). Formula 2: infusion volume of 650 mL (flow rate 46 mL/h, lipid concentration 1.85%).

solution. Table 36 presents the concentration of DEHP leached from tubing into etoposide infusion solutions as a function of time of exposure for two tubing lengths. The results showed that fast and considerable leaching of DEHP occurred, even when the PVC component had no direct contact with the solution. DEHP leaching was greatest for the tubing made only of PVC, but it also occurred with the coextruded (PE + PVC) and triple-layered tubes, despite their claims to prevent such leaching. The authors concluded that either DEHP was present in the internal PE layer or it migrated rapidly through the other polymer layers.

The kinetics of DEHP extraction from triple-layered tubing was studied by these authors by varying tube length, flow rate, and drug concentration. Figure 13 shows

TABLE 36 Concentration of DEHP Leached from Tubing into Etoposide Infusion Solutions as a Function of the Time of Exposure

Tubing Length and Type	Mean ± SD DEHP Concentration (μg/mL)					
	0 h	1 h	2 h	3 h	4 h	6 h
25 cm						
PE[b]	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
PVC[b,c]	<0.5	22.59 ± 4.21	35.05 ± 2.51	49.88 ± 4.78	58.57 ± 1.03	73.51 ± 3.51
Triple layered[d]	<0.5	18.92 ± 2.23	31.49 ± 3.78	44.19 ± 5.38	54.83 ± 4.28	61.99 ± 1.25
PVC[e]	<0.5	19.93 ± 1.90	33.01 ± 1.87	46.82 ± 1.98	55.46 ± 3.15	66.10 ± 1.23
Coextruded[f]	<0.5	18.64 ± 1.38	28.77 ± 2.33	39.37 ± 3.47	48.13 ± 2.21	54.60 ± 2.53
50 cm						
PE[b]	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
PVC[b,c]	<0.5	51.67 ± 3.63	82.00 ± 2.65	112.69 ± 4.89	131.67 ± 3.65	155.22 ± 2.35
Triple layered[d]	<0.5	39.85 ± 0.49	59.73 ± 3.04	81.51 ± 1.57	85.01 ± 2.17	98.72 ± 2.33
PVC[e]	<0.5	45.38 ± 2.08	73.27 ± 0.96	94.65 ± 0.88	117.53 ± 3.43	143.41 ± 11.39
Coextruded[f]	<0.5	39.98 ± 0.74	59.61 ± 1.49	78.03 ± 0.52	82.47 ± 3.56	107.83 ± 9.68

Source: From ref. 77.

[b]Manufactured by Vycon, Ecouan, France.

[c]Tubing length 80 cm.

[d]Consisted of an outer layer of PVC, a middle layer of EVA, and an inner layer of PE.

[e]Manufactured by Cair, Civrieux d'Azergues, France.

[f]PVC and PE tubing.

DEHP: diethyhexylphthalate; PE: polyethylene; PVC: polyvinyl chloride.

the amount of DEHP leached with the volume of solution infused. As could be expected, higher drug concentrations and tubing lengths increased the amount of DEHP released. Similarly, the slower the flow rate was, the higher the migration of the additive.

As mentioned under plastic containers (Section 6.1.3.2), DEHP is not the only additive that can be leached from plastic materials. Jenke et al. [78] studied the composition of extractables from plastic tubing used in pharmaceutical production facilities. Eight tubing materials made of silicone and neoprene rubber were characterized by their extractable substances. The authors investigated not only organic but also inorganic extractables. Extracts were obtained by static experiments using both water and ethanol. The tubing was cut and autoclaved in water at 121°C for 1 h, or lengths of tubing were filled with 100% ethanol and kept at 55°C for 24 h. Metal determination was carried out by inductively coupled plasma–atomic emission spectroscopy (ICP-AES), which included 29 elements. Besides the elements listed in Table 37, Be, Co, Cr, Cd, Se, V, Ge, Pb, and Bi were also measured but not detected since they either presented a concentration not different from the blank sample or below the lowest quantity determinable (LQD). It is important to mention that the ICP-AES is not a very sensitive technique. LQD values for elements ranging between 0.01 and 0.1 mg/L are relatively high levels for contaminants such as Cd or Be. This means that these elements could possibly be present in the extracts but not detected using this technique. In general, all tested tubing materials presented metals that were extracted either by water or by ethanol, although water, due to its greater capability to interact with metallic ions, promoted higher extraction yields. All tubing materials contained extractable Ca, Mg, Zn, and B. Elevated

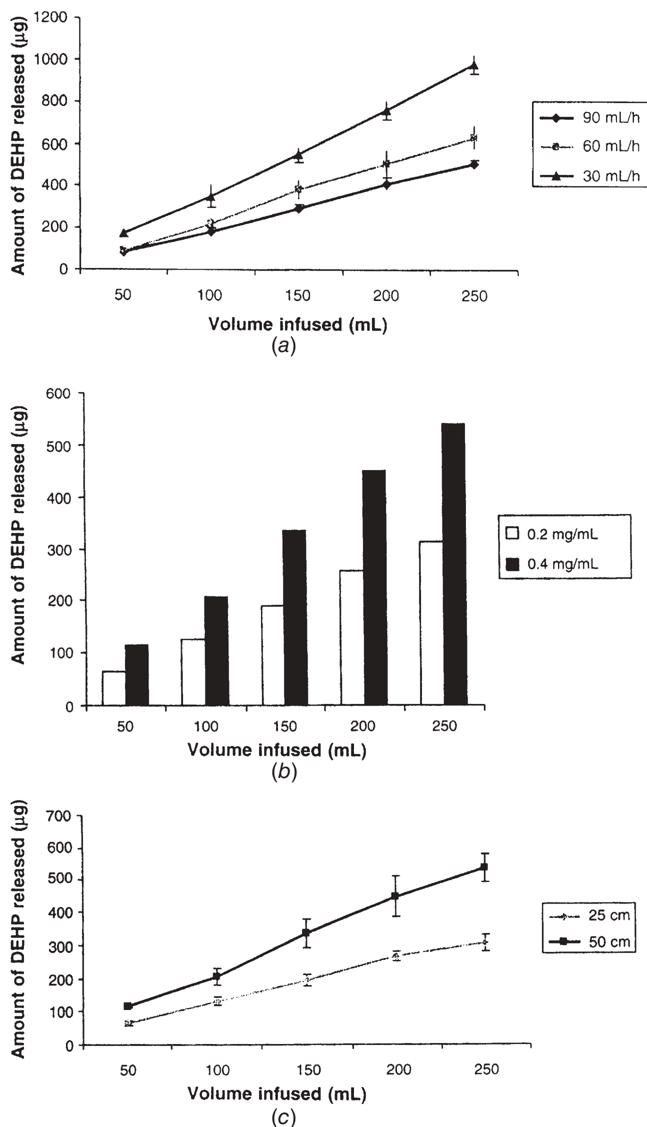


FIGURE 13 (*a*) Cumulative amount of DEHP leached from 50- and 80-cm PVC-only tubing after infusion of 0.4-mg/mL etoposide solution using various flow rates. (*b*) Cumulative amount of DEHP leached from 50- and 80-cm triple-layered tubing after infusion of 0.2 and 0.4 mg/mL etoposide solution with a flow rate of 30 mL/h. (*c*) Amount of DEHP leached from 50- and 80-cm triple-layered tubing after infusion of 0.4-mg/mL etoposide solution with a flow rate of 30 mL/h [77].

concentrations of other metals were also extracted from samples 3 and 5, probably due to the tubing's reinforced embedded metallic wire.

The authors also measured silicon and both organic and inorganic carbon. While inorganic carbon is indicative of the presence of carbonates (earth carbonates as additives), organic carbon is related to organic extractables. As could be expected,

TABLE 37 Levels of Total Carbon (Organic and Inorganic), and Silicone and Metals Extracted of the Tubing Materials with Water or Ethanol by Heating at 121°C for 1 h

Tubing Material	Total Inorganic Carbon (μg/g)	Total Organic Carbon (μg/g)	Si (μg/g) Ethanolic Extract	Si (μg/g) Aqueous Extract	Metal Concentration in Aqueous Extract		
					<0.5 μg/mL	0.5–1.0 μg/mL	>1 μg/mL
Silicone 1	0	14.0	765	101	B, Mg, Zn		
Silicone 2	0.9	38.1	1360	<0.2	Ca	B, Mg, Mn Mo, Ti, Zr. Sn, Zn, Sb, Li, Ag, Ni	
Silicone 3 (embedded wire)	2.3	250	1860	66.0	Ca, Ba, Mn, Mg, Al, Cu	—	B, Fe, Zn,
Silicone 4	0.2	34.0	1120	130	Ca, B, Fe, Mg, Al, Zn	—	—
Silicone 5 (embedded wire)	0	49.9	1300	87.3	Ca, B, Mn, Fe, Mg, Zn, Sb	—	—
Silicone 6	0	46.9	739	<0.2	Mn, Fe, Mg, Zn, Cu, Sb, Ni	B	Ca
Santoprene 1	10.1	16.6	— [a]	<0.2	Ca, Ba, B, Mg, Zn,	—	—
Santoprene 2	4.6	13.5	Not tested	0	Ca, Mo, Ti, Zr, Mn, Zn, Li, Ag, Ni	—	Mg

Source: From ref. 78.

[a]Tube disintegration.

Santoprene extracts contained higher concentrations of inorganic carbon and little, if any, extractable silicon. On the other hand, the amount of silicone in extracts of silicone polymers was very high. From Table 37, it is possible to see that these materials, though all made of silicone, are very different each other. While about 100 µg/g Si was found in the aqueous extract from sample 1, no measurable Si was detected in the same extracts from samples 2 and 6. As could be expected, silicone extractables were much higher in ethanolic extracts, being that 739 µg/g Si from sample 6 was the lowest amount leached.

The analysis of aqueous and ethanolic extracts by techniques such as liquid chromatography–mass spectrometry (LC–MS) and CG–MS showed that all silicon materials contained essentially the same peaks, that is, the same compounds were extracted. The distribution, however, varied from material to material. The primary organic extractables from silicone tubing were homologous series of silicone oligomers with the structural formula $[(CH_3)_2SiO]_n$. Direct matches with an MS spectral library allowed the identification of oligomers with $n = 5$ to $n = 25$. However, while in sample 3, the peaks were at the lower range of n, the oligomer distribution for sample 2 was shifted toward higher molecular weights, which are less soluble and therefore less extractable. Figure 14 illustrates the differences in the ethanolic extracts of materials 2 and 3.

Results of Santoprene extracts are quite different from those of silicone. GC–MS chromatograms for water extracts of Santoprene tubing show peaks associated with C8 and C9 acids, phthalates, and other organic substances. Figure 15 shows the GC–MS chromatograms of static ethanol extracts of Santoprene tubing materials, and Table 38 lists the compounds identified in Figure 14, based on matches of MS spectrum peaks.

6.1.3.4 Particulate Matter

Particulate contamination can be classified as intrinsic or extrinsic depending on its origin. Intrinsic contaminants arise from the manufacturing, packaging, transport, and storage of solutions; extrinsic contaminants are introduced or generated during drug reconstitution and administration to the patient.

The presence of particulate matter, while undesirable in any pharmaceutical product, is truly a problem in intravenous and ophthalmic preparations. Most common particulates in intravenous preparations are glass fragments, from the opening of glass ampoules, particles from rubber stoppers and intravenous equipment, and particles from plastic syringes, providing that the manipulation of such solutions is carried out in a controlled clean area. Air in a controlled area, in the immediate proximity of exposed sterilized containers/closures and filling/closing operations is appropriate when it has a particle count of no more than 3520 in a size range of 0.5 µm per cubic meter. This corresponds to a Class 100 air cleanliness level and is the regular environmental condition for manipulating sterile preparations.

Pharmacopoeial compendia prescribe the examination of particulate contamination for injections and infusion solutions and consider particulate contamination as the presence of extraneous mobile undissolved particles, other than gas bubbles, unintentionally present in the solution. They limit the number of particles according to their size and the volume of the preparation.

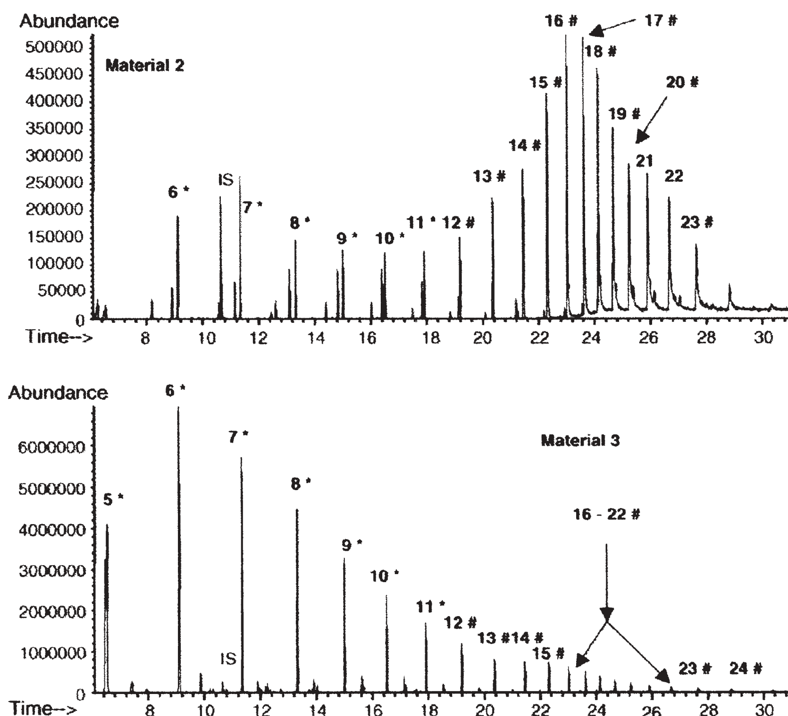


FIGURE 14 GC–MS chromatograms of the static ethanol extracts of silicone tubing materials (underivatised). The chromatograms from all the silicone materials were similar (same peaks but different relative sizes), and thus chromatograms from two different silicones are shown. A majority of the peaks are attributable to silicone oligomers. Peaks associated with cyclic oligomers are identified by the number of repeating units, *n*: e.g., $[(CH_3)_2SiO]_n$. Peaks denoted with * produced exact compound matches versus a library of mass spectra, while peaks denoted by # produced a library match to the right compound class (cyclic oligomer) but wrong specific oligomer. Small peaks at 7.95, 10.32, and 11.93 min were linked to 5–7 member linear silicone oligomers. IS = internal standard (dimethyl phthalate) [78].

Generally, particulate matter is eliminated by filtration, but the word *elimination* or even the concept of *being substantially free of*, as appear in some compendia, are levels practically impossible to reach. In fact, complying with pharmacopeia prescription is limited to a removal of particles above a certain size, since the elimination of particles becomes increasingly more difficult the smaller the particle is. Elimination difficulty seemingly increases in an exponential fashion as the size decreases.

Pharmacopeial count limits for particulates in parenteral solutions is given in Table 39. The limit depends on the method used for the determination and also on the volume of the sample. Two different procedures for the determination are generally proposed: light obscuration particle count test (LO) and microscopic particle count test (M), since neither is applicable to all kinds of samples.

It is possible to display the relationship between size and number of particles graphically. Groves [79] reported a logarithmic relationship between the number of

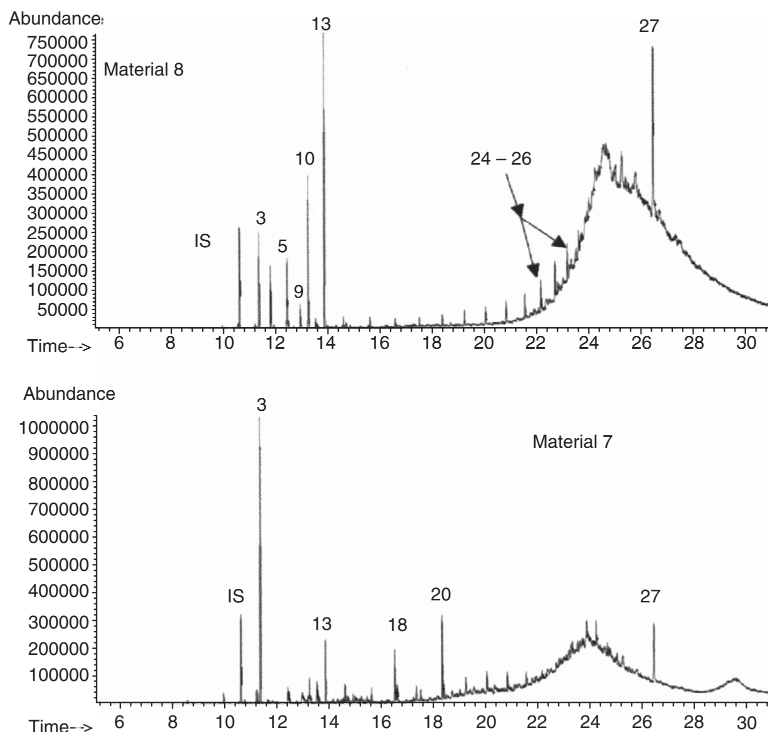


FIGURE 15 GC–MS chromatograms of the static ethanol extracts of Santoprene tubing materials (underivatized). The chromatograms from these two Santoprene materials were quite different from those of the silicone materials (Figure 14). IS = internal standard (dimethyl phthalate). See Table 39 for the tentative peak identifications. Only those peaks with recorded spectral library matches are noted for each sample, although retention times and patterns may suggest some additional peak identifications [78].

particles and their diameter in a solution. Thus, using the limits prescribed by BP, the graphical representation would be as shown in Figure 16.

The author, however, considers that if the distribution number/size of particles follows a random pattern, graphically represented in Figure 16, it cannot be considered contamination. On the other hand, if the solution no longer contains random identities but rather is dominated by particles such as starch grains from a stopper composition, glass shards, from a delaminating container, skin fragments, or clothing fibers, a nonrandom behavior would occur and be defined as "contamination." In other words, only positive deviation of the log-log particle size distribution curve, constructed using, for example, pharmacopoeial limits, would be considered contamination. Figure 17 graphically displays the particulate matter found in 5% dextrose solutions. The smooth curve represents the BP specification and the two others represent the samples. One product is within the specification (2G) and the other is not (2AL). According to the author, while in the 2G sample carbon black particles with sizes between 1 and 3 μm were identified, in the 2AL sample, besides carbon black (1–5 μm), some other particles such as starch grains, lacquer flakes, and rubber were found [80].

TABLE 38 Peak Identification in Ethanol Extracts of Santoprene Tubing Samples 7 and 8

Peak Number	Tentative Compound Identification	Present in Material
3	2,4-di-*t*-Butylphenol	7, 8
5	4-(1,1,3,3-Tetramethylbutyl)phenol	8
6	Isomer of octyl phenol (TMS)	8
7	Hexadecane	7
8	Isomer of octyl phenol (TMS)	8
9	4-Methyl-6-*tert*-octyl phenol	8
10	Isomer of decyl phenol	7, 8
11	Isomer of nonyl phenol (TMS)	8
12	Heptadecane	7
13	Isomer of undecyl phenol	7, 8
14	Octadecane	7
15	Isomer of decyl phenol (TMS)	8
16	Nonadecane	7
17	Isomer of undecyl phenol (TMS)	7, 8
18	Hexadecanoic acid, ethyl ester	7
19	Cyclohexadecane, heneicosane	7
20	Octadecanoic acid, ethyl ester	7
21	Docosane	7
22	Tetratriacontane, 9-methyl-nonadecane	7, 8
23	Tetracosane	7
24	Pentacosane	7, 8
25	Hexacosane, nonadecane	7, 8
26	Heptacosane	8
27	Irganox 1076	7, 8

Source: From ref. 78.

TABLE 39 Limits for Particulate Contamination in Infusion Solutions Established by Pharmacopeial Compendia

Compendia	Volume	Particle Size (μm)	LO Limit	M Limit
BP	SVP	≥10	6000/container	3000/container
		≥25	600/container	300/container
	LVP	≥10	25/mL	12/mL
		≥25	3/mL	2/mL
USP	SVP	≥10	6000/container	—
		≥25	600/container	—
	LVP	≥10	25/mL	12/mL
		≥25	3/mL	2/mL
IP	SVP, LVP	—	One or more particles in more than one container	—

Note: The limits are related to the method used for the determination: LO = light obscuration particle count test, M = microscopic particle count test, IP = International Pharmacopeia.

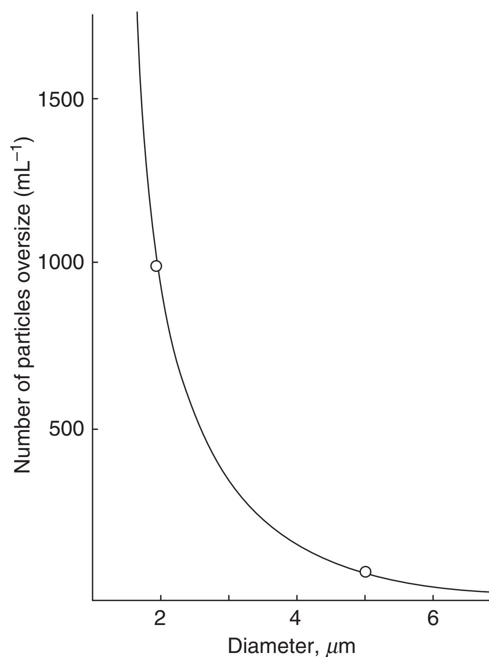


FIGURE 16 Coordinate plot of log (number of particles oversize per milliliter) versus log (particle diameter) for BP specifications.

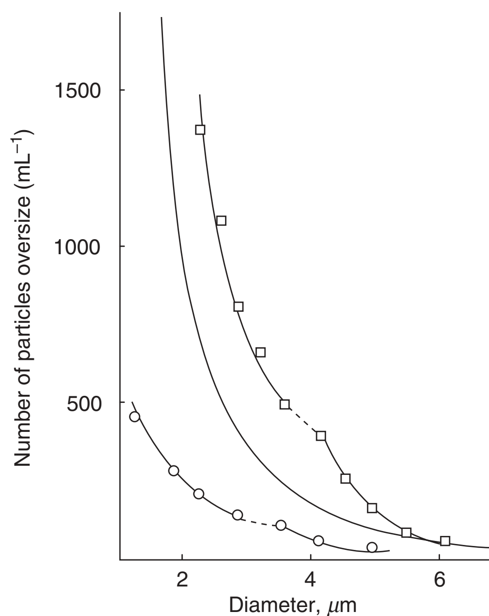


FIGURE 17 Cumulative particle size distribution in 5% dextrose solutions for injection: (□) product 2AL packed in 500-mL glass containers with lacquer-coated rubber stopper; (○) product 2G packed in 500-mL plastic bags; (smooth curve), BP specification [80].

Techniques for counting particulate matter allow a discrimination of the particles by sizes even lower than the pharmacopoeial limits, and though not prescribed, it is also possible to characterize particulate matter by its composition. Table 41 displays the amount of particles found in infusion solutions classified according to size.

Since the results correspond to different studies, they are displayed in different fashions. Nevertheless, all solutions present particulate matter as contaminants, and it is possible to observe the exponential behavior of the relationship between the number and size of the particles.

Foroni et al. [81] measured and characterized inert particles both in individual solutions and in the final ternary mixture prepared by the sterile transfer technique. The results are presented in Table 40 and also in Figure 18. The difference between the number of particles greater than 2 and 5 μm is higher than 50. The histograms in Figure 18 show the number of particles of the individual components (Figure 18*a*) and the number of particles after transferring the components into an EVA bag (Figure 18*b*). The results show that the contamination of 10% KCl, 30% dextrose, amino acids, and lipid emulsion is significantly higher than of the other components (Figure 18*a*). The results also show that the concentration of particles in the components packaged in glass ampoules, KCl and disodium phosphate is also higher than in the other components. The identification of contaminants was also carried out, where particles composed of silica, aluminum, and sodium were identified in a KCl solution, and particles containing sulfur and silicate were identified in all bags. The authors consider the presence of sulfur to be related to rubber particles and attribute the presence of silicate to contact with talcum from gloves used by manipulators during the manufacturing of the bags.

Kamiya et al. [83] evaluated particulate contamination in 199 samples of admixed and un-admixed parenteral nutrition solution bags from 10 hospitals in Japan. Seven samples were used as controls since they had not been mixed with ampoules or vials (un-admixed samples). Size and number of particles were measured using a particle counter, and the identification of elements was carried out by scanning electron microscopy coupled to energy dispersion spectroscopy. The authors collected the residual volume of the samples (10–60 mL) after their usage. The results are presented in Table 40.

Figure 19 shows the scanning electron micrograph of two types of particles on the filter after filtration (0.22 μm membrane filter) of 50 mL of an admixture containing 1700 mL glucose/electrolytes/amino acids plus one vial of vitamin complex (Maltamin), two glass ampoules of 1 mL panthol (Pantol), two glass ampoules of 20 mL sodium chloride (Conclyte-Na), two glass ampoules of 2 mL metoclopramide (Primperan), and two glass ampoules of 10 mL potassium L-aspartate (Aspara K). Figure 19 also shows the identification of these particles. Their composition suggests that they are particles of glass (Figure 19*a*) and particles of rubber (Figure 19*b*).

A similar study was carried out by Ball et al. [82] in New Zealand and the United Kingdom. The authors analyzed 20 samples of adult and 20 samples of the pediatric PN admixtures, collecting the first and second fractions drawn from the infusion sets. The number of particles greater than 5 μm was 50 times higher than the number of particles greater than 40 μm in all solutions (Table 40). The analysis of the particulate matter allowed for a characterization of particles such as rubber and glass fragments (Table 41).

TABLE 40 Mean (Range) of Particles Found in Mixed and Unadmixed Parenteral Nutrition Solutions Classified According to Their Size

			Particle Size										
			>1.3 µm		>5 µm		>10 µm		>25 µm		>50 µm		
Sample	Volume (mL)	*n*	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Reference
30 % Dextrose	25	3	452	±127	n.d.[a]	—	—	—	—	—	—	—	81
50 % Dextrose	25	3	2831	±278	—	±90	—	—	—	—	—	—	81
Amino acids	25	3	3715	±184	—	±58	—	—	—	—	—	—	81
All-in-one solution (adult)[b]	1	20	3.47	±1.24	1.40	±0.73	0.96	±0.45	1.06	±0.39	—	—	82
Two-in-one (pediatric)[b]	1	20	7.59	±2.56	11.77	±7.38	1.43	±1.09	0.38	±0.26	—	—	82
Lipid emulsion (syringe packed)	1	20	16.72	±10.85	8.98	±4.63	1.27	±1.23	0.76	±0.34	—	—	82
Unadmixed samples	10–60	7	62.7	8–146	1.70	0–4	0.4	0–2	—	—	0		83
Admixed samples[c]	10–60	192	960.9	30–9539	42.8	0–587	6.4	1–146	—	—	0.09	0–1	83
Solutions mixed using 1–3 glass ampoules[c]	10–60	29	862.1	30–5707	31.3	0–176	4.4	0–24	—	—	0.1	0–1	83
Solutions mixed during 4–13 glass ampoules[c]	10–60	63	1163.4	142–9539	66.2	4–587	10.6	1–146	—	—	0.08	0–1	83

[a]n.d. = not detected.
[b]First milliliters collected.
[c]residual volume collected.

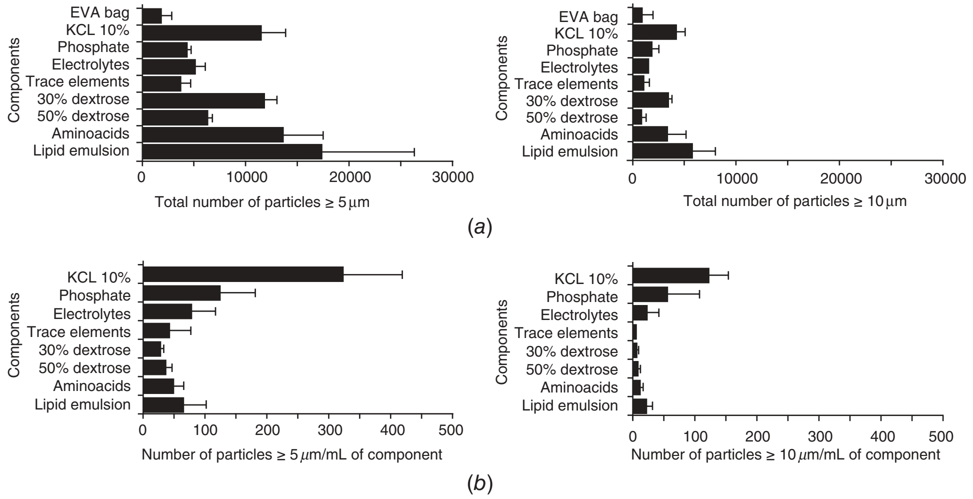


FIGURE 18 (*a*) Total number of particles in each component of the admixture analyzed by filtration-observation method. (*b*) Number of particles per milliliter of each component analyzed by filtration-observation method.

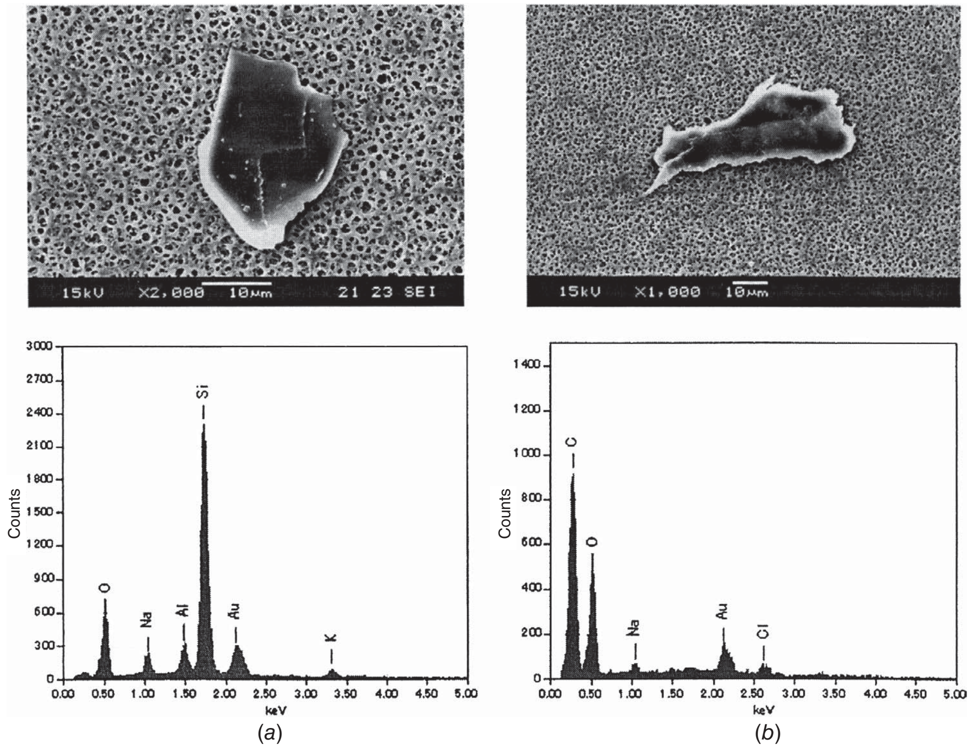


FIGURE 19 Identification of two types of particles by scanning electron microscopy coupled to energy dispersion spectroscopy: (*a*) suggests glass particles; (*b*) suggests rubber particles [83].

Roseman et al. [84] used scanning electron microscopy to analyze flakes found in solutions stored in glass ampoules (type I glass ampoules). They observed that all flakes had similar characteristics (colorless, platelike, <1 μm in thickness), but their sizes ranged from a few micrometers to a 100 μm in length. The elemental analysis of four flakes gave the results presented in Table 42. Because the flakes presented the same elements of glass, the authors broke a glass ampoule and analyzed the fragments (results also presented in Table 42). Since the composition of the flakes and glass fragments were similar to each other, the authors believed that the flakes originated in the ampoule due to an attack on the glass surface by some chemical substance. It has been shown that, besides strong alkali or hydrofluoric acid, other substances such as EDTA and other organic acids such as citric acid, as well as phosphate salts are able to attack glass (see Section 9.1.3.2). The existence of such particulates in glass ampoules was attributed to a delamination process that could occur just above the bottom of the ampoule, a region of thermal stress because of the intense heat used to form the bottom of the container.

The major problem associated with particulate contamination in infusion fluids is not related to the composition itself (since they are mostly pieces of the container and therefore innocuous elements) but to the potential of each particle to cause

TABLE 41 Elements in Particulate Matter Found in PN Solutions

Most Frequent Elements	Frequency of Appearance	Probable Origin	Reference
Oxygen	1	Glass; rubber	82, 83
Silicon	1	Glass; rubber	81, 82, 83
Carbon	3	Rubber	82, 83
Aluminum	1	Glass	81, 82, 83
Magnesium	2	Talc	81, 82
Sodium	2	Glass, rubber	81, 83
Chlorine	3	Rubber	83
Potassium	3	Glass	83

Note: Frequency of appearance: 1 = high; 2 = medium; 3 = low.

TABLE 42 Elemental Analysis of Flakes Found in Solution Stored Glass Ampoule and of Glass Fragments Resulting of a Broken Glass Ampoule

	Composition (%)				
Element	Flake 1	Flake 2	Flake 3	Flake 4	Broken glass[a]
Silicon	25–30	35–45	30–35	25–30	30–40
Alumnum	10–12	3–5	8–10	10–11	2–5
Potassium	4–6	n.d.[b]	3–5	3–4	n.d.
Calcium	1–2	n.d.	n.d.	1–2	n.d.
Boron	3–8	2–6	3–8	Present	4–10
Sodium	1–3	1–2	1–3	1–2	4–6

Source: From ref. 84.

Note: All ampoules were of type I glass.

[a]Mean of four samples; n.d. = not detected.

adverse reactions. The probability of an adverse reaction occurring is proportional to the number (and size) of particles introduced in the circulatory system. Turco et al. [85] suggest that responses resulting from the infusion of particles include physical occlusion, inflammatory responses, neoplastic responses, and antigenic responses. Animal studies have shown that the distribution of particles in tissues is related to their diameter. Particles larger than 8 μm are trapped in the lung capillaries, 3–6-μm particles are lodged in the spleen and hepatic lymph nodes, and 1-μm particles in the liver [86].

6.1.4 CONCLUDING REMARKS

The safety of drug therapy is closely related to the quality of drugs. All steps in drug processing may contribute to increasing the presence of foreign species in the final product. The requirements with respect to active ingredients, excipients, residual solvents, containers, and closures, although set up guidelines, are not a guarantee of products free of contaminants.

Although pharmacopeial biological reactivity tests for containers are good indicators of the toxicity of extractables, there are species that do not cause an acute toxic reaction but rather a chronic reaction, as is the case of phthalates (DEHP) and metallic species such as aluminum. Moreover, there are species that are extractable from packaging materials only by action of formulation constituents and therefore are not present in the extracts obtained by conventional pharmacopeial compendia tests.

The degradation of formulations due to lipid and vitamins peroxidation is a contamination problem not foreseen in pharmacopeial compendia since it is the exposure of the solutions to air and ambient light that induce peroxide generation.

Particulate contamination has been found in PN solutions and other intravenous drugs and fluids. Administration of particles through infusion solutions can result in adverse effects. The probability of these effects to occur increases proportionally with the amount of fluid administrated.

The presence of contaminants, though undesirable in all kind of drug formulations, is really critical in that intent for parenteral administration. Patients who require intensive or prolonged parenteral therapy, the immunocompromised, and neonates and infants might have increased susceptibility to the detrimental effect of contaminants.

REFERENCES

1. U.S. Pharmacopeia (2005), USP 27, U.S. Pharmacopeial Convention, Rockville, MD.
2. World Health Organization (WHO) (2004), *Guidelines for Drinking-water Quality*, Vol. 1: *Recommendations*, WHO, Geneva.
3. Water programs, Environmental Protection Agency, National Interim Primary Drinking Water Regulations, *Code of Federal Regulations*, Part 141, (1985).
4. ASTM International (2005), *ASTM Book of Standards*, Vol. 11.02: *Water and Environmental* Technology: *Water (II)*, ASTM International, West Conshohocken, PA.

5. BP (2003), British Pharmacopoeia Commission, London.
6. Deutsche Arzneibuch (DAB) (2005), Deutschen Apotheker Verlag, Stuttgart.
7. European Pharmacopoeia (Ph.Eur.) (2004), 4th ed., European Directorate for the Quality of Medicines (EDQM), Ph.Eur., Strasbourg.
8. The International Pharmacopoeia (IP) (2003), 3rd ed., World Health Organization, Geneva.
9. Küster, F. W., and Thiel, A. (1985), *Rechentafeln für die Chemische Analytik*, 103 Auflage, Walter de Gruyter, Berlin, p. 258.
10. Koo, W. W., Kaplan, L. A., Horn, J., Tsang, R., and Steichen, J. (1986), Aluminum in parenteral nutrition solutions—sources and possible alternatives, *J. Parenteral Enteral Nutr.*, 10, 591–595.
11. Recknagel, S., Brätter, P., Chrissafidou, A., Gramm, H.-J., Kotwas, J., and Rösick, U. (1994), Parenteral aluminum loading in critical care medicine. Part I: Aluminum content of infusion solutions and solutions for parenteral nutrition, *Infusionsther. Transfusionsmed.,* 21, 266–273.
12. Bohrer, D., do Nascimento, P. C., Becker, E., de Carvalho L. M., and Dessuy, M. (2005), Arsenic species in solutions for parenteral nutrition, *J. Parenteral Enteral Nutr.*, 29, 1–7.
13. PluhatorMurton, M. M., Fedorak, R. N., Audette, P. J., Marriage, B. J., and Yatscoff, R. W. (1996), Extent of trace-element contamination from simulated compounding of total parenteral nutrient solutions, *Am. J. Health-Syst. Pharm.*, 53, 2299–2303.
14. Bohrer, D., Bortoluzzi, F., Nascimento, P. C., Carvalho, L. M., and Ramirez, A. G. (2008), Silicate release from glass for pharmaceutical preparations, *Int. J. Pharm.*, in press.
15. Bohrer, D., do Nascimento, P. C., Binotto, R., Becker, E., and Pomblum, S. G. (2002), Contribution of the raw material to the aluminum contamination in parenterals, *J. Parenteral Enteral Nutr.*, 26, 382–388.
16. Bohrer, D., Becker, E., do Nascimento, P. C., de Carvalho L. M., and Marques, M. S. (2006), Arsenic release from glass containers by action of intravenous nutrition formulation constituents, *Int. J. Pharm.*, 315, 24–29.
17. Thompson, J. E., and Davidow, L. (2003), *A Practical Guide to Contemporary Pharmacy Practice*, Lippincott Williams & Wilkins, Philadelphia.
18. Wade, A., and Weller, P. J. (1994), *Handbook of Excipients*, 2nd ed., Pharmaceutical Press, London.
19. Neuzil, J., Darlow, B. A., Inder, T. E., Sluis, K. B., Winterbourn, C. C., and Stocker, R. (1995), Oxidation of parenteral lipid emulsion by ambient and phototherapy lights: Potential toxicity of routine parenteral feeding, *J. Pediatr.*, 126, 785–790.
20. Steger, P. J. K., and Mühlebach, S. F. (1997), In vitro oxidation of IV lipid emulsions in different All-in-One admixture bags assessed by an iodometric assay and gas-liquid chromatography, *Nutrition*, 13, 133–140.
21. Murray, R. K., Granner, D. K., Mayes, P. A., and Rodwe, V. W. (2000), *Harper's Biochemistry*, 25th ed., McGraw-Hill, New York, p. 169.
22. Helbock, H. J., Motchnik, P. A., and Ames, B. N. (1993), Toxic hydroperoxides in intravenous lipid emulsions used in preterm infants, *Pediatrics*, 91, 83–87.
23. Picaud, J. C., Steghens, J. P., Auxenfans, C., Barbieux, A., Laborie, S., and Claris, O. (2004), Lipid peroxidation assessment by malondialdehyde measurement in parenteral nutrition solutions for newborn infants: A pilot study, *Acta Paediatr.*, 93, 241–245.
24. Pironi, L., Guidetti, M., Zolezzi, C., Fasano, M. C., Paganelli, F., Merli, C., Bersani, G., Pizzoferrato, A., and Miglioni, M. (2003), Peroxidation potential of lipid emulsions after compounding in all-in-one solutions, *Nutrition*, 19, 784–788.

25. Steger, P. J. K., and Mühlebach, S. F. (1998), Lipid peroxidatoin of IV lipid emulsions in TPN bags: The influence of tocopherols, *Nutrition*, 14, 179–185.
26. Burton, G. W., and Ingold, K. U. (1986), Vitamin E as an in vitro and in vivo antioxidant, *Ann. NY Acad. Sci.*, 570, 7–22.
27. Bowry, V. W., Ingold, K. U., and Stocker, R. (1992), Vitamin E in human low-density lipoprotein. When and how this antioxidant becomes a pro-oxidant, *Biochem. J.*, 288, 341–344.
28. Bowry, V. W., and Stocker, R. (1993), Tocopherol-mediated peroxidation. The pro-oxidant effect of Vitamin E on the radical-initiated oxidation of human low-density lipoprotein, *J. Am. Chem. Soc.*, 115, 6029–6040.
29. Steger, P. J. K., and Mühlebach, S. F. (2000), Lipid peroxidatoin of intravenous lipid emulsions and all-in-one admixtures in total parenteral nutrition bags: The influence of trace elements, *J. Parenteral Enteral Nutr.*, 24, 37–41.
30. Laborie, S., Lavoie, J-C., Rouleau, T., and Chessex, P. (2002), Multivitamin solutions for enteral supplementation: A source of peroxides, *Nutrition*, 18, 470–473.
31. Laborie, S., Lavoie, J-C., Pineaut, M., and Chessex, P. (2000), Contribution of multivitamins, air and light in the generation of peroxides in adult and neonatal parenteral nutrition solutions, *Ann. Pharmacother.*, 34, 440–445.
32. Helbock, H. J., Motchnick, P. A., and Ames, B. N. (1993), Toxic hydroperoxides in intravenous lipid emulsions used in preterm infants, *Pediatrics*, 91, 83–88.
33. Lavoie, J-C., Bélanger, S., Spalinger, M., and Chessex, P. (1997), Admixture of a multivitamin preparation to parenteral nutrition: The major contributor to in vitro generation of peroxides, *Pediatrics*, 99, 61–70.
34. Lavoie, J. C., Chessex, P., Rouleau, T., Migneault, D., and Comte, B. (2004), Light-induced byproducts of vitamin C in multivitamin solutions, *Clin. Chem.*, 50, 135–140.
35. Knafo, L., Chessex, P., Rouleau, T., and Lavoie, J-C. (2005), Association between hydrogen peroxide-dependent byproducts of ascorbic acid and increased hepatic acetyl-CoA carboxylase activity, *Clin. Chem.*, 51, 1462–1471.
36. Balet, A., Cardona, D., Jane, S., Molins-Pujol, A. M., Sanchez Quesada, J. L., Gich, I., and Mangues, M. A. (2004), Effects of multilayered bags vs ethylvinyl-acetate bags on oxidation of parenteral nutrition, *J. Parenteral Enteral Nutr.*, 28, 85–91.
37. Baker, M., Gregerson, M. S., Martin, S. M., and Buettner, G. R. (2003), Free radical and drug oxidation products in an intensive care unit sedative: Propofol with sulfite, *Crit. Care Med.*, 31, 787–792.
38. Lavoie, J-C., Lachance, C., and Chessex, P. (1994), Antiperoxide activity of sodium metabisulfite. A double-edged sword, *Biochem. Pharmacol.*, 47, 871–876.
39. Hayon, E., Treinin, A., and Wilf, J. (1972), Electronic spectra, photochemistry, and autoxidation: Mechanism of the sulfite-bisulfite-pyrosulfite systems. The SO_2^-, SO_3^-, SO_4^- radicals, *J. Am. Chem. Soc.*, 94, 47–57.
40. International Conference on Harmonization (ICH) (1997), Guideline on residual solvents, ICH, Geneva.
41. Bohrer, D., do Nascimento, P. C., Becker, E., Bortoluzzi, F., depoi, F., and de Carvalho, L. M. (2004), Critical evaluation of the standard hydrolytic resistance test for glasses used for containers for blood and parenteral formulations, *PDA J. Pharm. Sci. Technol.*, 58, 96–105.
42. Scholze, H. (1988), *Glas, Natur, Struktur und Eigenschaften*, 3rd ed., Springer, Heidelberg, pp. 128, 324.

43. Bacon, F. R. (1986), Glass containers for parenterals, in Avis, K. E., Lachman, L., and Lieberman, H. A., Eds., *Pharmaceutical Dosages Forms: Parenteral Medications*, Vol 2, Marcel Dekker, New York, pp. 55–110.
44. Hak, E. B., Storm, M. C., and Helms, R. A. (1998), Chromium and zinc contamination of parenteral nutrient solution components commonly used in infants and children, *Am. J. Health-Syst. Pharm.*, 55, 150–154.
45. Borg, C., Constant, H., Fusselier, M., and Aulagner, G. (1994), Zinc, copper and iron in total parenteral nutrition mixtures: A contamination study, *Nutrition*, 13, 325–326.
46. Buchman, A. L., Neely, M., Grossie Jr, B., Truong, L., Lykissa, E., and Ahn, C. (2001), Organ heavy-metal accumulation during parenteral nutrition is associated with pathologic abnormalities in rats, *Nutrition*, 17, 600–606.
47. do Nascimento, P. C., Marques, M. S., Hilgemann, M., de Carvalho L. M., Bohrer, D., Pomblum, S. G., and Schirmer, S. (2006), Simultaneous determination of cadmium, copper, lead, and zinc in amino acid parenteral nutrition solutions by anodic stripping voltammetry and sample digestion by Uvirradiation, *Anal. Lett.*, 39, 1–14.
48. Alfrey, A. C., Le Gendre, G. R., and Kaenhy, W. D. (1976), The dialysis encefalophathy syndrome, possible aluminum intoxication, *N. Eng. J. Med.*, 294, 184–188.
49. Driscoll, W. R., Cummings, J. J., and Zorn, W. (1997), Aluminum toxicity in preterm infants, *N. Eng. J. Med.*, 337, 1090–1091.
50. Bohrer, D., do Nascimento, P. C., Binotto, R., and Pomblum, S. G. (2001), Influence of the glass packing on the contamination of pharmaceutical products by aluminium. Part I: Salts, glucose, heparin and albumin, *J. Trace Elem. Med. Biol.*, 15, 95–101.
51. Bohrer, D., do Nascimento, P. C., Martins, P., and Binotto, R. (2002), Availability of aluminum from glass and an Al-form exchanger in presence of complexing agents and amino acids, *Anal. Chim. Acta*, 459, 267–276.
52. Bohrer, D., do Nascimento, P. C., Binotto, R., and Becker, E. (2003), Influence of the glass packing on the contamination of pharmaceutical products by aluminium. Part III: Interaction container-chemicals during heating for sterilisation, *J. Trace Elements Med. Biol.*, 17, 107–115.
53. Bohrer, D., do Nascimento, P. C., Binotto, R., and Carlesso, R. (2001), Influence of the glass packing on the contamination of pharmaceutical products by aluminium. Part II: Amino acids for parenteral nutrition, *J. Trace Elements Med. Biol.*, 15, 103–108.
54. Popinska, K., Kierkus, J., Lyszkowska, M., Socha, J., Pietraszek, E., Kmiotek, W., and Ksiazky, J. (1999), Aluminum contamination of parenteral nutrition additives, amino acid solutions, and lipid emulsions, *Nutrition*, 15, 683–686.
55. Baydar, T., Aydin, A., Duru, S., Isimer, A., and Sahin, G. (1997), Aluminum in enteral nutrition formulas and parenteral solutions, *Clin. Toxicol.*, 35, 277–281.
56. Guidance for industry, container closure systems for packaging human drugs and biologics, chemistry, manufacturing and control documentation, Food and Drug Administration, 1999.
57. *Ullmann's Encyclopedia of Industrial Chemistry*, Vol. A5, 5th ed., VCH, Weinheim, 1993, p. 334.
58. Solomon, D. D., Jurgens, R. W., and Wong, K. L. (1986), Plastic containers for parenterals, in Avis, K. E., Lachman, L., and Lieberman, H. A., Eds., *Pharmaceutical Dosages Forms: Parenteral Medications*, Vol. 2, Marcel Dekker, New York, pp. 111–153.
59. U.S. Food and Drug Administration (FDA) (1999), Safety assessment of di-2-ethylhexylphthalate (DEHP) released from PVC medical devices, FDA, Center for Devices and Radiological Health, Rockville, MD.

60. Arabin, A., and Östelius, J. (1980), Determination by electron-capture gas chromatography of mono(2-ethylhexyl) phthalate and di(2-ethylhexyl) phthalate in intravenous solutions stored in poly(vinyl chloride) bags, *J. Chromatogra. B*, 193, 405–412.
61. Arabin, A., Jacobsson, S., Hagman, A., and Östelius, J. (1986), Studies on contamination of intravenous solutions from poly(vinyl chloride) bags with dynamic headspace gas chromatography–mass spectrometry and gradient liquid chromatography diode array techniques, *Int. J. Pharm.*, 28, 211–218.
62. Faouzi, M. A., Khalfi, F., Dine, T., Luyckx, M., Brunet, C., Gressier, B., Goudaliez, F., Cazin, M., Kablan, J., Belabed, A., and Cazin, J. C. (1999), Stability, compatibility and plasticizer extraction of quinine injection added to infusion solutions and stored in polyvinyl chloride (PVC) containers, *J. Pharm. Biomed. Anal.*, 21, 923–930.
63. Kambia, K., Dine, T., Gressier, B., Bah, S., Germe, A.-F., Luyckx, M., Brunet, C., Michaud, L., and Gottrand, F. (2003), Evaluation of childhood exposure to di(2-ethylhexyl) phthalate from perfusion kits during long-term parenteral nutrition, *Int. J. Pharm.*, 262, 83–91.
64. Allwood, M. C., and Martin, H. (1996), The extraction of diethylhexylphthalate (DEHP) from polyvinyl chloride components of intravenous infusion containers and administration sets by paclitaxel injection, *Int. J. Pharm.*, 127, 65–71.
65. Sautou-Miranda, V., Brigas, F., Vanheerswynghels, S., and Chopineau, J. (1999), Compatibility of paclitaxel in 5% glucose solutions with ECOFLAC low-density polyethylene containers-stability under different storage conditions, *Int. J. Pharm.*, 178, 77–82.
66. Marcato, B., Guerra, S., Vianello, M., and Scalia, S. (2003), Migration of antioxidants additives from various polyolefinic plastics into oleaginous vehicles, *Int. J. Pharm.*, 257, 217–225.
67. Smith, E. J., and Nash, R. J. (1986), Elastomeric closures for parenterals, in Avis, K. E., Lachman, L., and Lieberman, H. A., Eds., *Pharmaceutical Dosages Forms: Parenteral Medications*, Vol. 2, Marcel Dekker, New York, pp. 155–215.
68. Accardi-Dey, A., and Gschwend, P. M. (2003), Reinterpreting literature sorption data considering both absorption into organic carbon and adsorption onto black carbon, *Environ. Sci. Technol.*, 37, 99–106.
69. U.S. Food and Drug Administration (FDA) (1999), Guidance for industry, container closure systems for packaging human drugs and biologics, FDA, Rockville, MD.
70. Parenteral Drug Association (PDA) (1998), Pharmaceutical package integrity, Technical Report 27, PDA Bethesda, MD.
71. Mannermaa, J. P., Räisänen, J., Hyvönen-Dabek, M., Spring, E., and Yliruusi, J. (1994), Use of proton-induced X-ray emission (PIXE) analysis in the evaluation of large volume parenteral rubber stoppers, *Int. J. Pharm.*, 103, 125–129.
72. Delaunay-Bertoncini, N., van der Wielen, F. W. M., De Voogt, P., Erlandsson, B., and Schoenmakers, P. J. (2004), Analysis of low-molar-mass materials in commercial rubber samples by Soxhlet and headspace extractions followed by GC-MS analysis, *J. Pharm. Biomed. Anal.*, 35, 1059–1073.
73. Jenke, D. R. (1997), Utilization of extraction profiles to estimate the accumulation of extractables from polymeric materials, *J. Appl. Polym. Sci.*, 63, 843–848.
74. Loff, S., Kabs, F., Witt, K., Sartoris, J., Mandl, B., Niessen, K. H., and Waag, K. L. (2000), Polyvynilchloride infusion lines expose infants to large amounts of toxic plasticizers, *J. Pediatr. Surg.*, 35, 1775–1781.
75. Haishima, Y., Seshimo, F., Higuchi, T., Yamazaki, H., Hasegawa, C., Izumi, S., Makino, T., Nakahashi, K., Ito, R., Inoue, K., Yoshimura, Y., Saito, K., Yagami, T., Tsuchiya, T., and Nakasawa, H. (2005), Development of a simple method for predicting the levels of DEHP

migrated from PVC medical devices into pharmaceutical solutions, *Int. J. Pharm.*, 298, 126–142.

76. Kambia, K., Dine, T., Gressier, B., Germe, A.-F., Luyckx, M., Brunet, C., Michaud, L., and Gottrand, F. (2001), High-performance liquid chromatographic method for the determination of DEHP in total parenteral nutrition and in plasma, *J. Chromatogr. B*, 755, 297–303.
77. Boithias, S-B., Sautou-Miranda, V., Bourdeaux, D., Tramier, V., Boyer, A., and Chopineau, J. (2005), Leaching of DEHP from multilayer tubing into etoposide infusion solutions, *Am. J. Health-Syst. Pharm.*, 62, 182–188.
78. Jenke, D. R., Story, J., and Lalani, R. (2006), Estractables/leachables from plastic tubing used in product manufacturing, *Int. J. Pharm.*, 315, 75–92.
79. Groves, M. J. (1991), Particulate contamination in parenterals: Current issues, *Boll. Chim. Farma.*, 130, 347–354.
80. Bikhazi, A. B., Shiatis, J. A., and Haddad, A. F. (1977), Quantitative estimation of particulate matter in pharmaceutical preparations intended for intravenous administration, *J. Pharm. Sci.*, 66, 181–186.
81. Foroni, L. A., Rochat, M. H., Trouiller, P., and Calop, J. Y. (1993), Particle contamination in a ternary nutritional admixture, *J. Parenteral Sci. Technol.*, 47, 311–314.
82. Ball, P. A., Bethune, K., Fox, J., Ledger, R., and Barnett, M. (2001), Particulate contamination in parenteral nutrition solutions: Still a cause for concern? *Nutrition*, 17, 926–929.
83. Oie, S., and Kamiya, A. (2005), Particulate and microbial contamination in in-use admixed parenteral nutrition solutions, *Biol. Pharm. Bull.*, 28, 2268–2270.
84. Roseman, T. J., Brown, J. A., and Scothorn, W. W. (1976), Glass for parenteral products: A surface view using the scanning electron microscope, *J. Pharm. Sci.*, 65, 22–29.
85. Turco, S. J., and Davis, N. M. (1971), Detrimental effects of particulate matter on the pulmonary circulation, *J. Am. Med. Assoc.*, 217, 81–82.
86. Hearse, D. J., Sonmez, B., Saldanha, C., Braimbridge, M. V., Maxwell, M. P., and Erol, C. (1986), Particle-induced coronary vasoconstriction in the rat heart: Pharmacological investigation of underlying mechanisms, *Thorac. Cardiovasc. Surg.*, 34, 316–325.

6.2

QUANTITATION OF MARKERS FOR GRAM-NEGATIVE AND GRAM-POSITIVE ENDOTOXINS IN WORK ENVIRONMENT AND AS CONTAMINANTS IN PHARMACEUTICAL PRODUCTS USING GAS CHROMATOGAPHY–TANDEM MASS SPECTROMETRY

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6.2.1 INTRODUCTION

Endotoxins are bacterial cell envelope constituents that, when present in pharmaceutical products, cause pyrogenic reactions sometimes resulting in lethality. The toxicity of endotoxins is directly related to their chemical composition. However, the viability of the organism is irrelevant since endotoxin derived from dead or live microbes is equally active. The classical endotoxin is lipopolysaccharide (LPS). However, peptidoglycan (PG) also displays endotoxin-like activities. LPS is found only in gram-negative bacterial outer membranes, while PG is present in the cell

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wall of both gram-positive and gram-negative bacteria. The *Limulus* amoebocyte lysate (LAL) test is widely used as a biological assay for LPS levels but has poor sensitivity for detection of PG. Substances are found in LPS and PG [3-hydroxy fatty acids (3-OH FAs) and muramic acid (Mur), respectively] that are rarely found elsewhere in nature and serve as chemical markers. Both markers for LPS and PG can be detected using gas chromatography–tandem mass spectrometry (GC–MS/MS) and this latter technology is the focus of this chapter.

A recently published book provides an excellent survey of issues that relate to contamination with endotoxins (present in both viable and nonviable bacteria), their released cell wall constituents, and also viable bacteria in the pharmaceutical industry [1]. It is important to know both the content of the work environment (e.g., indoor air) and the pharmaceutical products themselves. The former provides information on possible sources of microbial contamination and the latter the purity of the final commercial product (or precursors in various stages in its preparation). In some cases it is vital to know the actual bacterial species involved in contamination; culture-based methods are standard microbiological techniques which were the focus of Jimenez [1] and thus will not be discussed further. Any contamination (e.g., with endotoxins), regardless of the species of origin, is of utmost of importance (e.g., in determining the safety of a batch of antibiotics to be administered intravenously). This is determined optimally by non-culture-based methods.

Endotoxic reactions result from inflammatory responses to both culturable and nonculturable organisms. Thus, traditional culture-based methods often significantly underestimate the risk of endotoxin present. Culture- and non-culture-based monitoring methods differ greatly in their characteristics. Importantly, results from culture-based methods are strongly affected by variability in culture conditions (e.g., media selected, culture time, and temperature), making it difficult to standardize protocols for quantitation of contamination and only detecting those organisms that grow under the conditions selected. However, the use of non-culture-based procedures certainly does not eliminate the need for information obtained by culture.

Real-time polymerase chain reaction (PCR) methods have become the primary alternative to bacterial culture, measuring the levels of characteristic genes originating from particular species (whether culturable or not). PCR has proven to be highly amenable in the clinical microbiology laboratory, but its utility in more diverse environmental or pharmaceutical products can be more problematic. Of course, PCR will not detect an endotoxin. PCR enzymatically amplifies a genetic region, with a characteristic deoxyribonucleic acid (DNA) sequence; a flanking set of primers (short pieces of complementary DNA) focuses the enzyme on the DNA region of interest. Classical PCR involves detection of a PCR product by electrophoretic mobility on a gel, which is time consuming. Real-time PCR is distinct from classical PCR in that electrophoresis is avoided and the PCR product is detected simply by an increase in fluorescence; this can be performed with or without prior culture. This approach is of great utility, but there can be variation depending on the sample matrix (e.g., in some cases total inhibition of the reaction by metal ions or humic acids). Assessment of total bioload (e.g., by measuring the levels of chemical markers for bacterial endotoxins by GC–MS/MS) is much less subject to matrix-based variation and is more readily standardized.

An overview of the relative advantages (and disadvantages) of modern non-culture-based methods to each other and versus culture was also provided by

Jimenez [1], including discussing measurement of adenosine triphosphate (ATP) levels, microscopy/fluorescent-activated cell sorting, molecular biology (e.g., PCR and microarrays), and immunology-based methods. The endotoxin section focused on the LAL bioassay method, which is still the most common method for measuring such contamination; modern alternatives, including bioassays for cytokines and analytical chemical methods (GC–MS), were also discussed. The LAL assay measures biological activity which can vary with slight changes in structure among endotoxins affecting determination of levels; this is not the case for chemical analysis [2]. Furthermore biological assays, including the LAL assay, often suffer from false positives from cross-reactivity with other contaminants. As mentioned above, the LAL assay only detects the gram-negative endotoxin LPS, not the gram-positive endotoxin PG.

Chemical assays for endotoxin are considerably more selective than the LAL assay but until recently lacked the sensitivity required for routine detection of gram-positive and gram-negative endotoxins. This relates to the introduction of advanced but still user-friendly GC–MS/MS instrument which are rapidly replacing GC–MS for trace analysis in the pharmaceutical industry and elsewhere. This chapter will thus focus on GC–MS/MS methodology for detection of 3-OH FAs (markers for LPS) and Mur (a marker for PG), which are each well established [3–8]. Ergosterol is also used as a marker for fungal contamination using GC–MS/MS [9].

Endotoxicity results from the interaction of a bacterial cell envelope component (e.g., LPS or PG with a cell surface receptor constituting part of the nonspecific immune system, (i.e., a toll-like receptor on white blood cells). This results in the production of cytokines [e.g., interleukin 1 (IL-1) or tumor necrosis factor (TNF)] as part of an intracellular enzyme cascade which can cause severe tissue injury. Bioassays or immunoassays can be used to detect such reactions respectively. As noted above the most widely used bioassay is the LAL assay. A lysate of amoebocytes of the horseshoe crab (*Limulus*) contains an enzymatic clotting cascade which is activated by extremely low levels of LPS (nanogram levels or lower). There are variants of this assay that can detect PG, but they are not as widely used. As noted above, other bioassays employ cultured cell lines that respond to LPS or PG, respectively. Unfortunately bioassays are highly amenable to false positives (from the presence of cross-reactive substances) or false negatives from inhibition (by contaminants present in the sample) [10]. A detailed discussion of these assays is beyond the scope of this chapter and has been reviewed elsewhere [1].

6.2.2 ANALYSIS OF GC–MS/MS MARKERS FOR LPS AND PG AND APPLICATIONS

For groups experienced in trace analysis of chemical markers for bacteria, currently samples are analyzed almost exclusively by GC–MS/MS. GC–MS/MS assays are now well established and a wide range of clinical and environmental samples have been analyzed. However, application in the pharmaceutical industry requires further evaluation. For example, Mur is released by hydrolysis and analyzed as an alditol acetate; 3-OH FAs after methanolysis are converted to methyl trimethylsilyl derivative. Detailed analytical procedures have been described elsewhere for Mur [3, 4, 7, 8, 11] and for 3-OH FAs [2, 5, 12, 13]. The compounds of interest contain active

groups (e.g., OH or CO_2H) that interact with GC columns. Derivatization is necessary to convert the samples into a suitable form for analysis. Prederivatization and postderivatization clean-up yields simple chromatograms. Quantitation employs stable-isotope-labeled forms of the markers (e.g., ^{13}C-labeled Mur derived from labeled blue-green algae).

The 3-OH FAs have had great utility in the determination of LPS levels in indoor air. However, in tissues and body fluids it has been determined that 3-OH FAs are naturally present at low levels as products of mammalian metabolism (mitochondrial fatty acid β oxidation). Due to this background GC–MS/MS for 3-OH FAs is not recommended as a general marker to determine trace LPS levels in clinical samples [14]. However, in certain situations the assessment of 3-OH FAs has been successfully used, for example, in the diagnosis of chronic peridontitis [15]. There is great potential for the utility of 3-OH FAs as markers for LPS contamination in pharmaceutical products, where often the background matrix would be anticipated to be much less complex.

Chemical analysis of Mur levels has proved effective in both clinical and environmental samples, since Mur is not synthesized by eukaryotic cells. For example, it is readily detected in infected human body fluids, for example, synovial fluids from patients with staphylococcal arthritis and spinal fluids from those with pneumococcal pneumonia [7, 16]. However, the most widely used method for its analysis, as an alditol acetate, is time consuming. A large number of derivatives have been tested in order to develop a simpler alternative. Unfortunately the limit of detection for these alternative approaches has not been optimal [17, 18].

The marker monomer is chemically converted into a volatile form suitable of passage through a gas chromatograph. The samples then pass into the tandem mass spectrometer where the ionized molecules are detected. GC–MS/MS employs GC separation coupled with the exquisite selectivity of MS/MS. In MS (monitoring/quantitation mode), background peaks are screened out. MS/MS screens out background a second time, thus dramatically lowering the detection limit. Alternatively, in the identification mode (MS/MS), a chemical fingerprint of the compound of interest allows definitive identification. The analysis of Mur serves as an example: natural ^{12}C muramic acid is first released from PG polymers (present as a minor component in a complex sample matrix) by hydrolysis. Conversion of ^{12}C muramic acid to a volatile derivative, muramicitol lactam pentaacetate molecular weight (MW) 445, is essential for GC–MS/MS analysis.

Maximal selectivity, in GC–MS analysis, requires selected ion monitoring (SIM). In SIM, one or more prominent ions characteristic of a given compound are monitored exclusively, thereby ignoring background or contaminant ions. Similarly, maximum selectivity in GC–MS/MS analysis involves twofold SIM, or multiple-reaction monitoring (MRM). In quadropole mass spectrometers, the first stage of MRM involves selective transmission of a molecular ion from the first mass spectrometer to the collision cell. This instrumental clean-up removes all nonidentical molecular weight contaminants produced from the initial ionization. The selected ion is then fragmented by collision with an inert gas (e.g., argon). In the second mass spectrometer the selected fragment ion is monitored. If either sensitivity or specificity is adversely affected, then the limit of detection is affected. GC–MS/MS provides much greater specificity than GC–MS. Using GC–MS it was impossible to reliably visually differentiate chromatograms that contained the lowest concentrations of

Mur from negative controls (including plants and fungi). GC–MS/MS chromatograms of dust were always readily differentiated from controls. However, these analyses were clearly performed at the current limits of sensitivity for GC–MS/MS. Current developments in MS technology may lead to dramatically improved sensitivity by GC–MS/MS.

Tandem mass spectrometry consists of two stages. For example, in Mur analysis, in the first stage, the molecule is isolated essentially intact with a MW of 403 due to the loss of a ketene (loss of 42). Coeluting molecules of different MW are largely but not totally eliminated; that is, only molecules with a MW of 403 are permitted to pass into the next stage. In the second stage, molecules with MW of 403 are broken into characteristic fragments including one that contains the original lactam with a MW of 198. Thus, in the second stage, only molecules with a MW of 198 are detected. Thus, for a second time, background molecules of different MW that coelute are essentially eliminated.

The use of a stable-isotope-labeled (^{13}C) analog of muramic acid, in each sample, verifies that there is no false-negative result. This assures that muramic acid is not lost during the sample preparation or hidden within the background in the instrumental analysis. Although ^{12}C muramic acid and ^{13}C muramic acid have the same retention time on GC analysis, they can be discriminated in the tandem mass spectrometer. GC–MS/MS analysis of ^{13}C muramic acid is identical to natural (^{12}C) muramic acid. However, the MWs in the first and second stages are correspondingly higher, 412 and 205, respectively. Thus in the tandem mass spectrometer, it is possible to monitor two separate windows simultaneously, one for ^{13}C muramic acid (top window) and one for natural muramic acid (bottom window). Accurate quantitation is readily accomplished by comparing the ratio of the area of ^{13}C versus ^{12}C muramic acid (see Figure 1).

A chemical fingerprint is generated consisting of scission products, in the MS/MS instrument, characteristic of the compound of interest. The parent molecule has a MW of 403. In each case the same major fragments are observed: masses 361, 301,

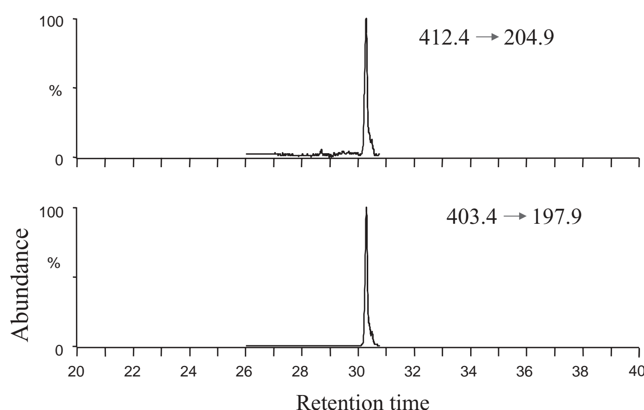

FIGURE 1 GC–MS/MS monitoring chromatogram. The upper window depicts the internal standard (^{13}C muramic acid) and the lower natural (^{12}C) muramic acid isolated from dust. The peak areas in the two separate windows are normalized relative to the highest peak in that window. Medical samples appear similar

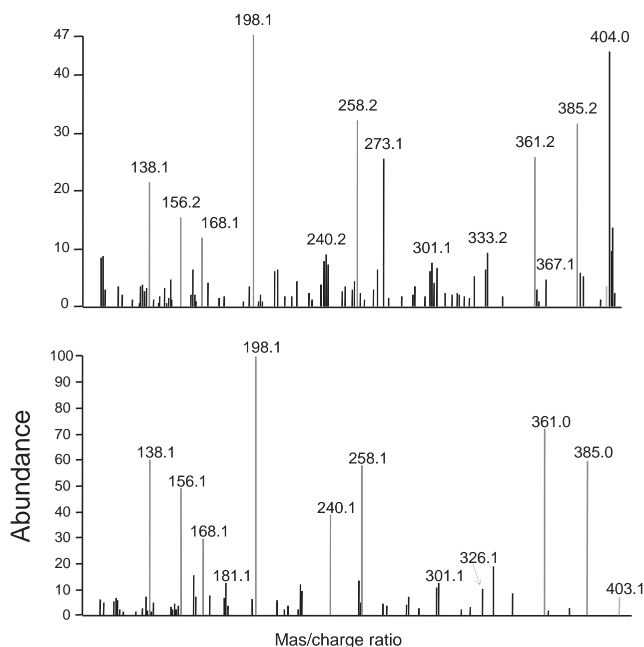

FIGURE 2 GC-MS/MS chemical fingerprint (product ion spectrum) of (*a*) standard muramic acid (2 ng total in sample) and (*b*) muramic acid isolated from dust. Medical samples appear similar.

258, 240, 198, 156, and 138: 361 results from the loss of a ketene (loss of 42) and 301 from a subsequent loss of acetic acid (loss of 60). Breakage at C4–C5 (loss of 145) would generate 258 and further loss of acetic acid (loss of 60) would generate 198. Loss of ketene or acetic acid from 198 results in 156 and 138, respectively (see Figure 2).

6.2.3 OPERATIONAL PARAMETERS

Every marketed product has a level of endotoxin tolerated based on the minimum pyrogenic dose and amount of the drug to be administered as per Food and Drug Administration (FDA) guidelines [19]. However, there are none for the more advanced chemical assays described here. Indeed there are only a few highly specialized university laboratories that currently have experience in trace chemical analysis of LPS and PG. There are no commercial testing laboratories. Simplification and automation will allow more widespread availability of these methods.

At the current time it is recommended that 1–10 mg of solid sample be analyzed. In environments that are not heavily contaminated with airborne dust it takes several days for sample collection. In an unoccupied or minimally occupied room around 1 mg of dust needed for analysis can be collected in 72 h. In a heavily occupied room the levels of dust increase dramatically and collection takes around 6–8 h.

Chemically inert membranes (e.g., Teflon) are preferably used for dust collection since they are not affected by heating in sulfuric acid, the first stage of the chemical analysis.

Large (milligram-to-gram) quantities of dust can readily be collected from air conditioners or surfaces. The concentration of dust in air varies from micrograms to milligrams pero cubic meter. Low concentrations are more typical of office buildings and laboratories, while the high range is more typical of dusty environments such as barns or chicken houses. Since commonly used pumps collect a few liters per minute, except in grossly contaminated environments, only microgram amounts of dust can be collected without extended air-sampling periods. Chemical markers of interest are only one component of bacteria and the bacteria constitute only a small fraction of the dust. Mur is present at between 5 and 50 ng/mg (corresponding to approximately 50–500 ng PG/mg dust) in normal house and air conditioning dust. Somewhat higher levels have been found in airborne dust (over 100 ng Mur/mg). LPS is present at between 500 and 5000 ng/mg dust. Solids (e.g., pharmaceutical products) are more simply analyzed, generally without any sample pretreatment. Generally, for the optimal level of sensitivity, around 10 mg should be analyzed.

As noted above, sensitive and specific GC–MS/MS methods for the determination of 3-OH FAs and Mur have been developed. MS is an alternative to the classical LAL assay for determination of LPS, while no other regulated approach exists for PG assessment. These chemical methods are reproducible and provide quantitative, accurate determination of microbial biocontamination. At the present time mass spectrometric measurement of LPS and PG have matured sufficiently to be used for routine assessment of air quality. Numerous products of medical and environmental origin have been analyzed. However, use for assessment of pharmaceutical products remains limited.

The precision of MS assays is in the range typical of most clinical assays (i.e., under 5–15%). The best choice of internal standard is the stable-isotope-labeled form (preferably ^{13}C) of the compound of interest (e.g., β-hydroxy myristic acid or muramic acid). Specific trace detection of chemical markers in complex matrices requires appropriate negative controls. Procedures are often described that do not employ the mass spectrometer and false positives are often reported. The mere analysis of blank filters or water blanks is not satisfactory since chemical noise contributed by the sample is much greater and is not accounted for with this form of control.

As noted above the predominant technique for measuring endotoxin levels is the LAL assay. This assay involves assessing activation of a clotting cascade in amoebocyte lysates (from the horseshoe crab). The LAL primarily detects LPS, and the sensitivity of detection of PG by LAL is extremely poor. The LAL bioassay and MS measurements of LPS sometimes correlate poorly. LAL measures biological activity while MS measures total quantity. Thus the two techniques are not strictly comparable. However, some differences (between results with LAL versus MS) may relate to the superior specificity of tandem mass spectrometry. It is worthy of note that GC–MS/MS can provide some information on the population of gram-negative bacteria present since distribution of hydroxy FAs varies among bacterial species. The 2- and 3-OH fatty acids with 10–18 carbon atoms are all common in organic dust.

6.2.4 CONCLUDING REMARKS

User-friendly commercial GC–MS/MS instruments have been available since the mid-1990s. Unfortunately there has been limited development in instruments that perform automated processing of samples for GC–MS/MS analysis, although a prototype automated derivatization has been built [20]. The pharmaceutical industry is well versed in the use of other types of mass spectrometry detection methods for high-throughput drug analysis [e.g., liquid chromatography–tandem mass spectrometry (LC-MS/MS) which eliminates the necessity for posthydrolysis derivatization by employing electrospray ionization mass spectrometry]. Unfortunately there has been limited interest in applying these methods for detection of LPS or PG markers [21]. Thus analysis of markers for endotoxins remains technically demanding and this has inhibited the widespread use of these techniques outside of a few specialist laboratories. However, there is great potential in the application of GC–MS/MS methods for assessing contamination with gram-positive and gram-negative bacterial endotoxins in the pharmaceutical industry. Tandem mass spectrometers are still expensive. However, such instruments are widely available in the pharmaceutical industry for general drug analysis. The most modern machines are run by Windows-based PC programs. Thus they can be readily operated by individuals, after appropriate training, with little prior experience of mass spectrometry or indeed analytical chemistry.

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REFERENCES

1. Jimenez, L. (2004), *Microbial Contamination Control in the Pharmaceutical Industry*, Marcel Dekker, New York.
2. Saraf, A., Larsson, L., Burge, H., and Milton, D. (1997), Quantification of ergosterol and 3-hydroxy fatty acids in settled house dust by gas chromatography–mass spectrometry: Comparison with fungal culture and determination of endotoxin by a *Limulus* amoebocyte lysate assay, *Appl. Environ. Microbiol.*, 63, 2554–2559.
3. Fox, A., Wright, L., and Fox, K. (1995), Gas chromatography tandem mass spectrometry for trace detection of muramic acid, a peptidoglycan chemical marker in organic dust, *J. Microbiol. Meth.*, 22, 11–26.
4. Fox, A., Krahmer M., and Harrleson, D. (1996), Monitoring muramic acid in air (after alditol acetate derivatization) using a gas chromatograph–ion trap tandem mass spectrometer, *J. Microbiol. Meth.*, 27, 129–138.
5. Saraf, A., and Larsson, L. (1996), Use of gas chromatography ion trap tandem mass spectrometer for the determination of microorganisms in organic dust, *J. Mass Spectrom.*, 31, 389–396.
6. Larsson, L., and Saraf, A. (1997), Use of gas chromatography–ion trap tandem mass spectrometry for the detection and characterization of microorganisms in complex samples, *Mol. Biotechnol.*, 7, 279–287.

7. Kozar, M., Krahmer, M. T., Fox, A., and Gray, B. M. (2000), Failure to detect muramic acid in normal rat tissues but detection in cerebrospinal fluid from patients with pneumococcal meningitis, *Infect. Immun*., 68, 4688–4698.
8. Kozar, M., Laman, J. D., and Fox, A. (2002), Muramic acid is not generally present in human spleen as determined by gas chromatography–tandem mass spectrometry, *Infect. Immun*., 70, 741–748.
9. Sebastian, A., and Larsson, L. (2003), Characterization of the microbial community in indoor environments: A chemical-analytical approach, *Appl. Environ. Microbiol*., 69, 3103–3109.
10. Kaneko, T., Goldman, W. E., Mellroth, P., Steiner, H., Fucase K., Kusumoto, S., Harley, W., Fox, A., Golenbock, D., and Silverman, N. (2004), Monomeric and polymeric Gram-negative peptidoglycan but not purified LPS stimulate the *Drosophila* IMD pathway, *Immun*., 20, 1–20.
11. Fox, A., Harley, W., Feigley, C., Salzberg, D., Toole, C., Sebastian, A., and Larsson, L. (2005), Large particles are responsible for elevated bacterial marker levels in school air upon occupation, *J. Environ. Monit*., 7, 450–456.
12. Mielniczuk, Z., Mielniczuk, E., and Larsson, L. (1993), Gas chromatography–mass spectrometry methods for analysis of 2- and 3-hydroxylated fatty acids: Application for endotoxin measurement, *J. Microbiol. Meth*., 17, 91–102.
13. Mielniczuk, Z., Mielniczuk, E., and Larsson, L. (1995), Determination of muramic acid in organic dust by gas chromatography–mass spectrometry, *J. Chromatogr. B*, 670, 167–172.
14. Sponazr, B., Norin, E., Midvedt, T., and Larrson, L. (2002), Limitations in the use of 3-hydroxy fatty acids to determine endotoxin in mammalian tissues, *J. Microbiol. Meth*., 50, 283–289.
15. Ferrando, R., Szponar, B., Sánchez, A., Larsson, L., and Vaero-Guillén, P. L. (2005), 3-Hydroxy fatty acids in saliva as diagnostic markers in chronic peridontitis, *J. Microbiol. Meth*., 62, 285–291.
16. Fox, A., Fox, K., Christensson, B., Krahmer, M., and Harrelson, D. (1995), Absolute identification of muramic acid at trace levels in human septic fluids *in vivo* and absence in aseptic fluids, *Infect. Immun*., 64, 3911–3955.
17. Kozar, M., and Fox, A. (2002), Analysis of a stable halogenated derivative of muramic acid by gas chromatography–negative ion chemical ionization tandem mass spectrometry, *J. Chromatogr*., 946, 229–238.
18. Sebastian, A., Harley, W., Fox, A., and Larsson, L. (2004), Evaluation of the methyl ester *O*-methyl acetate derivative of muramic acid for the determination of peptidoglycan in environmental samples by ion-trap GC-MS-MS, *J. Environ. Monit*., 6, 1–6.
19. U.S. Department of Health and Human Services (DHHS) (1987), FDA guidelines on validation of the *Limulus* amoebocyte lysate test as an end product test for human and animal parenteral drugs, biological products and medical devices, DHHS, Rockville, MD.
20. Steinberg, P., and Fox, A. (1999), Automated derivatization instrument: Preparation of alditol acetates for analysis of bacterial carbohydrates using gas chromatography–mass spectrometry, *Anal. Chem*., 71, 1914–1917.
21. Shahgholi, M., Ohorodnik, S., Callahan, J., and Fox, A. (1997), Trace detection of underivatized muramic acid in environmental dust samples by microcolumn liquid chromatography–electrospray tandem mass spectrometry, *Anal. Chem*., 69, 1956–1960.

6.3

MICROBIOLOGY OF NONSTERILE PHARMACEUTICAL MANUFACTURING

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6.3.1 INTRODUCTION

In order to comply with general microbiological quality attributes for nonsterile pharmaceuticals and established monograph or drug application specifications for

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raw materials and finished products, manufacturers need to follow the good manufacturing practice (GMP) regulations, with specific reference to contamination control stated in various sections of GMPs. For example, GMP regulations promulgated by the U.S. Food and Drug Administration (FDA) for finished products in the *Code of Federal Regulations* state (21 CFR Part 211.113): "Appropriate written procedures, designed to prevent objectionable microorganisms in drug products not required to be sterile, shall be established and followed" [1]. To meet this regulatory requirement, the microbial bioburden of nonsterile pharmaceutical raw materials and finished-product manufacturing environment (air, walls, and floors), equipment used for manufacturing, as well as the finished raw materials and drug products should be monitored and controlled to ensure that they have acceptable amount of total microbial bioburden and are free of objectionable microorganisms. Historically, the microbial contamination of nonsterile pharmaceuticals has been a concern, with a number of products recalled due to microbial contamination with undesirable microorganisms due to their suspected health hazards. As early as 1979, Dunnigan of the Bureau of Medicines at the FDA, commenting on the acceptable levels and contamination of microbiological contamination in drug products, expressed his concern that topical preparations contaminated with gram-negative organisms are a probable moderate to serious health hazard [2].

The U.S. Pharmacopeia (USP), in a recent revision of general chapter <1111> (585), "Microbiological Examination of Nonsterile Products: Acceptance Criteria for Pharmaceutical Preparations and Substances for Pharmaceutical Use," stated: "The presence of certain microorganisms in nonsterile preparations may have the potential to reduce or even inactivate the therapeutic activity of the product and has a potential to adversely affect the health of the patient. Manufacturers have therefore to ensure low bioburden of finished dosage forms by implementing current guidelines on GMPs during the manufacture, storage and distribution of pharmaceutical preparations" [3].

Pharmaceutical manufacturers and their trade associations have been proactive in addressing the concerns related to microbial contamination in nonsterile manufacturing environment. In an article in the March 1997 issue of *Pharmaceutical Technology*, the Pharmaceutical Research and Manufacturers Association of America (PhRMA) Environmental Monitoring Work Group provided a realistic assessment of the need for understanding and controlling microbial bioburden contributed by raw materials, primary packaging components, the manufacturing process, and the manufacturing environment. The work group emphasized in this article that, except for sterile products, most pharmaceutical products are neither intended to be nor represented as being sterile, but because they are administered to people who are ill and in a weakened state, the microbial content should be minimized [4]. The Australian Pharmaceutical Manufacturers' Association (APMA) published a guideline on nonsterile pharmaceuticals for human use emphasizing the need for control of microbial contamination and the importance of adhering to GMPs to control microbiological levels [5].

Besides GMP regulations for finished dosage forms (which also include regulatory requirements for inactive ingredients, active ingredients, containers, and closures used for finished products) published by the FDA [1], the International Conference on Harmonization (ICH) [6] (GMPs for active ingredients), authorities in Australia [7, 8] (pharmaceutical products), as well as the World Health Organization (WHO) [9] (pharmaceutical products), International Pharmaceutical

Excipients Council (IPEC) [10] (excipients), and the USP [11] have also published GMP guidelines. These guidelines require manufacture of pharmaceutical products with acceptable microbial bioburden and free of objectionable microorganisms.

This chapter discusses regulatory aspects of microbiology of nonsterile pharmaceutical manufacturing, including manufacturing environment, raw materials, and finished products. In addition, the microbiological bioburden control of pharmaceutical raw materials and products is reviewed, with particular reference to the type of finished dosage forms. Assessment of microbial bioburden requirements for nonsterile pharmaceutical manufacturing by the pharmaceutical industry is discussed. The microbiology of the pharmaceutical manufacturing environment for production of sterile pharmaceuticals or microbiological quality expectations for sterile products is not discussed in this chapter.

6.3.2 GLOBAL REGULATIONS AND REGULATORY GUIDANCES RELEVANT TO MICROBIAL BIOBURDEN CONTROL DURING NONSTERILE MANUFACTURING

GMP regulations for finished pharmaceutical drugs and components of finished drugs promulgated by the FDA [1], GMP guidelines for active pharmaceutical ingredients (Q7A) from the ICH [6], and GMP guidelines for bulk pharmaceutical excipients published by the USP [11] are discussed to illustrate the GMP requirements for pharmaceutical finished drug products, active (drug substances) and inactive (excipients) components of the drug products, respectively. In the context of this discussion on GMP regulations relevant to the microbiology of nonsterile manufacturing, *contamination* is defined as "the undesired introduction of impurities of a chemical or microbiological nature, or of foreign matter, into or on to a raw material, intermediate, or API during production, sampling, packaging, or repackaging, storage, or transport" [6]. A similar definition of contamination applies with reference to drug products. Microbiological bioburden refers to the level and type of microorganisms that may be present. The drug product, drug product components, manufacturing equipment, or environment can be considered contaminated when bioburden exceeds established specifications or acceptance criteria.

6.3.2.1 GMPs for Finished Products and Components of Finished Products

The FDA enforces the implementation of current good manufacturing practice (cGMP, GMP) regulations (21 CFR Parts 210 and 211) [1] for drug products manufactured in the United States (also applicable to drugs and drug components manufactured abroad and imported into the United Sates), under the Food, Drug, and Cosmetic Act (FD&C Act). Under FD&C Act 501(a)(2)(B), failure to comply with any GMP regulation shall render such drug to be adulterated and such drug as well as the person who is responsible for failure to comply shall be subjected to regulatory action [1].

It is important to understand the regulatory definitions of finished drug product, active ingredient, and inactive ingredient [1] in the context of the discussions on the subject presented here. Under GMP regulations 21 CFR Part 210.3(b)(4), drug product is defined as a finished dosage form, for example, tablets, capsule, and solution, that contains an active ingredient generally, but not necessarily, in association

with inactive ingredients. The term also includes a finished dosage form that does not contain an active ingredient but is intended to be used as a placebo. Placebo is used especially during safety and efficacy studies during drug development and in preclinical animal studies or human clinical trials. Active ingredient (also known as active drug substance) means any component that is intended to furnish pharmacological activity or other direct effect on the diagnosis, cure, mitigation, treatment, or prevention of disease or to affect the structure or any function of the body of humans or other animals [21 CFR Part 210.3(b)(7)]. Inactive ingredient (also known as excipient) means any component other than an active ingredient [21 CFR Part 210.3(b)(8)].

One of the important aspects of GMP regulations is the quality control during manufacturing to ensure that the drug products are free of contamination from objectionable microorganisms. GMPs [1] require that the personnel engaged in drug product manufacture wear protective apparel such as head, face, hand, and arm covering (garbing) as necessary to protect drug products from contamination [Part 211, Section 211.28(a)]. The regulations also require that the flow of components, drug product containers, closures, labeling, in-process materials, and drug products through the building or buildings be designed to prevent contamination [211.42(b)]. Contamination prevention also includes designation of separate or defined areas or such other control systems for the manufacturing operations as are necessary to prevent contamination [211.42(c)]. Appropriate equipment is required for adequate control over microorganisms [211.46(b)]. Adequate exhaust systems or other systems adequate to control contaminants are required where air contamination is likely to occur during production [211.46(c)]. Water is used in many areas of pharmaceutical manufacturing operations. GMPs require [211.48(a)] that incoming potable water into the manufacturing plant should comply with the U.S. Environmental Protection Agency's (EPA's) Primary Drinking Water Regulations (40 CFR Part 141), where control of microbiological contamination in potable water is defined. GMPs require written procedures be designed to prevent contamination of equipment, components, drug product containers, closures, packaging, and labeling materials or drug products and that such procedures be followed [21 CFR Part 211.56(c)]. Equipment cleaning and maintenance are also required to prevent contamination [21 CFR Part 211.67(a)]. Components and drug product containers and closures should at all times be handled and stored in a manner to prevent contamination [21 CFR Part 211.80(b)]. GMPs also require testing and release as described in 21 CFR Part 211.84(d)(6), which states that each lot of component, drug product container, or closure that is liable to microbiological contamination that is objectionable in view of its intended use should be subjected to microbiological tests before use. Appropriate written procedures, designed to prevent objectionable microorganisms in drug products not required to be sterile, should be established and followed [21 CFR Part 211.113(a)]. GMP regulations in 21 CFR Part 211.165(b) require appropriate laboratory testing be performed, as necessary, of each batch of drug product required to be free of objectionable microorganisms.

6.3.2.2 GMPs for Active Pharmaceutical Ingredients

The ICH Q7A guidance for GMPs for active pharmaceutical ingredients (APIs) [6] illustrates GMP expectations for microbial control during API production. The ICH

guideline specifies that where microbiological specifications have been established for intermediates during API production, or for an API, facilities should be designed to limit exposure to objectionable microbiological contaminants, as appropriate (ICH Q7A Section 4.1). Adequate ventilation, air filtration, and exhaust systems have been suggested to minimize risk of contamination due to microorganisms (Q7A Section 4.2). If tighter microbiological specifications for water used for API manufacture are called for, appropriate specifications for total microbial counts, objectionable microorganisms, and/or endotoxins should be established (Q7A Section 4.2). The guideline also states that where the manufacturer of a nonsterile API either intends or claims that it is suitable for use for further processing to produce a sterile drug product, water used in the final isolation and purification steps for such API should be monitored and controlled for total microbial counts, objectionable microorganisms, and endotoxins (Q7A Section 4.3). The guideline recommends cleaning of equipment assigned for continuous production or campaign production of successive batches of the same intermediate or API at appropriate intervals to prevent buildup and carryover of objectionable levels of microorganisms (Q7A Sections 5.2 and 8.5). Under the laboratory controls section of the guideline, ICH recommends that if the API has a specification for microbiological purity, appropriate action limits for total microbial counts, objectionable organisms, and endotoxins should be established and met (Q7A Section 11.1). Appropriate microbiological specification tests should be conducted on each batch of intermediate or API where microbial quality is specified (Q7A Section 11.2). The guidelines specify that equipment cleaning/sanitation studies should address microbiological and endotoxin contamination for those processes where there is a need to reduce total microbiological count or endotoxins in the API, where a nonsterile API is used to manufacture sterile products (Q7A Section 12.7). For APIs manufactured by cell culture, fermentation techniques, raw materials used such as media, and buffer components may have potential for microbiological contamination. For example, in such cases, depending on the intended use of the API or intermediate, control of bioburden, viral contamination, and/or endotoxins during manufacturing and monitoring of the process at appropriate stages may be necessary (Q7A Section 18.1). When the quality of the API can be affected by microbial contamination, manipulations using open vessels should be performed in a biosafety cabinet or similarly controlled environment (Q7A Section 18.3).

6.3.2.3 GMPs for Bulk Pharmaceutical Excipients

The USP general chapter <1078> outlines the GMP guidelines for bulk pharmaceutical excipients [11]. The guideline states that building and facilities should be designed so that operations performed within do not contribute to an actual or potential contamination of the excipient. An effective and regular cleaning program of equipment used is recommended to remove product residues and dirt, which may also contain microorganisms and act as a source of contamination. Further, the guideline states that all equipment that has been in contact with contaminated material should be thoroughly cleaned and disinfected before coming in contact with an excipient. A controlled environment may be necessary to avoid microbial contamination. Potable water used for production of excipients should be compliant with chemical and microbiological standards, including freedom from pathogenic

organisms. Purified water is also used for production of pharmaceutical excipients, and the systems used to produce purified water from potable water (deionizers, ultrafiltration, or reverse-osmosis systems) may have potential for microbial growth. Appropriate specifications for chemical and microbiological quality of water used for pharmaceutical production and periodic testing to demonstrate compliance with specifications are recommended. When excipient product specifications require it to be endotoxin or pyrogen-free, and water is used for production of that excipient, validation of the purified water systems to produce endotoxin and pyrogen-free water is required.

6.3.3 PHARMACOPEIAL GUIDELINES RELEVANT TO MICROBIOLOGY OF NONSTERILE MANUFACTURING

United States, European, and Japanese pharmacopeia have described general requirements, specifications, and tests for monitoring microbial bioburden in nonsterile pharmaceutical products. Although there are minor differences in the expectations of the pharmacopeia, the general principles for microbial bioburden monitoring remain similar as described below.

6.3.3.1 United States Pharmacopeia

In the general chapter “Microbiological Examination of Nonsterile Products” [3], the USP provides guidance for microbial examination of various nonsterile pharmaceutical dosage forms (Table 1). Enumeration of the total aerobic counts and total yeasts and molds in products are determined using procedures stated in USP general chapter <61> [12], and tests for specified microorganisms (*Staphylococcus aureus*, and *Pseudomonas aeruginosa, Escherichia coli, Salmonella* sp., and *Candida albicans*) are performed using procedures stated in USP general chapter <62> [13]. Acceptance criteria for microbiological quality of nonsterile dosage forms for various routes of administration are also provided. In addition, in pharmacopeial monographs, microbiological specifications are described where applicable in monographs for excipients, actives, and drug products. Microbiological best laboratory practices are described in general chapter <1117> [14], which provides guidance on implementing good laboratory practice standards when examining various sample matrixes for microbial bioburden in the laboratory. Alternative microbiological testing methods other than those specified in USP can be used provided such methods are appropriately validated. The procedures for validation of alternative microbiological methods are described in USP general chapter <1223> [15].

The USP also provides guidance on reduced microbial limits testing for product release and stability evaluation when drug products have reduced water activity [ratio of vapor pressure of H_2O in product (P) to vapor pressure of pure H_2O (P_o) at the same temperature] well below 0.75. Water is required for microbial growth in the pharmaceutical products [16]. However, the more resistant microorganisms, including spore-forming *Clostridium* sp., *Bacillus* sp., *Salmonella* sp., and filamentous fungi, which may not proliferate in the drug product with low water activity, may persist in the dormant state in the product. For example, a water activity of 0.61 is required for the growth of the fungus *Xeromuces bisporus*, a water activity

TABLE 1 Acceptance Criteria for Microbial Bioburden Control of Nonsterile Dosage Forms

Route of Administration	Total Aerobic Count Limit (CFU/g, CFU/mL)	Total Combined Yeasts and Molds Limit (CFU/g, CFU/mL)	Specified Microorganism/s (in 1 g or 1 mL)
Nonaqueous preparations for oral use	1000	100	Absence of *E. coli*
Aqueous preparations for oral use	100	10	Absence of *E. coli*
Rectal use	1000	100	
Oromucosal use (gingival, cutaneous, nasal, auricular)	100	10	Absence of *S. aureus* and *P. aeruginosa*
Vaginal use	100	10	Absence of *S. aureus, P. aeruginosa*, and *C. albicans*
Transdermal patches (one patch including adhesive layer and backing)	100	10	Absence of *S. aureus* and *P. aeruginosa*
Inhalation use (other than liquid preparations for nebulization)	100	10	Absence of *S. aureus, P. aeruginosa*, and bile-tolerant gram-negative bacteria

Source: From ref. 3.

of 0.62 is required for the growth of the yeast *Zygosaccharomyces rouxii*, while the majority of other fungi and yeasts require water activity higher than 0.75 for growth. Water activity of 0.75 is adequate for the growth of the bacterium *Halobacterium halobium*, but the majority of other bacteria require higher water activity for growth. The guidance also provides for a microbial limit testing strategy for pharmaceutical products for various routes of administration based on water activity. For example, for compressed tablets and liquid-filled capsules with representative water activities of 0.36 and 0.30, respectively, reduced testing is recommended, while for topical creams and nasal inhalants, with water activity of 0.97 and 0.99, respectively, testing for total aerobic counts, total yeasts and molds, *S. aureus*, and *P. aeruginosa* is recommended. Antimicrobial preservatives are added to nonsterile pharmaceutical dosage forms to protect them from microbial growth or from microorganisms that are introduced inadvertently during or subsequent to the manufacturing process. USP general chapter <51> describes how to perform antimicrobial effectiveness testing [17]. In this chapter, it is emphasized that antimicrobial preservatives should not be used as a substitute to GMPs or solely to reduce the viable microbial population of a nonsterile product. It is recommended that antimicrobial effectiveness of preservatives must be demonstrated for dosage forms such as multiple-dose topical and

oral dosage forms, ophthalmic, otic, nasal, and irrigation as described in the USP general chapter on pharmaceutical dosage forms [18].

The USP also provides guidance on water for pharmaceutical purposes [19]. The guidance points out that the major exogenous source of microbial contamination of bulk pharmaceuticals is the source water. Source water (potable water) should meet quality attributes of drinking water, in which coliform levels are controlled. Other microorganisms present in the incoming water, although not objectionable in nature, may compromise subsequent purification steps. Microorganisms present in source water may absorb to carbon beds, deionized resin beds, and filter membranes (used in the processing of potable water to purified water) and initiate the formation of a biofilm. Microorganisms in a biofilm are adapted to the prevailing low-nutrient environment. Microorganisms in the biofilm may also move downstream when they are shed from the existing biofilm and carried to other areas of the water system. A detailed account of biofilm formation in the pipelines and methods for detection and quantitation of biofilms is provided by Olson (1997) [20]. Microbial contamination may also come from unprotected vents, faulty air filters, ruptured disks in the water system, and backflow from contaminated outlets, among others. Several categories of microorganisms may be problematic to the pharmaceutical water systems and as a result to the pharmaceutical products, which are manufactured using water from these systems. The microorganisms may include opportunistic or overt pathogens, nonpathogenic indicators of potentially undetected pathogens, or microorganisms that could be resistant to a preservative in a drug product or which can degrade an active or inactive ingredient in a drug product. Defining alert and action levels of microorganisms in water systems and monitoring these levels by microbiological testing at scheduled intervals can serve as an early warning that will allow remedial actions to occur and prevent a system from producing water unfit for pharmaceutical use. For purified water, for example, maximum action levels are considered to be 100 colony-forming units of microorganisms per milliliter (CFUs/mL) [19].

6.3.3.2 European Pharmacopoeia

For pharmaceutical products, the European Pharmacopoeia describes sampling plans and tests for quantitative enumeration of bacteria and fungi that can grow under aerobic conditions [21]. Enterobacteria and certain other gram-negative bacteria, *E. coli, Salmonella* sp., *P. aeruginosa*, and *S. aureus* and Clostridia and counts for *Clostridium perfringens* [22] are described under tests for specified microorganisms. In the general chapter on microbiological quality of pharmaceutical preparations [23], pharmaceutical dosage forms are classified into three categories: Category 1 is for sterile pharmaceutical preparations, which is not the subject of this chapter, and categories 2 and 3 describe requirements for various nonsterile dosage forms. For example, for category 2, preparations for topical use, testing for total aerobic count and fungi, enterobacteria and other gram-negative bacteria, *P. aeruginosa*, and *S. aureus* is performed. Under category 3A, preparations for oral rectal administration, testing for total viable aerobic count and *E. coli* is performed, whereas under category 3B, where raw materials of natural origin are included in the drug products, testing for total viable aerobic count, nitrobacteria and other gram-negative bacteria, *Salmonella* sp., *E. coli*, and *S. aureus* is performed. Microbiological specifications

are detailed wherever applicable under each monograph. Antimicrobial preservative effectiveness tests are recommended, where antimicrobial preservatives are used, such as for multidose containers where there is a potential for microbiological contamination, or aqueous or topical preparations, which also have potential for microbial contamination with high moisture content in these dosage forms. Under the general chapter on efficacy of antimicrobial preservation [24], both the rationale for using antimicrobial preservatives and the tests for demonstrating effectiveness of antimicrobial preservatives are described.

6.3.3.3 Japanese Pharmacopoeia

In the general chapter on microbial attributes of nonsterile pharmaceutical products, the guidance suggests that the presence of microbial contaminants in nonsterile products [25] can reduce or inactivate the therapeutic activity of the product and has the potential to adversely effect the health of the patients and recommends manufacturers to ensure that contamination levels are as low as possible for finished dosage forms. Microbial enumeration limits for raw materials (total aerobic microbial count and total combined yeasts and molds count) and finished dosage forms are described. For inhalation, nasal, and topical routes of administration, tests for total aerobic microbial count and total combined and yeast and mold count, *P. aeruginosa*, and *S. aureus* are recommended. For vaginal preparations, testing for total aerobic microbial count and total combined yeast and mold count, *E. coli, S. aureus*, and *C. albicans* is recommended, while for oral liquids and solids, testing for total aerobic microbial count and total combined and yeast and mold count and *E. coli* is recommended. The microbiological assessment of preservatives is required when preservatives are used in a pharmaceutical product to control microbial bioburden. The test microorganisms and methods for evaluating the efficacy of the preservative in pharmaceutical products are described in the general chapter on preservative effectiveness tests [26].

6.3.4 CURRENT REGULATORY EXPECTATIONS FOR MICROBIAL BIOBURDEN CONTROL DURING NONSTERILE MANUFACTURING

Regulatory expectations for microbial bioburden for nonsterile pharmaceutical products are reviewed using the FDA guide to inspections of microbiological quality control laboratories [2], purified water systems [27], topical products [28], and oral solutions and suspensions [29].

In the guide to inspections of microbiological quality control laboratories [2], a number of problems associated with microbiological contamination of topical drug products, nasal solutions, and inhalation products have been emphasized. In that guidance, Dunnigan of the FDA is quoted as saying that topical preparations contaminated with gram-negative organisms are a probable moderate to serious health hazard.

In the guide to inspections of topical products [28], it is indicated that water deionizers are usually excellent breeding areas for microorganisms, where flow rates, temperature, surface area of resin beds, and microbial quality of the feed water all influence microbial growth. Since topical products (e.g., creams, ointments)

generally contain purified water, the microbial integrity of the product is contingent on the microbial bioburden of purified water. The guidance also points out that in assessing the significance of microbial contamination of the product, both the identification of isolated microorganisms and the number of organisms found are significant. When high numbers of nonpathogenic microorganisms are present, they may affect product efficacy and/or physical and chemical stability. Topical creams may tend to separate on standing. For topical products, data should be available to show continued effectiveness of preservatives throughout the product's shelf life.

Purified water is normally used for the manufacture of nonsterile pharmaceutical products. The guide to inspections of high-purity water systems [27] primarily discusses microbiological aspects and suggests that an action limit of over 100 CFU/mL for a purified water system is unacceptable. The purpose of establishing an action limit is to assure that the water system is under control. Purified water used in the manufacture of drug products should be free of "objectionable microorganisms," which are defined as organisms that when present can cause infections when the drug product is used as directed. The definition can also include organisms capable of growth in the drug product. Microorganisms may exist in water systems as either free floating or attached to the walls of pipes and tanks (biofilms). Microorganisms continuously slough off from biofilms and contribute to movement of microorganisms upstream or downstream in the water systems. The guidance emphasizes that establishing the level of contamination allowed in high-purity water systems used in the manufacture of a nonsterile product requires an understanding of the use of the product, the formulation (preservative system), and the manufacturing process. In the manufacture of antacids, which do not have an effective preservative system, an action limit below 100 CFU/mL is required. The inspection guide also points out that equipment used in purified water systems may be liable to contamination. Pumps used in water systems, if not continuously used, may have still (stagnant) water, which is the site of microbial growth. *Pseudomonas* sp. contamination reported in the water system was attributed to a pump which was operational only periodically. Deadlegs and fittings are a source of microbial bioburden in water systems. Threaded fittings should not be used in pharmaceutical water systems, as they are a source of microbial growth. Filters are another source of contamination, as microbes tend to accumulate and grow on their surface. While a 0.2-µm point-of-use filter can mask the level of microbiological contamination, it will not necessarily stop endotoxin contamination, since the toxins can pass through the filter. Filters should be frequently changed to prevent contamination.

In a water system, contamination by *Pseudomonas* sp. was found after FDA testing and the same species was also found in a topical steroid product after FDA testing [27]. The inspection resulted in product recall and issuance of a warning letter. The root cause for microbial bioburden was found to be a one-way water system that employ ultraviolet (UV) light to control microbiological contamination, where the UV light is turned on only when water is needed, and when the UV lights are not on, sterilization is ineffective and the standing water is a source of microbial growth. A flexible hose used in this water system that was difficult to sanitize may also have contributed to the observed microbial bioburden.

In the guide to inspection of oral solutions and suspensions [29], it is stated that in some oral liquids microbiological contamination can present significant health hazard. For instance, microbiological contamination with gram-negative

microorganisms is objectionable in some oral liquids, especially those used in infants and immunocompromised patients. In oral liquids such as antacids, contamination with *Pseudomonas* sp. is objectionable. In general, contamination of any preparations with gram-negative organisms is not desirable. Such contamination may suggest a deficient manufacturing process, inadequate preservative system, and potential use of contaminated raw materials.

6.3.5 INDUSTRY PERSPECTIVE ON MICROBIAL BIOBURDEN CONTROL FOR NONSTERILE PHARMACEUTICAL MANUFACTURING

The PhRMA Environmental Monitoring Work Group published an article in the March 1997 issue of *Pharmaceutical Technology* on microbiological monitoring of environmental conditions for nonsterile manufacturing [4]. This publication is a compilation of survey results of nonsterile manufacturing facilities within the United States and recommendations based on the survey results. In this section, a summary of the survey results and recommendations published in this article are reviewed with a few comments from this author based on experiences in this area.

6.3.5.1 Survey Results

Most manufacturers conducted the monitoring of nonsterile pharmaceutical manufacturing environments. Companies producing topicals, liquids, and aerosols tend to have more extensive programs than those companies manufacturing tablets or other solid oral dosage forms. Among the nonsterile manufacturing environments monitored were air, processing equipment, and water. Air quality was monitored using centrifugal air samplers, settling plates, and so on. Product contact surfaces of equipment were monitored using swabs or contact plates, with swabs being preferred because of better access of irregular surfaces. Since swabs used are presterilized, they are less likely to contaminate the surface being sampled, whereas contact plates which contain nutrient agar media may encourage growth of microorganisms after sampling, unless the sampled area is thoroughly cleaned with ethanol or other sterilizing agent. However, contact plates are easier to use on plain flat surfaces and also help provide an accurate assessment of in situ microbial status.

Companies with monitoring programs have established alert and action limits. Alert levels of microorganisms may indicate a change from normal operating procedures but may not require any corrective action, whereas action levels may suggest a significant change from normal operating procedures and require corrective actions to control further proliferation of microbes.

6.3.5.2 Recommendations

Specific Recommendations The work group recommended that the routine monitoring of microbiological activity in nonsterile environments should not be mandatory but should be determined by the type of nonsterile dosage form manufactured. In general, cleaning and sanitization procedures, good maintenance schedules, well-defined process control programs, raw material quality, facility design and control, and training of personnel involved in various aspects of manufacturing can

contribute to microbial bioburden control. A defined monitoring program can provide data on historical trends and such data can help identify deviations, root-cause analysis, troubleshooting, and early and effective actions. Among nonsterile dosage forms, inhalation products are of particular concern, followed by liquids and topical formulations, with a lower priority given to solid oral dosage forms. When monitoring programs are instituted, sampling should include those areas that are most likely to be contaminated, such as product contact surfaces of processing equipment, ventilation systems, process gases and purified water, and water systems. Sampling frequency can be based on historical trends and types of dosage forms manufactured, focusing in particular on those products more likely to be susceptible to microbial contamination. Manufacturing process, cleaning, and utilities validations should include microbial sampling to ensure microbial bioburden control. Holding times for process steps such as coating solutions and wet granulation should be determined by sampling and testing these matrices for microbial bioburden during validation and subsequent manufacturing.

General Recommendations Complying with GMP regulations specified in 21 CFR Part 211 [1] is the most effective way of controlling microbial bioburden in a nonsterile manufacturing environment as (as described under Section 6.3.2.1). Appropriate written procedures should be established to control microbial bioburden and prevent objectionable microorganisms. Microbiologists should be well trained in sampling and testing of the pharmaceutical manufacturing environment, so that microbial bioburden is not introduced into product or environmental samples during sampling and testing. Identification of isolates should be undertaken as applicable when action levels (or above acceptance criteria) are seen during quantitation of microbial bioburden.

6.3.6 MICROBIAL BIOBURDEN CONTROL DURING SHELF LIFE OF PHARMACEUTICAL PRODUCTS

GMPs require that the stability of pharmaceutical products be tested to evaluate the product integrity throughout the shelf life of the product [1, 6, 11]. The ICH guidance Q1A (R2), stability testing of drug substances and drug products [30], emphasizes that the stability testing should cover, as appropriate, the physical, chemical, biological, and microbiological attributes. In addition, for drug products, if antimicrobial preservatives are used, preservative content should also be investigated.

6.3.7 SUMMARY AND CONCLUSIONS

In nonsterile pharmaceutical manufacturing, control of microbial bioburden through appropriate design of manufacturing facilities and implementation of GMPs during manufacturing would help control microbial bioburden in pharmaceutical raw materials and finished products. The GMPs for finished product and its components (active and inactive ingredients) are discussed in this chapter to highlight expectations for contamination control during manufacturing. In support of GMP

expectations for contamination control and testing required to establish such control, pharmacopeia in the United States, Europe, and Japan provide test methods and procedures for determination of microbial bioburden, which are also summarized in this chapter. Manufacturers of pharmaceutical products through their trade associations have taken a proactive approach to microbial bioburden control in nonsterile manufacturing by publishing results from surveys of member companies and have made general recommendations for implementation of microbial bioburden control by member companies. A brief summary of the industry work group survey results on current industry practices and general recommendations for implementation is presented. The requirement for microbial bioburden monitoring throughout the shelf life of the products is also emphasized.

REFERENCES

1. U.S. Food and Drug Administration (FDA) (2007), Current good manufacturing practice regulations, 21 CFR Parts 210 and 211, FDA, Rockville, MD.
2. U.S. Food and Drug Administration (FDA) (1993), Guide to inspections of microbiological pharmaceutical quality control laboratories, FDA, Rockville, MD.
3. U.S. Pharmacopeia (USP) (2007), <1111> Microbiological examination of nonsterile products: Acceptance criteria for pharmaceutical preparations and substances for pharmaceutical use, U.S. Pharmacopeial Convention, Rockville, MD.
4. Pharmaceutical Research and Manufacturers Association of America (PhRMA) (1997, March), Microbiological monitoring of environmental conditions for nonsterile manufacturing, *Pharm. Technol.*, 58–74.
5. Australian Pharmaceutical Manufacturers' Association (APMA) (1990), *The Control of Microbial Contamination in Nonsterile Pharmaceutical Products for Human Use*, APMA, Canberra, Australia.
6. International Conference on Harmonization (ICH) (2001), Guidance for industry. Q7A good manufacturing practice guidance for active pharmaceutical ingredients, ICH, Brussels, Belgium.
7. Therapeutic Goods Administration Laboratories of Australia (TGAL) (1990), *Guidelines for Assessing the Results of Microbiological Tests on Nonsterile Pharmaceuticals for Human Use*, TGAL, Canberra, Australia.
8. Tang, S. (1998), Microbial limits reviewed—Basis for unique Australian regulatory requirements for microbial quality of nonsterile pharmaceuticals, PDA, *J. Pharm. Sci. Technol.*, 52, 100–109.
9. World Health Organization (WHO) (2003), Good manufacturing practices for pharmaceutical products, main principles, WHO Technical Report Series No. 908, WHO, Geneva, Switzerland.
10. International Pharmaceutical Excipients Council (IPEC) (2006), *Good Manufacturing Practices*, IPEC, Washington DC.
11. U.S. Pharmacopeia (USP) (2006), Good manufacturing practices for bulk pharmaceutical excipients, general chapter <1078>, U.S. Pharmacopeial Convention, Rockville, MD.
12. U.S. Pharmacopeia (USP) (2007), Microbiological examination of nonsterile products: Microbial enumeration tests <61>, U.S. Pharmacopeial Convention, Rockville, MD.
13. U.S. Pharmacopeia (USP) (2007), Microbiological examination of nonsterile products: Tests for specified microorganisms <62>, U.S. Pharmacopeial Convention, Rockville, MD.

14. U.S. Pharmacopeia (USP) (2007), Microbiological best laboratory practices, general chapter <1117>, U.S. Pharmacopeial Convention, Rockville, MD.
15. U.S. Pharmacopeia (USP) (2007), Validation of alternative microbiological methods, general chapter <1223>, U.S. Pharmacopeial Convention, Rockville, MD.
16. U.S. Pharmacopeia (USP) (2007), Application of water activity determination to nonsterile pharmaceutical products, general chapter <1112>, U.S. Pharmacopeial Convention, Rockville, MD.
17. U.S. Pharmacopeia (USP) (2007), Antimicrobial effectiveness testing, general chapter <51>, U.S. Pharmacopeial Convention, Rockville, MD.
18. U.S. Pharmacopeia (USP) (2007), Pharmaceutical dosage forms, general chapter <1151>, U.S. Pharmacopeial Convention, Rockville, MD.
19. U.S. Pharmacopeia (USP) (2007), Water for pharmaceutical purposes, general chapter <1231>, U.S. Pharmacopeial Convention, Rockville, MD.
20. Olson, P. W. (1997), Biofilms in the pipeline and in the patient, *J. Pharm. Sci. Technol.*, 51(6), 252–261.
21. European Pharmacopoeia (Eur. Ph.) (2005), Microbiological examination of nonsterile products (total viable aerobic count), general chapter 2.6.12, Council of Europe, Strasbourg Cedex, France.
22. European Pharmacopoeia (Eur. Ph.) (2005), Microbiological examination of nonsterile products (tests for specified microorganisms), general chapter 2.6.13, Council of Europe, Strasbourg Cedex, France.
23. European Pharmacopoeia (Eur. Ph.) (2005), Microbiological quality of pharmaceutical preparations, general chapter 5.1.4, Council of Europe, Strasbourg Cedex, France.
24. European Pharmacopoeia (Eur. Ph.) (2005), Efficacy of antimicrobial preservation, general chapter 5.1.3, Council of Europe, Strasbourg Cedex, France.
25. Japanese Pharmacopoeia (JP) (2001), Microbiological attributes of nonsterile pharmaceutical products, general information chapter 7, Society of Japanese Pharmacopoeia, Tokyo.
26. Japanese Pharmacopoeia (JP) (2001), Preservatives—Effectiveness tests, general information chapter 12, Society of Japanese Pharmacopoeia, Tokyo.
27. U.S. Food and Drug Administration (FDA) (1993), Guide to inspections of high purity water systems, FDA, Rockville, MD.
28. U.S. Food and Drug Administration (FDA) (2000), Guide to inspections of topical drug products, FDA, Rockville, MD.
29. U.S. Food and Drug Administration (FDA) (1994), Guide to inspection of oral solutions and suspensions, FDA, Rockville, MD.
30. International Conference on Harmonization (ICH) (2003), Guidance for industry, Q1A (R2) stability testing of new drug substances and products, ICH, Brussels, Belgium.

SECTION 7

DRUG STABILITY

7.1

STABILITY AND SHELF LIFE OF PHARMACEUTICAL PRODUCTS

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7.1.1 INTRODUCTION

Pharmaceutical drug products (e.g., tablets, capsules, creams, injectables, and inhalation products) and the components in a drug product, that is, the active pharmaceutical ingredient (API, drug substance), and the inactive ingredients (excipients) should be stable in the drug product for the proposed shelf life duration of the drug product and the proposed duration of the shelf life of the individual components. Shelf life (retest or expiration date) is the time period during which excipients, APIs, and drug products are expected to remain within approved shelf life specification, provided that they are stored under the conditions defined on the container label. Stability and storage conditions for excipients, APIs, and drug products are determined by evaluation of their quality parameters with time under the influence of a variety of environmental factors such as temperature, humidity, and light.

Stability applies to chemical, physical, microbiological, therapeutic, and toxicological properties. The U.S. Pharmacopeia (USP) [1] defined *stability* as the extent to which a product retains, within specified limits and throughout its period of storage and use (i.e., its shelf life), the same properties and characteristics that it possessed at the time of manufacture. For example, chemical stability implies that each active ingredient in a drug product retains its chemical integrity and label potency within the specified limits during the shelf life. Physical stability is assured if the original physical properties, including appearance, palatability, uniformity, dissolution, and suspendability, are retained during the shelf life. Microbiological stability is demonstrated if the sterility or resistance to microbial growth is retained according to the specified requirements and antimicrobial agents that are present retain effectiveness within the specified limits. The therapeutic effect of the drug should remain unchanged and no significant increase in toxicity should occur [1] during the shelf life if the product is stable.

Good manufacturing practice (GMP) regulations and guidelines allude to the importance of stability and shelf life of pharmaceutical products. The U.S. Food and

Drug Administration's (FDA's) GMP regulations for finished products require stability testing of drug products to assess the stability characteristics, the results of which are used for determining appropriate storage conditions and expiration dates [2]. The FDA's GMP guidance for APIs requires that a documented, on-going testing program should be established to monitor the stability characteristics of APIs and the results should be used to confirm the appropriate storage conditions and retest or expiry dates [3]. The USP guidance on GMPs for bulk pharmaceutical excipients suggests that there should be a documented testing program designed to assess the stability characteristics of excipients and the results of such stability testing should be used in determining appropriate storage conditions and their reevaluation (retest) or expiration date [4]. The GMP guideline for pharmaceutical products from the World Health Organization (WHO) [5] alludes to the stability of finished pharmaceutical products and starting materials.

In addition to GMP requirements, several guidelines are published specifically to address pharmaceutical stability. Organizations with international mandates such as the WHO [6] and International Conference on Harmonization [ICH, comprising principally the European Union (EU), Japan, and the United States] [7] have published stability guidelines. The stability guidance of the WHO [6] defines stability data package requirements for pharmaceutical products. In addition to addressing APIs and drug products, the WHO guidance also emphasizes that the stability of excipients that may contain or form reactive degradation products should be considered for stability testing. The ICH Q1A (R2) guidance published by the FDA [7] states that the purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light and to establish a retest period for the drug substance or a shelf life for the drug product and recommend storage conditions. The ICH has published a comprehensive series of stability guidances covering different aspects of stability: for example, ICH Q1A (R2), Stability Testing of New Drug Substances and Products [7]; ICH Q1B, Photostability Testing of New Drug Substances and Products [8]; ICH Q1C, Stability Testing of New Dosage Forms [9]; ICH Q1D, Bracketing and Matrixing Designs for Stability Testing of New Drug Substances and Products [10]; and ICH Q1E, Evaluation of Stability Data [11]. Some of these guidelines have been adopted and published by the FDA (see References).

In this chapter, the stability and shelf life of pharmaceutical excipients, APIs, and finished drug products are reviewed, with information derived from GMP regulations and guidelines as well as technical and regulatory guidance documents on stability. The importance of photostability studies for stability-indicating method development and for appropriate container closure selection to assure stability of the marketed product through shelf life is discussed. The evaluation and assessment of stability results for the determination of shelf life are discussed. The use of validated stability-indicating analytical methods for assessment of the potency of drug substance and the amount of degradation products and impurities through the shelf life is emphasized. Recommended storage conditions for various climatic zones are discussed. The testing parameters/attributes for evaluation of stability of various drug dosage forms are outlined. (The mechanisms and pathways of degradation of pharmaceutical products are not discussed in this chapter.)

7.1.2 STABILITY REQUIREMENTS IN GMP REGULATIONS/GUIDELINES

7.1.2.1 Finished Products

GMPs require assessment of stability characteristics of the pharmaceutical drug product, and the principles of such assessment are outlined in 21 CFR Part 211, Section 211.166 (a)(b) [2], and summarized in this section. The drug product stability samples should be stored in the same container closure as that in which the drug product is marketed. Appropriate storage conditions for samples designated for stability evaluation and reliable, specific, and meaningful test methods should be used. If a drug product is reconstituted at the time of dispensing as directed on the label, testing of the material for compliance with stability specifications is required pre- and post-reconstitution. Results from stability testing should be used to determine the appropriate storage conditions and expiration dates. The product stability estimates should be valid based on statistical evaluation of each stability attribute. Shelf life, expiration date, and storage conditions assigned for a drug product should be supported by long-term stability storage and testing of an adequate number of batches. When drug applications are submitted to the FDA, long-term storage stability studies may be in progress and data may not be available for the full proposed shelf life duration to support shelf life, expiration dating, or storage conditions. In such cases, tentative expiration dates can be established based on the accelerated stability studies, which are designed to increase the rate of chemical degradation or physical change by using exaggerated temperature and humidity conditions. In addition, other basic stability information on known physical and chemical stability attributes of excipients and APIs and container closure systems is also relevant for shelf life determination. Tentative expiration dates thus established must be supported by long-term storage stability studies.

7.1.2.2 Excipients

The GMPs for bulk pharmaceutical excipients [4] emphasize that the stability of excipients is an important contributing factor to the stability of the finished dosage form. Changes in raw materials used or changes in manufacturing procedures of excipients may affect their stability. In addition, the container closure packaging for excipients may vary widely to include but not be limited to metal and plastic drums, plastic bottles, and tank cars, which can affect the stability of the product inside. The stability characteristics of the excipient should be assessed using appropriate storage conditions and storage intervals and the testing should be performed to determine compliance with the established stability specifications. Results of such testing should be used to determine appropriate storage conditions and reevaluation or retest dates. Samples should be examined at or before retest date to ensure that the material is still in compliance with the specification and thus is suitable for its intended use. The excipient stability testing program should be ongoing and should be documented through a stability protocol that will include number of lots tested per year, sample size, test intervals, storage conditions (e.g., temperature, humidity), and test methods that are stability indicating. The materials used for container closures for stability sample storage (if the containers and closures are smaller than

those used for the marketed product) should be similar to the packaging materials used for marketed products. Storage time and conditions should support the conditions that are likely to prevail during the designated shelf life of the product. A model approach can be used for excipients that are available in different grades such as various molecular weights of a polymer or different monomer ratios or mixtures or blends of other excipients. Stability data available on model products can be used to determine the theoretical stability for similar products. In addition, for excipients that have been on the market for a long time, historical data may be used to assign the shelf life, retest dates, and storage conditions [4].

7.1.2.3 Active Pharmaceutical Ingredients

GMPs for APIs [3] require a documented stability storage and testing program to monitor the stability characteristics of APIs against established stability specifications. The results from stability studies are used to establish storage conditions and retest or expiry dates. For APIs, retest rather than expiration date terminology is commonly used. The GMP guideline also emphasizes that the assay procedures (for drug substance and degradation products) in stability samples should be validated and it should be stability indicating. A stability-indicating assay is defined as "a validated quantitative analytical procedure that can detect the changes with time in the pertinent properties of the drug substance and measures the active ingredient accurately without interference from degradation products, process impurities or other potential impurities" [12; Section III C].

The GMP API guideline also emphasizes that stability samples should be stored in containers and closures (the sum of packaging components that together contain and protect API or drug product dosage forms) that simulate the market container and should be consistent with storage conditions specified in the FDA stability guidance [7]. The GMP guideline requires that the first three commercial production batches should be placed on the stability program to confirm the retest or expiry date. If the API is expected to remain stable for at least two years, fewer than three batches are allowed. The stability program should be ongoing with at least one batch of API manufactured each year tested annually to confirm stability, with testing done more frequently for APIs with shorter shelf lives.

The WHO GMPs for pharmaceutical products [5] require that quality control evaluate the stability of finished pharmaceutical products and, when necessary, of starting materials and intermediate products. The guideline requires that a written stability program be developed and implemented to generate the stability data. Expiration dates and shelf life specifications should be based on stability data generated under defined storage conditions. The WHO guideline also specifies that stability should be determined prior to marketing and following any significant change in, for example, process, equipment, and packaging materials.

7.1.3 STABILITY REQUIREMENTS FOR EXCIPIENTS

In the absence of specific stability guidance from the FDA and ICH (and limited guidance from the WHO) for excipients, the USP GMP guidance for bulk pharmaceutical excipients [4] is used to review stability requirements for excipients. A

description of stability requirements for excipients is provided in Section 7.1.2.2. The principles underlying the evaluation of stability data and determination of shelf life (retest dates) for excipients is similar to that described for APIs and drug products in Section 7.1.7.

7.1.4 STABILITY REQUIREMENTS FOR DRUG SUBSTANCES (APIs)

The guidance for industry Q1A (R2) [7] defines requirements for API stability data package submission with drug applications in the EU, Japan, and the United States.

7.1.4.1 Selection of Batches and Container Closure System

Stability data should be generated on at least three primary batches, which should be manufactured to a minimum of pilot scale by the same synthetic route and manufacturing process as the production batches. The quality of the API placed on a formal stability program should be similar to the quality of the material to be made on a commercial production scale. The container closure system must be the same or simulate the packaging proposed for storage and distribution of marketed product.

7.1.4.2 Storage Conditions and Testing Frequency

The guidance ICH Q1A (R2) [7] provides information on storage conditions and testing frequency for APIs under four intended storage conditions: (1) general cases, (2) Intended for storage in a refrigerator, (3) Intended for storage in a freezer, and (4) drug products intended for storage below –20°C (for this storage no specific guidance is provided except to be treated on case-by-case basis). The storage conditions are summarized in Table 1.

Marketed API Intended for Room Temperature Storage Conditions The storage conditions defined in the guidance document [7] as a *general case* has no definition assigned in the guidance. In this chapter, the author is interpreting this term as requiring room temperature storage (vs. storage in refrigerator and freezer) conditions for the marketed API. The storage conditions (temperature and humidity) and lengths of storage for stability studies should be sufficient to cover the following stages after manufacture: storage, shipment, and subsequent use. For submission of drug application, the data for long-term storage should cover a minimum of 12 months on three primary batches. The testing should, however, continue for the duration of the proposed shelf life and retest period. The accelerated and, when necessary, intermediate (storage condition designed to moderately increase rate of chemical degradation or physical change) storage conditions should be carried out for 6 months. In this scenario, long-term storage can be conducted at either at 25°C ± 2°C; 60% relative humidity (RH) ± 5% RH or 30°C ± 2°C, 65% RH ± 5% RH. If the long-term stability study is conducted at 30°C ± 2°C, 65% RH ± 5% RH, no intermediate condition storage is required. If long-term storage studies are conducted at 25°C ± 2°C, 60% RH ± 5% RH and a significant change occurs in the test

TABLE 1 Storage Conditions for Stability Evaluation of APIs

Stability Study Type	Stability Storage Conditions	Minimum Time Period Covered by Data at Submission (months)
Marketed API Intended for Room Temperature (General Case) [7] Storage Conditions		
Long term	25°C ± 2°C, 60% RH ± 5% RH or 30°C ± 2°C, 65% RH ± 5% RH	12
Intermediate	30°C ± 2°C, 65% RH ± 5% RH	6
Accelerated	40°C ± 2°C, 75% RH ± 5% RH	6
Marketed API Intended for Storage in Refrigerator		
Long term	5°C ± 3°C	12
Accelerated	25°C ± 2°C, 60% RH ± 5% RH	6
Marketed API Intended for Storage in Freezer		
Long term	–20°C ± 5°C	12

Source: From ref. 7.

result at any time during the 6 months testing at the accelerated storage condition (40°C ± 2°C, 75% RH ± 5% RH), testing for established stability specifications at the intermediate storage condition (30°C ± 2°C, 65% RH ± 5% RH) should be conducted and evaluated against significant change criteria. The guidance document [7] defines *significant change* for an API as failure to meet its specification. If intermediate storage condition and testing become a necessity, a minimum of 6 months of data from this study should be submitted with the application.

The sampling frequency and testing in long-term stability studies should be targeted to generate data sufficient to establish a stability profile for the API. The guidance [7] recommends testing every 3 months over the first year, 6 months over the second year, and annually thereafter throughout the retest period under long-term storage condition. For a 6-month accelerated storage stability condition, sampling at 0, 3, and 6 months is recommended. When significant change to established test specification occurs under accelerated storage condition, sampling at time 0, 6, 9, and 12 is recommended for a 12-month intermediate storage condition.

Marketed API Intended for Storage in Refrigerator For an API intended for storage in a refrigerator (5°C ± 3°C), if a significant change occurs between three and six months testing at the accelerated storage condition of 25°C ± 2°C, 60% RH ± 5% RH, the proposed retest period should be based on the real-time data available at the long-term storage condition of 5°C ± 3°C [7]. On the other hand, if a significant change occurs within three months at the accelerated storage condition, the effect of short-term excursions outside the label storage condition during shipping or handling should be discussed. When significant change occurs during the first three months storage at accelerated condition, consideration of further storage or testing at this condition is not necessary. However, testing on a single batch of API for a period shorter than three months, with more frequent sampling during

three months may be necessary to narrow the period in which the API can be demonstrated to be stable [7].

Marketed API Intended for Storage in Freezer For an API with intended storage in a freezer (–20°C ± 5°C), the retest period should be based on the real-time data available at the long-term storage condition of –20°C ± 5°C [7]. Since no accelerated storage condition for a freezer-stored API is proposed [7], storage and testing of a single batch at elevated temperatures of 5°C ± 3°C or 25°C ± 2°C for an appropriate time period should be considered to understand the effect of short-term excursions outside the label storage condition during shipping or handling. Other storage conditions may be considered with appropriate justification for their selection.

7.1.4.3 Stress Studies and Stability-Indicating Methods for Analysis of API Stability

Appropriate physical, chemical, biological, and microbiological attributes of the API that are likely to be susceptible to change during storage and are likely to influence the quality, safety, and efficacy should be tested. The analytical testing performed to evaluate the stability of the API should be stability indicating as defined above under GMPs for APIs [3; Section 11.5]. The stability-indicating assay accurately measures the active ingredient without interference from degradation products, process impurities, excipients, or other potential impurities. In order to develop a stability-indicating method, it is necessary to conduct stress studies on an API. Stress testing is carried out on a single batch of the drug substance and the stresses can include acid (e.g., 0.2*N* HCl) and base (e.g., 0.2*N* NaOH) hydrolysis, temperature (10°C increments above the accelerated stability storage temperature of 40°C, e.g., 50°C, 60°C), humidity (75% RH or greater), photolysis (see Section 7.1.6), and oxidation (e.g., 10% H_2O) of the API [7]. Hydrolysis is a chemical transformation process whereby an organic molecule RX reacts with water (H_2O), resulting in direct replacement of X by OH. Among various stresses stated above, hydrolysis could be the most common stress leading to degradation of an API since amides, esters, and salts of weak acids and strong bases are well known to hydrolyze [13]. Photolysis is the process whereby chemicals are altered directly as a result of irradiation or indirectly through interaction with products of irradiation [13]. Carbonyl, nitroaromatic, N-oxide function, C—C double bond, weak C—H bond, suplfides, alkenes, polyenes, and phenols are functional groups that may react with light [14]. Oxidation reactions depend on several factors, including temperature, light, pH, oxygen concentration, impurities including metal ions (e.g., cupric, ferric), and the oxidizable component of the molecule [15].

The stress studies should demonstrate that impurities and degradants from the active ingredient do not interfere with the quantitation of the API [12]. Stress testing of the API, in addition to validating the stability-indicating power of the analytical method, can also help establish the degradation pathways and the intrinsic stability of the molecule [7].

7.1.4.4 Evaluation of API Stability Results

When the stability data for all three batches on stability at various sampling intervals show little variability from initiation of the stability study, the API can be considered

stable and no statistical analysis of the data are required. Where the data show API degradation with time, the time at which the 95%, one-sided confidence limit for the mean curve intersects the acceptance criteria should be defined. When the analysis shows small batch-to-batch variation, combining the data into an overall estimate is recommended by applying appropriate statistical tests to the slopes of regression lines and zero time intercepts for the individual batches. The overall retest period should be based on the minimum time a batch can be expected to remain within acceptance criteria. Appropriate statistical methods should be considered to test the goodness of the fit of the data on all batches and where applicable combined batches to the assumed degradation line or curve [7]. The stability evaluation should cover API content, levels of degradation products, and other appropriate attributes.

7.1.4.5 Stability Commitment

When the submission includes long-term stability studies on three batches of API covering the proposed retest period, a postapproval commitment is unnecessary. If, on the other hand, when at the time of approval long-term stability data for the primary batches do not cover the proposed retest period granted, a commitment should be made to continue the stability studies postapproval to firmly establish the retest period. If the submission includes data from stability studies on fewer than three production batches, a commitment should be made to continue these studies through the proposed retest period and to place additional production batches for a total of three and generate data through the proposed retest period. If the submission does not include stability data on the production batches, a commitment should be made to place the first three production batches on long-term stability studies through the proposed retest period.

7.1.4.6 Storage Statement and Labeling

A storage statement for the finished API should be prepared in compliance with the national and regional requirements. The established retest period, which is supported by the stability data, should be displayed on the certificate of analysis (COA) and as appropriate on the container label also.

7.1.5 STABILITY REQUIREMENTS FOR DRUG PRODUCTS

The guidance for industry Q1A (R2) [7] defines requirements for stability data package submission with drug applications in the EU, Japan, and the United States for drug products. Information on the chemistry of the API molecule and its degradation behavior from the stability studies of the API (see Section 7.1.4) should be useful in designing the stability program for the drug product, since one of the key measures of shelf life determination of the drug product is the stability of the API in the drug product formulation.

7.1.5.1 Selection of Batches and Container Closure System

Stability data should be generated on at least three primary batches of the drug product, with the manufacturing process simulating the production batches, with

formulation and quality specifications similar to those batches intended for marketing. The three batches of the drug product manufactured should use different batches of the drug substance where possible, at least two of three batches should be pilot scale (batch manufactured by a procedure fully representative of and simulating that applied to a full production-scale batch), and the third batch can be of the smaller size when justified [7].

The drug product for the stability studies should be packaged in the same container closure system as proposed for marketing of the drug product, and each individual strength and container size of the proposed packaging configuration should be placed on stability, unless bracketing and matrixing designs are used in compliance with ICH guidance for stability testing [10].

7.1.5.2 Bracketing and Matrixing

During the design of stability studies, bracketing and matrixing [10] may be used to achieve reduced testing while at the same time generating enough stability data for evaluation of shelf life.

In bracketing, the design may include reduction in storage and sampling of dosage strengths or container closure configuration. For example, in a three-batch stability study with dosage strengths of 50, 75, and 100 mg in 15-, 100-, and 150-mL high-density polyethylene (HDPE) containers, testing for 50- and 100-mg strengths in 15- and 150-mL container sizes may be adequate with no testing proposed for the 75-mg strength.

Matrixing design [10] may involve elimination of some stability sample pull time points to achieve reduced testing strategy. For example, a one-half reduction in time points eliminates one in every two time points from full study design, and one-third reduction eliminates one in every three time points. However, such a scenario must include full testing at initial, 12-month, and final time points under a 36-month shelf life study [10].

7.1.5.3 Storage Conditions and Testing Frequency

The drug product guidance Q1A (R2) [7] provides information on storage conditions and testing frequency for the drug products under six intended storage conditions: (1) general case (room temperature), (2) drug products packaged in impermeable containers, (3) drug products packaged in semipermeable containers, (4) drug products intended for storage in a refrigerator, (5) drug products intended for storage in a freezer, and (6) drug products intended for storage below –20°C (for this storage no specific guidance is provided except to be treated on a case-by-case basis). The storage conditions are summarized in Table 2.

Marketed Drug Product Intended for Room Temperature Storage Conditions The storage condition defined in the guidance document [7] as a *general case* has no definition assigned in the guidance and in this chapter is interpreted as room temperature storage (other than refrigerator and freezer) condition for the marketed drug product. The storage conditions (temperature and humidity) and lengths of storage for stability studies should be sufficient to cover the following stages after manufacture: storage, shipment, and subsequent use.

TABLE 2 Storage Conditions for Stability Evaluation of Drug Products

Stability Study Type	Stability Storage Conditions	Minimum Time Period Covered by Data at Submission (months)
Marketed Drug Product Intended for Room Temperature Storage Conditions		
Long term	25°C ± 2°C, 60% RH ± 5% RH or 30°C ± 2°C, 65% RH ± 5% RH	12 12
Intermediate	30°C ± 2°C, 65% RH ± 5% RH	6
Accelerated	40°C ± 2°C, 75% RH ± 5% RH	6
Marketed Drug Product Packaged in Semipermeable Containers		
Long term	25°C ± 2°C, 40% RH ± 5% RH or 30°C ± 2°C, 35% RH ± 5% RH	12
Intermediate	30°C ± 2°C, 65% RH ± 5% RH	6
Accelerated	40°C ± 2°C, no more than 25% RH	6
Marketed Drug Product Intended for Storage in Refrigerator		
Long term	5°C ± 3°C	12
Accelerated	25°C ± 2°C, 60% RH ± 5% RH	6
Marketed API Intended for Storage in Freezer		
Long term	–20°C ± 5°C	12

Source: From ref. 7.

For submission of drug application, the data for long-term storage should cover a minimum of 12 months on at least three primary batches. The primary batches are those used in a formal stability study from which the stability data are derived and submitted in a registration application for the purpose of establishing a retest period or shelf life/expiration date. The testing should, however, continue for the duration of the proposed shelf life and retest period. The accelerated and, when necessary, intermediate storage conditions should be carried out for 6 months. In this scenario, long-term storage can be conducted at either 25°C ± 2°C, 60% RH ± 5% RH or 30°C ± 2°C, 65% RH ± 5% RH. If the long-term stability study is conducted at 30°C ± 2°C, 65% RH ± 5% RH, no intermediate condition storage is required. If long-term storage studies are conducted at 25°C ± 2°C, 60% RH ± 5% RH and a significant change occurs in the test result at any time during the 6 months testing at the accelerated storage condition (40°C ± 2°C, 75% RH ± 5% RH), testing for established stability specifications at the intermediate storage condition (30°C ± 2°C, 65% RH ± 5% RH) should be conducted and evaluated against significant change criteria (Table 2).

The guidance document [7] defines *significant change* for drug product as one or more of the following: (1) a 5% change in assay from initial value or failure to meet the acceptance criteria for potency when using biological or immunological procedures; (2) any degradation product exceeding its acceptance criterion; (3) failure to meet the acceptance criteria for appearance, physical attributes, and functionality test (e.g., color, phase separation, resuspendability, caking, hardness, and dose delivery per actuation; under accelerated storage conditions, some changes in physical attributes may be expected, e.g., softening of suppositories and melting and possible phase separation in of creams); (4) failure to meet acceptance criterion for pH; and (5) failure to meet acceptance criteria for dissolution for 12 dosage units.

If intermediate storage and testing become a necessity, a minimum of 6 months of data from this study should be submitted with the application.

The sampling frequency and testing in long-term stability studies should be targeted to generate data sufficient to establish a stability profile of the drug product. For long-term storage conditions, the guidance [7] recommends testing every 3 months over the first year, 6 months over the second year, and annually thereafter through the proposed shelf life for drug products when the proposed shelf life is at least 12 months.

For a 6-month accelerated storage stability condition, sampling at 0, 3, and 6 months is recommended. When significant changes to established test specifications are likely to occur under the accelerated storage condition, increased testing is required with inclusion of a fourth sampling point. When significant changes to established test specifications occur under the accelerated storage condition, testing at the intermediate storage condition for 12 months with sampling at time 0, 6, 9, and 12 is recommended.

Drug Products Packaged in Impermeable Containers For drug products packaged in impermeable containers that provide a permanent barrier, moisture or solvent loss is not a concern and for such products stability studies can be conducted under any controlled or ambient humidity conditions.

Drug Products Packaged in Semipermeable Containers Stability studies for aqueous-based drug products packaged in semipermeable containers (containers that allow the passage of solvent, usually water, while preventing solute loss) should be conducted under conditions of low relative humidity and temperatures specified in Table 2. Stability attributes such as potential water loss and physical, chemical, biological, and microbiological stability should be evaluated.

If long-term storage studies are conducted at 25°C ± 2°C, 40% RH ± 5% RH and a significant change other than water loss occurs during the six months testing at the accelerated storage condition (40°C ± 2°C, 75% RH ± 5% RH), testing for established stability specifications at the intermediate storage condition (30°C ± 2°C, 65% RH ± 5% RH) should be conducted to evaluate the effect of 30°C (Table 2). While a significant change in water loss (5% water loss from initial value) alone under accelerated storage conditions need not prompt testing of samples under the intermediate storage condition, water loss through the proposed shelf life should be monitored to ensure that the drug product has no significant water loss during long-term storage at 25°C ± 2°C, 40% RH ± 5% RH.

Marketed Drug Product Intended for Storage in Refrigerator For a drug product intended for storage in a refrigerator, if a significant change occurs between three and six months testing at the accelerated storage condition of 25°C ± 2°C, 60% RH ± 5% RH, the proposed retest period should be based on the real-time data available at the long-term storage condition of 5°C ± 3°C. On the other hand, if a significant change occurs within three months at the accelerated storage condition, the effect of short-term excursions outside the label storage condition during shipping or handling should be discussed. When significant change occurs during the first three months storage at the accelerated condition, consideration of further storage or testing is not necessary. However, testing on a single batch of drug product for a period shorter than three months with more frequent sampling during the three months may be necessary to narrow the period in which the drug product can be demonstrated to be stable.

Marketed Drug Products Intended for Storage in Freezer For a drug product with intended storage in a freezer, the retest period should be based on the real-time data available at the long-term storage condition of –20°C ± 5°C. Since no accelerated storage condition for a freezer-stored API is proposed, storage and testing of a single batch at elevated temperatures of 5°C ± 3°C or 25°C ± 2°C for an appropriate time period should be considered to understand the effect of short-term excursions outside the label storage condition during shipping or handling. Other storage conditions may be considered with appropriate justification for selection.

7.1.5.4 Stress Studies and Stability-Indicating Methods for Analysis of Drug Product Stability

Appropriate physical, chemical, biological, and microbiological attributes, antimicrobial preservative and antioxidant content, and dosage functionality test (e.g., dose delivery system) of the drug product that are likely to be susceptible to change during storage and are likely to influence quality, safety, and efficacy should be tested. The analytical testing performed to evaluate the stability of the drug product should be validated and stability indicating [12]. The stability-indicating assay accurately measures the active ingredient in drug product without interference from degradation products, process impurities, excipients, or other potential impurities. In order to develop a stability-indicating method, it is necessary to conduct stress studies. Stress testing (studies undertaken to elucidate the intrinsic stability of the drug substance) can be carried out on the drug product similar to that described for APIs in Section 7.1.4. Degradation information obtained from stress studies for the active ingredient in the drug product should demonstrate the specificity of the assay, that is, that impurities and degradants from the active ingredient and drug product excipients do not interfere with the quantitation of the active ingredient in the drug product [12].

7.1.5.5 Evaluation of Stability Results

Data on stability of the drug products should be presented for all testing intervals and evaluated with physical, chemical, and microbiological, microbial preservative effectiveness, antioxidant effectiveness, and functionality tests as appropriate to the

drug product dosage form and all established specifications for the attributes being considered.

When the stability data for all three batches on stability at various sampling intervals show little variability from initiation of the stability study, the drug product can be considered stable and no statistical analysis of the data is required. Where the data show change in quantitative attributes (API and degradation amounts, dissolution rates), the time at which the 95% one-sided confidence limit for the mean curve intersects the acceptance criteria should be defined. When the analysis shows small batch-to-batch variation, combining the data into an overall estimate is recommended by applying appropriate statistical tests to the slopes of regression lines and zero-time intercepts for the individual batches. The overall shelf life should be based on the minimum time during the stability study a drug product can be expected to remain within acceptance criteria. Appropriate statistical methods should be considered to test the goodness of fit of the data on all batches and where applicable combined batches to the assumed degradation line or curve [7]. The stability evaluation should cover the levels of degradation products and other appropriate attributes. The mass balance, that is, whether the addition of assay value and degradation products add up to 100% of the initial value, should be considered taking into account the margin of analytical error of the method used. Details on stability evaluation are discussed in Section 7.1.7.

7.1.5.6 Stability Commitment

When the submission includes long-term stability studies on three batches of drug product covering the proposed shelf life, a postapproval commitment is unnecessary. If, on the other hand, when at the time of approval long-term stability data for the primary batches of drug product do not cover the proposed shelf life granted, a commitment should be made to continue the long-term stability studies (and six-month accelerated stability study if not performed), postapproval, to firmly establish the shelf life. If the submission includes data from stability studies on fewer than three production batches (batches manufactured at production scale by using production equipment in a production facility as specified in the application), a commitment to continue long-term studies through proposed shelf life and accelerated studies for six months for a total of three batches should be made. If the submission does not include stability data on the production batches, a commitment should be made to place the first three production batches on long-term stability studies through the proposed shelf life and accelerated studies for six months. When significant change occurs in the accelerated stability study on the primary batches (requiring a study under intermediate conditions), stability of commitment batches can be conducted at either the accelerated or intermediate condition. On the other hand, if a significant change occurs for the commitment batches under the accelerated condition, testing at the intermediate storage condition is also required for commitment batches. A formal stability commitment protocol should be in place for primary and commitment batches [7].

7.1.5.7 Storage Statement and Labeling

A storage statement for the finished drug product should be established in compliance with the national and regional requirements, which should be based on the

conclusions derived from stability evaluation. For drug products that cannot tolerate freezing, specific instructions should be provided. The established expiration date (shelf life) should be displayed on the COA and as appropriate on the container label [7].

7.1.6 PHOTOSTABILITY STUDIES

The stability guidance for drug substances and drug products [7] suggests that photostability testing should be an integral part of stress testing. The guidance on photostability testing [8] suggests that intrinsic stability of new drug substances and drug products should be evaluated to demonstrate that light exposure does not result in unacceptable change. The guidance [8] also recommends a systematic approach to stability testing, including on drug substance by direct exposure, and tests on the exposed drug product outside the the immediate (primary) pack. In the immediate (primary) pack, the packaging material is in contact with the drug substance or drug product and also includes any label associated with the primary pack. If change after exposure of drug product outside the immediate pack is not acceptable, testing on the immediate pack should be conducted. If change in the immediate pack exposed to light is not acceptable, photostability testing on the marketing pack (combination of immediate pack and other secondary packaging such as carton) should be conducted. If the change in the marketed pack exposed to light is not acceptable, the packaging configuration should be redesigned or the product may require reformulation and photostability testing performed under the redesigned scenario.

The photostability guidance [8] provides recommendations for the light sources to which the API or drug product should be exposed. Two light exposure options are suggested. Option 1 includes light output similar to internationally recognized standards for outdoor light (D65) or indoor indirect light (ID 65), defined in the Internation Organization for Standardization (ISO) 10977. The light sources covered under option 1 are the artificial daylight fluorescent lamp with a combination of ultraviolet (UV) and visible outputs, xenon, or metal halide lamps. In option 2, it is recommended that the sample should be exposed to both the cool white fluorescent and near-UV lamp. The cool white fluorescent lamp should comply with the outputs specified in ISO 10977. It is recommended that the near-UV fluorescent lamp having a spectral distribution from 320 to 400 nm with a maximum energy emission between 350 and 370 nm be used with a significant portion of UV in both bands, that is, 320–360 and 360–400 nm. For confirmatory studies, the samples should be exposed to light providing an overall illumination of not less than 1.2 million lux hours and an integrated near-UV energy of not less than 200 Wh/m^2. Use of a validated chemical actinometric system is recommended to ensure that the samples are exposed to desired light exposure by exposing actinometer solutions to light side by side with the drug substance or drug product samples. Light exposure also should be measured using calibrated radiometers or lux meters. Dark controls (covered with an aluminum foil to protect from light) should also be placed side by side with the light-exposed samples. The guidance provides important references on chemical actinometers [16], interlaboratory studies by industry and FDA [17], and perspectives from FDA scientists [18] on photostability testing of pharmaceutical products. In addition, a reference on forced degradation testing (including but not limited to light stress) of ibuprofen bulk drug and tablets by Farmer et al. [19] illustrates the

differences in degradation of ibuprofen as neat API and ibuprofen in association with excipients in drug product. A reference is also provided to illustrate how different light sources and intensities effect the degradation of a light-sensitive compound valerophenone [20].

7.1.6.1 Photostability of APIs

Photostability studies of a drug substance can provide an overall evaluation of the sensitivity of the drug substance to light. Because the exposure levels under options 1 and 2 described above can facilitate worst-case exposure (forced degradation or light stress) of a drug substance, extensive degradation is likely to be seen, especially if the drug substance has functional groups that are sensitive to photooxidation. If degradation of APIs occurs during photostability study, that may present an opportunity for development of a stability-indicating method. Photostability of the drug substance may be carried out on its neat form or in solutions with samples placed in chemically inert and transparent containers for exposure (e.g., quartz glass). Depending on the known photosensitivity of the drug substance (i.e., based on the functional groups that are susceptible to oxidation or known absorbance since compounds in the 290–800-nm range are likely to undergo degradation [13]), the intensity of the light exposure and length exposed can be controlled to achieve the desired degradation in a forced degradation study. It is also important to partition the temperature effects on degradation of the drug substance from light effects since elevated temperatures seen under light exposure may influence degradation independent of light exposure. Appropriate temperature controls should be used. Changes in physical state such as melting, sublimation, and evaporation should be minimized. Use of quartz glass containers are recommended for photostability studies. An appropriate amount of solid APIs can be spread in a container with thickness of approximately 3 mm. Liquids can be exposed in appropriate containers that are closed tightly. Appropriate dark controls (containers covered with aluminum foil and placed side by side along the exposed samples) should be used. Analysis of samples should include both the exposed and dark controls and the analysis should be performed concomitantly. Parameters examined can include physical properties such as appearance and color of solids and, if a solution is exposed, the clarity and color of solution. The assay and degradation products should be evaluated using validated stability-indicating methods that clearly separate the degradation products and impurities from the drug substance in the sample chromatograms.

The confirmatory studies with drug substance should be designed to identify precautionary measures needed to protect the API from light during formulation or manufacturing of the drug product, especially if the drug substance is found to degrade under light exposure. These studies also should help in the design of primary and secondary packaging material for commercial packing of the API.

7.1.6.2 Photostability of Drug Products

Drug product should be exposed to light conditions as stated above. The testing should be done in a sequential manner with fully exposed drug product and as necessary followed by testing the product in the immediate pack and then in the

marketing pack [8]. Results of the photostability study should demonstrate that the drug product is adequately protected from light during the shelf life. The testing should include one batch during the developmental phase followed by confirmation of the photostability characteristics using one batch. Additional two batches should be conducted if the results in the confirmatory study with one batch are similar to the developmental study. Confirmatory studies should establish the photostability characteristics of the drug product under standardized conditions. Results from these studies should help in (1) identification of precautionary measures needed during manufacture and packaging, (2) container closure design for protection from light, and (3) storage conditions and light protection required during shelf life of the marketed product. While keeping the light exposure constant during the exposure period, care should be taken to control the temperature to ensure that the degradation products observed are light exposure related. Physical characteristics of the samples under test conditions should be monitored to ensure that changes in the physical state of the drug product are minimized. Dark controls (unexposed controls) should be tested side by side with the light-exposed samples. Maximum light exposure under direct exposure for solid oral dosage forms such as tablets and capsules can be achieved by spreading them in a single layer. Quartz glass containers are recommended as containers for direct exposure. Based on the results obtained during direct exposure of the drug product, if immediate and marketing pack exposure becomes necessary, samples should be placed in such a manner as to facilitate uniform exposure. Key physical parameters to be examined should include appearance, clarity and color of solutions, dissolution, and disintegration. Quantitation of drug substance, degradation products of drug substance (known and unknown), impurities, and excipients present in the samples of drug product from photostability studies should be performed using validated stability-indicating methods. It is important to sample representative exposed or dark control samples to make a realistic assessment of the photostability of the various dosage forms. Solutions, suspensions, and creams should be examined to ensure that there is no settling or partitioning of phases and that uniform and homogenous samples are analyzed.

7.1.7 EVALUATION OF STABILITY DATA AND SHELF LIFE DETERMINATION

Requirements for stability data evaluation and shelf life determination are described in the guidance for industry—ICH Q1E [11], which covers evaluation of stability data that should be submitted in registration applications for new molecular entities and associated drug products. It also provides recommendations for the establishment of retest periods and shelf lives for drug substances and drug products intended to be stored at room temperature, in refrigerator or freezer, and below –20°C. In addition to the guidance document, studies by Grimm [21, 22] should be reviewed which laid the foundation for testing and evaluation of stability data.

7.1.7.1 Shelf Life Estimation for Drug Substances or Drug Products Intended for Room Temperature Storage

The stability data evaluation for drug substance or drug product intended for storage at room temperature should include evaluation of any significant change at

the various time points during accelerated stability study and at intermediate storage conditions when they are used and evaluation of long-term stability data. Different scenarios for retest period and shelf life determination are presented based on the results observed under accelerated, intermediate, and long-term storage conditions [11], as described below.

Long-Term and Accelerated Stability Data Show Little or No Change over Time and Little or No Variability The drug substance or drug product can be considered stable if the long-term and accelerated stability data show little or no change or little no variability over time. In this scenario, no statistical analysis is required but a justification should include a discussion of the pattern of change or variability or lack of change or variability, supportive accelerated stability data, mass balance of drug substance in samples, and/or other supporting data. The retest period or shelf life in this scenario can be up to twice as long as but not more than 12 months beyond the period covered by long-term storage stability data. However, extrapolation of the retest period or shelf life beyond the period covered by long-term data can be proposed.

Long-Term and Accelerated Stability Data Show Change over Time and/or Variability In this scenario, statistical analysis of the long-term data can be useful in establishing a retest period or shelf life. When there are differences, for example, among batches or among dosage strengths, container sizes, and/or fill or any combination thereof that preclude combining the data, the proposed retest date or shelf life should not exceed the shortest period supported by long-term studies of any batch, other factors, or combinations of factors. However, when differences are attributed, for example, to strength of the dosage form, different shelf lives can be assigned to different strengths within the dosage form of the drug product, with appropriate discussion of the data generated. If the statistical analysis is performed on long-term data and the statistical and other relevant data are supportive, it can be appropriate to extrapolate and propose a retest period or shelf life up to twice as long but not more than 12 months beyond the period covered by long-term storage stability data. When long-term stability data are not amenable to statistical analysis, the proposed retest period or shelf life can be up to one and a half as long as but not more than 6 months beyond the period covered by long-term data.

Accelerated Stability Data Show Significant Change When a significant change occurs at the accelerated storage condition, stability results at the intermediate condition and the long-tem storage condition will determine the retest period or shelf life. The guidance [11] suggests two exceptions to the observations at accelerated conditions: (1) physical change such as softening of a suppository designed to melt at 37°C if the melting point is clearly demonstrated and (2) failure to meet acceptance criteria for dissolution for 12 units of gelatin capsules or gel-coated tablets if the failure can be unequivocally attributed to gelatin cross-linking. These exceptions do not apply to physical change as exemplified by phase separation of a semisolid dosage form, creams, or other occurrences at accelerated conditions, and if such a change occurs, testing at the intermediate condition should be performed.

When no significant change is observed at the intermediate condition, extrapolation beyond the period covered by long-term data can be proposed when statistical analysis data and relevant supporting data back up such extrapolation. The proposed retest period or shelf life can be up to one and a half as long as but not more than six months beyond the period covered by long-term data. When long-term stability data are not amenable to statistical analysis but the relevant supportive data are available, the proposed retest period can be up to three months beyond the period covered by long-term data.

In a scenario where significant change occurs at the intermediate condition, the retest period or shelf life shorter than the period covered by long-term data is appropriate but should not exceed the period covered by long-term data.

7.1.7.2 Shelf Life Estimation for Drug Substances or Drug Products Intended for Storage in a Refrigerator

Long-Term and Accelerated Stability Data Show Little Change over Time and/or Variability Under this scenario, the proposed retest period or shelf life can be up to one and a half times as long as but not more than six months beyond the period covered by long-term data without the support of statistical analysis.

Long-Term and Accelerated Stability Data Show Change over Time and/or Variability Under this scenario, the proposed retest period and shelf life can be up to one and a half times as long as but not more than six months beyond the period covered by long-term stability data when backed by statistical analysis and supporting data. On the other hand, the proposed retest period and shelf life can only be up to three months beyond the period covered by long-term stability data if the long-term data are amenable to statistical analysis but the statistical analysis is not performed or the long-term data are not amenable to statistical analysis but data supporting the proposed retest date and shelf life are available.

Accelerated Stability Data Show Significant Change or Variability Under this scenario, if significant change occurs during accelerated stability study between three and six months, the proposed retest period or shelf life should be based on the long-term data, and extrapolation is not considered appropriate, but the retest period or shelf life shorter than that supported by long-term stability data is considered appropriate. In the case of variability in the long-term stability data, verification of the proposed retest period or shelf life by statistical analysis is considered appropriate. On the other hand, if significant change occurs during accelerated stability study within the first three months, the proposed retest period or shelf life should be based on the long-term data, and extrapolation is not considered appropriate, but the retest period or shelf life shorter than that supported by long-term stability data is considered appropriate. Conducting a stability study at accelerated conditions for shorter than three months should be considered under this scenario at least for one batch with more frequent sampling and analysis.

7.1.7.3 Shelf Life Estimation for Drug Substances or Drug Products Intended for Storage in a Freezer

In this scenario, the retest period or shelf life should be based on long-term data.

7.1.7.4 Shelf Life Estimation for Drug Substances or Drug Products Intended for Storage below –20°C

In this scenario, the retest period or shelf life should be based on long-term data.

7.1.8 ASSIGNMENT OF CLIMATIC ZONES AND RECOMMENDED STORAGE CONDITIONS

In June 2004, the FDA issued a guidance document to the industry on stability data packaging for registration applications in climatic zones III and IV [23] (adopted from the ICH guidance Q1F). The guidance provided an update to the stability storage conditions for climatic zones I and II and provided new guidance for climatic zones III and IV based on recommendations provided in the WHO stability guidance [24]. The WHO guidance [24] described stability testing recommendations and storage conditions for all four climatic zones, considering Grimm's [25] recommendation for climatic zones III and IV. The QIF guidance [23] clarified that the long-term storage condition for climatic zones I and II is 25°C ± 2°C, 60% RH ± 5% RH, and the intermediate storage condition for climatic zones I and II is 30°C ± 2°C, 65% RH ± 5% RH. It also stated [23] that the storage condition of 30°C ± 2°C, 65% RH ± 5% can also be a suitable alternative for the long-term storage condition of 25°C ± 2°C, 60% RH ± 5% RH for climatic zones I and II, in which case no intermediate condition is required. For climatic zones III and IV, the Q1F guidance [23] suggested using 30°C ± 2°C, 65% RH ± 5% (for products intended to be stored at room temperature; general case stated in the guidance) as the long-term storage condition (12 months) with accelerated storage at 40°C ± 2°C, 75% RH ± 5% (6 months), and further no intermediate storage condition was recommended for climatic zones III and IV. For aqueous-based drug products packaged in semipermeable containers, the guidance [23] recommended 30°C ± 2°C, 35% RH ± 5% RH for long-term (12 months) and 40°C ± 2°C and not more than 25% RH ± 5% RH for accelerated storage (6 months). The WHO Expert Committee on Specifications for Pharmaceutical Preparations in its fortieth meeting, held at Geneva, Switzerland, in October 2005, recommended to split the climatic zone IV into zone IVA with long-term storage condition remaining at 30°C, 65% RH, whereas for climatic zone IVB, the long-term storage condition recommended was 30°C, 75% RH [26]. In Annex 1 of working document QAS/06.179 (2006) [26], the WHO defined the five climatic zones and proposed long-term testing conditions for each zone, which are summarized in Table 3. Following the WHO guidance [26] publication in 2006, the FDA Q1F [23] was withdrawn in 2006, with future revision of Q1F possible.

7.1.9 STABILITY TESTING PARAMETERS FOR DIFFERENT DOSAGE FORMS

The testing parameters published by the WHO as Annex 2 to working document QAS/06.179 in 2006 [27] is the basis for the information summarized in this section for various dosage forms. Although the parameters listed here provide guidance for various dosage forms, pharmacopeial and those approved by regulatory authorities

TABLE 3 Definition of Climatic Zones and Recommended Long-Term Stability Conditions

Climatic Zone	Definition	Criteria [Mean Annual Temperatures Measured in Open Air (°C) and Mean Annual Partial Vapor Pressure (hPa)]	Long-Term Testing Condition [Temperature (°C) and RH]
I	Temperate climate	≤15°C, ≤11 hPa	21°C, 45% RH
II	Subtropical and mediterranean climate	>15–22°C, >11–18 hPa	25°C, 60% RH
III	Hot and dry climate	>22°C, ≤15 hPa	30°C, 35% RH
IVA	Hot and humid climate	>22°C, >15–27 hPa	30°C, 65% RH
IVB	Hot and very humid climate	>22°C, >27 hPa	30°C, 75% RH

Source: From ref. 26.

in drug applications should be considered in applying the information described below for various dosage forms.

7.1.9.1 Stability Tests Common to All Dosage Forms

Appearance, assay, and degradation products, preservative, and antioxidant content as applicable should be considered for all dosage forms. The microbial bioburden of sterile dosage forms must be controlled and tested in compliance with pharmacopeial and/or internal specifications. The microbial bioburden of nonsterile dosage forms should be controlled, with appropriate sampling and testing.

7.1.9.2 Stability Tests Specific to Dosage Forms

Tablets Dissolution (or disintegration, if justified), water content, hardness/friability.

Hard Gelatin Capsules Brittleness, dissolution (or disintegration, if justified), water content, and microbial bioburden.

Soft Gelatin Capsules Dissolution (or disintegration, if justified), microbial bioburden, pH, leakage, and pellicle formation.

Emulsions Phase separation, pH, viscosity, microbial bioburden, mean size and distribution of dispersed globules.

Oral Solutions and Suspensions Formation of precipitate, clarity for solutions, pH, viscosity, microbial bioburden, extractables, and polymorphic conversion when applicable. Additional tests for suspensions include redispersability, rheological properties, mean size, and distribution of particles.

Powders for Oral Solutions and Suspensions Water content, reconstitution time, and reconstituted solutions and suspensions should be tested as above for oral solutions and suspensions.

Topical, Ophthalmic, and Otic Preparations Clarity, homogeneity, pH, resuspendability (for lotions), consistency, viscosity, microbial bioburden, and water loss should be tested. For ophthalmic and otic products additional attributes should include sterility, particulate matter, and extractables.

Suppositories Softening range, dissolution at 37°C.

Transdermal Patches In vitro release rates, leakage, microbial bioburden/sterility, and peel and adhesive forces.

Metered-Dose Inhalers and Nasal Aerosols Content uniformity, aerodynamic particle size distribution, microscopic evaluation, water content, leak rate, microbial bioburden, valve delivery, extractables, leachables from plastic and elastomeric components.

Nasal Sprays Clarity, microbial bioburden, pH, particulate matter, unit spray medication content uniformity, droplet and/or particle size distribution, weight loss, pump delivery, microscopic evaluation of suspensions, particulate matter, extractables, leachables from plastic and elastomeric components of container closure and pump.

Small-Volume Parenterals Color, clarity of solutions, particulate matter, pH, sterility, endotoxins. Powders for injection solutions include clarity, color, reconstitution time and water content, pH, sterility, endotoxins/pyrogens, and particulate matter. Suspensions for injection should include additional particle size distribution, redispersability, and rheological properties. Emulsion for injection should include phase separation, viscosity, mean size, and distribution of dispersed globules.

Large-Volume Parenterals Color, clarity, particulate matter, pH, sterility, endotoxin/pyrogen, and volume.

7.1.10 SUMMARY AND CONCLUSIONS

Studies on the stability of pharmaceutical products provide information on how the quality of excipients, APIs, and drug products varies with time under the influence of various environmental factors such as temperature, humidity, and light and help determine shelf life and recommended storage conditions for the life cycle of products. Good manufacturing practice regulations and GMP guidelines require that stability studies on pharmaceutical products be conducted and shelf life be determined based on the results from stability studies. Accelerated stability studies at elevated temperature and humidity and long-term stability studies at more moderate temperature and humidity conditions are performed during drug development and approval process to predict the shelf life of pharmaceutical products. The proposed shelf life is then confirmed by performing long-term studies for the duration of the shelf life or longer. Stress studies using acid, base, temperature, oxidation, and light stresses are conducted to predict the degradation products that may be formed during the accelerated and/or long-term studies and to develop stability-indicating methods required for stability evaluation. Photostability and temperature studies also help determine the packaging configuration as well as make recommendations for storage conditions during the shelf life. For a realistic assessment of shelf life in diverse climates that exist around the world, five climatic zones are identified and

the long-term storage condition for shelf life determination in these climatic zones is based on humidity and temperature conditions likely to prevail in those climatic zones. Quality parameters for evaluation of stability of pharmaceutical products depend on the chemical nature of the active ingredient being studied as well as the type of dosage form of the drug product.

REFERENCES

1. U.S. Pharmacopeia (2006), Stability considerations in dispensing practices, general chapter ⟨1191⟩, U. S. Pharmacopeial Convention, Rockville, MD.
2. U.S. Food and Drug Administration (FDA) (2007) Current good manufacturing practice regulations, 21 CFR Parts 210 and 211, FDA, Rockville, MD.
3. U.S. Food and Drug Administration (FDA) (2001), Guidance for industry, Q7A Good manufacturing practice guidance for active pharmaceutical ingredients, FDA, Rockville, MD.
4. U.S. Pharmacopeia (2007), Good manufacturing practices for bulk pharmaceutical excipients, general chapter ⟨1078⟩, U.S. Pharmacopeial Convention, Rockville, MD.
5. World Health Organization (WHO) (2003), Good manufacturing practices for pharmaceutical products: Main principles, WHO Technical Report Series 908, WHO, Geneva.
6. World Health Organization (WHO) (2006), Stability testing of active substances and pharmaceutical products, Working Document QAS/06.179, WHO, Geneva.
7. U.S. Food and Drug Administration (FDA) (2003), Guidance for industry, ICH Q1A (R2), Stability testing of new drug substances and products, FDA, Rockville, MD.
8. U.S. Food and Drug Administration (FDA) (1997), Guidance for industry, ICH Q1B, Photostability testing of new drug substances and products, FDA, Rockville, MD.
9. International Conference on Harmonization (ICH) (1997), Guidance for industry, ICH Q1C, Stability testing of new dosage forms, ICH, Brussels.
10. International Conference on Harmonization (ICH) (2003), Guidance for industry, ICH Q1D, Bracketing and matrixing designs for stability of drug substances and drug products, ICH, Brussels.
11. U.S. Food and Drug Administration (FDA) (2004), Guidance for industry, ICH Q1E, Evaluation of stability data, FDA, Rockville, MD.
12. U.S. Food and Drug Administration (FDA) (2000), Guidance for industry, Analytical procedures and methods validation—Chemistry, manufacturing, and controls documentation, FDA, Rockville, MD.
13. Lymann, W. J., Reehl, W. F., and Rosenblatt, D. H. (1982), *Handbook of Chemical Property Estimation Methods*, McGraw-Hill, New York.
14. Albini, A., and Fasani, E. (1998), *Photochemistry of Drugs: An Overview of Practical Problems, Drugs, Photochemistry and Photostability*, The Royal Society of Chemistry, London.
15. Carstensen, J. T. (1990), *Drug Stability—Principles and Practices*, Marcel Dekker, New York.
16. Drew, H. D., Brower, J. F., Juhl, W. E., and Thornton, L. K. (1998), Quinine photochemistry: A proposed chemical actinometer system to monitor UV exposure in photostability studies of pharmaceutical drug substances and drug products, *Pharmacopeial Forum*, 24(3), 6334.

17. Drew, H. D., Thornton, L. K., Juhl, W. E., and Brower, J. F. (1998), An FDA/PhRMA interlaboratory study of the International Conference on Harmonization's proposed photostability testing procedures and guidelines, *Pharmacopeial Forum*, 24(3), 6317.
18. Sager, N., Baum, R. G., Wolters, R. J., and Layloff, T. (1998), Photostability studies of pharmaceutical products, *Pharmacopeial Forum*, 24(3), 6331.
19. Farmer, S., anderson, P., Burns, P. K., and Velagaleti, R. (2002, May), Forced degradation of ibuprofen in bulk drug and tablets and determination of specificity, selectivity, and the stability-indicating nature of the USP ibuprofen assay method, *Pharm. Technol.*, 28–42.
20. Farmer, S., McCauslin, L., Burns, P. K., and Velagaleti, R. (2004, Aug.), Photosensitivity of internal standard valerophenone used in USP ibuprofen bulk drug and tablet assay and its effect on quantitation of ibuprofen and its impurities, *Pharm. Technol.*, 68–74.
21. Grimm, W. (1985), Storage conditions for stability testing—Long term testing and stress tests (part I), *Drugs Made in Germany*, 28, 196–202.
22. Grimm W. (1986), Storage conditions for stability testing—Long term testing and stress tests (part II), *Drugs Made in Germany*, 29, 39–47.
23. U.S. Food and Drug Administration (FDA) (2004), Guidance for industry, ICH Q1F, Stability data package for registration applications in climatic zones III and IV, FDA, Rockville, MD.
24. World Health Organization (WHO) (2001), Stability testing of pharmaceutical products containing well established drug substances in conventional dosage form, WHO Technical Report Series 863, Annex 5, WHO, Geneva.
25. Grimm W. (1998), Extension of International Conference on Harmonization tripartite guideline for stability testing of new drug substances and products to countries of climatic zones III and IV, *Drug Dev. Ind. Pharm.*, 24, 313–325.
26. World Health Organization (WHO) (2006), Assignment of climatic zones and recommended storage conditions, Working Document QAS/06.179, Annex 1, WHO, Geneva.
27. World Health Organization (WHO) (2006), Testing parameters (for dosage forms), Working Document QAS/06.179, Annex 2, WHO, Geneva.

7.2

DRUG STABILITY

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References

Pharmaceutical Manufacturing Handbook: Regulations and Quality, edited by Shayne Cox Gad
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7.2.1 OVERVIEW OF STABILITY

7.2.1.1 Introduction

Every drug product on the market requires the expiration date to be specified on the immediate container label [1]. Regulations from the U.S. Food and Drugs Administration (FDA) and the International Conference on Harmonization (ICH) require that pharmaceutical companies present factual evidence supporting the shelf life for either existing or new products. The presence of the right amount of the active ingredients in a pharmaceutical formulation is extremely important for the drug product to be an effective medicament. A rigorous protocol is usually implemented to measure the amount of the active ingredient through time for a given drug product and the collected data are analyzed to estimate the shelf life, which leads to the calculation of the expiration date. Thus, the expiration date provides the consumer the confidence that the drug product will retain its identity, strength, quality, and purity throughout the expiration period.

The main purpose of this chapter is to describe a set of practical tools to design a stability study as well as to discuss and illustrate how to determine the shelf life for a given drug product. This chapter is organized as follows. This section presents a general overview of the stability studies and considers the current regulatory requirements. The stability study not only characterizes the degradation of the active ingredient for a drug product over time but also provides the basis to establish the shelf-life period. This section will also present a brief description of short- and long-term stability studies and the important statistical requirements and issues stated in the FDA and ICH guidelines. Section 7.2.2 presents the fundamentals for designing a stability study. The design of a stability study intends to establish a procedure to extract reliable data to determine the shelf life, which is based on testing a limited number of batches of a drug product and will be applicable to all future batches of the drug product that will be manufactured under similar conditions. Designing long-term stability studies usually involves the determination of the following factors: number of batches, strength, packaging configurations, and storage conditions. Essentially, the stability process design includes the application of matrixing and bracketing design. Section 7.2.3 presents a description of the conventional procedure to determine the shelf life, which is established based on long-term stability studies that are conducted under normal storage conditions. Calculation of a drug product shelf life for single and multiple batches will be discussed and illustrated by examples. A set of computer programs are included in the Appendix to carry out the stability calculations. Section 7.2.4 discusses the accelerated stability testing procedure, which is often used to provide a tentative shelf life. At the early stage of a drug product development, the primary goal of the stability studies is to determine the rates of chemical and physical reactions and their relationships with storage conditions such as temperature, moisture, light, and others. A short-term stability study is conducted under stressed conditions to increase the rate of chemical and physical degradation of the drug product. This section will discuss the chemical reaction kinetics and the statistical procedure to establish a preliminary shelf life. This section will cover the regression techniques to estimate the parameters of the Arrhenius equation.

7.2.1.2 Regulatory Requirements

The FDA requires pharmaceutical industries to establish a stability testing program for each drug product [1–3]. Matthews [4] reviews the regulatory situation in Europe. The purpose of the stability program is to design the appropriate procedure to extract the assay data and calculate the shelf life to determine the expiration date of a drug product. The program should be described in a written protocol including all the requirements established by the regulation. The FDA establishes that the requirements to assess stability are the following [1]:

1. A sample size and test intervals for each attribute to be tested should be specified based on statistical rationale.
2. The storage conditions for the samples should be specified. The storage conditions should be the same as those specified in the drug product labeling.
3. All test methods used for each of the drug product attributes need to be qualified to demonstrate adequacy and reliability.
4. The stability samples should be stored in the same packaging configuration used for the final drug product to be marketed.
5. If the drug product needs to be reconstituted, testing of the drug product before and after reconstitution should be provided.
6. The number of batches to be used for the stability program for a new drug product is at least three. Different batches of the drug substance should be used.
7. The pharmaceutical industry should maintain records for all data, protocols, and reports related to the stability program.

7.2.1.3 Stability and Shelf Life

The shelf life is the period of time for which the drug product is assured to maintain its identity, strength, quality, and purity when stored at the conditions specified on the labeling. The expiration date marks the end of the shelf life period. The shelf life and the expiration date for a drug product are determined by carrying out a stability study. The stability of the drug product is assessed by testing all the attributes required to release the product to the market. The tests are carried out at specified time intervals for a determined period of time. The expiration date is usually included on the label of the drug product.

7.2.1.4 Short- and Long-Term Stability Studies

The short-term stability study is conducted over extreme environmental conditions to increase the rate of chemical degradation. The data obtained from short-term stability studies are normally used to evaluate longer term chemical effects at non-extreme storage conditions, but they are also helpful to assess the effect of short-term excursions outside the recommended storage conditions that might occur during shipping. However, the results from short-term stability studies cannot always

be used to predict physical changes of the drug product. The recommended testing frequency should be a minimum of three time points, including the initial and final time points, for a six-month study. If significant changes are expected, additional or more frequent testing should be conducted as well as stability testing at intermediate conditions. The actual extreme and intermediate conditions depend on the recommended storage conditions for drug product, for example, room temperature or under refrigeration. A significant change occurs if one or more of the following events take place:

1. A 5% change in assay from its initial value or failure to meet the acceptance criteria for potency when using biological or immunological procedures.
2. Presence of any impurity at a level exceeding its acceptance criterion.
3. Failure to meet an acceptance criterion for appearance, physical attribute, or functionality test (e.g., color, phase separation, resuspendibility, caking, hardness, or dose delivery per actuation). However, some changes in physical attributes (e.g., softening of suppositories, melting of creams) may be expected under accelerated conditions.
4. Failure to meet the acceptance criterion for pH.
5. Failure to meet the acceptance criteria for dissolution of 12 dosage units.

The long-term stability study is performed under the recommended storage conditions and packaging configuration to be used for marketing the drug product and during the shelf life period, which is displayed on the product label. The recommended testing frequency should be every three months over the first year, every six months over the second year, and annually thereafter. The general storage conditions used for long-term, intermediate, and short-term stability studies for climatic regions I and II, which includes Europe, Japan, and the United States [5, 6], are shown in Table 1. Alternate storage conditions for long-term, intermediate, or short-term stability study could be used if justified.

TABLE 1 Storage Conditions for Stability Studies

Study Condition	Storage Conditions	Minimum Time Period Covered at Submission (Months)
	Product Stored at Room Temperature	
Long Term	25°C ± 2°C, 60% RH ± 5% RH	12
Intermediate	30°C ± 2°C, 60% RH ± 5% RH	6
Accelerated	40°C ± 2°C, 75% RH ± 5% RH	6
	Product Stored in a Refrigerator	
Long Term	5°C ± 3°C	12
Accelerated	25°C ± 2°C, 60% RH ± 5% RH	6
	Product Stored in a Freezer	
Long Term	–20°C ± 5°C	12

Note: RH, relative humidity.

7.2.1.5 Statistical Considerations

It is recommended to use an appropriate statistical method to analyze the data generated during a stability study. The purpose of using statistics is to establish, with a high degree of confidence, the shelf life, that is, the period during which a quantitative attribute of the drug product remains within acceptance criteria for all future batches manufactured, packaged, and stored under similar conditions. Stability studies are expensive and time consuming and statistics can certainly help in this aspect. Statistical design principles can be applied to reduce the amount of testing required [7].

The shelf life for a single batch is usually computed based on regression techniques. An appropriate approach to shelf life estimation when using regression analysis is by calculating the earliest time at which the 95% confidence limit for the mean intersects the proposed acceptance criterion [8]. A detailed description of shelf life calculations is provided in Sections 7.2.3 and 7.2.4.

The analysis of covariance is the conventional statistical tool to determine whether or not the degradation lines from several batches belong to a single population. If the degradation lines are statistically different, the minimum criterion is used to determine the shelf life for the current and future batches. According to the minimum criterion, the shelf life of future batches corresponds to the shortest of all shelf lives from the tested batches. On the other hand, if the batches belong to a single population, regression analysis is used to develop a single degradation line based on pooled data of the tested batches.

A short-term stability study is conducted under stressed conditions to increase the rate of chemical and physical degradation of the drug product. The classical statistical procedure to establish a preliminary shelf life is based on regression techniques, which are used to estimate the parameters of the Arrhenius equation.

A systematic approach for stability data evaluation should be used to determine whether extrapolation beyond the time period covered in the stability study for the long-term data is appropriate to calculate the shelf life period. The approach consists of evaluating any significant change at the accelerated condition and, if applicable, at the intermediate condition in order to follow a guideline on how to establish the shelf life period by extrapolation. The required relevant supporting data and the commitment to place stability batches to be tested until the end of the extrapolated shelf life should be provided to the regulatory agency. The relevant supporting data include satisfactory long-term data from development batches manufactured with close related formulation in a small scale and stored in a packaging configuration similar to the primary stability batches. The data evaluation approach is executed as follows [8].

Product to be Stored at Room Temperature For products to be stored at room temperatures the extent of extrapolation will depend on the evaluation of the results from the short-term, intermediate, and long-term data. If no significant change at the accelerated (short-term) condition is observed, then the long-term data are evaluated for change and variability:

- *Long-Term Data with Small or No Change over Time and Small or No Variability* In this case a statistical analysis may be considered unnecessary

but a justification should be provided. Extrapolation can be proposed and the resulting shelf life can be up to twice the period covered in the stability study for the long-term data but not more than 12 months beyond the period tested.

- *Long-Term Data with Change over Time and Variability* In this case the statistical analysis of the long-term data can be useful to determine the shelf life of the drug product. If there is statistical difference among batches, among factors, or among factor combinations, it is not possible to perform data pooling and the shelf life will correspond to the shortest of all shelf lives obtained from all batches, factors, or factor combinations. If the statistical difference is related to a particular factor (e.g., strength or packaging), different shelf lives can be assigned to each level of that factor. The extent of extrapolation depends on whether statistical analysis is applicable to the stability data:
  1. Data for which statistical analysis does not apply: The proposed shelf life can be up to 1.5 times but should not be more than 6 months beyond the period covered in the stability study for the long-term data. This will require relevant supporting data to show that the drug product will meet all attribute acceptance criteria by the end of the proposed shelf life period.
  2. Data for which statistical analysis does apply: When statistical analysis is applicable to the long-term data and it is not performed, a justification is required and the extent of extrapolation is the same as when statistical analysis does not apply. If the statistical analysis is performed, the extent of extrapolation can be up to twice but not more than 12 months beyond the period covered in the stability study for the long-term data.

If significant change at any time during the 6-month period of the accelerated (short-term) condition is observed, then stability testing at an intermediate condition is required and the extent of extrapolation will depend on the outcome of both the long-term and intermediate conditions:

- *Data with No Significant Change at Intermediate Conditions* In this case extrapolation of the long-term data can be proposed and the extent of extrapolation will depend on whether a statistical analysis is applicable.
  - Data for which statistical analysis does not apply: The proposed shelf life can be up to 3 months beyond the period covered in the stability study for the long-term data. This will require relevant supporting data to show that the drug product will meet all the attribute acceptance criteria by the end of the proposed shelf life period.
  - Data for which statistical analysis does apply: When statistical analysis is applicable to the long-term data and it is not performed, a justification is required and the extent of extrapolation is the same as when statistical analysis does not apply. If the statistical analysis is performed, the extent of extrapolation can be up to 6 months beyond the period covered in the stability study for the long-term data. This will require relevant supporting data to show that the drug product will meet all the attribute acceptance criteria by the end of the proposed shelf life period.

- *Data with Significant Change at Intermediate Conditions* In this case extrapolation of the long-term data is not allowed and the shelf life should not exceed the period covered by the long-term stability study.

Product to be Stored In a Refrigerator For products to be stored in a refrigerator the same data evaluation approach but with more restrictive extent of extrapolation as for product to be stored at room temperature will be used unless otherwise specified below.

If no significant change at the the accelerated (short-term) condition is observed, then the long-term data is evaluated for change and variability:

- *Long-Term Data with Small or No Change over Time and Small or No Variability* Extrapolation can be proposed and the resulted shelf life can be up to 1.5 times but should not be more than six months beyond the period covered in the stability study for the long-term data.
- *Long-Term Data with Change over Time and Variability* The extent of extrapolation depends on whether statistical analysis is applicable to the stability data:
    - Data for which statistical analysis does not apply: The proposed shelf life can be up to three months beyond the period covered in the stability study for the long-term data.
    - Data for which statistical analysis does apply: When statistical analysis applies and is not performed, a justification is required and the extent of extrapolation is the same as when statistical analysis does not apply. If the statistical analysis is performed, the extent of extrapolation can be up to 1.5 times but not more than six months beyond the period covered in the stability study for the long-term data.

If significant change at any time during the six-month period of the accelerated (short-term) condition is observed, then extrapolation is not considered appropriate. If the long-term data show variability, the proposed shelf life can be verified by statistical analysis.

Product to be Stored in a Freezer For a drug product to be stored in a freezer the shelf life is determined from the long-term stability data and no extrapolation is allowed.

7.2.2 DESIGN OF STABILITY STUDIES

7.2.2.1 Introduction

The FDA guidelines establish, first, that the stability study protocol must describe how the stability study is designed and carried out and, second, the statistical methods to be used to analyze the data. The design of a stability study is aimed at establishing an expiration dating period. The expiration dating period is based on testing on a

limited number of batches of a drug product, and the results of these tests are applied to future batches of the drug product manufactured under similar conditions. Therefore, the study design should reduce the bias and identify and control any expected or unexpected source of variations. An appropriate stability design should provide the highest accuracy and precision of the established shelf life.

In this section, the fundamentals for designing a stability study are described. A stability study will characterize the degradation of the active ingredient with respect to time and is required to determine the shelf life period used to calculate the expiration date of a drug product. The shelf life is the maximum allowable period of time for the drug product to be stored in its final packaging while maintaining the therapeutic amount of the active pharmaceutical ingredient (API). The expiration date marks the end of the shelf life period. The expiration date is calculated by adding the shelf life to the manufacturing end date of the drug product. The determined shelf life is applicable to all future batches of the drug product manufactured under similar conditions as the batches used in the stability study.

The design of a stability study should be based on knowledge of the behavior and properties of the drug substance obtained from stability studies on the drug substance and data generated during clinical formulation studies. The stability study is performed by establishing and executing a protocol. The stability study protocol should specify all the aspects to be considered (e.g., sample size, test methods, and acceptance criteria) while carrying out the stability study. It is important to comply with all FDA (or ICH) regulations when performing a stability study.

Designing a stability study is based on a factorial design of experiments where a systemic procedure is used to determine the effect on the response variable of various factors and factor combinations. A linear model is used to represent the relationship between the factors and factor combinations with the response variable. Once the experimental design is established, the assays are conducted and stability data are saved to finally estimate the shelf life period.

Typically, the following factors are involved in the design of stability studies: number of batches, strength, package configuration, and storage conditions. The typical response variable of the stability study is the API amount. However, any other response variables (drug product attributes) that are susceptible to change during storage and are likely to influence quality, safety, and efficacy of the drug product (e.g., physical appearance, sterility, drug release rate, impurities, and degradation products) should also be considered. The testing procedure should include, as appropriate, the physical, chemical, biological, and microbiological attributes, preservative contents (e.g., antioxidant or antimicrobial preservatives), and functionality tests (e.g., for a dose delivery system).

Section 7.2.2 describes practical guidelines to design stability studies that are aligned with the guidance for industries provided by the FDA [9]. A different approach can be used depending on the characteristics of the drug product and with an appropriate scientific justification.

7.2.2.2 Basic Design Considerations

When designing a stability study, the following aspects should be taken into account:

Preliminary Stability Data During the drug product design phase, stability data are generated to choose the final package configuration and storage conditions and characterize the product. Such data are helpful in the development of a good stability study design that can offer as much information as possible with as few data as possible. Also, the preliminary stability data may provide scientific justification to the selection of the type of stability study design.

Drug Product Attributes All attributes that may affect the quality of the drug product have to be included in design of a stability study. These attributes have to be tested at every time period and, thus, the number of samples needed at each time should be set accordingly.

Drug Product Specifications The specification for each attribute of the drug product is required to define the acceptance criteria of the stability study.

Test Methods All test methods used in the stability study should be previously qualified.

Preliminary Testing Data Any data generated during the developmental and clinical trial phases of the drug may be useful to determine the expected manufacturing process variability. The process variability is an important factor to define the sampling plan to be used in the stability study.

Design Factors Identification of the design factors is crucial when choosing the design of the stability study. It is important to identify the most relevant design factors that may affect the stability of the drug product during storage. Failure to identify a relevant design factor may cause a significant delay in the stability study completion and submission to the regulatory agency, for example, the FDA.

Full Design versus Reduced Design A full design requires testing the drug product for factor combinations and at all time periods. The full design provides the largest amount of information to determine the shelf life of a drug product. However, the number of required combinations increases exponentially with the number of factors. On the other hand, a reduced design requires drug product testing only at a fraction of factor combinations. The reduced design requires less testing effort but entails the potential risk to result in a shorter shelf life than the one obtained from a full design due to the reduced amount of data collected.

7.2.2.3 Design of Stability Studies

Full Stability Study Design Assume that a stability study includes three factors: batch, strength (denoted S1, S2, and S3 in what follows), and packaging size (denoted P1, P2, and P3). Each value of a factor is normally referred to as *level*. Therefore, if there are four packaging sizes, the factor called packaging size has four levels. It should be noted that the FDA requires at least three batches and consequently the batch factor will have three levels. If the other two factors have three levels each, then the required number of experiments is 27. Table 2 shows the 27 experiments that should be performed at each point in time. The number of combinations, C, can be readily calculated by multiplying the number of levels of each factor:

TABLE 2 Factor Combinations for Three Factors with Three Levels Each

	S1			S2			S3		
Batch	P1	P2	P3	P1	P2	P3	P1	P2	P3
1	C1	C2	C3	C10	C11	C12	C19	C20	C21
2	C4	C5	C6	C13	C14	C15	C22	C23	C24
3	C7	C8	C9	C16	C17	C18	C25	C26	C27

$$C = \mathrm{LF}_1 \times \mathrm{LF}_2 \times \mathrm{LF}_3 \times \cdots \times \mathrm{LF}_n$$

where LF_1 = number of levels of factor 1
LF_2 = number of levels of factor 2
LF_3 = number of levels of factor 3
LF_n = number of levels of factor n

Once the factor combinations are defined, the number of samples required for the study can be determined. Assuming that the only testing required for the stability study design specified in Table 2 is amount of drug and that the test requires one package of the drug product, the amount of samples required for the study is 27 packages per time period. A drug product package for each of the 27 combinations has to be included in the study for each time period.

For a proposed shelf life of 36 months, testing of the drug product has to be carried out at the following time periods (t): 0, 3, 6, 9, 12, 18, 24, and 36 months. Considering the full design shown in Table 2, a total of 216 samples are needed to perform all required testing. The first 27 samples are tested at the beginning of the study to obtain data for $t = 0$. All other samples are placed in an environmental chamber that will maintain the temperature and relative humidity as specified in the stability study protocol. At each testing time, 27 additional samples are pulled from the chamber for testing, as shown in Table 3. The data analysis for stability studies will be discussed in section 7.2.3.

Reduced Stability Study Designs There are occasions where it is not possible to obtain the total number of required samples to perform a full stability study design or it is simply desired to reduce the sampling requirement; this is done by selecting a reduced stability study design. To perform a reduced stability study design, some assumptions are needed which should be fully justified; the justifications should be provided to the regulatory agency. Usually, a reduced design applies when a preliminary stability study is performed.

Bracketing and matrixing are the two reduced designs recommended by the FDA [9]. Each of these methods applies to different situations. Using both of them simultaneously may reduce the ability of the study to determine the shelf life since factor combinations can be confounded due to the aliasing effect [10].

Bracketing The bracketing design consists in testing only two levels: the highest and lowest values of the factor. Bracketing requires showing that the selected levels are the extremes of the factor range. If the stability of the extreme levels is different,

TABLE 3 Testing Schedule for Full Stability Study Design

	By Time in Months							
Factor Combination	0	3	6	9	12	18	24	36
C1	T	T	T	T	T	T	T	T
C2	T	T	T	T	T	T	T	T
C3	T	T	T	T	T	T	T	T
C4	T	T	T	T	T	T	T	T
C5	T	T	T	T	T	T	T	T
C6	T	T	T	T	T	T	T	T
C7	T	T	T	T	T	T	T	T
C8	T	T	T	T	T	T	T	T
C9	T	T	T	T	T	T	T	T
C10	T	T	T	T	T	T	T	T
C11	T	T	T	T	T	T	T	T
C12	T	T	T	T	T	T	T	T
C13	T	T	T	T	T	T	T	T
C14	T	T	T	T	T	T	T	T
C15	T	T	T	T	T	T	T	T
C16	T	T	T	T	T	T	T	T
C17	T	T	T	T	T	T	T	T
C18	T	T	T	T	T	T	T	T
C19	T	T	T	T	T	T	T	T
C20	T	T	T	T	T	T	T	T
C21	T	T	T	T	T	T	T	T
C22	T	T	T	T	T	T	T	T
C23	T	T	T	T	T	T	T	T
C24	T	T	T	T	T	T	T	T
C25	T	T	T	T	T	T	T	T
C26	T	T	T	T	T	T	T	T
C27	T	T	T	T	T	T	T	T

Note: T = sample tested.

TABLE 4 Factor Combinations for Bracketing of Three Factors with Three Levels

	S1			S2			S3		
Batch	P1	P2	P3	P1	P2	P3	P1	P2	P3
1	C1	—	C3	—	—	—	C19	—	C21
2	C4	—	C6	—	—	—	C22	—	C24
3	C7	—	C9	—	—	—	C15	—	C27

it is expected that the stability of any intermediate level should be higher than the stability of the least stable extreme. Table 4 shows the bracketing design for the full design example presented in Table 2.

Note that bracketing was not applied to the *batch* factor because the FDA regulation requires testing at least three batches to determine a drug product shelf life. Even so, the sampling required for the bracketing design was reduced substantially. The sample size required per time period is 12, a small number when compared to

TABLE 5 Testing Schedule for Bracketing Stability Study Design

	By Time in Months							
Factor Combination	0	3	6	9	12	18	24	36
C1	T	T	T	T	T	T	T	T
C2	—	—	—	—	—	—	—	—
C3	T	T	T	T	T	T	T	T
C4	T	T	T	T	T	T	T	T
C5	—	—	—	—	—	—	—	—
C6	T	T	T	T	T	T	T	T
C7	T	T	T	T	T	T	T	T
C8	—	—	—	—	—	—	—	—
C9	T	T	T	T	T	T	T	T
C10	—	—	—	—	—	—	—	—
C11	—	—	—	—	—	—	—	—
C12	—	—	—	—	—	—	—	—
C13	—	—	—	—	—	—	—	—
C14	—	—	—	—	—	—	—	—
C15	—	—	—	—	—	—	—	—
C16	—	—	—	—	—	—	—	—
C17	—	—	—	—	—	—	—	—
C18	—	—	—	—	—	—	—	—
C19	T	T	T	T	T	T	T	T
C20	—	—	—	—	—	—	—	—
C21	T	T	T	T	T	T	T	T
C22	T	T	T	T	T	T	T	T
C23	—	—	—	—	—	—	—	—
C24	T	T	T	T	T	T	T	T
C25	T	T	T	T	T	T	T	T
C26	—	—	—	—	—	—	—	—
C27	T	T	T	T	T	T	T	T

Note: T = sample tested.

the 27 samples required for the full design. As a result, the amount of samples required for the whole stability study is 8×12, or 96, whereas the full-design study required 216 samples. The execution procedure of stability testing for both a complete and a reduced stability study is the same, as shown in Table 5.

Matrixing The matrixing design consists in selecting a fraction of the total number of possible combinations included in the full design. At each time, a different fraction will be tested. The main assumption is that the stability of each fraction represents the stability of all factor combinations at that particular time. The matrixing design can be applied across the factors or across the time points of the stability design. The degree of reduction (e.g., one-half or one-third) from a full design depends on the quantity of factors to be considered. The larger the number of factors and levels included in the full design, the larger the degree of reduction that can be implemented.

Applying a matrixing design on time points only, all factor combinations (full factorial design) should be tested at the initial and final points in time, while a

fraction of the full factorial design is tested at intermediate points in time. If the full long-term stability data required for the proposed shelf life will not be available for review before submission to the regulatory agency, the full factorial design should be tested at 12 months and at the last scheduled point in time prior to submission. Therefore, the FDA regulations require testing full factorial design at 0, 12, and 36 months for a matrixing design. In addition, data from at least three time points, including initial, should be available for each factor combination through the first 12 months of the study. For matrixing at accelerated storage conditions, testing should occur at a minimum of three time points, including initial and final, for each factor combination.

The matrixing design should be as balanced as possible so that each factor combination is tested to the same extent over the duration of the study. A matrixing design is applicable when the supporting data indicate predictable product stability and small variability. A statistical justification for using a matrixing design could be based on evaluating the proposed matrixing design with respect to its power to detect differences in the degradation rates among the factors or the level of accuracy on estimating the shelf life.

A matrixing design performed across factors other than time points generally has less precision in shelf-life estimation and yields a shorter shelf life than the corresponding full design due to the confounding and aliasing effects [10]. Matrixing design may have insufficient power to detect main factors or factor interaction effects. Thus an excessive reduction in the number of factor combinations may produce an unreliable estimation of the shelf life due to the missing factor combinations. On the other hand, a matrixing design on time points would often have similar ability as the full design to detect differences in rates of change among factors and to establish a reliable shelf life. This is because full testing of all factor combinations would still be performed at both the initial and the last time points. For illustration purposes, matrixing design will be implemented over time points, factors, and in both time points and factors. The reader should be wise when choosing a design for a particular stability study and should have in mind that supporting data and justifications have to be provided.

Matrixing in Time Points When matrixing in time points design, a fraction of the combinations is selected using the fractional factorial design procedure. Note that the full design presented above is equivalent to a 3^k factorial design, where k is the number of factors and 3 is the number of levels of each factor. The total number of combinations for three factors and three levels is 3^3, or 27. Assuming that a one-third reduction for the matrixing design is desired, the required sample size per time period is 18 compared to 27 for the full design. As a result, the number of samples needed for the whole stability study is $5 \times 18 + 3 \times 27$, or 171, whereas the full design required 216 samples. Table 6 shows the schedule to implement a matrixing design over time points for 36 months of stability study. This table shows a full design on times 0, 12, and 36 and two-thirds on the remaining times. The sampling shown in Table 6 has been designed to maintain a proper balance. If bracketing was applied to one of the factors (either strength or packaging size) or the design has only two levels on one of those factors, the full experiment design will be reduced, as shown in Table 7. Table 8 shows a two-thirds matrixing design with three strength

TABLE 6 Testing Schedule for Matrixing in Time Point Stability Study Design

	By Time in Months							
Factor Combination	0	3	6	9	12	18	24	36
C1	T	—	T	T	T	—	T	T
C2	T	T	—	T	T	T	—	T
C3	T	T	T	—	T	T	T	T
C4	T	T	—	T	T	T	—	T
C5	T	T	T	—	T	T	T	T
C6	T	—	T	T	T	—	T	T
C7	T	T	T	—	T	T	T	T
C8	T	—	T	T	T	—	T	T
C9	T	T	—	T	T	T	—	T
C10	T	T	T	—	T	T	T	T
C11	T	—	T	T	T	—	T	T
C12	T	T	—	T	T	T	—	T
C13	T	—	T	T	T	—	T	T
C14	T	T	—	T	T	T	—	T
C15	T	T	T	—	T	T	T	T
C16	T	T	—	T	T	T	—	T
C17	T	T	T	—	T	T	T	T
C18	T	—	T	T	T	—	T	T
C19	T	T	—	T	T	T	—	T
C20	T	T	T	—	T	T	T	T
C21	T	—	T	T	T	—	T	T
C22	T	T	T	—	T	T	T	T
C23	T	—	T	T	T	—	T	T
C24	T	T	—	T	T	T	—	T
C25	T	—	T	T	T	—	T	T
C26	T	T	—	T	T	T	—	T
C27	T	T	T	—	T	T	T	T

Note: T = sample tested.

TABLE 7 Factor Combination Considering Two Levels in Packaging

	S1		S2		S3	
Batch	P1	P2	P1	P2	P1	P2
1	C1	C2	C7	C8	C13	C14
2	C3	C4	C9	C10	C15	C16
3	C5	C6	C11	C12	C17	C18

levels and two packaging size levels. Thus, 18 factor combinations are scheduled for time equal to 0, 12, and 36 months and 12 combinations for the remaining time points.

For this matrixing design, the total number of samples needed for the whole stability study is $3 \times 18 + 5 \times 12$, or 114, compared to 216 samples needed for the full 3^k design. Furthermore, for the case where both strength and packaging size have

TABLE 8 Testing Schedule for Matrixing in Time Points (Two Levels for Packaging Size)

	By Time in Months							
Factor Combination	0	3	6	9	12	18	24	36
C1	T	—	T	T	T	—	T	T
C2	T	T	T	—	T	T	T	T
C3	T	T	—	T	T	T	—	T
C4	T	—	T	T	T	—	T	T
C5	T	T	T	—	T	T	T	T
C6	T	T	—	T	T	T	—	T
C7	T	T	T	—	T	T	T	T
C8	T	T	—	T	T	T	—	T
C9	T	—	T	T	T	—	T	T
C10	T	T	T	—	T	T	T	T
C11	T	T	—	T	T	T	—	T
C12	T	—	T	T	T	—	T	T
C13	T	T	—	T	T	T	—	T
C14	T	—	T	T	T	—	T	T
C15	T	T	T	—	T	T	T	T
C16	T	T	—	T	T	T	—	T
C17	T	—	T	T	T	—	T	T
C18	T	T	T	—	T	T	T	T

Note: T = sample tested.

TABLE 9 Factor Combination Considering Two Levels in Packaging Size and Strength

	S1		S2	
Batch	P1	P2	P1	P2
1	C1	C2	C7	C8
2	C3	C4	C9	C10
3	C5	C6	C11	C12

only two levels or bracketing is applied to both factors, the full experiment design is reduced, as shown in Table 9. In this case, the total number of factor combinations is 12 rather than 27. Table 10 shows the design when bracketing and matrixing are implemented. Bracketing is applied to factors while matrixing is applied to time points. Table 10 shows that there are 12 factor combinations for times 0, 12, and 36 and 8 factor combinations on the remaining time points. Thus, for this matrixing design, the number of samples required for the whole stability study is $3 \times 12 + 5 \times 8$, or 76, compared to 216 samples needed for the full 3^k design.

Matrixing in Factors For the matrixing-in-factors design, the factor combinations are eliminated in a systematic way as shown in Table 11. As a result, not all factor combinations are tested during the stability study. Such a design can be used when the factor combination eliminated exhibits a similar behavior as the other factor

TABLE 10 Testing Schedule for Matrixing in Time Points (Two Levels for Packaging Size and Strength)

	By Time in Months							
Factor Combination	0	3	6	9	12	18	24	36
C1	T	—	T	T	T	—	T	T
C2	T	T	T	—	T	T	T	T
C3	T	T	—	T	T	T	—	T
C4	T	—	T	T	T	—	T	T
C5	T	T	T	—	T	T	T	T
C6	T	T	—	T	T	T	—	T
C7	T	T	—	T	T	T	—	T
C8	T	—	T	T	T	—	T	T
C9	T	T	T	—	T	T	T	T
C10	T	T	—	T	T	T	—	T
C11	T	—	T	T	T	—	T	T
C12	T	T	T	—	T	T	T	T

Note: T = sample tested.

TABLE 11 Matrixing in Factor Combination Elimination

	S1			S2			S3		
Batch	P1	P2	P3	P1	P2	P3	P1	P2	P3
1	—	C2	C3	C10	C11	—	C19	—	C21
2	C4	—	C6	—	C14	C15	C22	C23	—
3	C7	C8	—	C16	—	C18	—	C26	C27

combinations within the study. The implementation of this matrixing design is shown in Table 12. All factor combinations are tested at times 0, 12, and 36 months, and just 18 factor combinations at the remaining times. For this matrixing design, the number of samples needed for the whole study is $3 \times 27 + 18 \times 5$, or 171, while 216 samples were needed for the full 3^k design.

Matrixing in Factors and Time Points Matrixing in both factors and time points is a combination of the two previously mentioned matrixing designs and the schedule is shown in Table 13. In this matrixing design, all factor combinations are tested at times 0, 12, and 36 and fractional factorial at the remaining times. The total number of experiments to be conducted in this stability study is $3 \times 27 + 5 \times 12$, or 141, while 216 samples were required for the full 3^k design.

7.2.3 LONG-TERM STABILITY ANALYSIS

7.2.3.1 Introduction

This section considers the stability analysis of the long-term studies. The purpose of this section is to provide a set of fundamental statistical tools to calculate the shelf life for single and multiple packages and strengths. The statistical methods will be

TABLE 12 Testing Schedule for Matrixing in Factor Stability Study Design

	By Time in Months							
Factor Combination	0	3	6	9	12	18	24	36
C1	T	—	—	—	T	—	—	T
C2	T	T	T	T	T	T	T	T
C3	T	T	T	T	T	T	T	T
C4	T	T	T	T	T	T	T	T
C5	T	—	—	—	T	—	—	T
C6	T	T	T	T	T	T	T	T
C7	T	T	T	T	T	T	T	T
C8	T	T	T	T	T	T	T	T
C9	T	—	—	—	T	—	—	T
C10	T	T	T	T	T	T	T	T
C11	T	T	T	T	T	T	T	T
C12	T	—	—	—	T	—	—	T
C13	T	—	—	—	T	—	—	T
C14	T	T	T	T	T	T	T	T
C15	T	T	T	T	T	T	T	T
C16	T	T	T	T	T	T	T	T
C17	T	—	—	—	T	—	—	T
C18	T	T	T	T	T	T	T	T
C19	T	T	T	T	T	T	T	T
C20	T	—	—	—	T	—	—	T
C21	T	T	T	T	T	T	T	T
C22	T	T	T	T	T	T	T	T
C23	T	T	T	T	T	T	T	T
C24	T	—	—	—	T	—	—	T
C25	T	—	—	—	T	—	—	T
C26	T	T	T	T	T	T	T	T
C27	T	T	T	T	T	T	T	T

Note: T = sample tested.

described first under the context of a general application and then numerical examples will be described step by step to illustrate particular applications. A set of computer programs are provided in the Appendix to facilitate the implementation of the stability analysis procedures. Chen et al. [11] pointed out that the stability analysis usually consists of three steps. The first step is to collect the assay results at several time intervals for the sample stored under appropriate conditions. The second step is to select the appropriate model to describe the relationship between assay results and sampling times, and the third step is to establish the expiration dating period. The first step was described in Section 7.2.2 and the second and third steps are described in Section 7.2.3.

7.2.3.2 Drug Shelf Life for Single Batch

Calculation of the shelf life for a drug product in a single package type will be illustrated assuming that assay results are obtained from a single batch. The FDA guideline establishes that the expiration dating period for a drug product consists

TABLE 13 Testing Schedule for Matrixing in Factor and Time Point Stability Study Design

	By Time in Months							
Factor Combination	0	3	6	9	12	18	24	36
C1	T	—	T	T	T	T	T	T
C2	T	T	—	T	T	—	T	T
C3	T	—	—	—	T	—	—	T
C4	T	—	—	—	T	—	—	T
C5	T	T	T	—	T	T	—	T
C6	T	—	T	T	T	T	T	T
C7	T	T	T	—	T	T	—	T
C8	T	—	—	—	T	—	—	T
C9	T	T	—	T	T	—	T	T
C10	T	T	—	T	T	—	T	T
C11	T	—	—	—	T	—	—	T
C12	T	—	T	T	T	T	T	T
C13	T	T	T	—	T	T	—	T
C14	T	—	T	T	T	T	T	T
C15	T	—	—	—	T	—	—	T
C16	T	—	—	—	T	—	—	T
C17	T	T	—	T	T	—	T	T
C18	T	T	T	—	T	T	—	T
C19	T	—	—	—	T	—	—	T
C20	T	—	T	T	T	T	T	T
C21	T	T	—	T	T	—	T	T
C22	T	—	T	T	T	T	T	T
C23	T	—	—	—	T	—	—	T
C24	T	T	T	—	T	T	—	T
C25	T	T	—	T	T	—	T	T
C26	T	T	T	—	T	T	—	T
C27	T	—	—	—	T	—	—	T

Note: T = sample tested.

of determining the time at which the 95% one-sided lower confidence interval for the mean degradation curve intersects the lower acceptable specification limit, which is usually adopted by the FDA as 90% of the label claim (LC). Assuming that concentration of a drug product decreases linearly with time and it can be expressed as

$$y_i = \alpha + \beta x_i + \varepsilon_i \qquad i = 1, \ldots, n \tag{1}$$

where y_i is the percentage of the label claim (assay result) at a given time x_i for the ith sample, x_i is the time at which the ith sample was analyzed, and α and β are the regression parameters. The coefficient α represents the percentage label claim when $x_i = 0$ and is usually known as the batch effect, while coefficient β is known as the degradation rate, and the product βx_i is the stability loss over time. It is assumed that the random variable ε_i follows a normal distribution with zero mean and a constant variance σ^2 and n is the total number of samples. In Section 7.2.4, the

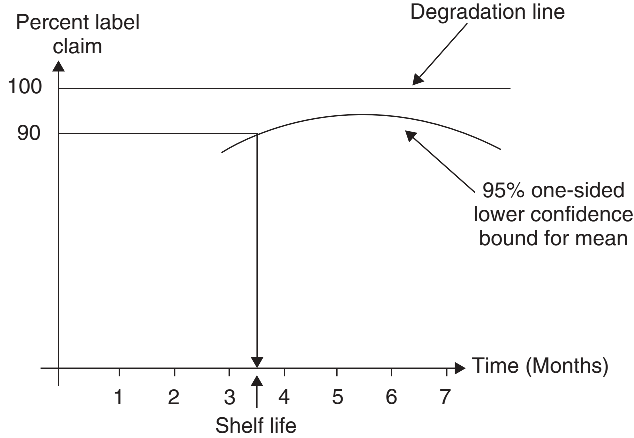

FIGURE 1 Graphical representation of shelf life.

various degradation kinetic models will be presented. In this context, Equation (1) describes a zero-order degradation.

The drug expiration date for a single batch exhibits a 95% confidence that the average drug characteristic of the dosage units in the batch is within specifications up to the end of the expiration date. The 95% one-sided lower confidence bounds for the mean degradation line is shown in Figure 1.

The 95% confidence interval can be expressed by the probability statement

$$P(t \leq t_{0.05,n-2}) = 0.95 \tag{2}$$

where $t_{0.05,n-2}$ is the upper five percentile of the t student distribution with $n - 2$ degrees of freedom. Assuming that the percentage of the label claim (%LC) follows a normal distribution, the statistic t is defined as

$$t = \frac{z}{\sqrt{x_m^2/m}} = \frac{[\hat{y} - (\alpha + \beta x)] \Big/ \sqrt{\sigma^2[1/n + (x - \bar{x})^2/S_{xx}]}}{\sqrt{\text{SSE}/[\sigma^2(n-2)]}} \tag{3}$$

where

$$S_{xx} = \sum_{i=1}^{n}(x - \bar{x})^2 \qquad \text{SSE} = \sum_{i=1}^{n}(y_i - \hat{y})^2 \tag{4}$$

where SSE is the sum-of-squares error, z is a random variable that follows the standard normal distribution, χ_m^2 is a random variable that follows the chi-square distribution with m degrees of freedom, ŷ is the estimated %LC at time x, a and b are the estimates of the parameters α and β, respectively, and $\bar{x}$ is the average sampling time.

The t statistic was used to eliminate the unknown parameter σ^2. Thus, Equation (3) can be written as

$$t = \frac{\hat{y} - (\alpha + \beta x)}{S_{\hat{y}}} \tag{5}$$

$$S_{\hat{y}} = \sqrt{\text{MSE}\left[\frac{1}{n} + \frac{(x - \bar{x})^2}{S_{xx}}\right]} \qquad \text{MSE}=\frac{\text{SSE}}{n-2} \tag{6}$$

where MSE is the mean-square error.

Thus, Equation (2) can be now written as

$$P\left(\frac{\hat{y} - (\alpha - \beta x)}{S_{\hat{y}}} \le t_{0.05,n-2}\right) = 0.95 \tag{7}$$

and after arranging terms, Equation (7) can be expressed as

$$P(\hat{y} - t_{0.05,n-2} S_y \le \alpha + \beta x) = 0.95 \tag{8}$$

Therefore, the 95% one-sided lower confidence bound is

$$L(x) = \hat{y} - t_{0.05,n-2} S_{\hat{y}} = a - bx - t_{0.05,n-2}\sqrt{\text{MSE}\left[\frac{1}{n} + \frac{(x - \bar{x})^2}{S_{xx}}\right]} \tag{9}$$

The points where $L(x)$ intersects the acceptable lower specification limit δ can be obtained by finding the roots of the following equation: $\delta - L(x) = 0$. It should be pointed out that this equation can also be written in the form

$$f(x) = [\delta - (a + bx)]^2 - t^2_{0.05,n-2}\ \text{MSE}\left[\frac{1}{n} + \frac{(x - \bar{x})^2}{S_{xx}}\right] = 0 \tag{10}$$

The quadratic equation (10) has two roots and the shelf life is obtained by computing the root of Equation (10) that is smaller than a reference point, which is defined as

$$x_{\text{ref}} = \frac{b_0 - \delta}{-b_1} \tag{11}$$

A numerical example is presented below to illustrate, step by step, the shelf-life calculation procedure.

Example 1 Shelf Life for Single Batch The assay results for a batch of a drug product are given in Table 14. Based on these results, determine the shelf life for this batch.

TABLE 14 Single-Batch Assay Results (%LC) for Example 1

Sampling time, months	0	3	6	9	12	18	24	36
Percent label claim	103.5	97.3	97.2	94.2	94.8	94.1	91.3	88.7

Solution First, regression techniques are used to estimate the expected degradation line [12]. This yields

$$\hat{y} = 99.64 - 0.3335x$$

It is well known that conventional statistical computer programs such as MINITAB, STATGRAPHICS, or SAS will conduct model fitting and provide the following additional information: $t_{0.05,n-2} = t_{0.05,6} = 1.943$, MSE = 4.1665, $n = 8$, $\bar{x} = 13.5$, and $S_{xx} = 1008$. For the reader's convenience, a computer program to perform model regression fitting and to compute all the statistics required to determine the shelf life is given in the Appendix.

Thus, the 95% one-sided lower confidence bound for the mean degradation rate is

$$L(x) = 99.64 - 0.3335x - 1.943\sqrt{4.1665\left[\frac{1}{8} + \frac{(x-13.5)^2}{1008}\right]}$$

Consequently, the shelf life can be obtained by using the equation

$$f(x) = [90 - (99.64 - 0.3335x)]^2 - 1.943^2(4.1665)\left[\frac{1}{8} + \frac{(x-13.5)^2}{1008}\right] = 0 \qquad (12)$$

To compute the shelf life, we compute first the reference point as

$$x_{\text{ref}} = \frac{b_0 - \delta}{-b_1} = \frac{99.64 - 90}{0.3335} = 28.90$$

Therefore, the shelf life is the root smaller than 28.90. A simple and practical tool to compute the roots of Equation (12) is perhaps solving the following equivalent problem. Find x such that it minimizes the absolute value of $f(x)$. This root is obtained by using the quasi-Newton line search (QNLS) algorithm [13]. The computer program requires an initial point and we recommend using the value

$$x(0) = x_{\text{ref}} - d \qquad (13)$$

where d is a positive value that should be explored by using a trial-and-error method until the program converges to a local minimum, usually the range of d is between 0 and 10. For instance, when $d = 0$, the QNLS method converges to $x_R = 23.6989$. The shelf life x_L is the integer part of the root x_R and, in this case, the expiration dating period of this batch is $x_L = 23$ months since $f(x_R) = 0$, and $x_R < x_{\text{ref}}$. The QNLS method is implemented in MATLAB software and the computer program is also given in the Appendix.

7.2.3.3 Drug Shelf Life for Multiple Batches

Test for Poolability of Batches The FDA guidelines establish that at least three batches must be used to determine the batch-to-batch variability. Thus, if the statistical procedure shows evidence that the three batches belong to the same population, a single shelf life for all batches will be determined by pooling data from all batches. The FDA guidelines also established that batch similarity of the degradation lines can be assessed by the equality of slopes and the equality of intercepts of individual batches.

Assuming that the degradation decreases linearly with time (zero-order degradation), it can be represented by the following model, which is analogous to Equation (1):

$$y_{ij} = \alpha_i + \beta_i x_{ij} + \varepsilon_{ij} \qquad i = 1, \ldots, I \qquad j = 1, \ldots, n_i \tag{14}$$

where y_{ij} is the assay result (%LC) of the ith batch for a drug product sampled at time x_{ij}, n_i is the number of sampling times for the ith batch, I is the total number of batches, α_i and β_i are the intercept and slope of the degradation line for the ith batch, respectively, and ε_{ij} is assumed to be a random variable with zero mean and constant variance. It is worth mentioning that α_i is considered as the batch effect and β_i as the degradation rate for the ith batch.

The FDA guidelines indicate that the tests for the equality slopes and the equality of intercepts should be performed at the 0.25 level of significance, as was suggested by Bancroft [14]. It would be desirable to test whether or not the batches belong to a single population and if that is the case to derive a model with a single intercept and slope for the entire population of batches. Thus, a poolability test is implemented to determine if a single population should be used. The poolability test is implemented in two steps: (1) testing for equality of slopes and (2) testing for equality of intercepts. The conventional procedure to test these hypotheses is accomplished by using the analysis of covariance for a completely randomized design [15, 16]. An alternative method that can easily be generalized for studying multifactor is using a regression model with indicator variables [17].

Analysis of Covariance (ANCOVA) for Testing Similarity of Slopes The first step is determining whether or not the degradation rates for all batches behave in a similar fashion. The following hypothesis will be tested:

$$H_0: \quad \beta_i = \beta_j \quad \text{for all } i \neq j, \quad i = 1, \ldots, I \qquad j = 1, \ldots, I \tag{15}$$

where I is the number of batches.

To develop the F statistics and be able to test the above hypothesis, it is required to compute different sum of squares and cross products for %LC and also for sampling times [15, 16, 18]. The aggregated sum of squares of the sampling times is defined as

$$Z_{xx} = \sum_{i=1}^{I} S_{xx}(i) \tag{16}$$

where

$$S_{xx}(i) = \sum_{j=1}^{n_i}(x_{ij} - \bar{x}_{i\bullet})^2 = \sum_{j=1}^{n_i} x_{ij}^2 - \frac{x_{i\bullet}^2}{n_i}$$
$$x_{i\bullet} = \sum_{j=1}^{n_i} x_{ij} \qquad \bar{x}_{i\bullet} = \frac{x_{i\bullet}}{n_i} \tag{17}$$

The aggregated sum of squares of %LC is defined as

$$Z_{yy} = \sum_{i=1}^{I} S_{yy}(i) \tag{18}$$

where

$$S_{yy}(i) = \sum_{j=1}^{n_i}(y_{ij} - \bar{y}_{i\bullet})^2 = \sum_{j=1}^{n_i} y_{ij}^2 - \frac{y_{i\bullet}^2}{n_i}$$
$$y_{i\bullet} = \sum_{j=1}^{n_i} y_{ij} \qquad \bar{y}_{i\bullet} = \frac{y_{i\bullet}}{n_i} \tag{19}$$

The aggregated sum of cross products between the %LC and sampling times is given as

$$Z_{xy} = \sum_{i=1}^{I} S_{xy}(i) \tag{20}$$

where

$$S_{xy}(i) = \sum_{j=1}^{n_i}(x_{ij} - \bar{x}_{i\bullet})(y_{ij} - \bar{y}_{i\bullet}) = \sum_{j=1}^{n_i} x_{ij} y_{ij} - \frac{x_{i\bullet} y_{i\bullet}}{n_i} \tag{21}$$

Thus, the sum-of-squares error based on the aggregated sums is computed as

$$\mathrm{SSE}_a = Z_{yy} - \frac{Z_{xy}^2}{Z_{xx}} \tag{22}$$

The aggregated sum-of-squares error for each batch, which is also needed, is calculated as

$$\mathrm{SSE} = \sum_{i=1}^{I} \mathrm{SSE}(i) \tag{23}$$

where

$$\mathrm{SSE}(i) = S_{yy}(i) - \frac{S_{xy}^2(i)}{S_{xx}(i)} \tag{24}$$

The mean-square errors due to slope contribution and the total mean-square errors are defined as

$$MS_{\text{slope}} = \frac{SSE_a - SSE}{I - 1} \tag{25}$$

$$MSE = \frac{SSE}{N - 2I} \qquad \text{where} \quad N = \sum_{i=1}^{I} n_i \tag{26}$$

Finally, the F statistics to test hypothesis (15) can be written as

$$F(\text{slope}) = \frac{MS_{\text{slope}}}{MSE} \tag{27}$$

It should be noted that hypothesis (15) is rejected when $F(\text{slope}) > F_{0.25,I-1,N-2I}$, where $F_{0.25,I-1,N-2I}$ is the upper percentile of the F distribution with $I - 1$, and $N - 2I$ degrees of freedom. If hypothesis (15) is not rejected (i.e., the slopes are similar), it can be proceeded with the next step: to test whether or not the intercepts of the involved batches are similar. The computational procedure for this is described next.

ANCOVA for Testing Intercept Similarities The second step is to test whether or not the intercepts for the individual degradation lines are equal given that the degradation lines from the considered batches have similar slopes. The hypothesis of interest can be written as

$$H_0: \quad \alpha_i = \alpha_j \quad \text{for all } i \neq j \qquad i = 1, \ldots, I \qquad j = 1, \ldots, I \tag{28}$$

Since differences in slopes were not identified, model (14) reduces to a model with a single degradation rate as follows:

$$y_{ij} = \alpha_i + \beta x_{ij} + \varepsilon_{ij} \qquad i = 1, \ldots, I \qquad j = 1, \ldots, n_i \tag{29}$$

The intercepts can be decomposed into two elements: the common intercept and the batch effect. That is, $\alpha_i = \alpha + \tau_i$, where $\alpha = \mu - \beta \bar{x}_{..}$ is the common intercept and τ_i is the batch effect; μ is the expected value of y_{ij}, and $\bar{x}_{..}$ is the average of x_{ij} and was defined by Equation (32). It should be noted that the deviation of each intercept from the common intercept is called the batch effect. Thus, model (29) can be written as

$$y_{ij} = \mu + \tau_i + \beta(x_{ij} - \bar{x}_{..}) + \varepsilon_{ij} \tag{30}$$

A model without a batch effect is compared with a model that includes the batch effect to be able to measure the intercept effect. Equation (30) includes the batch effect and will be called the complete model. The model with no batch effect will be called a reduced model and can be expressed as

$$y_{ij} = \mu + \beta(x_{ij} - \bar{x}_{..}) + \varepsilon_{ij} \qquad i = 1, \ldots, I \qquad j = 1, \ldots, n_i \tag{31}$$

The sums of squares, cross products for totals, and errors for the reduced model are computed as [12, 18]

$$R_{xx} = \sum_{i=1}^{I}\sum_{j=1}^{n_i}(x_{ij} - \bar{x}_{..})^2 = \sum_{i=1}^{I}\sum_{j=1}^{n_i}x_{ij}^2 - \frac{x_{..}^2}{N} \qquad \bar{x}_{..} = \frac{1}{N}\sum_{i=1}^{I}\sum_{j=1}^{n_i}x_{ij} \qquad x_{..} = N\bar{x}_{..} \tag{32}$$

$$R_{yy} = \sum_{i=1}^{I}\sum_{j=1}^{n_i}(y_{ij} - \bar{y}_{..})^2 = \sum_{i=1}^{I}\sum_{j=1}^{n_i}y_{ij}^2 - \frac{y_{..}^2}{N} \qquad \bar{y}_{..} = \frac{1}{N}\sum_{i=1}^{I}\sum_{j=1}^{n_i}y_{ij} \qquad y_{..} = N\bar{y}_{..} \tag{33}$$

$$R_{xy} = \sum_{i=1}^{I}\sum_{j=1}^{n_i}(x_{ij} - \bar{x}_{..})(y_{ij} - \bar{y}_{..}) = \sum_{i=1}^{I}\sum_{j=1}^{n_i}x_{ij}y_{ij} - \frac{x_{..}y_{..}}{N} \tag{34}$$

where N was defined in Equation (26).

The sum of squares for the complete model can be computed as

$$T_{xx} = \sum_{i=1}^{I}n_i(\bar{x}_{i.} - \bar{x}_{..})^2 = \sum_{i=1}^{I}\frac{x_{i.}^2}{n_i} - \frac{x_{..}^2}{N} \tag{35}$$

$$T_{yy} = \sum_{i=1}^{I}n_i(\bar{y}_{i.} - \bar{y}_{..})^2 = \sum_{i=1}^{I}\frac{y_{i.}^2}{n_i} - \frac{y_{..}^2}{N} \tag{36}$$

$$T_{xy} = \sum_{i=1}^{I}n_i(\bar{x}_{i.} - \bar{x}_{..})(\bar{y}_{i.} - \bar{y}_{..}) = \sum_{i=1}^{I}\frac{x_{i.}y_{i.}}{n_i} - \frac{x_{..}y_{..}}{N} \tag{37}$$

$$E_{xx} = \sum_{i=1}^{I}\sum_{j=1}^{n_i}(x_{ij} - \bar{x}_{i.})^2 = R_{xx} - T_{xx} \tag{38}$$

$$E_{yy} = \sum_{i=1}^{I}\sum_{j=1}^{n_i}(y_{ij} - \bar{y}_{i.})^2 = R_{yy} - T_{yy} \tag{39}$$

$$E_{xy} = \sum_{i=1}^{I}\sum_{j=1}^{n_i}(x_{ij} - \bar{x}_{i.})(y_{ij} - \bar{y}_{i.}) = R_{xy} - T_{xy} \tag{40}$$

The sum-of-squares errors for the reduced model can be computed as

$$\mathrm{SSE_r} = R_{yy} - \frac{R_{xy}^2}{R_{xx}} \tag{41}$$

The sum-of-squares errors for the complete model can be computed as

$$\text{SSE}_c = E_{yy} - \frac{E_{xy}^2}{E_{xx}} \tag{42}$$

The mean-square errors for the intercept effect and the mean-square errors for the complete model can be written as

$$\text{MSE}_{\text{int}} = \frac{\text{SSE}_r - \text{SSE}_c}{I-1} \qquad \text{MSE}_c = \frac{\text{SSE}_c}{N-I-1} \tag{43}$$

Finally, the F statistics to measure whether or not the intercepts are different from batch to batch is given as

$$F(\text{int}) = \frac{\text{MSE}_{\text{int}}}{\text{MSE}_c} \tag{44}$$

The hypothesis (28) is rejected if $F(\text{int}) > F_{0.25,I-1,N-I-1}$, where $F_{0.25,I-1,N-I-1}$ is the upper percentile of the F distribution with $I-1$ and $N-I-1$ degrees of freedom. Thus, if the null hypotheses (15) and (28) are not rejected at the 0.25 level of significance, the batches can be considered to come from a single population or pool and a single shelf life is computed based on the studied batches. If that is the case, model (14) reduces to the expression

$$y_{ij} = \alpha + \beta x_{ij} + \varepsilon_{ij} \qquad i = 1, \ldots, I \qquad j = 1, \ldots, n_i \tag{45}$$

where α and β are the common intercept and slope, respectively, for model (45).

The approach to estimate the shelf life of a single batch can be applied to the pooled stability data from all batches. Thus, the shelf life is obtained by finding the minimum root of Equation (10), where a and b are the estimates of α and β from model (45), respectively, $\bar{x}$ is the average sampling time, and S_{xx} is defined by Equation (4) and considering for all sampling times.

If hypothesis (15) is not rejected and hypothesis (28) is rejected, model (14) reduces to the expression

$$y_{ij} = \alpha_i + \beta x_{ij} + \varepsilon_{ij} \qquad i = 1, \ldots, I \qquad j = 1, \ldots, n_i \tag{46}$$

Assuming a common slope with different intercepts for different batches, the expiration dating period is computed for each individual batch. The minimum of the expiration dating periods of the individual batches is the expiration dating period of the drug product.

A third alternative may occur if hypothesis (15) is rejected; it is concluded in this case that the batches do not belong to a single population and the shelf life should be computed for each individual batch. The minimum of the expiration periods of the individual batches is the shelf life of the drug product.

Example 2 Slopes and Intercepts Are the Same This example illustrates the application of the poolability test for batch similarity. Assay data from three batches, expressed as %LC, are shown in Table 15. Determine whether or not the three batches belong to a single population.

TABLE 15 Assay Results (%LC) for Example 2

Sampling time, months	0	3	6	9	12	18	24	36
Batch 1	102.4	98.1	99.2	97.5	95.0	96.1	—	—
Batch 2	101.1	101.2	99.0	97.2	96.4	95.5	94.3	—
Batch 3	104.1	102.1	99.5	98.1	95.7	94.1	94.0	93.5

Solution The poolability test is implemented in two steps.

Step 1. The first test will determine whether or not the slopes from the three batches are similar. From Table 15 the following information can be extracted: $I = 3, n_1 = 6, n_2 = 7, n_3 = 8$, and $N = 21$. The first hypothesis to be tested is

$$H_0: \quad \beta_1 = \beta_2 = \beta_3 = \beta \tag{47}$$

Using Equations (16)–(21), the sum of squares and cross products for %LC and sampling time are computed as follows:

$$S_{xx}(1) = \sum_{j=1}^{6} x_{1j}^2 - \frac{x_{1\bullet}^2}{6} = 3^2 + 6^2 + \ldots + 18^2 - \frac{48^2}{6} = 210$$

$$S_{xx}(2) = \sum_{j=1}^{7} x_{2j}^2 - \frac{x_{2\bullet}^2}{7} = 3^2 + 6^2 + \ldots + 24^2 - \frac{72^2}{7} = 429.43$$

$$S_{xx}(3) = \sum_{j=1}^{8} x_{3j}^2 - \frac{x_{3\bullet}^2}{8} = 3^2 + 6^2 + \ldots + 36^2 - \frac{108^2}{8} = 1008$$

$$S_{yy}(1) = \sum_{j=1}^{6} y_{1j}^2 - \frac{y_{1\bullet}^2}{6} = 102.4^2 + 98.1^2 + \ldots + 96.1^2 - \frac{588.3^2}{6} = 33.65$$

$$S_{yy}(2) = \sum_{j=1}^{7} y_{2j}^2 - \frac{y_{2\bullet}^2}{7} = 101.1^2 + 101.2^2 + \ldots + 94.3^2 - \frac{684.7^2}{7} = 43.75$$

$$S_{yy}(3) = \sum_{j=1}^{8} y_{3j}^2 - \frac{y_{3\bullet}^2}{8} = 104.1^2 + 102.1^2 + \ldots + 93.5^2 - \frac{781.1^2}{8} = 111.98$$

$$S_{xy}(1) = \sum_{j=1}^{6} x_{1j} y_{1j} - \frac{x_{1\bullet} y_{1\bullet}}{6} = 3(98.1) + 6(99.2) + \ldots + 18(96.1) - \frac{48(588.3)}{6} = -69.6$$

$$S_{xy}(2) = \sum_{j=1}^{7} x_{2j} y_{2j} - \frac{x_{2\bullet} y_{2\bullet}}{7} = 3(101.2) + 6(99) + \ldots + 24(94.3) - \frac{72(684.7)}{7} = -131.23$$

$$S_{xy}(3) = \sum_{j=1}^{8} x_{3j} y_{3j} - \frac{x_{3\bullet} y_{3\bullet}}{8} = 3(102.1) + 6(99.5) + \ldots + 36(93.5) - \frac{108(781.1)}{8} = -294.45$$

The total sums of squares are computed as

$$Z_{xx} = \sum_{i=1}^{3} S_{xx}(i) = 210 + 429.43 + 1008 = 1647.43$$

$$Z_{yy} = \sum_{i=1}^{3} S_{yy}(i) = 33.65 + 43.75 + 111.98 = 189.38$$

$$Z_{xy} = \sum_{i=1}^{3} S_{xy}(i) = -69.6 - 131.23 - 294.45 = -495.28$$

Using Equation (22), the aggregated sum-of-squares error is computed as

$$\mathrm{SSE_a} = Z_{yy} - \frac{Z_{xy}^2}{Z_{xx}} = 189.38 - \frac{495.28^2}{1647.43} = 40.48$$

The sum-of-squares errors for each batch is computed using Equation (24):

$$\mathrm{SSE}(1) = S_{yy}(1) - \frac{S_{xy}^2(1)}{S_{xx}(1)} = 33.65 - \frac{69.6^2}{210} = 10.58$$

$$\mathrm{SSE}(2) = S_{yy}(2) - \frac{S_{xy}^2(2)}{S_{xx}(2)} = 43.75 - \frac{131.23^2}{429.43} = 3.65$$

$$\mathrm{SSE}(3) = S_{yy}(3) - \frac{S_{xy}^2(3)}{S_{xx}(3)} = 111.98 - \frac{294.45^2}{1008} = 25.97$$

The aggregate sum-of-squares error is obtained using Equation (23):

$$\mathrm{SSE} = \sum_{i=1}^{3} \mathrm{SSE}(i) = 10.58 + 3.65 + 25.97 = 40.2$$

Thus, the mean-square error due to the slope is computed using Equation (25):

$$\mathrm{MS_{slope}} = \frac{\mathrm{SSE_a} - \mathrm{SSE}}{I - 1} = \frac{40.48 - 40.2}{3 - 1} = 0.14$$

The mean-square error for the regression model (14) is

$$\mathrm{MSE} = \frac{\mathrm{SSE}}{N - 2I} = \frac{40.2}{21 - 2(3)} = 2.68$$

Finally, the F statistic for testing equal degradation rate [hypothesis (47)] is

$$F(\text{slope}) = \frac{\text{MS}_{\text{slope}}}{\text{MSE}} = \frac{0.14}{2.68} = 0.052$$

The critical value to test for equality on slopes is $F_{0.25,I-1,N-2I} = F_{0.25,2,15} = 1.52$. Since $F(\text{slope}) < F_{0.25,2,15}$, hypothesis (47) for equal degradation rate cannot be rejected at the 0.25 level of significance. It is concluded that there is no significant difference among the slopes of model (14), that is, the model represented by Equation (14) reduces to model (29).

Step 2. The second step consists in testing for equality of intercepts among the batches. To derive the appropriate statistic for testing the underlying hypothesis, it is required to compute the following sum and cross products for the %LC and the sampling time. The hypothesis to be tested is

$$H_0: \quad \alpha_1 = \alpha_2 = \alpha_3 = \alpha \tag{48}$$

The sum and cross products are computed by using equations (32)–(40):

$$R_{xx} = \sum_{i=1}^{3}\sum_{j=1}^{n_i} x_{ij}^2 - \frac{x_{\cdot\cdot}^2}{N} = 3^2 + 6^2 + \ldots + 36^2 - \frac{228^2}{21} = 1754.5$$

$$R_{yy} = \sum_{i=1}^{3}\sum_{j=1}^{n_i} y_{ij}^2 - \frac{y_{\cdot\cdot}^2}{N} = 102.4^2 + 98.1^2 + \ldots + 93.5^2 - \frac{2054.1^2}{21} = 189.97$$

$$R_{xy} = \sum_{i=1}^{3}\sum_{j=1}^{n_i} x_{ij}y_{ij} - \frac{x_{\cdot\cdot}y_{\cdot\cdot}}{N} = 3(98.1) + 6(99.2) + \ldots + 36(93.5) - \frac{228(2054.1)}{21} = -503.06$$

$$T_{xx} = \sum_{i=1}^{3} \frac{x_{i\cdot}^2}{n_i} - \frac{x_{\cdot\cdot}^2}{N} = \frac{48^2}{6} + \frac{72^2}{7} + \frac{108^2}{8} - \frac{228^2}{21} = 107.14$$

$$T_{yy} = \sum_{i=1}^{3} \frac{y_{i\cdot}^2}{n_i} - \frac{y_{\cdot\cdot}^2}{N} = \frac{588.3^2}{6} + \frac{684.7^2}{7} + \frac{781.1^2}{8} - \frac{2054.1^2}{21} = 0.59$$

$$T_{xy} = \sum_{i=1}^{3} \frac{x_{i\cdot}y_{i\cdot}}{n_i} - \frac{x_{\cdot\cdot}y_{\cdot\cdot}}{N} = \frac{48(588.3)}{6} + \frac{72(684.7)}{7} + \frac{108(781.1)}{8} - \frac{228(2054.1)}{21} = -7.78$$

$$E_{xx} = \sum_{i=1}^{3}\sum_{j=1}^{n_i} (x_{ij} - \bar{x}_{i\cdot}) = R_{xx} - T_{xx} = 1754.5 - 107.14 = 1647.36$$

$$E_{yy} = \sum_{i=1}^{3}\sum_{j=1}^{n_i} (y_{ij} - \bar{y}_{i\cdot})^2 = R_{yy} - T_{yy} = 189.97 - 0.59 = 189.38$$

$$E_{xy} = \sum_{i=1}^{3}\sum_{j=1}^{n_i}(x_{ij} - \bar{x}_{i\bullet})(y_{ij} - \bar{y}_{i\bullet}) = R_{xy} - T_{xy} = -503.06 + 7.78 = -495.28$$

The sum-of-squares errors for the reduced and complete models are given by Equations (41) and (42), respectively:

$$\text{SSE}_\text{r} = R_{yy} - \frac{R_{xy}^2}{R_{xx}} = 189.97 - \frac{503.06^2}{1754.5} = 45.73$$

$$\text{SSE}_\text{c} = E_{yy} - \frac{E_{xy}^2}{E_{xx}} = 189.38 - \frac{495.28^2}{1647.36} = 40.47$$

The mean-square errors for measuring intercept effects are calculated using Equation (43) as

$$\text{MSE}_\text{int} = \frac{\text{SSE}_\text{r} - \text{SSE}_\text{c}}{I - 1} = \frac{45.73 - 40.47}{3 - 1} = 2.63$$

$$\text{MSE}_\text{c} = \frac{\text{SSE}_\text{c}}{N - I - 1} = \frac{40.47}{21 - 3 - 1} = 2.38$$

Finally the F statistic for testing the equality of intercepts is given by Equation (44) and computed as

$$F(\text{int}) = \frac{\text{MSE}_\text{int}}{\text{MSE}_\text{c}} = \frac{2.63}{2.38} = 1.105$$

The critical value to test the equality of the intercept is $F_{0.25,I-1,N-I-1} = F_{0.25,2,17} = 1.51$. Since $F(\text{int}) < F_{0.25,2,17}$, there is not enough evidence to reject the null hypothesis expressed by Equation (47) at the 25% of level of significance. It can be concluded that the intercepts are not statistically different.

In summary, the null hypotheses for equality of slopes and intercepts are not rejected at the 0.25 significance level and, consequently, all batches are considered from the same population. Therefore, the expiration dating period can be computed by using model (45).

After pooling the data from the three batches, the regression line can be written as

$$\hat{y}_{ij} = a + bx_{ij} = 100.93 - 0.2867x_{ij}$$

The regression subroutine also provides the following calculations: MSE = 2.407, $\bar{x} = 10.8571$, and $S_{xx} = 1.754.6$. Thus, to determine the shelf life, the following equation can be used:

$$f(x) = [90 - (100.93 - 0.2867x)]^2 - 1.7291^2(2.407)\left[\frac{1}{21} + \frac{(x - 10.86)^2}{1754.6}\right] = 0$$

To find the shelf life, the root of the above equation has to be found that is smaller than the reference value; the reference value is given by

$$x_{\text{ref}} = \frac{a - 90}{-b} = \frac{100.93 - 90}{0.2867} = 38.11$$

The expiration dating period must be smaller than 38.11 months. Using the initial point $x(0) = x_{\text{ref}} - d = 38.11 - 8$, the QNLS algorithm converges to $x_R = 32.801$. Therefore, the expiration dating period for the underlying production batches is 32 months.

Minimum Approach for Multiple Batches When the hypothesis for equality of slopes is rejected at the 0.25 significance level, the minimum approach should be implemented. This is because the degradation lines of individual batches cannot be considered the same since they have different degradation rates. In this situation the FDA guideline establishes that the overall expiration dating period has to ensure that the product will remain within acceptable limits regardless of the batch from which it comes. Thus, the shelf life for each batch is calculated and the expiration dating period is based on the lowest of all shelf lives. Mathematically, this can be expressed as

$$\min\{x_L(1), \ldots, K, x_L(k)\} \tag{49}$$

where the $x_L(i)$ is the shelf life of the ith batch, and i is the total number of batches.

Since the minimum of all the expiration dating periods is the shortest shelf life among all batches, this estimate will provide a 95% confidence that the strength of the drug product will remain above the acceptable lower specification limit.

Example 3 Slopes and Intercepts Are Different A stability study provides the assay results shown in Table 16. Based on this information, determine the shelf life for this drug product.

TABLE 16 Assay Results (%LC) for Example 3

Sampling time, months	0	3	6	9	12	18	24
Batch 1	99.2	97.1	96.1	95.2	93.8	93.1	92.4
Batch 2	98.7	97	96.2	95.1	94.2	93.3	—
Batch 3	102.5	98.9	97.1	95.6	94.1	93.1	—

Solution The conventional approach to determine the shelf life will require to test whether or not the slopes and the intercepts of the straight lines associated with the degradation rate of the considered batches are the same. ANCOVA is also used to test equalities on both slopes and intercepts.

The strategy consists of testing first the null hypothesis of equalities of slopes, which is given by Equation (14). To determine whether or not this hypothesis is rejected, the statistics defined by Equation (27) is computed:

$$F(\text{slope}) = \frac{\text{MS(slope)}}{\text{MSE}} = \frac{4.0507}{0.8032} = 5.04$$

The null hypothesis, expressed by Equation (15), is rejected at the 0.25 level of significance because the critical value ($F_{0.25,2,13} = 1.55$) of this test is smaller than F(slope). It is concluded that the slopes of the degradation lines of these batches are different and consequently the minimum approach applies. Thus, the shelf life for each batch is computed and the one that exhibits the minimum shelf life is applied to all manufactured batches.

The shelf life for the first batch is obtained by finding the root which is smaller than the reference point of the following equation:

$$[90-(98.0461-0.2898x)]^2 - 2.015^2(0.6689)\left[\frac{1}{7}+\frac{(x-10.28)^2}{429.43}\right]=0$$

The reference point of this equation is

$$x_{\text{ref}} = \frac{a-\delta}{-b} = \frac{98.0461-90}{0.2698} = 29.82$$

Using as an initial point $x(0) = x_{\text{ref}} - 8$, the root is $x_R(1) = 24.93$ and the shelf life for the first batch is $x_L(1) = 24$ months.

Using data for the second batch, the following equation is derived:

$$[90-(98.1157-0.2957x)]^2 - 2.1318^2(0.2328)\left[\frac{1}{6}+\frac{(x-8)^2}{210}\right]=0$$

The reference point for this equation is $x_{\text{ref}} = 27.44$ and the initial point to accomplish convergence is $x_{\text{ref}} - 5$. The corresponding root is $x_R(2) = 23.44$ and the shelf life for batch 2 is $x_L(2) = 23$ months.

The associated equation for the third batch is

$$[90-(100.91-0.5033x)]^2 - 2.1318^2(1.5415)\left[\frac{1}{6}+\frac{(x-8)^2}{210}\right]=0$$

The reference point for this equation is $x_{\text{ref}} = 21.67$ and the initial point to accomplish convergence is $x_{\text{ref}} - 5$. The root for this equation is $x_R(3) = 17.59$ and the shelf life for batch 3 is $x_L(3) = 17$ months.

Therefore, the shelf life that should be applied to future batches is

$$\min\{24 \quad 23 \quad 17\} = 17 \quad \text{months.}$$

Example 4 Equality of Slopes and Different Intercepts A stability study provides the assay results shown in Table 17. Based on this information, determine the shelf life for this drug product.

Solution The ANCOVA method is used for testing whether or not the batches have a common slope. The statistics that determines whether or not this hypothesis is rejected is given by Equation (27) and yields

$$F(\text{slope}) = \frac{\text{MS(slope)}}{\text{MSE}} = \frac{0.9891}{2.7134} = 0.3645$$

The null hypothesis, expressed by Equation (15), cannot be rejected at the 0.25 significance level because the critical value ($F_{0.25,2,17} = 1.51$) of this test is larger than F(slope). It is concluded that the slopes of the degradation lines of these batches are similar.

It is necessary to test whether or not the intercepts of batches are similar. The statistic to test intercept similarities is given by Equations (43) and (44) and provides the following results:

$$\text{MSE}_{\text{int}} = \frac{\text{SSE}_{\text{r}} - \text{SSE}_{\text{c}}}{I-1} = \frac{172.19 - 48.10}{2} = 62.04 \qquad \text{MSE}_{\text{c}} = \frac{\text{SSE}_{\text{c}}}{N-I-1} = \frac{48.10}{19}$$

$$F(\text{int}) = \frac{\text{MSE}_{\text{int}}}{\text{MSE}_{\text{c}}} = \frac{62.04}{2.53} = 24.51$$

The null hypothesis, expressed by Equation (28), is rejected at the 0.25 significance level because the critical value ($F_{0.25,2,19} = 1.49$) of this test is larger than F(int). It is concluded that the intercepts are different at the 0.25 significance level and the shelf life is estimated for each batch and the lowest value is applied to all the manufactured batches.

TABLE 17 Assay Results (%LC) for Example 4

Sampling time, months	0	3	6	9	12	18	24	36
Batch 1	98.4	96.1	94.2	93.5	90	89.1	89.2	87.3
Batch 2	99.1	97.2	96.3	95.2	93.4	91.5	90.3	—
Batch 3	104.1	102.1	99.5	98.1	95.7	94.1	94.0	93.5

The model that describes the degradation line is given in Equation (46), where $I = 3$, $n_1 = 8$, $n_2 = 7$, and $n_3 = 8$. To estimate the common slope and the three different intercepts of model (46), it has to be expanded as follows:

$$
\begin{aligned}
y_{11} &= \alpha_1 + \beta x_{11} + \varepsilon_{11} \\
y_{12} &= \alpha_1 + \beta x_{12} + \varepsilon_{12} \\
&\vdots \\
y_{18} &= \alpha_1 + \beta x_{18} + \varepsilon_{18} \\
y_{21} &= \alpha_2 + \beta x_{21} + \varepsilon_{21} \\
y_{22} &= \alpha_2 + \beta x_{22} + \varepsilon_{22} \\
&\vdots \\
y_{27} &= \alpha_2 + \beta x_{27} + \varepsilon_{27} \\
y_{31} &= \alpha_3 + \beta x_{31} + \varepsilon_{31} \\
y_{32} &= \alpha_3 + \beta x_{32} + \varepsilon_{32} \\
&\vdots \\
y_{38} &= \alpha_3 + \beta x_{38} + \varepsilon_{38}
\end{aligned}
\tag{50}
$$

A matrix representation of model (50) is given by the expression

$$\mathbf{y} = \mathbf{X}\mathbf{b} + \mathbf{e} \tag{51}$$

where the elements of **y, X, b**, and **e** are given in Table 18.

Regression techniques are used to estimate the parameter of model (51). Thus the intercepts for batches 1, 2, and 3 are $a_1 = 96.369$, $a_2 = 97.871$ and $a_3 = 101.78$, respectively; the common slope is $b = -0.3069$. All p values of the regression models are close to zero, indicating that the intercept of the batches are highly significant and the coefficient of multiple determination is very high, $R^2 = 0.9996$, indicating very good model fitting.

TABLE 18 Description of the Matrix and Vectors of Model (50)

Vector **y**	Matrix **X**				Vector **b**	Vector **e**
y_{11}	α_1	ε_{11}	0	x_{11}	α_1	ε_{11}
y_{12}	α_2	ε_{12}	0	x_{12}	α_2	ε_{12}
$\vdots$	α_3	$\vdots$	$\vdots$	$\vdots$	α_3	$\vdots$
y_{18}	β	ε_{18}	0	x_{18}	β	ε_{18}
y_{21}	0	ε_{21}	0	x_{21}		ε_{21}
y_{22}	0	ε_{22}	0	x_{22}		ε_{22}
$\vdots$	$\vdots$	$\vdots$	$\vdots$	$\vdots$		$\vdots$
y_{27}	0	ε_{27}	0	x_{27}		ε_{27}
y_{31}	0	ε_{31}	1	x_{31}		ε_{31}
y_{32}	0	ε_{32}	1	x_{32}		ε_{32}
$\vdots$	$\vdots$	$\vdots$	$\vdots$	$\vdots$		$\vdots$
y_{38}	0	ε_{38}	1	x_{38}		ε_{38}

TABLE 19 Information Required for Quadratic Equation

Batch	SSE	MSE	$\bar{x}$	S_{xx}	n_i
1	18.8860	3.1476	13.5	1008	8
2	3.0331	0.6061	10.28	429.43	7
3	26.187	4.3646	13.5	1008	8

The expiration dating period will be estimated by solving the corresponding quadratic equation (10) for each batch. The major difficulty of using the quadratic equation is the estimation of the MSE for each batch. The MSE estimate for each batch may be computed by performing a regression analysis for each batch; however, this approach may not be correct because there is no guarantee that each regression provides the same slope. Thus, we recommend implementing the following approach. Use results from the regression analysis of model (51) and compute the residuals for the entire model. Extract the corresponding errors for each batch and compute the SSE for each batch using Equation (4). Use this result to calculate the MSE for each batch using Equation (6) and the sample size for the corresponding batch. In addition, extract the corresponding values of x_{ij} for each batch and compute $\bar{x}$, and S_{xx} using Equation (4). Results from the described procedure are shown in Table 19.

The quadratic equation for batch 1 can be written as

$$[90-(96.369-0.3069x)]^2-1.9432^2(3.1476)\left[\frac{1}{8}+\frac{(x-13.5)^2}{1008}\right]=0$$

The reference point for this equation is x_{ref} = 20.7492 and the initial point to accomplish convergence is $x_{\text{ref}} - 6$. The root for this equation is $x_{\text{R}}(1)$ = 16.62 and the shelf life for batch 1 is $x_{\text{L}}(1)$ = 16 months.

Following a similar procedure the quadratic equation associated with batch 2 is

$$[90-(97.871-0.3069x)]^2-2.015^2(0.6061)\left[\frac{1}{7}+\frac{(x-10.2857)^2}{429.43}\right]=0$$

The reference point for this equation is x_{ref} = 25.6451 and the initial point to accomplish convergence is $x_{\text{ref}} - 6$. The root for this equation is $x_{\text{R}}(2)$ = 22.1394 and the shelf life for batch 2 is $x_{\text{L}}(2)$ = 22 months.

The corresponding quadratic equation for batch 3 is

$$[90-(101.78-0.3069x)]^2-1.9432^2(4.3646)\left[\frac{1}{8}+\frac{(x-13.5)^2}{1008}\right]=0$$

The reference point for this equation is x_{ref} = 38.38 and the initial point to accomplish convergence is $x_{\text{ref}} - 5$. The root for this equation is $x_{\text{R}}(3)$ = 30.0519 and the shelf life for batch 3 is $x_{\text{L}}(3)$ = 30 months.

Therefore, the shelf life that should be applied to all manufactured batches is

$$\min\{16 \quad 22 \quad 30\}=16 \quad \text{months}$$

7.2.3.4 Shelf Life Estimation for Multiple Factors

Most drug products are manufactured with more than one strength and are marketed in more than one package and consequently the stability analyses must be carried out for every combination of package and strength. For instance, suppose that a drug product is available in two strengths and four containers sizes. Thus, eight sets of data from the 2×4 strength size combinations can be analyzed and eight separate shelf lives should be estimated to calculate the shelf life for the drug product.

A general analysis-of-covariance model for a stability design with several batches and packages can be expressed as

$$y_{ijk} = \alpha_{ij} + \beta_{ij} x_{ijk} + \varepsilon_{ijk} \qquad i = 1, \ldots, I \qquad j = 1, \ldots, J \qquad k = 1, \ldots, n \tag{52}$$

where y_{ijk} is the response from the kth time point of the ith batch and the jth package of a drug product, x_{ijk} is the sampling time at which the y_{ijk} response was obtained, α_{ij} is the intercept for the ith batch and the jth package, β_{ij} is the degradation rate of the ith batch and the jth package, and ε_{ijk} is the random error assumed to be independently and normally distributed with zero mean and constant variance σ^2.

Chen et al. [11] show that there are 16 different models of Equation (52); however, these models reduce to 9. The general procedure consists in identifying the class of model that is associated to a given assay information. Once the model is determined, the appropriate procedure is implemented to determine the shelf life of the drug product. Chen et al. [11] established the procedure for estimating the shelf life.

Identification of Analysis of Covariance Model A general procedure, based on regression analysis, to identify the analysis-of-covariance model that applies to a given set of assay results to determine the shelf life is introduced here. We call this procedure the regression model with indicator variables for testing poolability of batches and packages.

To introduce the fundamental concepts and facilitate the understanding, we consider first a simple case, second a general model, and finally the methodology as an example.

Suppose we have three batches and the corresponding indicator variables model is defined as

$$y_{ij} = \beta_0 + \delta_1 u_1 + \delta_2 u_2 + x_{ij}(\beta_1 + \Delta_1 u_2 + \Delta_2 u_2) + e_{ij} \tag{53}$$

where y_{ij} is the assay result from the jth time point of the ith batch of a drug product, x_{ij} is the time at which the assay sample y_{ij} was obtained, u_1 and u_2 are indicator variables that assign a binary code to each factor combination, β_0 is part of the intercept of the regression line, and β_1 is part of the slope of the regression line. It should be noted that the intercept of model (53) is $\beta_0 + \delta_1 u_1 + \delta_2 u_2$ and the slope is given by $\beta_1 + \Delta_1 u_2 + \Delta_2 u_2$. The parameters δ and Δ are estimated from assay data; e_{ij} is the random error assumed to be independently and normally distributed with zero mean and constant variance σ^2.

TABLE 20 Binary Codification for Indicator Variables

Batch	u_1	u_2	Assay Result
B1	1	0	y_{1j}
B2	0	1	y_{2j}
B3	0	0	y_{3j}

Let B1, B2, and B3 be codes that represent batches 1, 2, and 3, respectively. The indicator variables are used to indicate at which batch (level of factor) is assigned the response variable. For instance, a response variable associated with B1 is represented by $u_1 = 1$ and $u_2 = 0$. Similarly, a response variable from B2 is represented by $u_1 = 0$ and $u_2 = 1$. Also a response variable from B3 is represented by $u_1 = 0$ and $u_2 = 0$. Thus, the values of u_1 and u_2 define in a unique manner the factor combination. The indicator variables for this model are summarized in Table 20.

The response variable from batch 1 can be written as follows [after replacing the values of indicator variables in model (53)]:

$$\begin{aligned}
y_{11} &= \beta_0 + \delta_1 + x_{11}(\beta_1 + \Delta_1) + e_{11} \\
y_{12} &= \beta_0 + \delta_1 + x_{12}(\beta_1 + \Delta_1) + e_{12} \\
&\vdots \\
y_{1n_1} &= \beta_0 + \delta_1 + x_{1n_1}(\beta_1 + \Delta_1) + e_{1n_1} \\
y_{21} &= \beta_0 + \delta_2 + x_{21}(\beta_1 + \Delta_2) + e_{21} \\
y_{22} &= \beta_0 + \delta_2 + x_{22}(\beta_1 + \Delta_2) + e_{22} \\
&\vdots \\
y_{2n_2} &= \beta_0 + \delta_2 + x_{2n_2}(\beta_1 + \Delta_2) + e_{2n_2} \\
y_{31} &= \beta_0 + x_{31}\beta_1 + e_{31} \\
y_{32} &= \beta_0 + x_{32}\beta_1 + e_{32} \\
&\vdots \\
y_{3n_3} &= \beta_0 + x_{3n_3}\beta_1 + e_{3n_3}
\end{aligned} \tag{54}$$

where n_i is the number of sampling times in the ith batch.

It should be noted that the system of linear equations expressed by (54) represents the response variable from the three batches. The required condition for the three batches to have the same intercept is that $\delta_1 = \delta_2 = 0$. The three batches will have the same slope if and only if $\Delta_1 = \Delta_2 = 0$. Thus, the problem for testing poolability reduces to fit the regression model (54) and test the following hypotheses: h_1: $\delta_1 = \delta_2 = 0$ and h_2: $\Delta_1 = \Delta_2 = 0$. Therefore, if the null hypothesis h_2 is not rejected at the 0.25 significance level, it implies that the slopes of the three batches are the same, that is, $\beta_1 = \beta_2 = \beta_3 = \beta$. Similarly, if the null hypothesis h_1 is not rejected, the intercepts of the three batches are the same, that is, $\alpha_1 = \alpha_2 = \alpha_3 = \alpha$. If that is the case, the shelf life is determined by a model with a single intercept and a single slope.

This procedure was applied to data from Examples 2 and 3 and the results from the regression analysis are given in Table 21.

TABLE 21 Testing for Poolability of Batches

Example 2: Equality of Slopes and Intercepts[a]				Example 3: Slopes and Intercepts Are Different[b]			
Parameter	Estimate	*t* Statistic	*p* Value	Parameter	Estimate	*t* Statistic	*p* Value
β_0	101.58	112.20	0.0000	β_0	100.91	163.99	0.0000
δ_1	–0.87	–0.61	0.5513	δ_1	–2.86	–3.44	0.0044
δ_2	–0.62	–0.46	0.6543	δ_2	–2.79	–3.21	0.0068
β_1	–0.29	–5.66	0.0000	β_1	–0.50	–8.14	0.0000
Δ_1	–0.04	–0.32	0.7559	Δ_1	0.23	3.09	0.0085
Δ_2	–0.01	–0.14	0.8883	Δ_2	0.21	2.37	0.0337

Example 2:
[a]h_1: $\delta_1 = \delta_2 = 0$. *P* Values of this table show that this hypothesis is not rejected at the 0.25 significance level and batches have common intercepts.
h_2: $\Delta_1 = \Delta_2 = 0$. This hypothesis is not rejected at the 0.25 significance level and batches have common slopes.
Example 3:
[b]h_1: $\delta_1 = \delta_2 = 0$. This hypothesis is rejected at the 0.25 significance level and batches have different intercepts.
h_2: $\Delta_1 = \Delta_2 = 0$. This hypothesis is rejected at the 0.25 significance level and batches have different slopes.

The indicator variables model to perform a poolability test for two factors (packages and batches) can be expressed as follows:

$$\begin{aligned} y_{ijk} = \beta_0 + \beta_1 x_{ijk} + \delta_1 u_1 + \delta_2 u_2 + \ldots + \delta_r u_r + \phi_1 v_1 + \phi_2 v_2 \\ + \ldots + \phi_s v_s + x_{ijk}(\Delta_1 u_1 + \Delta_2 u_2 + \ldots + \Delta_r u_r + \Phi_1 v_1 \\ + \Phi_2 v_2 + \ldots + \Phi_s v_s + \omega_{11} u_1 v_1 + \omega_{12} u_1 v_2 + \ldots \\ + \omega_{rs} u_r v_s) + e_{ijk} \end{aligned} \quad (55)$$

where $i = 1, \ldots, I$, $j = 1, \ldots, J$, and $k = 1, \ldots, n_{ij}$ and I is the number of batches, J is the number of packages, n_{ij} is the sample size of the ith batch and the jth package, and u_i and v_j are indicator variables that are defined as shown in Table 22.

The first column of Table 22 shows the factor combinations. For instance, B1P1 represents the first batch and the first package, B1P2 indicates the first batch and the second package, and BIPJ represents the last batch and the last package. The subscripts r and s are defined as follows: $r = I - 1$, $s = J - 1$.

Testing for poolability requires fitting the regression model with indirect variables to the assay data and testing four possible hypotheses. Table 23 shows the hypotheses to be tested, the corresponding interpretation to whether or not the hypothesis is accepted or rejected, the code of the model, and the model established by the FDA [8, 11]. In Table 23 the letters A and R represent whether the considered hypotheses are accepted or rejected, respectively.

Rules for Determining Shelf Life Once the analysis-of-covariance model has been identified, a set of rules for computing the shelf life must be implemented. This section describes the rules to follow to determine the expiration dating period for each of the nine representative models described in the previous section [8, 11]:

TABLE 22 Binary Code for Factor Combinations

Factor Combinations	u_1	u_2	…	u_r	v_1	v_2	…	v_r	y_{ijk}
B1P1	1	0	…	0	1	0	…	0	y_{11k}
B2P1	0	1	…	0	1	0	…	0	y_{21k}
⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮
BrP1	0	0	…	1	1	0	…	0	y_{r1k}
BIP1	0	0	…	0	1	0	…	0	y_{I1k}
B1P2	1	0	…	0	0	1	…	0	y_{12k}
B2P2	0	1	…	0	0	1	…	0	y_{22k}
⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮
BrP2	0	0	…	1	0	1	…	0	y_{r2k}
BIP2	0	0	…	0	0	1	…	0	y_{I2k}
⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮
B1PJ	1	0	…	0	0	0	…	1	y_{1Jk}
B2PJ	0	1	…	0	0	0	…	1	y_{2Jk}
⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮
BrPJ	0	0	…	1	0	0	…	1	y_{rJk}
BIPJ	0	0	…	0	0	0	…	1	y_{IJk}

1. C0: The expiration dating period is computed separately for each batch and package combination. The minimum shelf life of the individual batches (at least three batches) from the same package is the shelf life of that package.
2. C1: The data from the individual batches are combined within each package. For each package:
   - M1: Assuming a common slope with different intercepts for different batches, the shelf lives are computed for individual batches. The lowest of all shelf lives of the individual batches is the expiration dating period of that package.
   - M3: Assuming a common slope and a common intercept for all batches, a single shelf life is computed as the expiration dating period of that package.
3. C2: The data from individual packages are combined within the batch:
   - M2: For each batch, assuming a common slope with different intercepts for different packages, the shelf lives are computed for individual packages. The lowest shelf life of the individual batches from the same package is used as the expiration dating period of that package.
   - M4: For each batch, assuming a common slope and a common intercept for all packages, a single shelf life is computed. The lowest expiration dating period of the individual batches is used as the shelf life for every package.
4. C3: The data for all packages and batches are combined:
   - M5: Assuming different intercepts but a common slope for all batches and package combinations. The lowest shelf life of the individual batches from the same package is used as the expiration dating period of that package.
   - M6: Assuming a common intercept for all batches with a common slope for all batch and package combinations, a single expiration dating period is computed for each package to be used as the expiration dating period of that package.

TABLE 23 Procedure for Identifying Analysis of Covariance Model

Code	Hypotheses	A	R	Interpretation	ANCOVA Model
1 C0: M0a	H_1: $\delta_1 = \delta_2 = \ldots = \delta_r = 0$	X		Intercepts of all batches are the same, $\alpha_{1j} = \alpha_{2j} = \ldots = \alpha_{Ij} = \alpha_j$	$y_{ijk} = \alpha_j + \beta_{ij}x_{ijk} + e_{ijk}$
2 C1: M1	H_2: $\Delta_1 = \Delta_2 = \ldots = \Delta_r = 0$, $\omega_{11} = \omega_{12} = \ldots = \omega_{rs} = 0$	X		Slopes of all batches are the same, $\beta_{1j} = \beta_{2j} = \ldots = \beta_{Ij} = \beta_j$	$y_{ijk} = \alpha_{ij} + \beta_j x_{ijk} + e_{ijk}$
2 C1: M3	H_1 and H_2	X		All batches have the same intercept and the same slope, $\alpha_{1j} = \alpha_{2j} = \ldots = \alpha_{Ij} = \alpha_j$ $\beta_{1j} = \beta_{2j} = \ldots = \beta_{Ij} = \beta_j$	$y_{ijk} = \alpha_j + \beta_j x_{ijk} + e_{ijk}$
1 C0: M0b	H_3: $\phi_1 = \phi_2 = \ldots = \phi_s = 0$	X		Intercepts of all packages are the same, $\alpha_{i1} = \alpha_{i2} = \ldots = \alpha_{iJ} = \alpha_i$	$y_{ijk} = \alpha_i + \beta_{ij}x_{ijk} + e_{ijk}$
3 C2: M2	H_4: $\Phi_1 = \Phi_2 = \ldots = \Phi_s = 0$, $\omega_{11} = \omega_{12} = \ldots = \omega_{rs} = 0$	X		Slopes of all packages are the same, $\beta_{i1} = \beta_{i2} = \ldots = \beta_{iJ} = \beta_i$	$y_{ijk} = \alpha_{ij} + \beta_i x_{ijk} + e_{ijk}$
3 C2: M4	H_3 and H_4	X		Intercepts and slopes of all packages are the same, $\alpha_{i1} = \alpha_{i2} = \ldots = \alpha_{iJ} = \alpha_i$ $\beta_{i1} = \beta_{i2} = \ldots = \beta_{iJ} = \beta_i$	$y_{ijk} = \alpha_j + \beta_i x_{ijk} + e_{ijk}$
3 C3: M5	H_2 and H_4	X		All batches and all packages will have a common slope, $\beta_{1j} = \beta_{2j} = \ldots = \beta_{Ij} = \beta_j$ $\beta_{i1} = \beta_{i2} = \ldots = \beta_{iJ} = \beta_i$ $\beta_i = \beta_j = \beta$	$y_{ijk} = \alpha_{ij} + \beta x_{ijk} + e_{ijk}$
4 C3: M6	H_1, H_2, and H_4	X		All batches and packages have common slope and the intercepts of all batches are the same, $\alpha_{1j} = \alpha_{2j} = \ldots = \alpha_{Ij} = \alpha_j$ $\beta_{1j} = \beta_{2j} = \ldots = \beta_{Ij} = \beta_j$ $\beta_{i1} = \beta_{i2} = \ldots = \beta_{iJ} = \beta_i$ $\beta_i = \beta_j = \beta$	$y_{ijk} = \alpha_j + \beta x_{ijk} + e_{ijk}$
4 C3: M7	H_2, H_3, and H_4	X		All batches and packages have common slope and the intercepts of all packages are the same, $\alpha_{i1} = \alpha_{i2} = \ldots = \alpha_{iJ} = \alpha_i$ $\beta_{1j} = \beta_{2j} = \ldots = \beta_{Ij} = \beta_j$ $\beta_{i1} = \beta_{i2} = \ldots = \beta_{iJ} = \beta_i$ $\beta_i = \beta_j = \beta$	$y_{ijk} = \alpha_i + \beta x_{ijk} + e_{ijk}$

TABLE 23 ***Continued***

Code	Hypotheses	A	R	Interpretation	ANCOVA Model
4 C8: M8	H_1, H_2, H_3, and H_4	X		Slopes and intercepts of all batches and packages are the same, $\alpha_{1j} = \alpha_{2j} = \ldots = \alpha_{Ij} = \alpha_j$ $\alpha_{i1} = \alpha_{i2} = \ldots = \alpha_{iJ} = \alpha_i$ $\alpha_i = \alpha_j = \alpha$ $\beta_{1j} = \beta_{2j} = \ldots = \beta_{Ij} = \beta_j$ $\beta_{i1} = \beta_{i2} = \ldots = \beta_{iJ} = \beta_i$ $\beta_i = \beta_j = \beta$	$y_{ijk} = \alpha + \beta x_{ijk} + e_{ijk}$
1 C0	H_1, H_2, H_3, and H_4		X	Intercepts and slopes of batches and packages are different	$y_{ijk} = \alpha_{ii} + \beta_{ij} x_{ijk} + e_{ijk}$

TABLE 24 Assay Results (%LC)

		By Sampling Time in Months					
Package	Batch	0	3	6	9	12	18
Bottle	1	104.8	102.5	101.5	102.4	99.4	96.5
	2	103.9	101.9	103.2	99.6	100.2	98.8
	3	103.5	102.1	101.9	100.3	99.2	101.0
	4	101.5	100.3	101.1	100.6	100.7	98.4
	5	106.1	104.3	101.5	101.1	99.4	98.2
Blister	1	102.0	101.6	100.9	101.1	101.7	97.1
	2	104.7	101.3	103.8	99.8	98.9	97.1
	3	102.5	102.3	100.0	101.7	99.0	100.9
	4	100.1	101.8	101.4	99.9	99.2	97.4
	5	105.2	104.1	102.4	100.2	99.6	97.5

- M7: Assuming a common intercept for all packages and a common slope for all batches and package combinations, the expiration dating periods are computed for each batch. The minimum of the individual batches is used as the expiration dating period for every package.
- M8: Assuming a common slope and a common intercept for all packages and batch combinations, a single expiration dating period is computed to be used as the shelf life for every package.

Example 5 Multiple-Factor Stability Study A well-known example introduced by Shao and Chow [19] is used to illustrate the application of shelf life calculations for a multifactor case. A stability study was conducted on a 300-mg tablet of a drug product to establish the shelf life for each of the two types of packages used for this product: bottle and blister. The results are shown in Table 24. Each type of package includes five batches. The tablets were tested for potency at 0, 3, 6, 9, 12, and 18 months. Determine the shelf life based on these stability data.

Solution Based on these data, we can extract the following information to build the regression model with indicator variables: I = 5 batches, J = 2 packages, r = 4, s = 1, and n = 6 sampling times for all batches: 0, 3, 6, 9, 12, and 18 months. The indicator variables are shown in Table 25 and the indicator variables model for this case is

$$\begin{aligned} y_{ijk} = \beta_0 + \beta_1 x_{ijk} + \delta_1 u_1 + \delta_2 u_2 + \delta_3 u_3 + \delta_4 u_4 + \phi_1 v_1 \\ + x_{ijk}(\Delta_1 u_1 + \Delta_2 u_2 + \Delta_3 u_3 + \Delta_4 u_4 + \Phi_1 v_1 + \omega_{11} u_1 v_1 \\ + \omega_{21} u_2 v_1 + \omega_{31} u_3 v_1 + \omega_4 u_4 v_1) + e_{ijk} \end{aligned} \tag{56}$$

where subscript i refers to the batch, subscript j to the package type, and subscript k to the sampling time.

A conventional computer package for regression analysis was used to estimate the parameters for the model described by Equation (56) and the results are summarized in Table 26.

TABLE 25 Definition of Indicator Variables

Factor Combinations	u_1	u_2	u_3	u_4	v_1	y_{ijk}
B1P1	1	0	0	0	1	y_{11k}
B2P1	0	1	0	0	1	y_{21k}
B3P1	0	0	1	0	1	y_{31k}
B4P1	0	0	0	1	1	y_{41k}
B5P1	0	0	0	0	1	y_{51k}
B1P2	1	0	0	0	0	y_{12k}
B2P2	0	1	0	0	0	y_{22k}
B3P2	0	0	1	0	0	y_{32k}
B4P2	0	0	0	1	0	y_{42k}
B5P2	0	0	0	0	0	y_{52k}

TABLE 26 Parameter Estimation for Indicator Variables Model

Parameter	Estimate	t Statistics	p Value
β_0	104.95	185.76	0.0000
δ_1	–1.63	–2.23	0.0306
δ_2	–1.33	–1.83	0.0747
δ_3	–2.83	–3.88	0.0003
δ_4	–3.64	–4.99	0.0000
ϕ_1	0.44	0.96	0.3408
β_1	–0.43	–6.91	0.0000
Δ_1	0.15	1.73	0.0901
Δ_2	0.07	0.88	0.3823
Δ_3	0.31	3.62	0.0007
Δ_4	0.26	3.01	0.0043
Φ_1	–0.01	–0.18	0.8557
ω_{11}	–0.06	–0.65	0.5161
ω_{21}	0.05	0.56	0.5770
ω_{31}	–0.02	–0.21	0.8313
ω_{41}	0.04	0.43	0.6660

P Values of Table 26 show the hypothesis H_3: $\phi = 0$ and H_4: $\Phi_1 = \omega_{11} = \omega_{21} = \omega_{31} = \omega_{41} = 0$ are not rejected at the 0.25 significance level and, according to Table 23, the intercepts and slopes of all packages are the same, that is, $\alpha_{i1} = \alpha_{i2} = \alpha_i$ and $\beta_{i1} = \beta_{i2} = \beta_i$. Therefore, the analysis-of-covariance model that should be applied to the assay results has the code 3 C2: M4 and has the form $y_{ijk} = \alpha_i + \beta_i x_{ijk} + e_{ijk}$.

Since there is no difference between the packages, a simple analysis-of-covariance model is suitable. The rules for computing the expiration dating period is given in Section 7.2.3.3 and indicate that a shelf life should be computed for each batch and the minimum criterion is used to determine the expiration dating period for the underlying batches of the drug product. Thus, regression model fitting should be conducted using model 3 C2: M4 and the results are used to develop the quadratic equation.

The corresponding quadratic equation to determine the shelf life for the first batch is

$$[90-(103.54-0.3233x)]^2 - 1.8125^2(1.48)\left[\frac{1}{12}+\frac{(x-8)^2}{420}\right]=0$$

The reference point for this equation is $x_{\text{ref}} = 41.89$ and the initial point to accomplish convergence is $x_{\text{ref}} - 7$. The root for this equation is $x_{\text{R}}(1) = 33.25$ and the shelf life for batch 1 is $x_{\text{L}}(1) = 33$ months. Since the sampling times are the same for all batches, the following values remain invariant for all batches: $n = 12$, $\bar{x} = 8$, $S_{xx} = 420$, and $t_{0.05,10} = 1.8125$. The values required by the quadratic equation and the associated shelf life for each batch are given in Table 27. The computer program to perform this calculation is given in the Appendix.

Therefore, the shelf life that should be applied to current and future batches is

$$\min\{33 \quad 32 \quad 54 \quad 49 \quad 31\} = 31 \quad \text{months}$$

This approach provides a conservative estimate of the overall shelf life because it provides more than 95% confidence for all batches except the batch from which it is estimated. It is worth mentioning that the minimum approach has been highly criticized by several researchers. For instance, Chow and Shao [20] pointed out that the minimum approach lacks statistical justifications. Ruberg and Stegeman [21] and Ruberg and Hsu [22] described the drawbacks of this methodology. However, Chen et al. [11] show that the FDA procedure performs reasonably well for data from a typical stability design with three batches. The FDA procedure is based on the assumption that batch effects are fixed. If the analysis shows that batch-to-batch differences are small, it is advantageous to combine all the data to obtain one overall estimate. If the analysis shows evidence of batch-to-batch differences, then the FDA

TABLE 27 Required Parameters to Compute Shelf Life

batch	a	b	MSE	x_{ref}	x_{R}	Shelf Life
2	103.84	–0.3429	1.47	40.37	32.49	32
3	102.34	–0.1429	1.24	86.4	54.26	54
4	101.54	–0.1671	0.74	69.02	49.84	49
5	105.17	–0.4426	0.45	34.28	31.08	31

uses the minimum of all estimates obtained from individual batches based on the premise that the overall expiration dating period may depend on the minimum time a batch may be expected to remain within acceptable limits. It also shows that the smaller is the experimental variance, the higher is the expiration period. Thus, the FDA exhibits conservatism when the number of batches is large with small batch variabilities. The FDA established that the 0.25 significance level is used to compensate for the expected low power of the design due to the relatively limited sample size in a typical formal stability study [8]. We supported the FDA arguments for the 0.25 significance level and this handbook outlines the procedure approved by the FDA.

7.2.4 SHORT-TERM STABILITY ANALYSIS

7.2.4.1 Introduction

Assuring acceptable stability of drugs remains a challenge to industry. As mentioned in Section 7.2.2.1 the stability concept involves the classical aspect of API degradation as well as the presence of degradation products at levels that represent a risk to the patient [23]. In general, two stability studies are conducted to ensure that the market drug product is under the required specifications: short-term and long-term studies. A short-term stability study is an accelerated stability testing study, that is, a study under stressed storage conditions. The main goal of accelerated stability testing is not only to determine the chemical reaction kinetics but also to establish a tentative expiration date under the environment of marketing storage conditions.

The stability of a drug product depends on the storage conditions, temperature and relative humidity, at which the product is exposed during its shelf life period. The effect of the environment on the degradation depends on the packaging configuration used and the chemical characteristics of the drug product. The degradation of a drug product is mainly caused by the chemical reaction of the API with the excipients or with species in the environment (atmospheric oxygen and humidity) causing a decrease of the assay results over time. During the whole shelf life period, the API of a drug product is degrading at a certain rate. Other quality attributes (e.g., physical appearance, sterility, drug release rate, impurities) may also be changing with time, affecting the functionality, efficacy, or purity of the drug product. The magnitude of these changes during the expiration dating period should in no way represent a risk to the patient.

The rate of a chemical reaction is a function of temperature and concentration of the reactants present. Therefore, to induce a change in the reaction rate, a change in temperature or reactant concentration must be caused. The temperature can be readily changed by varying the storage temperature of the drug product. The reactants in a drug product are the API, the excipients, and the environment air and humidity. Of these, only the environment air and humidity can be changed because the API and the excipients within the drug product are specified and cannot be changed.

It is of interest for the pharmaceutical industry to know the degradation of its drug products at accelerated conditions to assess degradation for longer term storage at nonextreme conditions and short excursions outside of the recommended storage

conditions that might occur during shipping. The short-term stability study is the method to evaluate degradation of a drug product at accelerated conditions. As an example of this, Gil-Alegre et al. [24] studied the degradation kinetics of mitonafide, an antineoplastic agent, at temperatures between 60°C and 90°C at 10°C intervals. This is a very stable drug and, thus, rather high temperatures had to be used to have detectable concentration changes. Normally, accelerated testing is carried out at constant temperature. However, ramping temperatures have been used in non-isothermal testing [25]. This approach may lead to better prediction of the low-temperature kinetic constant and thus expiration dating periods [23].

7.2.4.2 Chemical Reaction Kinetics

To understand how degradation data are treated, it is convenient to mention the basics of chemical reaction kinetics. The principles of chemical reaction engineering can be found in any reaction engineering or reactor design textbook [26]. A chemical reaction is the process whereby one or more components are transformed into one or more different components. The rate of reaction is the velocity at which the component(s) are being transformed in a chemical reaction. For the chemical reaction

$$\mathrm{A} + \mathrm{B} \rightarrow \mathrm{E} \tag{57}$$

the reaction rate can be expressed as

$$\frac{dC}{dt} = -k_{n+m}[\mathrm{A}]^n[\mathrm{B}]^m \tag{58}$$

where C is the molar concentration of the component to be studied, that is, [A] or [B], the brackets represent the concentration of the reactants, and k is the rate constant, also known as the specific reaction rate. The rate of reaction is positive if it refers to a product and negative if it refers to a reactant. The reaction order is the sum of the exponents ($n + m$) in Equation (58). According to this, a reaction of order zero is represented with the equation

$$\frac{dC}{dt} = -k_0 \tag{59}$$

which can be integrated to give

$$C = C_0 - k_0 t \tag{60}$$

where C is the molar concentration at any time t, C_0 is the concentration at time $t = 0$, k_0 is the rate constant of the zero-order reaction, and t is the time. For a first-order reaction, the rate can be expressed as

$$\frac{dC}{dt} = -k_1 C \tag{61}$$

which integrates to

$$\ln\left(\frac{C}{C_0}\right) = -k_1 t \qquad \text{or} \qquad C = C_0 - C_0 e^{-k_1 t} \tag{62}$$

The temperature has a strong effect on the rate constant; this effect is represented by the Arrhenius equation:

$$k = A e^{-E/(RT)} \tag{63}$$

where A is the frequency factor and has the same units of k, E is the activation energy, R is the gas constant, and T is absolute temperature in kelvin. The logarithmic expression of the Arrhenius equation is

$$\ln k = -\frac{E}{RT} + \ln A \tag{64}$$

7.2.4.3 Degradation Data Evaluation at Accelerated Conditions

Chemical reaction kinetics can be used to evaluate degradation data at accelerated conditions and predict the drug product assay at normal conditions for periods longer than the proposed shelf life. This is applicable to limited cases because the reaction kinetics is often complex for drug products. The following example illustrates the procedure to follow to calculate the API concentration with time for a drug product stored at normal conditions when such data are not yet available.

Example 6 Estimation of Degradation from Accelerated Data: First-Order Case Consider the degradation data shown in Table 28, obtained at normal, intermediate, and accelerated storage conditions. All assay values are expressed as a percentage of the label claim (%LC). Ignoring the first two columns, that is, using only the data at higher temperatures (30°C and higher), estimate the assay values at 25°C as a function of time. [*Note:* The data in this example are artificial and will be used only for demonstration purposes. The normal-conditions data are displayed in the first two columns (25°C). Usually these data are not available when a new drug application (NDA) is submitted and that is why the procedure being presented here is important.]

TABLE 28 Assay Degradation Data for Drug Product

t (months)	Assay at 25°C	t (months)	Assay at 30°C	Assay at 40°C	Assay at 50°C
0	99.9	0	99.9	99.9	99.9
3	99.4	2	99.4	98.0	95.6
6	98.2	4	98.7	95.9	91.2
9	97.8	6	97.4	95.1	87.4
12	97.4				
18	95.6				
24	94.5				
36	91.7				

Solution The analysis can be done assuming that the reaction is first order (this is usually the case in real life) or zero order (simpler analysis with no significant error for degradation of less than 10%). Both cases are presented here. For first-order kinetics [refer to Equation (61)], the first step is to obtain the natural logarithm of the concentrations, which is done in Table 29. Then, these values are graphed as a function of time where straight lines should be obtained as shown in Figure 2. The slope of each line corresponds to the value of k_1, which is shown in the bottom part of Table 29. Next, a plot of $\ln(k_1)$ versus $1/T$ is prepared with the three values of k_1, one for each temperature as shown in Figure 3. Note that absolute temperature should be used here. From Equation (64), the value of k_1 can be extrapolated to $T = 25°C$ ($1/T = 0.003354\ K^{-1}$) to then predict the drug product assay as a function of time. The values of slope and intercept are shown in the inset of Figure 3; the calculation is

$$\ln k_{25} = -8237.4 \times 0.003354 + 21.639 = -5.990$$

$$k_{25} = 0.00250\,\text{month}^{-1}$$

Once the specific reaction rate is calculated, the drug product assay can be predicted using Equation (62). The results are shown in Table 30 along with the actual data from Table 28.

TABLE 29 Degradation Data Treated as First-Order Kinetics

t (months)	ln(assay) at 30°C	ln(assay) at 40°C	ln(assay) at 50°C
0	4.60	4.60	4.60
2	4.59	4.58	4.55
4	4.58	4.55	4.50
6	4.57	4.55	4.46
k_1	0.00415	0.00847	0.02224
$\ln(k_1)$	–5.483	–4.771	–3.798
1/T	0.00330	0.00319	0.00309

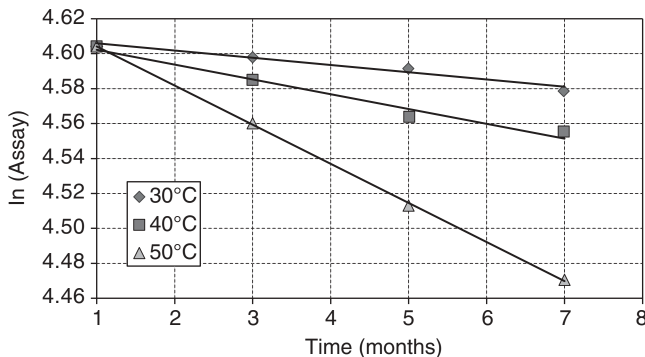

FIGURE 2 Degradation data treated as first-order kinetics.

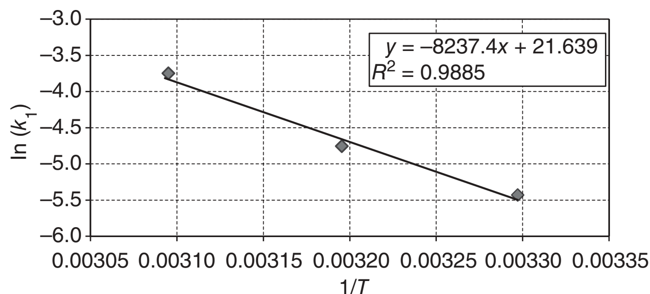

FIGURE 3 Arrhenius plot for first-order degradation.

TABLE 30 Predicted Assay Values Using Arrhenius and First-Order Kinetics

t (months)	Assay	Predicted Assay
0	99.0	100.0
3	98.5	98.3
6	97.3	97.5
9	96.9	96.8
12	96.5	96.1
18	94.7	94.6
24	93.6	93.2
36	90.8	90.5

Example 7 Estimation of Degradation from Accelerated Data: Zero-Order Case Repeat Example 6 assuming that the degradation is of zero order.

Solution For zero-order kinetics [refer to Equation (60)], the original concentrations can be graphed as a function of time. Straight lines can be drawn through the points even if the actual degradation order is 1 because the degradation is small (less than 10%), as seen in Figure 4. The slope of each line corresponds to the value of k_0. These values are shown in Table 31. Next, a plot of $\ln(k_0)$ versus $1/T$ is prepared with the three values of k_0, one for each temperature, which is shown in Figure 5.

As in Example 6, the value of k_0 can be extrapolated to $T = 25°C$ ($1/T = 0.003354$ K^{-1}) using Equation (64) to then predict the drug product assay as a function of time. The values of slope and intercept are shown in the inset of Figure 5; the calculation is

$$\ln k_{25} = -7974.8 \times 0.003354 + 25.369 = -1.379$$

$$k_{25} = 0.2519 \text{ month}^{-1}$$

Once the specific reaction rate is calculated, the drug product assay can be predicted using Equation (60). The results are shown in Table 32 along with the actual data from Table 28.

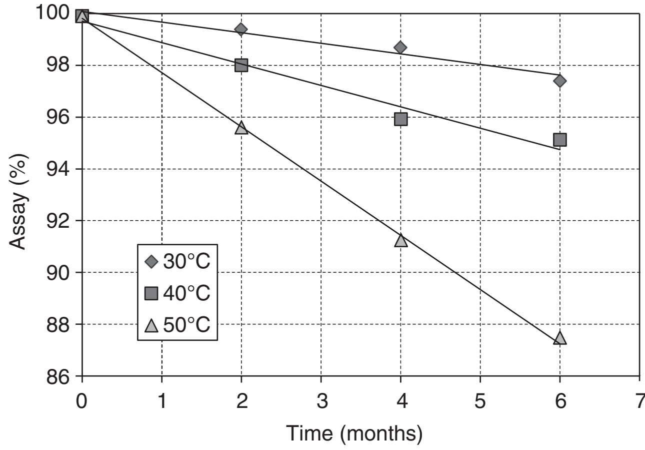

FIGURE 4 Degradation data treated as zero-order kinetics.

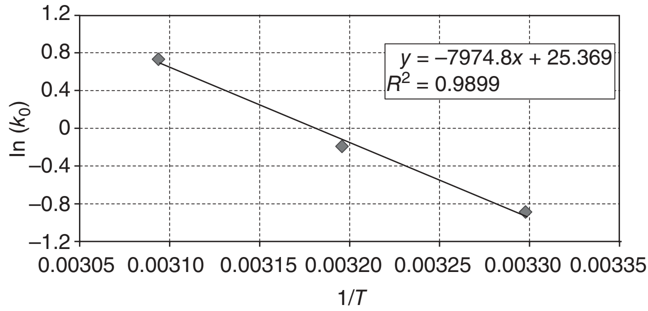

FIGURE 5 Arrhenius plot for zero-order degradation.

TABLE 31 Degradation Data Treated as Zero-Order Kinetics

	30°C	40°C	50°C
k_0	0.410	0.825	2.095
$\ln(k_0)$	–0.892	–0.192	0.740
$1/T$	0.00330	0.00319	0.00309

TABLE 32 Predicted Assay Values Using Arrhenius and Zero-Order Kinetics

t (months)	Assay	Predicted Assay
0	99.0	100.0
3	98.5	99.2
6	97.3	98.5
9	96.9	97.7
12	96.5	97.0
18	94.7	95.5
24	93.6	94.0
36	90.8	90.9
Shelf life	41	39

TABLE 33 Actual and Predicted Assay Values

		Predicted Assay	
t (months)	Assay	First Order	Zero Order
0	99.9	100.0	100.0
3	99.4	99.3	99.2
6	98.2	98.5	98.5
9	97.8	97.8	97.7
12	97.4	97.0	97.0
18	95.6	95.6	95.5
24	94.5	94.2	94.0
36	91.7	91.4	90.9

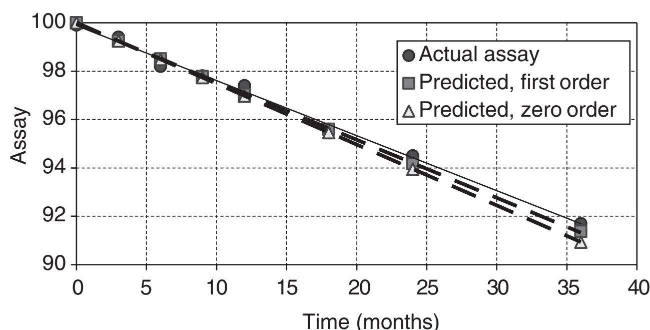

FIGURE 6 Actual and predicted assay values.

Comparison of Models To compare the predictions of both models (first- and zero-order kinetics), Table 33 and Figure 6 have been prepared. As expected, both predictions give quite similar results. The first-order model gives slightly better results, suggesting that the degradation is actually of first order. Both predictions yield similar results because degradation reactions are quite slow and therefore the time frame of these measurements is very small compared, say, to the half-life of the degradation (or the time it takes for the assay to reach the value of 50% LC). Although the discussion of half-life calculation is beyond the scope of this handbook, suffice it to say that based on the results of Examples 6 and 7, the half-life for first-order kinetics is about 23 years and for zero-order kinetics (probably less accurate) is 17 years. These values are about 40 times the time frame used in the accelerated (short-term) tests of 6 months (0.5 year). Thus, any kinetic model will work reasonably well.

7.2.4.4 Preliminary Shelf Life Calculation from Stressed Data

Preliminary shelf life can be calculated based on accelerated results. Regression techniques described in Section 7.2.3 applies to the estimation assays obtained from

accelerated data as illustrated in Examples 6 and 7. The procedure is the same as outlined in Section 7.2.3, that is, to develop the quadratic equation and determine the shelf life for each of the observed and predicted assay results.

The quadratic equation to determine the shelf life for the observed assay is

$$[90-(99.884-0.2275x)]^2-1.9432^2(0.0408)\left[\frac{1}{8}+\frac{(x-13.5)^2}{1008}\right]=0$$

The reference point for this equation is $x_{\text{ref}} = 43.44$ and the initial point to accomplish convergence is $x_{\text{ref}} - 1.5$. The root for this equation is $x_R(\text{obs}) = 41.78$ and the shelf life for observed assay results is $x_L(\text{obs}) = 41$ months. This value will be used for comparison purposes.

The quadratic equation for the predicted assay assuming a first-order chemical reaction applies is

$$[90-(99.95-0.2393x)]^2-1.9432^2(0.0032)\left[\frac{1}{8}+\frac{(x-13.5)^2}{1008}\right]=0$$

The reference point for this equation is $x_{\text{ref}} = 41.60$ and the initial point to accomplish convergence is $x_{\text{ref}} - 0.4$. The root for this equation is $x_R(\text{first}) = 41.17$ and the shelf life for the predicted assay assuming that the chemical reaction is first order is x_L (first) = 41 months.

Now, assuming that the chemical reaction is of zero order, the quadratic equation for the predicted assay is

$$[90-(99.99-0.2515x)]^2-1.9432^2(0.0013)\left[\frac{1}{8}+\frac{(x-13.5)^2}{1008}\right]=0$$

The reference point for this equation is $x_{\text{ref}} = 39.74$ and the initial point to accomplish convergence is $x_{\text{ref}} - 0.3$. The root for this equation is $x_R(\text{zero}) = 39.49$ and the shelf life for the predicted assay assuming that the chemical reaction is zero order is $x_L(\text{zero}) = 39$ months.

In summary, the shelf life from the actual data is 41 months, that estimated from first-order kinetics is also 41 months, and the one predicted from zero-order kinetics is 39 months. All three values are similar. However, the results reveal that the actual degradation reaction of the drug product is more likely to be of first order.

7.2.5 Concluding Remarks

A succinct description of the basics of chemical reaction engineering has been presented and its application to the estimation of shelf life has been outlined through examples. These techniques are of crucial importance in NDAs to regulatory agencies such as the FDA. Normally, at the time a new drug application is submitted, not enough data at low temperature are available since long-term studies take years. The tools presented here are the alternative approved by the FDA and ICH.

APPENDIX COMPUTER PROGRAMS

This Appendix contains four computer programs in MatLab that can be used by the reader to perform typical calculations related to shelf-life estimations.

This program computes the degradation line for single batch-and-save results to be input into the program called shelf life estimation:

```
% Regression Analysis for a single batch
clear, clc
close all
batch=3;
i=3;      % the number of the batch to be computed
n1(1)=7;
n1(2)=6;
n1(3)=6;
N=n1(i);
x=[0 3 6 9 12 18 24 36
   0 3 6 9 12 18 24 36
   0 3 6 9 12 18 24 36];
y=[99.2 97.1 96.1 95.2 93.8 93.1 92.4 0
   98.7 97 96.2 95.1 94.2 93.3 0 0
   102.5 98.9 97.1 95.6 94.1 93.1 0 0];
x1=[x(i,1:n1(i))];
y1=[y(i,1:n1(i))];
x1=x1';
X=[ones(N,1) x1];
Y=y1';
b=inv(X'*X)*X'*Y;
y_est=X*b;
e=Y-y_est;
SSE=e'*e;
SST=Y'*Y-N*(mean(Y)∧2);
SSR=SST-SSE;
t=tinv(0.95,N-2)
R2=SSR/SST
MSE=SSE/(N-2)
av_x=mean(x1)
Sxx=x1'*x1-N*(av_x)∧2
vec=[b' N MSE av_x Sxx]
save res vec
```

This program computes the degradation line after pooling data from several batches that have common slope and intercept:

```
.
% Regression Analysis for pooled data from several batches
clear, clc
close all
batch=3;
```

```
x=[0 3 6 9 12 18 24 36
   0 3 6 9 12 18 24 36
   0 3 6 9 12 18 24 36];
y=[102.4 98.1 99.2 97.5 95 96.1 95.2 94.3
   101.1 101.2 99 97.2 96.4 95.5 94.3 94.8
   104.1 102.1 99.5 98.1 95.7 94.1 94 93.5];
n(1)=6;
n(2)=7;
n(3)=8;
x1=[x(1,1:n(1)) x(2,1:n(2)) x(3,1:n(3))];
y1=[y(1,1:n(1)) y(2,1:n(2)) y(3,1:n(3))];
x1=x1';
N=sum(n);
X=[ones(N,1) x1];
Y=y1';
b=inv(X'*X)*X'*Y;
y_est=X*b;
e=Y-y_est;
SSE=e'*e;
SST=Y'*Y-N*(mean(Y)^2);
SSR=SST-SSE;
R2=SSR/SST
MSE=SSE/(N-2)
av_x=mean(x1)
Sxx=x1'*x1-N*(av_x)^2
vec=[b' N MSE av_x Sxx]
save res vec
```

This program computes the shelf life for data that have been input in either program 1 or 2:

```
clear, clc
close all
delete it* % para borrar archivos anteriores display('1:
  f = (90-(b0 - b1*x))^2-t^2*MSE*[1/n + (x- av_x)^2/Sxx] ')
flag_fun=input('Enter the number of the function ')
load res
b=vec(1:2);
n=vec(3);
MSE=vec(4);
av_x=vec(5);
Sxx=vec(6)
d=-5; % expiration date
reference=(b(1) - 90)/(-b(2))
if flag_fun==1
   x0=(b(1) - 90)/(-b(2))+d; % expiration date lower value
end
options = optimset('LargeScale','off');
```

```
cont=0;
save contador cont
[x,fval,exitflag,output] = fminunc(@(x)
obj_fun_expiration_date(x,flag_fun),x0,options)
```

This routine is required by program 3, shelf life estimation:

```
function f = obj_fun_expiration_date(x,flag_fun)
load contador
cont=cont+1
save contador cont
load res
b=vec(1:2);
n=vec(3);
MSE=vec(4);
av_x=vec(5);
Sxx=vec(6);
t=tinv(0.95,n-2);
if flag_fun==1
  f = abs((90-(b(1)+b(2)*x))∧2-(t∧2)*MSE*(1/n+(x-av_x)
    ∧2/Sxx));
end
save(['it' num2str(cont)],'f','x','flag_fun')
```

This program performs a statistical test for determining whether or not the slopes and/or intercepts of the degradation lines from several batches are equal:

```
% ANCOVA
clear, clc
close all
batch=3;
x=[0 3 6 9 12 18 24 36
   0 3 6 9 12 18 24 36
   0 3 6 9 12 18 24 36];
y=[99.2 97.1 96.1 95.2 93.8 93.1 92.4 0
   98.7 97 96.2 95.1 94.2 93.3 0 0
   102.5 98.9 97.1 95.6 94.1 93.1 0 0];
n(1)=7;
n(2)=6;
n(3)=6;
N=sum(n);
%%%%%%%%%%%%%%%%%%%
% testing slopes
%%%%%%%%%%%%%%%%%%
for i=1:batch
    sy=0;
    sx=0;
    sxy=0;
```

```
    for j=1:n(i)
        sy=sy+y(i,j)∧2;
        sx=sx+x(i,j)∧2;
        sxy=sxy+x(i,j)*y(i,j);
    end
    Syy(i)=sy-(sum(y(i,1:n(i))))∧2/length(y(i,1:n(i)));
    Sxx(i)=sx-(sum(x(i,1:n(i))))∧2/length(x(i,1:n(i)));
    Sxy(i)=sxy-(sum(y(i,1:n(i))))*(sum(x(i,1:n(i))))/
      length(y(i,1:n(i)));
end
SxxW=sum(Sxx);
SyyW=sum(Syy);
SxyW=sum(Sxy);
SSEW=SyyW-SxyW∧2/SxxW;
for i=1:batch
    sse(i)=Syy(i)-Sxy(i)∧2/Sxx(i);
end
SSE=sum(sse);
SS_slope=SSEW - SSE;
k=batch-1;
MS_slope=SS_slope/(batch-1);
MSE=SSE/(N-2*batch);
F_slope=MS_slope/MSE;
F_slope
df_num=batch-1
df_den=N-2*batch
F_cri=finv(0.75,df_num,df_den)
%%%%%%%%%%
% testing intercepts
%%%%%%%%%%
sy=0;
sy1=0;
sx=0;
sx1=0;
sxy=0;
for i=1:batch
    for j=1:n(i)
        sy=sy+y(i,j)∧2;
        sx=sx+x(i,j)∧2;
        sxy=sxy+x(i,j)*y(i,j);
    end
    sy1=sy1+sum(y(i,1:n(i)));
    sx1=sx1+sum(x(i,1:n(i)));
end
sy;
SY1=sy1∧2/N;
SYY=sy-SY1;
sx;
```

```
SX1=sx1^2/N;
SXX=sx-SX1;
sxy;
SXY1=sx1*sy1/N;
SXY=sxy-SXY1;
tx=0;
ty=0;
txy=0;
for i=1:batch
    ty=ty+(sum(y(i,1:n(i))))^2/length(y(i,1:n(i)));
    tx=tx+(sum(x(i,1:n(i))))^2/length(x(i,1:n(i)));
    txy=txy+(sum(x(i,1:n(i))))*(sum(y(i,1:n(i))))/
      length(y(i,1:n(i)));
end
my=sy1^2/N;
mx=sx1^2/N;
mxy=sx1*sy1/N;
Tyy=ty - my
Txx=tx - mx
Txy=txy - mxy
Eyy=SYY-Tyy
Exx=SXX-Txx
Exy=SXY-Txy
SSEr=SYY-SXY^2/SXX
SSEc=Eyy-Exy^2/Exx
SSb0=SSEr - SSEc
MSb0 = SSb0/(batch-1)
MSEc=SSEc/(N-batch-1)
F_b0=MSb0/MSEc
df_num=batch-1
df_den=N-batch-1
F_cri_inter=finv(0.75,df_num,df_den)
```

REFERENCES

1. Expiration Dating, Code of Federal Regulations, Title 21, Vol. 4, Part 211, 21CFR211.137.
2. American Medical Association (AMA) (2001), Report 1 of the Council on Scientific Affairs (A-01), Pharmaceutical expiration dates, available: http://www.ama-assn.org/ama/pub/category/print/13652.html, accessed on Feb. 19, 2007.
3. Bennett, B., and Cole, G., Eds. (2003), *Pharmaceutical Production. An Engineering Guide*, Institution of Chemical Engineers (IChemE), Rugby, Warwickshire, United Kingdom.
4. Matthews, B. R. (1999), Regulatory aspects of stability testing in Europe, *Drug. Dev. Ind. Pharm.*, 25, 831–856.
5. Dietz, R., Feilner, K., Gerst, F., and Grimm, W. (1993), Drug stability testing: Classification of countries according to climatic zone, *Drugs Made in Germany*, 36, 99–103.

6. U.S. Food and Drug Administration (FDA) (2001), Stability testing of new drug substances and products, ICH Q1A, FDA, Washington, DC.
7. Fairweather, W. R., Lin, T. Y., and Kelly, R. (1995), Regulatory, design, and analysis of complex stability studies, U.S. Food and Drug Administration, *J. Pharm. Sci.*, 84, 1322–1326.
8. U.S. Food and Drug Administration (FDA) (2004), Evaluation of stability data, ICH Q1E, FDA, Washington, DC.
9. U.S. Food and Drug Administration (FDA) (2003), Bracketing and matrixing designs for stability testing of new drug substances and products, ICH Q1D, FDA, Washington, DC.
10. Montgomery, D. G. (2004), *Design and Analysis of Experiments*, 6th ed., Wiley, New York.
11. Chen, J. J., Ahn, H., and Tsong, Y. (1997), Shelf-life estimation for multifactor stability studies, *Drug Inform. J.*, 31, 573–587.
12. Montgomery, D. C., Peck, E. A., and Vining, G. G., (2007), *Introduction to Linear Regression Analysis*, 3rd ed., Wiley, New York.
13. Reklaitis, G. V., Ravidran, A., and Ragsdell, K. M. (1983), *Engineering Optimization, Methods and Applications*, Wiley, New York.
14. Bancroft, T. A. (1964), Analysis of inference for incompletely specified models involving the use of preliminary test(s) of significance, *Biometrics*, 20, 427–442.
15. Snedecor, G. W., and Cochran, W. G. (1989), *Statistical Methods*, 8th ed., Iowa State University Press, Ames, IA.
16. Wang, S. G., and Chow, S. C. (1993), *Advanced Linear Model: Theory and Applications*, Marcel Dekker, New York.
17. Ramirez-Beltran, N. D., and Olivares, L. (1998), Drug Shelf-life Estimation Using Clustering Techniques. *Proceeding of the Computing Research Conference CRC'98*, edited by the University of Puerto Rico, Electrical and Computer Engineering Department. Mayaguez Puerto Rico, pp. 70–73.
18. Chow, S.-C., and Liu, J.-P. (1995), *Statistical Design and Analysis in Pharmaceutical Science*, Marcel Dekker, New York.
19. Shao, J., and Chow, S-Ch. (1994), Statistical inference in stability analysis, *Biometrics*, 50(3), 753–763.
20. Chow, S. C., and Shao, J. (1991), Estimating drug shelf-life with random batches, *Biometrics*, 47, 1071–1079.
21. Ruberg, S., and Stegeman, J. W. (1991), Pooling data for stability studies: Testing the equality of batch degradation slopes, *Biometrics*, 47, 1059–1069.
22. Ruberg, S., and Hsu, J. (1992), Multiple comparison procedures for pooling batches in stability studies, *Technometrics*, 34, 465–472.
23. Waterman, K. C., and Adami, R. C. (2005), Accelerated aging: Prediction of chemical stability of pharmaceuticals, *Int. J. Pharm.*, 293, 101–125.
24. Gil-Alegre, M. E., Bernabeu, J. A., Camacho, M. A., and Torres-Suárez, A. I. (2001), Statistical evaluation for stability studies under stress storage conditions, *Il Farm.*, 56, 877–883.
25. Oliva, A., Llabrés, M., and Fariña, B. (2006), Data analysis of kinetic modeling used in drug stability studies: Isothermal versus nonisothermal assays, *Pharm. Res.*, 23, 2595–2602.
26. Levenspiel, O. (1998), *Chemical Reaction Engineering*, 3rd ed., Wiley, Ney York.

7.3

EFFECT OF PACKAGING ON STABILITY OF DRUGS AND DRUG PRODUCTS

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7.3.1 INTRODUCTION

Stability is an essential attribute of drug products as evidenced by continuing national and international governmental interventions in the form of regulations. It is required that stability testing should demonstrate physical and chemical stability of a drug and its product(s) at a variety of environmental conditions such as temperature, humidity, and light [1]. Moreover, International Conference on Harmonization (ICH) guidelines provide requirements for stability studies [2, 3]. Pharmaceutical and biopharmaceutical companies are also making frantic efforts to improve on the development and manufacture of drug products with adequate stability profiles. All these activities (by governments and pharmaceutical and biopharmaceutical companies) are for the following purposes [4]: to deliver drug products of high quality (efficacious and safe) to the final consumer (the patient), to meet the legal requirements stipulated by the regulatory agencies, to provide the assurance to the manufacturer that the drug products will perform as intended, and to serve as a baseline for future drug development efforts.

7.3.1.1 Rationale for Concern with Stability of Drugs and Drug Products

Loss of Potency The effectiveness of any drug product (dosage form) is a function of its ability to deliver the required amount of the active therapeutic moiety to the biophase (site of action) for the intended length of time to achieve the purpose of therapy. Loss of the active ingredient in the dosage form, consequent upon chemical degradation, may result in poor performance of the drug product (i.e., the serum drug concentration may not reach the minimum effective concentration to elicit the desired therapeutic response). The potency of any drug product is not expected to remain constant ad *infinitum*; however it is expected that the quality of the drug product [the stability of the active pharmaceutical ingredients (APIs) among other attributes] is maintained until it either gets to the patients or reaches the expiration date, which is defined as the date placed on the immediate container label of the drug product that designates the date through which the product is expected to remain within specifications [5]. The specifications (the period within which the efficacy, safety, and esthetics of the drug product can be assured) are often defined in terms of the shelf life of the drug product. When the chemical degradation of the APIs is found to be the major contributor to the degradation process of the drug product, the shelf life is considered the time that elapses before the drug content falls below 90% of the label claim, with the assumption that the drug product is stored as directed on the label [4, 6].

Toxic Decomposition Products In situations where the content of the API in a drug product is well above 90% as indicated above, the formation of toxic degradation products within the shelf life (which may cause untoward effects to the patients) may warrant the reassignment of a different expiration date or recall of the drug product in question. Consequently, the pharmaceutical industry is often concerned with both the amount as well as the nature of the degradation products. The formation of toxic products is particularly problematic with protein drugs which may maintain therapeutic activity after deliberate modification or pertubation of molecular structure in a domain removed from that associated with therapeutic activity

but can result in acquired immunogenicity [4]. A discussion of the examples of products of drug degradation which are more toxic than the original (parent) drugs has been given by Guillory and Poust [7]. It has been reported that the concerns for the potential increase in the amount of toxic degradation products informed the reluctance of the regulatory agencies to approve the design of new drug products with stability overages [4].

Poor Bioavailability The bioavailability of a drug product is customarily defined in terms of the amount of API delivered to the blood (plasma concentration) and the rate at which it is delivered. For routes of administration other than intravascular, the extent of absorption is very important in that all the drug administered may not have a chance to get to the plasma and then get carried to the biophase. One of the most important problems of drug absorption and oral bioavailability is when the drug is not delivered from the drug product over an appropriate time frame (in solution form) to the sites in the gastrointestinal (GI) tract where it is well absorbed (i.e., problem of getting the drug into solution). This problem can be caused by changes in the quality attributes of the drug product on storage (especially the dissolution rate), though the potency is still acceptable and no toxic degradation product has been formed. The changes in quality attribute can be traced to changes in the physicochemical properties of the excipients with aging. The result is ineffectiveness of the drug product on administration.

Microbial Contamination The microbiological quality of all drug products is receiving a lot of attention in today's drug product design, unlike in the past, when the concern was for drug products that must remain sterile: parenteral and ophthalmic products. The control of total bioburden and the exclusion of pathogenic microbes are considered desirable [4]. For proper maintenance of the microbiological quality of drug products the quality of the raw materials and the manufacturing facility must be controlled and assured. Further, in assessing the suitability for use of the container closure system, protection is considered to be one of the most important considerations. Thus the container closure system is expected to protect the drug product from causes of degradation such as light, temperature, loss of solvent, oxygen, water vapor, and microbial contamination [8]. Package integrity must not be compromised from the manufacturer through the distribution channels and to the final consumer: the patient.

Changes in Physical Appearance of Drug Product Some of the physical changes may affect the efficacy of the drug products, while in others the potency may not change with the physical change but may affect pharmaceutical elegance such that patient acceptability suffers. Loss of an emulsifying agent may lead to breaking of an emulsion (phase separation) and caking of a suspension will lead to failure in resuspending the suspension on shaking. These changes will affect dose uniformity in the prescribed dosage regimen. Mottling of tablets may not affect the potency of the drug product, but such tablets will not be acceptable to patients and may affect compliance with dosage regimen. Moreover drug–excipient interactions may lead to changes in the physical appearance of the drug product as exemplified by the interaction between lactose and the amino functional group in drug which often

leads to the formation of yellow color on the surface of tablets with aging. Though the potency is still intact, such tablets will not appeal to patients.

Loss of Functional Stability It is one thing to design a very effective drug formulation; it is another important thing for it to be delivered properly by the container closure system during the shelf life of the drug product. Thus the performance of the container closure system for drug products (defined as the ability to function in the manner for which it was designed, i.e. ability to deliver the dosage form in the amount or at the rate described in the package insert [8]) is an important part of drug product stability. Reports have shown that early transdermal patches had problems of skin adhesion with aging (they showed loss of adhesion with the tendency to fall off the patient's skin) [4]. Other drug products for which consistency in drug delivery should be monitored over the entire shelf life are as follows: prefilled syringes, a metered tube, a dropper, a spray bottle, a powder inhaler, and a metered-dose inhaler.

7.3.2 FACTORS INFLUENCING STABILITY OF DRUGS AND DRUG PRODUCTS

The preceding section has elaborated on the importance of the stability of drugs and drug products. The general principle in the design and manufacture of drug products is that quality (defined as the physical, chemical, microbiological, biological, bioavailability, and stability attributes that a drug product should maintain if it is to be deemed suitable for therapeutic and diagnostic use [8]) should be built into all the processes or stages of drug manufacture rather than be inspected into the final product ready for distribution. Consequently, knowledge of the factors that can promote all forms of drug and drug product instability such as chemical degradation (formation of new chemical entities), physical degradation (drug loss without the production of distinctly different chemical products), and biological degradation (the most important is microbial degradation, though cognizance should be taken of the fact that nonmicrobiological organisms such as ants and rats may be important) during the shelf life of the drug product is very important. The awareness of these factors will help not only in the preformulation and formulation stages of drug development but also in the selection and appraisal of the protective capability of the appropriate container closure system for the drug product in question.

7.3.2.1 Moisture, Hydrolysis, and pH

Liquid Dosage Forms Most hydrolytic degradations take place in the presence of moisture. Thus hydrolysis together with the influence of pH on hydrolysis as a route of degradation of drugs is important for all types of dosage forms: liquid, solid, semisolid, and gases. Recognition of the chemical groups which are susceptible to hydrolysis will aid the drug formulation scientist to determine *a priori* the paths to take to achieve drug products of maximum stability. Examples of such chemical groups are as follows: ester linkages (acetyl salicylic acid, procaine, teracaine, and physostigmine), amides (cinchocaine, ergometrine, and chloramphenicol), lactams

(penicillins, cephalosporins, nitrazepam, and chlordiazepoxide), imides (glutethimide and ethosuximide), and lactones (pilocarpine and spironolactone) [7, 9]. The rate at which hydrolysis proceeds in each of the chemical groups highlighted above is a function of the chemical environment of the chemical group within the drug molecule. Substituent groups can exert electronic, steric, or hydrogen-bonding effects known to affect the susceptibility of the chemical groups to hydrolytic degradation. In fact, modification of drug chemical structure to control drug stability (though caution should be taken not to alter therapeutic efficacy) using appropriate substituents has been used to solve stability problems. The concept of Hammett linear free-energy relationship for the effects of substituents on the rates of aromatic side-chain reactions such as hydrolysis of esters has been reported and the principle has been used to produce and select the best substituents for allylbarbituric acids of optimum stability [9, 10].

While laboratory experiments together with the knowledge of chemical kinetics can be used to formulate a solution drug product at a pH of optimum stability (using appropriate buffers) for a drug whose hydrolytic degradation is catalyzed by hydrogen ion (specific acid catalysis) or hydroxyl ion (specific base catalysis), the known influence of buffer components (called general acid–base catalysis) in buffered drug products should be considered. Failure to evaluate complete stability of the drug by determining the catalytic coefficients for specific acid catalysis and base catalysis and the catalytic coefficients of buffer components will result in poor determination of the conditions for maximum stability of the drugs in solution. Furthermore, the ionization of the drug should be taken into account in the determination of the pH–rate profile for a complete determination of the conditions of maximum stability. Other strategies that have been used to stabilize drug products in solution involve the use nonaqueous solvents such as mono- and polyhydric alcohols (e.g., ethanol glycerin, and propyleneglycol) which can change the dielectric constant of the system. Micellar solubilization by surfactants and reduction in solubility (as exemplified by increase in the stability of penicillin in procaine penicillin in the presence of additives such as citrates, dextrose, sorbitol, and gluconate) are capable of reducing hydrolysis of drugs in solution [9].

The influence of packaging on the stability of drugs and drug products will be examined in greater detail later in this chapter, but suffice it to state here that the permeability of the packaging materials to gases and liquids should be considered for all types of dosage forms since it can lead to decomposition of the drug products due to oxidation and hydrolysis. In the case of solution, leachables from container closure systems can alter the predetermined stability of liquid dosage forms. For example, leaching of dioctyl phthalate, a plasticizer used in polyvinyl chloride (PVC) plastics, into intravenous solutions containing surfactants has been reported [11, 12]. The potential adverse effect of the leached compound is twofold: on the safety of the patient and on the stability of the drug administered intravenously. Under the consideration for the suitability for the intended use of any proposed packaging system, as indicated by the U.S. Food and Drug Administration (FDA), compatibility (packaging components are regarded compatible with the dosage form if there is no sufficient interaction to cause unacceptable changes in the quality of either the dosage form or the packaging component, i.e., leachable-induced degradation, precipitation, or changes in pH) and safety (packaging components should be constructed of materials that will not leach harmful or undesirable amounts of substances

to which a patient will be exposed when being treated with the drug product) are given prominent positions [8]. It is indicated clearly that for a drug product such as an injection, inhalation, ophthalmic, or transdermal, a comprehensive study should be carried out: extraction studies on the packaging component to determine which chemical species (and their concentrations) may migrate into the dosage form and a toxicological evaluation of those substances which are extracted to determine the safe level of exposure via the label-specified route of administration [8]. When the leached compound is an electrolyte, it can affect the ionic strength of the solution and hence the rate of degradation of the drug, which can be predicted by the Brönsted–Bjerrum model [9].

Liquid dosage forms which are disperse systems (colloidal, i.e., microspheres, nanoparticles, and micelles; suspensions; and emulsions) often contain preservatives which are methyl, ethyl, propyl, and butyl esters of *para*-hydroxybenzoic acid in various combinations. A typical example is the antacid suspensions which have high pH values which make the esters of the preservatives susceptible to hydrolysis. One way to circumvent this problem is to use several preservatives in combination with the hope that some quantities of the preservatives will remain to prevent the suspension from microbial attack. A report showing the assay of the four esters and the parent acid (one of the decomposition products) in drug products in which all the preservatives were used has been given [13].

Semisolid Dosage Forms The nature of the base (vehicle) used for the fabrication of semisolid dosage forms affects their hydrolytic stability. Increased degradation of benzylpenicillin sodium in hydrogels of various natural and semisynthetic polymers has been reported [14]. Also at pH 6 in Carbopol hydrogels, the percentage of undecomposed pilocarpine at equilibrium is a function of the apparent viscosity of the medium [15].

Solid Dosage Forms Moisture can affect the chemical stability as well as the physical stability of solid dosage forms. Reports have shown that the amount of moisture adsorbed by tablets in blister packages increased with increasing humidity and it resulted in decreased mechanical strength of the tablets [16]. Storage of prednisone and erythromycin tablets in moisture-permeable packaging changed drug release from the tablets [17, 18]. Drug release from enteric-coated and sugar-coated tablets was more susceptible to the effect of humidity than that from film-coated tablets. For example, storage of sugar-coated tablets changed the disintegration time, leading to increased or decreased dissolution rate [19]. Storage of two chloramphenicol capsules at high humidity prolonged the disintegration time and decreased the release rate [20]. Further, decrease in drug release from ampicillin capsules during storage at high humidity has been reported; it was attributed to the agglomeration of drug particles caused by moisture [21]. Gelatin-coated acetaminophen tablets exhibited a marked decrease in dissolution rate during storage at high humidity [30°C, 80% relative humidity (RH), and the effect was moderated by the addition of pancreatin to the dissolution medium. Pancreatin was believed to act by cleaving the cross-linked gelatin [22].

When solid dosage forms such as tablets adsorb moisture, drug present on the surface will be dissolved (if it is soluble). The drug in solution on the surface of the tablet will be subject to hydrolytic decomposition, and the process will be influenced

by the pH of the solution. It has been reported that an increase in the water vapor pressure significantly increases the decomposition of aminosalicylic acid [23]. A study on water permeation through rubber closures of injection vials indicates that rubber closures with a low permeability are often able to take significant amounts of water [24]. Thus freeze-dried products, which are highly hygroscopic and highly reactive with water, must be adequately protected against uptake of water in order to ensure the chemical stability of the active ingredient in the vial.

7.3.2.2 Oxygen and Oxidation

Semisolid and Solid Dosage Forms Whether the oxidative degradation process is by auto-oxidation (an uncatalyzed reaction which proceeds slowly under the influence of molecular oxygen) or by chain processes which involve initiation, propagation, and termination reactions, molecular oxygen is very important. Consequently, every effort should be made to determine and control oxygen concentration in an aqueous solution. Florence and Attwood [9] have given an oxidation scheme involving a chain reaction. The description of the scheme is in order to provide an appreciation of the influence of the interaction of molecular oxygen, components of the solution and even leachables from the packaging materials on oxidation. It is believed that initiation can take place through the free radicals formed by organic compounds consequent upon the action of light, heat, or transition metals (e.g., copper and iron) present in trace amounts in the buffer. The free radicals readily combine with molecular oxygen in the propagation step to form a peroxy radical which then abstracts hydrogen from a molecule of organic compound to form a hydroperoxide and then create another free radical. The termination occurs when the free radicals are destroyed by inhibitors or by side reactions which break the chain.

The presence of certain functional groups is known to make some drugs highly susceptible to oxidative degradation [7, 9]. The phenol functional group present in steroids is sensitive to oxidation. The ether group is susceptible to oxidation as reported for econazole nitrate and miconazole nitrate. Catecholamines such as dopamine and isoproterenol are susceptible to oxidation. Phenothiaxines possess a thioether functional group which is oxidized to sulfoxide in the presence of water. Many drug molecules possess carbon–carbon double bonds which can be easily attacked by peroxyl radicals, leading to oxidative degradation. In fact, the double bond is highly susceptible to singlet oxygen (which is highly oxidizing); the singlet oxygen is believed to form from the ground state of oxygen called triplet oxygen when excited by light. Consequently, the general term oxidation is more than mere exposure of a susceptible drug molecule to oxygen but also exposure to conditions that favor oxidation such as photolysis [6]. Amphotericin is a polyene antibiotic with seven conjugated double bonds. It is oxidized by peroxy radicals with a loss of activity and aggregation [25]. Addition of peroxyl radicals to simvastatin can result in oxidation with the formation of polymeric peroxides [26]. Carboxylic acid is another group highly susceptible to oxidation: The rate of oxidation of ascorbic acid depends on oxygen concentration [27].

Literature on pharmaceutical sciences is replete with oxidative degradation of drugs in solution. 5-Aminosalicylic acid undergoes oxidation and the product of oxidation forms polymeric compounds [28]. Further, morphine has been reported to be oxidatively degraded in solution [29] as well as hydrocortisone [30].

Semisolid and Solid Dosage Forms Since most oxidative reactions occur in solution, there is not much effort to investigate the effect of oxidative degradation in solid dosage forms. Auto-oxidation of tetrazepam tablets has been described [31].

7.3.2.3 Light

Photochemical decomposition is an important route of chemical degradation of drugs. Generalization on or a priori prediction of the effect of light on drug is very difficult because of the strong dependence of the decomposition on the spectral properties of drugs as well as the spectral distribution of the source of light [6]. Nevertheless, certain trends have been reported which might provide guidance on the effect of light on certain drug molecules so that efforts can be made at the early stage of drug development to circumvent the problems. Molecules with saturated bonds are not capable of interacting with visible or near-ultraviolet light. Those molecules with π electrons [aromatic hydrocarbons (nitroaromatic and aryl halide functional groups), heterocyclic aromatic compounds, aldehydes, ketones, sulfides, alkenes, and polyenes] absorb light throughout the wavelength range of visible or near-ultraviolet light and are very susceptible to photochemical decomposition. Sunlight can potentially affect drugs capable of absorbing light at wavelengths below 280 nm, and those capable of absorbing light strongly at wavelengths greater than 400 nm can be potentially degraded by light in both sunlight and room light [7, 32].

The importance of the excipients used in the development of drug products vis-à-vis photochemical decomposition has been stressed [9]. Photochemical degradation of drugs may occur by the direct interaction of the drug with the light of a particular wavelength (primary photochemical reaction) or the excipeints may absorb light radiation and then transfer the energy to the drug molecule (which cannot absorb the incident light) to cause decomposition. Such excipients are called photosensitizers. In fact, the ICH Harmonized Tripartite Guideline on Stability Testing (Photostability Testing of New Drug Substances and Products) [33] recognized the role that excipients in finished drug products can play on photostability and recommends that photostability testing should be carried out as follows: tests on drug substance, tests on the exposed drug product outside of the immediate pack, and, if necessary, tests on the drug product in the immediate pack and tests on the drug product in the marketing pack. As indicated earlier, oxidative degradation may be initiated by light; thus all the factors affecting the stability of drugs and drug products do not occur in isolation. Stabilization of drugs against photochemical decomposition often involves the use of colored containers (amber glass excludes light of wavelength less than 470 nm and can give a good measure of protection to drugs sensitive to ultraviolet light) and storage in the dark. Incorporation of ultraviolet absorbers in polymer film for tablet coating is another method of protecting drugs from photochemical degradation [34].

Liquid Dosage Forms Sodium nitropruside in aqueous solution for injection will remain stable for up to one year if protected from light; however, its shelf life is about 4 h when exposed to normal room light [35]. It has been reported that uric acid increases the photostability of sulfathiazole sodium in solutions [36]. Further *dl*-methionine increased the photostability of ascorbic acid in solution [37]. The

influence of light on the stability of molsidomine in infusion fluids has been reported [38]. It has been shown that the photochemical degradation of nifedipine is a function of light intensity (light source: high-pressure mercury lamp, sunlight, and fluorescent lamp) at room temperature: The amount of photodegraded nifedipine was proportional to the number of incident photons [39]. Stabilization of the photochemical degradation of daunorubicin in solution (at 290–700 nm) by the addition of various colorants has been reported: scarlet GN, amaranth, ponceau 6 R, and tartrazine [40].

Solid Dosage Forms A complex relationship was found between the discoloration rate of sulfisomide in tablets irradiated with a mercury lamp in comparison with ultraviolet light intensity [41]. The functional attributes of capsules such as drug release rate can change following the interaction of dyes and gelatin in capsule shell under the influence of light. The changes were reported to be enhanced by the interaction of light and humidity [42]. The solid-state photodecomposition of the drug, tretinoin tocoferil, has been shown to be highly temperature dependent. It was found that the rate constant for the photodegradation of tretinoin tocoferil has an Arrhenius-type temperature dependence [43]. A report has shown the stabilization of indomethacin coloration by the addition of titanium dioxide to gelatin capsule shells [44]. Moreover, incorporation of synthetic iron oxides resulted in the stabilization of tablets of nifedipine and sorivudine against photodegradation [45].

7.3.2.4 Temperature

The temperature dependence of the rate constant for a chemical reaction is often expressed using the Arrhenius equation:

$$k = Ae^{-E_a/RT} \tag{1}$$

where k is the kinetic rate constant, A is the preexponential factor, E_a is the activation energy, R is the ideal gas constant, and T is the temperature. As predicted by the equation, increasing the temperature increases the rate of reaction. Consequently, temperature is of considerable importance in consideration of the stability of drugs and drug products. Further, if the Arrhenius plot is linear, the degradation rates obtained at some temperature levels can be used to predict the rate of degradation at other temperatures. This equation is strictly valid for reactions that take place in solution, and it assumes there is no change in the degradation mechanism or the order of reaction; nevertheless, it has been used to predict the stability of dosage forms. For solid dosage forms, the effect of temperature on stability is complicated because of the effect of temperature on the excipients and the likely change in the properties of the excipients which may affect the stability of active ingredient. Though the Arrhenius-type equation has been used to model the dependence of reaction rate on temperature for solid dosage forms, the activation energy obtained could not be expected to have the same meaning as the one obtained with the solution of the drug [9].

Where the decomposition shows an approach to equilibrium, as found for vitamin E tablets [9, 46], the equilibrium concentrations of products of degradation and reactants are obtained at a series of temperatures. Then the logarithm equilibrium

constant K is plotted against the reciprocal of temperature according to the van't Hoff equation to model the dependence of temperature on drug breakdown:

$$\ln k = -\frac{\Delta H}{RT} + \text{const} \tag{2}$$

Liquid Dosage Forms The arrhenius equation has been used to model the discoloration of a liquid multisulfa preparation and the decomposition of liquid multivitamin preparations [47–49]. The degradation of codeine sulfate in solutions at several temperatures has been reported [50]. Further, the influence of pH on the Arrhenius plots for the hydrolysis of ciclosidomine has been studied [51]. The stability of a clofibride emulsion for oral administration was reduced (aggregation of the emulsion) when the storage temperature was increased from 25°C to 40°C. Moreover, storage at 4°C resulted in phase separation of the same emulsion [52]. It is believed that the stability of a vaccine can be predicted by estimating the loss of antigenicity during long periods of storage at different temperatures through accelerated stability studies. Though under certain conditions and for some vaccine products the Arrhenius equation can be used to make a prediction of real storage stability under accelerated stability data, at high temperatures many degradation processes of biomolecules are complicated by unfolding or other conformational or structural changes. Below the freezing point, the degradation rate can suddenly increase or decrease, showing no linear correlation with temperature [53]. Further the degradation rate constant k is not the only factor determining the residual antigenicity Pt of a vaccine. The time t during which a vaccine is stored at a given temperature and the initial antigenicity of the vaccine P_0 also have an influence which can be expressed by the relationship [53, 54]

$$k = \frac{P_0 - P_t}{t} \tag{3}$$

Semisolid Dosage Forms Heating easily brings about phase changes in semisolid drug products; consequently it is not feasible to use high temperatures to study the kinetics of degradation and hence the prediction of stability. Thus stability is often evaluated at the temperature in which the formulation is stored. This practice often takes a long time and stability problems may not be detected until the studies have been in progress for several months or years [55]. Storage temperature can bring about changes in the hardening of suppositories such that the time required for the suppository to melt is affected. It has been shown that the hardening effect increased with increased temperature up to 25°C but decreased at higher temperatures owing to partial melting of the suppository base [6].

Solid Dosage Forms The decomposition of aspirin in tablets made in a microcrystalline cellulose base was modeled by first-order kinetics and rate constants obtained adhered to an Arrhenius equation [56]. A tablet formulation containing polyvinylpyrrolidone (a disintegrant) showed a change in drug release when the temperature of storage was increased from 23 to 65°C [57]. Changes in the dissolution rate of hydrochlorothiazide tablets at room temperature have been estimated from changes

observed at 37, 50, and 80°C [58]. A report of the interaction of certain furoic acids when tableted with microcrystalline cellulose to cause the formation of carbon monoxide has been given [59]. The interaction was fast at 55°C and caused the tablets to crumble but was less pronounced at room temperature.

7.3.2.5 Microbes

Microbial stability is one of the factors affecting drug product reliability. Under design and interpretation of stability studies, the Center for Drugs and Biologics [5] has elaborated on the microbial quality of drug products. It is clearly indicated that drug products containing preservatives to control microbial contamination should have the preservative content monitored at least at the beginning and end of the projected expiration dating period of the product. Also adequacy of the preservative system under conditions of use for multiuse containers should be considered. Further, nonsterile products that require control of microbial quality and that do not contain preservatives should be tested at specific intervals throughout the projected dating period according to the release specification for bioburden. Topical preparations should also be tested for the presence of pathogens that may be identified as potentially harmful. These statements are also reinforced under the consideration of the suitability of a packaging system: The container closure system should provide the dosage form with adequate protection from factors that can cause degradation; microbial contamination is one of the factors listed [8].

The U.S. Pharmacopeia/National Formulary USP 28/NF23 [1] refers to the stability of a drug product as the extent to which a product remains within specified limits and throughout its period of storage and uses the maintenance of the same properties and characteristics that it possessed at the time of manufacture. Microbial stability (defined as the retention of sterility or resistance to microbial growth according to the specified requirements; antimicrobial agents that are present retain effectiveness within the specified limits) is one of the main types of stability recognized by USP 28/NF23. Thus in addition to efforts to stabilize drug products against chemical and physical decompositions brought about by environmental factors already discussed, liquid and semisolid drug products must be protected against microbial attack where necessary. The antimicrobial preservatives added to pharmaceutical products should be evaluated during stability studies by either chemical methods or microbial challenge test. The microbial challenge test is recommended at the last time point during stability testing.

Liquid Dosage Forms Both sterile products (consideration for the maintenance of sterility and preservative efficacy changes) and nonsterile products (consideration for the proliferation of microorganisms) are important. Ophthalmic and parenteral liquid products are often prepared in a sterile condition; nevertheless appropriative preservatives are often added so that they can remain sterile during the period of storage, distribution, and use. Further, other drug products that are not required to be sterile and therefore are not sterilized during their manufacture and may contain ingredients which can make then susceptible to microbial growth are also protected using antimicrobial preservatives [60]. Examples are aqueous products such as emulsions and suspensions. The use of methyl, ethyl, propyl, and butyl esters of *para*-hydroxybezoic acid in various combinations in drug products

has been mentioned previously [13]. The desire to allow certain amounts of the preservatives to remain in the suspension for preservation often informs the use of the preservatives in combination.

Semisolid Dosage Forms The FDA requires that all ophthalmic ointments should be sterile. It is also required that a suitable preservative or mixture of preservatives to prevent the growth of microorganisms must be added to ophthalmic ointments that are packaged in multiple-use containers; chlorobutanol and methyl- and propyl-*para*-hydroxybezoic acid are often used as preservatives in ointments [61]. Since the sterilization of finished ophthalmic ointment is fraught with difficulty that centers on the stability of the components, aseptic methods of processing are normally used to achieve the sterility requirements [62]. Ointments for topical applications, though not required to be sterile, are expected to meet acceptable standards for microbial content [1]. Creams and gels are often formulated to contain appropriate antimicrobial preservatives, as the high water content favors the growth of microbes.

Solid Dosage Forms At first sight one will expect solid dosage forms to be free of microbial contamination. However, reports have shown that tablet contamination could arise from the raw materials. Studies were carried out on the effects of the production, environment, method of production, and microbial quality of the starting materials on microbial loading during the various stages of tablet production [63]. High levels of microbial contamination were seen during the wet granulation process, which reduced significantly during the drying process.

7.3.2.6 Active Pharmaceutical Ingredients and Excipients

Physicochemical Properties of APIs

Hygroscopicity Hygroscopicity is the amount of moisture absorbed by a powder when it is exposed to an atmosphere of a known relative humidity. It is part of the preformulation program and its importance lies in the use of the information gained to decide whether or not a particular salt of the drug in question could be used for a dosage form design. Report has shown that flurazepam is used as a monosulfate rather than the disulfate which however has many other desirable characteristics: It is so hygroscopic that it will remove water from a hard-shell capsule and then render it very brittle [64]. Knowledge of the hygroscopicity of the APIs can aid in the selection of packaging materials.

Crystalline State or Amorphous State and Polymorphism Solid drug particles occur as pure crystalline substances of definite indentifiable shape or as amorphous particles without definite structure. The energy required for a molecule of a drug to leave the lattice in a crystal is very much greater than the energy required in an amorphous powder. Drugs in the crystalline state have low reactivity. A linear relationship has been found between the solid-state degradation rate constant of various vitamin A derivatives and the inverse of the melting point [65]. Moreover, results of studies on the relationships between degradation rate and crystallinity of β-lactam antibiotics such as cefazolin indicate that a drug with low crystallinity tends to have decreased chemical stability [66].

Polymorphism is the phenomenon by which a solid particle may exist in different crystalline forms called polymorphs. Medicinal compounds form different types of crystals, depending on the conditions (temperature, solvent, time) under which crystallization is induced. The molecules of the drug exhibit different space–lattice arrangements in the crystalline form from one polymorph to another. It should be emphasized that polymorphism exists only in the solid state. Only one form of a pure drug substance is stable at a given temperature and pressure with the other forms, called metastable forms, converting in time to the stable crystalline form. It is common for a metastable form of a medicinal agent to change form even when present in a completed pharmaceutical preparation, although the time required for a complete change may exceed the normal shelf life of the product itself. Although the drug is chemically indistinguishable in each form, polymorphic forms differ significantly with respect to a number of physical properties such as density, melting point, solubility, stability, and dissolution characteristics, which are of prime importance to the proper development of the dosage forms containing the drug. Solid-state hydrolysis of carbamazepine from needle-shaped crystals with a high crystalline order was found faster than that of beam-shaped and prismatic forms and the reactivity of carbamazepine to light is strongly dependent on the crystalline form of the drug [67, 68], and a similar report has been given for the photodegradation of furosemide [69].

Modification of Chemical Structure of Drug The use of a Hammett linear free-energy relationship to investigate the effects of substituents on the rates of aromatic side-chain reactions such as hydrolysis of esters has been alluded to earlier vis-à-vis attainment of optimum stability [9, 10]. Degradation of erythromycin under acidic pH conditions is inhibited by substituting a methoxy group for the C-6 hydroxyl as found for the acid stability of clathromycin, which is 340 times greater than that of erythromycin [70].

Vapor Pressure Active pharmaceutical ingredients with sufficiently high vapor pressures can become lost by their volatilization through the containers such that the stability and content uniformity suffer. Further such compounds can interact with other drug molecules and packaging components [71]. Nitroglycerin is notorious for this behavior and special packaging materials are needed for dispensing sublingual nitroglycerin tablets. When unstabilized nitroglycerin sublingual tablets were stored in enclosed glass containers, the high volatility of the drug gave rise to redistribution of nitroglycerin among stored tablets with concomitant deterioration in the uniformity of the tablets on storage [72].

Pharmaceutical Excipients Drugs are rarely administered to humans as pure chemical compounds. What is given is a preparation containing the drug and the materials of formulation called excipients. These excipients are added for various purposes to ensure adequate performance of the drug products. However, some of the excipients can exert deleterious effects on the stability of drug products. The degradation of codeine has been reported to be susceptible to the influence of buffer: Its hydrolytic rate constant in phosphate buffer at pH 7.0 is about 20 times faster than in buffered solution at the same pH [9]. Further, various phosphate species were found to enhance the degradation of bezypenicillin [73], cefadroxil

[74], carbenicillin [75], and spironolactone [76]. Some drugs may not be capable of absorbing light directly to undergo photolysis, but the excipients present in the formulation may absorb light radiation and transfer the absorbed energy to the drug to cause degradation [9]. Furthermore, transition metals such as copper and iron which are present in trace amounts in buffer are capable of initiating the chain process in oxidative degradation. Preservatives may affect the stability of dosage forms. Preservation of zinc insulin (in a multidose vial) with phenol can cause physical instability of the suspension. In the case ophthalmic pharmaceutical products, all raw materials used in the preparation should be of the highest quality available. Consequently, the practice by some drug manufacturers is to compound all ophthalmic drugs using water for injection.

Among other factors, the stability of APIs in the ointment base is of utmost importance in the selection of the appropriate base [60]. Studies have shown that the stability of hydrocortisone in ointment fabricated using polyethylene glycol base is very poor [77, 78]. Semisolid products often turn yellow or brown with age because of oxidative decompositions involving the base (especially natural fats and oils) used in the formulation of the products [55]. Extensive oxidation of natural fatty materials, termed rancidification, is associated with the development of a disagreeable odor. Various phase transitions, crystallizations, and transesterification reactions of suppository bases are believed to be responsible for the hardening of suppository on storage, which may adversely affect the quality of the suppository, such as poor availability of the active ingredients [6]. Moreover, aspirin was found to decompose in polyethylene glycols used as components of suppository bases; the decomposition was attributed to a transesterification reaction which gave rise to salicylic acid and acetylated polyethylene glycol [79]. Degradation of aspirin was also found when cocoa butter was used as the suppository base [80]. Polyethylene glycol esters of indomethacin were found in stored suppositories fabricated with polyethylene glycol bases [81].

Reports have shown that magnesium strearate (a lubricant) accelerated the discoloration of tablets containing amines and lactose [82]. Excipients such as binders (e.g., povidone) and disintegrants such as crosspovidone containing phenolic impurities can impact negatively the photostability of tablets by participating in the free-radical reaction [83]. Enhancement of the oxidation of phenylbutazone by dyes via the production of singlet oxygen that participates in chain reactions has been reported [84]. Excipients with high moisture content can increase the decomposition of drugs by increasing the amount of moisture associated with the drug. The percentage decomposition of aminosalicylic acid increased with increase in the water vapor pressure [85].

7.3.3 DRUG PACKAGING AND PACKAGING MATERIALS

7.3.3.1 Introduction

A packaging system, often called a container closure system, comprises packaging components that all together contain and protect the drug product [8]. It is customary to refer to two types of drug product packaging components: primary packaging component (the package components that are directly in contact with the drug

product) and the outer packaging components (usually a series of enclosures) often referred to as secondary packaging components, which include cartons, corrugated shippers, and pallets [86]. Contrary to the general belief that, with the possible exception of labeling, outer packaging components do not require any special consideration when applied to drug products, reports have shown that the outer package can extend the shelf life of drug products considerably: A blister pack exposed naked at 37°C, 90% RH had a shelf life of 21 days (suggesting that a blister might not be suitable for packaging the product). However, when the same blister pack was put in a carton and the carton in turn placed in a display outer overwrap and stored under the same conditions in the company's warehouse, the product was still in specification after 6 years [87].

The traditional definition of pharmaceutical packaging as a system capable of containing the drug product such that it remains safe and efficacious within its shelf life has shifted to a definition that recognizes the importance of packaging in drug product performance. Consequently, a pharmaceutical packaging has been defined as the combination of components necessary to contain, preserve, protect, and deliver a safe, efficacious drug product [87]. In fact, this definition echoes the statement in the guidance for industry Container Closure Systems for Packaging Human Drugs and Biologics [8] that the container closure system must be suitable for the intended use (it should adequately protect the dosage form; it should be compatible with the dosage form, and it should be composed of materials that are considered safe for use with the dosage form and the route of administration). It goes further to indicate that if the packaging system has a performance feature in addition to containing the product, the assembled container closure system should be shown to function properly. Thus drug products such as prefilled syringes, transdermal patches, metered-dose inhalers, and nasal sprays all contain formulations whose successful delivery to the final consumer (the patient) depends on the proper functioning of the packaging system. The requirements for protective, compatibility, safety, and performance characteristics of packaging systems vis-à-vis suitability for intended use vary from one type of dosage form and from one route of administration to another and a table that serves as a guide to the pharmaceutical industry on the suitability considerations for common classes of drug products has been provided elsewhere [8].

7.3.3.2 Materials of Fabrication of Packaging Components

The science of packaging materials vis-à-vis pharmaceutical and cosmetic products is very broad. The selection of a package often begins with a determination of the product's physical and chemical characteristics, its protective needs, and its marketing requirements. The materials selected must have the following characteristics: They must protect the preparation from various hazards (physical, climatic, chemical, and biological) [87]; they must not be reactive with the product (the current good manufacturing practice (CGMP) regulations [88]); they must not impart to the product tastes or odors; they must be nontoxic; they must be FDA approved (it should be indicated that the FDA approves only the materials used in the container and not the containers as such and an FDA publication lists substances generally recognized as safe (GRAS)]; they must meet applicable tamper resistance

requirements; and they must be adaptable to commonly employed high-speed packaging equipment [89].

Glass Containers Glass is commonly used in pharmaceutical packaging because it possesses superior protective qualities, it is economical, and containers are readily available in a variety of sizes and shapes. It is essentially chemically inert, impermeable, strong, and rigid and has FDA clearance. Glass does not deteriorate with age, and with a proper closure system, it provides an excellent barrier against practically every element except light. Colored glass, especially amber, can give protection against light when it is required. The major disadvantages of glass as a packaging material are its fragility and its weight.

The USP/NF [1] recognizes four major types of glass containers: Type I borosilicate glass containers are used for preparations that are intended for parenteral administration. Type II glass containers which are made of soda-lime glass that is suitably dealkanized are usually employed for packaging acidic and neutral parenteral preparations (type I glass may also be used for this purpose, but it is used for packaging alkaline parenteral preparations). Type III soda–lime glass containers are not used for parenteral preparations except if stability data indicate otherwise. The containers of the fourth category (type NP glass) are intended for packaging nonparenteral articles (i.e., those intended for oral and topical use).

Plastic Containers Plastics in packaging have proved useful for a number of reasons: The ease with which they can be formed, their high quality, and the freedom of design to which they lend themselves; plastic containers are extremely resistant to breakage and thus offer safety to consumers along with reduction of breakage losses at all levels of distribution and use [89]. Plastic-coated glass bottles are used in aerosol containers to protect from flying glass in the event of glass shattering; further, plastic coating around the neck of the container serves to absorb some of the shock from the crimping operation and decreases the danger of breaking around the neck.

Plastic containers for pharmaceutical products are primarily made from the following polymers:

(a) Polyethylene is a good barrier against moisture but a relatively poor one against oxygen and other gases; most solvents do not attack polyethylene, and it is unaffected by strong acids and alkalis; both high- and low-density polyethylene are used and they are long-chain polymers. The density of polyethylene directly determines the four basic physical characteristics of the blow-molded container—stiffness, moisture–vapor transmission, stress cracking, and clarity or translucency—and these characteristics determine the suitability of polyethylene used in containers for packaging drugs. The determinants of suitability of polyethylene have been identified by the USP/NF [1] as follows: oxygen and moisture permeability, modulus of elasticity, melt index, environmental stress crack resistance, and degree of crystallinity after molding).

(b) Polypropylene polymers are long-chain polymers synthesized from propylene or propylene and other olefins under controlled conditions of heat and pressure with the aid of catalysts. Polypropylene does not stresscrack under any conditions. Except for hot aromatic or halogenated solvents, which soften it, this polymer has

good resistance to almost all types of chemicals, including strong acids, alkalis, and most organic materials. Its high melting point makes it suitable for boilable packages and for sterilizable products. Lack of clarity is still a drawback, but improvement is possible with the construction of thinner walls. Polypropylene is an excellent gas and vapor barrier. Its resistance to permeation is equivalent to or slightly better than that of high-density or linear polyethylene, and it is superior to low-density or branched polyethylene [1, 89]. One of the biggest disadvantages of polypropylene is its brittleness at low temperatures.

(c) Polyethylene terephthalate (PET) is a condensation polymer typically formed by the reaction of terephthalic acid or dimethyl terephthalate with ethylene glycol in the presence of a catalyst. PETG resins are high-molecular-weight polymers prepared by the condensation of ethylene glycol with dimethyl terephthalate or terephthalic acid and 15–34 mole % of 1,4-cyclohexanedimethanol. PET and PETG resins and other ingredients used in the fabrication of these bottles conform to requirements in the applicable sections of the *Code of Federal Regulations*, Title 21, regarding use in contact with food and alcoholic beverages: PET and PETG bottles that are interchangeably suitable for packaging liquid oral dosage forms [1]. Other polymers that have been used or proposed for use in packaging of drug products include polycarbonate, polyvinyl chloride, and polystyrene and to a lesser extent polymethyl methacrylate, polyethylene terephthalate, polytrifluoroethylene, and polyamides [89].

Over the years efforts have been made to understand the various parameters to consider in the choice of plastics for packaging of drug products. Drug–plastic considerations have been divided into five separate categories: permeation, leaching, sorption, chemical reaction, and alteration in the physical properties of plastics or products [89].

Metals Metals are used as collapsible tubes and in aerosol containers. The most common metals in use are tin, aluminum, and lead. Tin is the most expensive, while lead is the cheapest. Laminates of tin-coated lead provide the appearance and oxidation resistance of straight tin at lower prices [89]. Tin is the most chemically inert of all collapsible tube metals. It offers a good appearance and compatibility with a wide range of products. Aluminum tubes provide the attractiveness of tin at relatively lower cost. Lead has the lowest cost of all tube metals and is widely used for nonfood products such as adhesives. However, with internal linings, lead tubes are used for such products as fluoride toothpaste. If the product is not compatible with bare metal, the interior can be flushed with wax-type formulations or with resin solutions.

Aluminum is used as the material of construction for some aerosol drug products. Also a three-piece tin-plated steel container finds use in topical pharmaceutical aerosols, and to decrease the compatibility problems, an internal organic coating is often used [90].

Rubber Stoppers, cap liners, and bulbs for dropper assemblies used in the pharmaceutical industry are made form rubber. The rubber stopper is used primarily for multiple-dose vials and disposable syringes. The rubber polymers most commonly used are natural, neoprene, and butyl rubber. As certain performance expectations

are expected from rubber, certain ingredients are commonly found in a rubber closure: for example, rubber vulcanizing agent, accelerator/activator, extended filler, reinforced filler, softener/plasticizer, antioxidant, pigment, and waxes. The complexity of rubber necessitates caution, especially when used in packaging parenteral products: When the rubber stopper comes in contact with parenteral solution, it may absorb active ingredient, antibacterial preservative, or other materials, and one or more ingredients of the rubber may be extracted into the liquid [89].

7.3.4 EFFECT OF PACKAGING ON DRUG PRODUCT STABILITY

7.3.4.1 Introduction

The purpose of stability testing is to investigate drug product (with and without container closure system) changes with time under the influence of various hazards such as temperature, humidity, and light and to establish a shelf life for the drug product and to recommend storage conditions. Generally, packaging suitability is based on four attributes: protection, safety, compatibility, and performance (function and/or drug delivery). Certain factors that must be considered in evaluating container closure systems are as follows: materials of construction of the container closure system, surface treatments and/or processing aids, dosage form active ingredients and excipients, sterilization and/or other related processing, and storage conditions. Guidelines are available in official compendia for the evaluation of container closure system. First guidelines are given for the evaluation of the container or the package materials: physicochemical and biological tests to evaluate glass and plastic bottles, metal closures, elastomeric closures, flexible and blister materials, syringe components, and aerosol packaging. Second, guidelines are available for stability studies (accelerated, short-term tests and long-term tests) to characterize the effects of the package component on the product. The general belief is that it is impossible to have a completely inert container closure system; thus the evaluation is often designed to identify, characterize, and monitor these interactions to achieve a safe, unadulterated, stable, and efficacious drug product [86]. At the end of the evaluation of a container closure system in the pharmaceutical industry, a technical report is issued which indicates the approach, results, and the conclusion.

7.3.4.2 Solid Dosage Forms

A stability study of losartan/hydrochlorothiazide tablets was carried out in three stages [91]. First, a stress test (forced-degradation study) was carried out without immediate packaging by exposing the tablets to severe storage conditions (separately 50 and 50°C, 80% RH). After four weeks it was concluded that the tablets are sensitive to moisture. Second, preliminary testing was carried out to select the packaging system. Two packaging systems with different barrier properties were studied: polyvinyl chloride 250 μm–polyethylene 25 μm–polyvinylidenechloride 60 g/m^2 foil (PVC–PE–PVdC–Al blisters capable of protecting the tablets partially from water vapor and gases) and oriented polyamide 25 μm–aluminum foil 45 μm–polyvinyl chloride 60 μm foil (OPA–AL–PVC–Al blisters capable of giving

absolute protection to the tablets). Drug assay, impurities, disintergration, and appearance were monitored. Following six months storage at 40°C, 75% RH, it was concluded that the protection against moisture offered by PVC–PE–PVdC–Al blisters was not sufficient and that losartan/hydrochlorothiazide tablets should be packaged in OPA–AL–PVC–Al blisters. Finally formal stability testing of the tablets packaged in OPA–AL–PVC–Al blisters was carried out. The parameters monitored were as follows: assay, impurities, dissolution, disintegration, hardness, water, appearance, and microbiological quality. The results obtained during 6 months of accelerated and 12 months of long-term stability testing showed that losartan/hydrochlorothiazide tablets packaged in OPA–AL–PVC–Al blisters were chemically, physically, and microbiologically stable and a shelf life of 24 h was proposed.

Studies were carried out to use the drug product and package characterization data (tablet equilibrium moisture content, degradation rate of unpacked product, and moisture barrier properties of the packages) to predict and subsequently confirm the package material that provided adequate stability for a moisture-sensitive compound (PGE-7762928) [92]. The physical and chemical stability [high-performance liquid chromatography (HPLC) assay] of the products were determined at 2, 4, 6, 8, 12, and 24 weeks at ICH conditions. At 6 months at 40°C, 75% RH, the percentage of active ingredient was 84% in polyvinyl chloride blisters, 91% in cyclic olefin blisters, 97% in aclar (polychlorotrifluoroethylene) blisters, 100% in cold-form aluminum blisters, and 99% in a high-density polyethylene bottle with a foil induction seal. The stability results for the packaged product were fairly consistent with the predictions based on the moisture sensitivity of the product and moisture barrier properties of the respective package. Based on the goal of 90% assay at 6 months, cold-form aluminum and Aclar blister packaging and high-density polyethylene bottles with foil induction seals provide acceptable PGE-7762928 tablet stability. Though inert atmosphere packaging is a common practice in the parenteral sector of the pharmaceutical industry, there are relatively few examples of solid dosage forms that are packaged under reduced oxygen levels. Model granule formulations which include a drug known to exhibit oxidative degradation were packaged in stoppered glass vials maintained at different head space oxygen concentrations and head space relative humidities and stored at 40°C. The oxidative degradation was quantified as a function of time and the data showed the dependence of oxidative degradation on head space oxygen concentration, relative humidity, drug loading, and time. It was recommended that the use of oxygen scavengers in bottles as well as inert atmospheric packaging foil–foil blister lines could be options for achieving pharmaceutical packages with low oxygen concentrations [93].

The effect of packaging and storage conditions on the in vitro performance of carbamazepine (CBZ) tablets was studied. Tablets used were Tegretol and the Egyptian generic Tegral, both presented in PVC–aluminum strip seals inside a carton and the German product Finlepsin dispensed in bottles of 50 tablets. In vitro performance was assessed through dissolution testing while chemical stability of CBZ was assessed via HPLC [94]. Tegral tablets were not affected by the tested stress conditions. Tegral tablets packaged in strips at 50 or 60°C and 75% RH showed fast disintegration and dissolution. The effect of 40°C, 97% RH for 6 months was similar to 1 month storage at 40°C, 97% RH; the tablets hardened and dissolved less than fresh Tegral tablets. Removal of Tegral tablets from their original strips

resulted in only 7% dissolution in 60 min. For Finlepsin, the effect of 97% RH at 40°C was more profound than 97% RH at 25°C, but both conditions caused a decrease in dissolution rate, the extent of which was found to be dependent on the tablet position in the bottle. However, all stressed tablets examined in this study showed no change in the chemical stability of CBZ tablets under all stressed conditions.

The model involving mass transfer characteristics of packaging materials and chemical stability has been used to characterize the diffusion of water through polyvinyl chloride blister packaging. It was found that the effect of moisture sorption on tablet crushing strength was dependent on the formulation and that it is necessary to consider the characteristics of the formulation when selecting a package [95]. Information on the tablet moisture sensitivity of compressed tablets together with package moisture permeability has been used to develop a physical model which predicted the changes in the crushing strength of tablet under a variety of storage and packaging conditions. The predictions are described as being useful in establishing long-term stability testing protocols. They are also useful in predicting what tablets stored in blister packages might reasonably be expected to experience in the marketplace where short- and long-term oscillating conditions are likely to occur. The theoretical predictions and experimental results emphasize the importance of matching the dosage formulation characteristics to the package material and testing conditions so that a more rational selection of packaging material and testing protocols can be made [96].

It is known that moisture transfer through packaging materials can affect hard gelatin capsules in two ways: changes in the properties of the shell and changes in the properties of the materials loaded in the capsule (contents of the capsules). Studies on capsules have recently examined the changes in both dissolution rates and potency of two brands of amoxicillin capsules packaged in PVC blister package (PVC blister having a thickness of 0.27 mm and aluminum base film of thickness 27 μm) and laminated–type package (the laminated package consisting of three layers: a nitrocellulose lacquer outer layer, a soft aluminum film intermediate layer, and a polyethylene inner layer). The two brands of amoxicillin stored outside the package at 76, 80, and 92% RH and inside the package at 92% RH showed no significant changes in dissolution profiles. Storage at 80 and 92% RH of the capsules of the two brands outside their packages resulted in a significant loss of amoxicillin potency. The laminated-type package afforded better protection compared with the PVC–aluminum blister package. Following 20 weeks storage at 92% RH at room temperature, only 6.4% loss in amoxicillin potency occurred for capsules packaged in the laminated package compared with 51.8% loss in the blister package [97].

The effects of two storage conditions (50°C, 50% RH and 40°C, 90% RH) on the properties (hardness, disintegration, tablet weight, dimensions, dissolution rate, and content of API) of two brands (A and B) of film-coated erythromycin stearate tablets and one brand (C) of enteric-coated tablets of erythromycin base were examined. The tablets were stored in paper bags, plastic dispensing bottles, and glass bottles. Aside from an increase in hardness by storage at 40°C, 90% RH for brand C tablets and a decrease in disintegration time for tablets of brand A under the same conditions, little or no changes were seen in most of the physical properties. However, dissolution rates significantly decreased for all the tablets by storage under the two conditions. Glass containers offered better protection for the tablets,

and consequently, the tablets retained higher dissolution rates as compared to tablets stored in plastic or paper containers [98].

The sorption of nitroglycerin by thermoplastic polymers and the stability of molded nitroglycerin tablets in strip packaging were studied. The polymers investigated varied greatly in their affinity for nitroglycerin, the order of decreasing affinity being vinyls > low-density polyethylene > ionomers > high-density polyethylene. With the proper choice of packaging, molded nitroglycerin tablets stabilized with povidone maintained acceptable potency for up to two years at 26°C when strip packaged in unit-dosage form. Chemical decomposition by hydrolysis of nitroglycerin was also investigated. Povidone accelerated the decomposition of nitroglycerin; at high temperature, decomposition was a significant factor in tablet stability for tablets containing povidone [99]. A liquid dispersion system of etodolac (20%) and Gelucire 44/14: D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) blend (80%) in different ratios was prepared. The capsule formulation was subjected to stability studies at different temperature humidity conditions based on ICH guidelines. Physical and chemical properties of the dispersion did not change during a period of storage at room temperature and at 4°C, 0% RH. However, the relative humidity and storage time exerted an effect on the dissolution behavior of etodolac. It was concluded that changes in dissolution behavior after storage under conditions of high humidity and temperature might be related to the formation of etodolac microcrystal and water absorption by the carrier during storage, thereby necessitating packaging in moisture-resistant packaging [100].

7.3.4.3 Nonsterile Liquid Dosage Forms

The effect of packaging materials on the stability of ultraviolet (UV) A, UVB, and infrared sunscreen emulsions after storage in different types of packaging materials (glass and plastic flasks; plastic and metallic tubes) has been reported [101]. The samples (emulsions containing benzophenone-3, octyl methoxycinnnamate, and Phycocorail) were stored at 10, 25, 35, and 45°C and representative samples were analyzed after 2, 7, 30, 60, and 90 days. Data showed that sample emulsions stored at different temperatures had similar rheological behaviors within 3 months and there were no significant changes in the physical and chemical stability of emulsions stored in different packaging materials. Glass and plastic packaging materials were found adequate for storing the products.

Reports have shown that chloroquine binds to glass but not to certain plastics. This information is very important in laboratory studies where significant reductions in chloroquine concentration may occur when the drug is prepared and stored in glass containers [102]. Further, buffered chloroquine diphosphate solutions of varying pH and concentration were stored in soda or borosilicate glass. Storage in soda glass showed a decrease in original drug concentration of up to 60 and 97% in test tubes and glass wools, respectively, Borosilicate glass did not show any binding. The highest binding recorded was at physiological pH. Thus borosilicate glass should be used when storing, assaying, or carrying out sensitivity tests for malaria in order to avoid loss of chloroquine from solution [103].

Oxygen is often used as a challenge to find out whether a particular drug is likely is to be affected by oxidative breakdown. The procedure involves storing the drug product in solution in ampoules purged with oxygen and then comparing their rate

of breakdown with similar solutions stored under nitrogen. Drug formulations shown to be susceptible to oxidation can be stabilized by replacing the oxygen in the storage containers with nitrogen or carbon dioxide; antioxidant can be added or the choice of container should aim at avoiding the presence of metal ions [9]. In the case of drug products (solution, semisolid, and solid) that are photolabile, the usual practice is to store them in containers which exclude UV light. It is believed that exposure to light in this wavelength range is the most frequent cause of photodecomposition. Amber glass is very effective in eliminating or reducing photodegradation because it is capable of excluding light of less than 470 nm [9]. Photolabile drugs can also be stored in the dark.

7.3.4.4 Sterile Liquid Dosage Forms

The use of prepackaged syringes in the hospital setting is increasing due to the advantages offered by their use and the current interest in unit-dose medication. It is known that all the various dosages and combinations prescribed in the hospital are not commercially available; consequently many hospital pharmacies are developing or operating a centralized parenteral admixture program. Packaging of the drug products requires consideration of the sterility and particulate matter content of single-syringe injections as well as compatibility between drugs and with the syringe components. Though plastic syringe packaging has a number of advantages, contact between plastic containers and drugs can present a number of problems, such as leaching, sorption, permeation, chemical reactivity of the plastic polymer, and alteration in physical properties of the plastic. Studies have been carried out on a commonly prescribed drug combination (hydroxyzine hydrochloride, meperidine hydrochloride, and atropine sulfate) used as preanesthetic medication to compare possible differences in stability between mixtures stored in glass and plastic containers. The combination drugs were stored in both glass and plastic syringes at 25 and 3°C for a 10-day period. Analyses were performed at intervals throughout the time period utilizing visual examination, pH determination, UV absorption spectra, and gas chromatography. No significant degradation of the syringe contents or appearance of additional constituents was detected in any of the admixture preparations. Thus the storage of such preparations in glass versus plastic syringes yielded no significant differences in product stability [104].

Report has shown the influence of the type of container on ceftazidime stability in intravenous solutions. Polypropylene (PP) and PVC (100 mL each) bags and 100-mL glass bottles were filled with 5% dextrose or 0.9% sodium chloride solutions containing ceftazidine (40 mg/mL) and stored at 20 and 35°C. One-milliliter samples were taken from each container at 0 and 20 h and assayed. Pyridine (the main degradation product) levels increased during storage and were higher in PVC and PP bags than in glass bottles in both diluents. Solutions stored in PP bags showed better stability than in PVC bags. Glass bottles seem to be the better container for storing ceftazidime solutions [105]. Studies have been carried out to evaluate the effect of diluent type, storage conditions, and nature of package on the stability of reconstituted Parecoxib sodium for injection (PSI) [106]. Results showed that the PSI reconstituted with normal saline, bacteriostatic normal saline, 5% dextrose injection, and 5% dextrose injection and half normal saline met visual acceptance criteria and showed almost no degradation under storage conditions, and no significant differ-

ences were seen between storage in glass vials or PP/glass syringes. However, PSI reconstituted with lactated Ringer's injection and lactated Ringer's and 5% dextrose injection showed visual precipitation in many vials as confirmed by HPLC assay values at all points.

The stability of an ophthalmic solution formulation of unoprostone isopropyl (UI) (a prostaglandin-like compound marketed as Rescula for treatment of elevated intraocular pressure in patients with primary open-angle glaucoma or ocular hypertension) was investigated in two types of packaging materials: PP and low-density polyethylene (LDPE). The concentrations of UI and its degradation products were monitored as a function of time. It was found that the rate of disappearance of drug was faster for the formulation stored in LDPE bottles than that stored in PP bottles. Further studies indicated that the inferior stability observed with LDPE packaging was primarily due to the sorption of UI to the packaging material and to a lesser degree chemical degradation. The sorption was found to be temperature dependent: Lowering the temperature reduced the sorption, thus improving the shelf life of the product [107].

The USP has proposed large-globule-size limits to ensure the physical stability of lipid injectable emulsions, expressed as the percent far greater than 5 µm, or $PFAT_5$, not exceeding 0.05%. Recent studies showed that injectable emulsions packaged in newly introduced plastic containers exceed the proposed USP $PFAT_5$ limits and subsequently become significantly less stable during simulated syringe-based infusion. Although modest growth in large-diameter fat globules was observed for the glass-based lipids, they remained within proposed USP globule size limits throughout the study. Thus glass-based lipids seem to be a more stable dosage form and potentially a safer way to deliver via syringe infusion to critically ill neonates [108].

Considerable interest has been shown, in recent years, in the clinical use of intravenous nitroglycerin for the treatment of myocardial infarction and in open heart surgery. In its intravenous use loss of the drug to PVC bags has been identified as a problem and has been characterized as a diffusionally controlled absorption process having a half-life for fractional absorption of 3.2 h at 30°C [109]. Moreover, in recent studies, administration set tubing has been implicated as the source for the observed loss of nitroglycerin from solution [110–112]. Quantitative studies have been reported on the loss of nitroglycerin to plastic intravenous tubing. It was found that in the short time periods the loss of nitroglycerin from normal saline solutions to PVC tubing could be treated as an adsorption process. The rate of adsorption was rapid and could be quantified as an apparent first-order process. The half-life of the loss was 2.6 min [113]. Recently a model describing the loss of nitroglycerin from the solution in the plastic bags as a rapid adsorption onto the plastic surface followed by partitioning into the plastic was proposed [114]. The mechanism of loss of nitroglycerin stored in plastic and glass containers was studied from an equilibrium and kinetic approach. Data showed that nitroglycerin was removed from aqueous solution by the plastic container material through an absorption process. Nitroglycerin loss from aqueous solution did not occur by hydrolysis since solutions stored in glass containers at pH 5.7 and 35°C retained potency for at least 48 h. Although experimental data supported the thesis that the uptake of nitroglycerin was migration of the drug into the plastic matrix, the finding did not exclude the possible adsorption of the drug on the surface: The rate of adsorption may be much

faster than the rate of absorption with the result that any adsorption may be obscured [115].

An investigation has been carried out to define the kinetics and mechanism of the interaction between various drugs and plastic infusion bags and to develop criteria that may be used in the prediction of such interactions. The sorption behavior of warfarin sodium, various benzodiazepines, and other drugs with PVC infusion bags was examined. The sorption of some compounds by PP infusion bags was also investigated. The sorption kinetics fro warfarin and diazepam could be accounted for by a diffusional model in which the loss of drug is determined primarily by the diffusivity of the compound in the plastic matrix. The rate and extent of sorption of warfarin showed a dependence on pH which could be interpreted in terms of ionization of the drug: Only the un-ionized form was sorbed. A rank-order relationship was found between the initial rate of uptake by the plastic bag and the hexane–water partition coefficients of the compounds. Thus hexane–water partition coefficient may be useful for the prediction of interactions between a drug substance and PVC infusion bags. Sorption of the compounds by infusion bags made from PP was insignificant, except for the highly lipophilic medazepam. Thus PP bags may be safer to use than PVC bags [116].

Drugs to be administered via the parenteral route which are unstable in water are often formulated as solids for reconstitution. Such drugs may be presented in vials or in two-compartment cartridges with powder in one side and diluent in the other. Rubber closures are used as primary packaging material because of their unique properties, such as elasticity for piercing and self-sealing which is maintained under freeze-drying conditions. Further, vial seals made from butyl or halogenated butyl rubber show low moisture vapor and gas transmission rate. During storage rubber closures are in intimate contact with vial contents and are a potential source of product contamination: shedding of particles, migration of benzothiazoles, leaching of metal ions, antioxidants and oligomers, silicone oil, sulfur, and paraffin wax. Reconstituted solutions in aqueous vehicles show haze formation which arises from precipitation in oversaturated solutions, polymerization of decomposed drugs, and release of nonpolar volatiles (saturated hydrocarbons, unchlorinated or chlorinated olefins, alkylbenzenes, and low-molecular-weight polydimethylsiloxanes) from rubber closures [117]. Studies have shown that high product surface area is a driving force for the adsorption of volatiles released from rubber stoppers (products with high surface areas show high levels of turbidity after reconstitution). Attempts to impede haze formation include the use of solubilizers and the use of closure materials such as bromobutyl closures instead of butyl and chlorobutyl rubber or Teflon-lined rubber stoppers. Reduction of migration of volatiles can be achieved by exposure of rubber stoppers to heat or vacuum within the sophisticated washing cycles [118].

Studies have been carried out on intravenous containers (plastic and glass) and plastic administration sets (with and without inline filters) on diazepam availability. No visual incompatibilities were observed and the pH remained constant. Solutions stored in glass bottles and infused through plastic sets retained greater than 90% of initial potency after 4 h. Solutions stored in plastic burette chambers and administered through plastic sets lost greater than 38% of potency after 2 h. The loss occurred principally in the plastic chamber and increased with increasing drug concentration and time. Drug availability was not affected by the type of intravenous

fluid, pH, flow rate, or filtration. It was recommended that if diazepam were to be infused, it should be diluted to at least 1:10 dilution in glass intravenous bottles and administered through plastic administration sets not having plastic burette chambers. Inline filters (0.45 μm) may be used without loss of potency [119]. Similar studies were carried out by Morris [120]. It was found that diazepam injection, in dilutions of 10 mg/dL or greater, was visually compatible and chemically stable in dextrose 5% in water, Ringer's injection, lactated Ringer's injection, and 0.9% sodium chloride in glass intravenous bottles when administered within 24 h. However, the same type of study carried out in plastic intravenous bags instead of glass bottles showed that the solutions had a potency loss of greater than 24% within 30 min after dilution. The percentage of diazepam lost from solution increased with increase in concentration and time [121].

Injectable lyophilized drug products are often presented in vials. The rubber stopper forms a critical barrier: Protection of the product against moisture or oxygen is strongly dependent upon the quality and functioning of this barrier. In fact, it has been reported that rubber not only is a barrier against moisture but also can be a source of water [122]. Studies have been carried out to investigate the speed of water uptake, the saturation, and finally the permeability of different types of rubber closures with a view to understanding the fundamental mechanisms which determine the barrier quality and obtaining the easy method to discriminate between different rubbers. Thirteen-millimeter closures of the same dimensions were used: the Helvoet FM 157 (grey) and FM 257 (grey); the Pharma Gummi PH 701/45 (red), PH 21/50 (red), and PH 4104/40 (grey); and the West Company W888 (grey). All rubbers were of the bromobutyl rubber type, with the exception of W888, which consists of a halobutyl/ polyisoprene blend. It was found that permeation through the closures is highly unpredictable owing to the concentration dependency of the diffusion coefficient D, which increases strongly with increasing RH. It was indicated that new types of rubbers could be evaluated with respect to their barrier properties by means of the absorption profiles under stress conditions such as 40°C 95% RH. Thus one does not require to make a selection on the basis of laborious permeation experiments [123].

7.3.4.5 Container Extractables and Leachables and Drug Product Stability

Containers and drug delivery devices for pharmaceutical and biological products may release low-molecular-weight compounds into the product. These compounds are called extractables and leachables. The toxicological concern is that an extractable or leachable compound from the container may affect the efficacy and biological safety of the drug-product. The drug-contacting packaging materials (synthetic polymeric formulations) may include antioxidants, colorants, slip agents, and plasticizers.

The subject of extractables and leachables in drug products is an area of active discussion in the pharmaceutical industry. Further the regulatory agencies have issued guidances on this subject in recent years. The FDA guidance on container closure systems defines extractables and leachables as follows [124]: *Extractables* are compounds that can be extracted from elastomeric or plastic components of the container closure system when in the presence of a solvent. *Leachables* are compounds that leach into the formulation from elastomeric or plastic components of

the drug product container closure system. Thus the term extractable refers to the removal of a compound from a container closure system under extractive conditions by exposure to solvents under nonstandard or atypical product handling conditions using a variety of solvents and/or stress conditions that are not used in the manufacturing process of the drug product (strong organic solvents or acid/alkaline solutions as well as elevated conditions of temperature and pressure). Leachable describes the removal of a compound from a container closure system under nonstressful solubilizing conditions (exposure of drug substance to surfaces and conditions defined specifically by the manufacturing process) [125]. The difference between the two terms is one of process and not related to a fundamental chemical property of the compound. An extractable or leachable is generated by partitioning of a given compound between two phases: the solid (container closure system) and liquid (drug product for leachables or a solvent for extractables).

It is necessary to test the extractables and/or leachables from each container or device followed by toxicological evaluation of the hazard potential. Toxicological risk assessment includes evaluating the toxicity of the packaging components and the specific extractable or leachable substance. Toxicological endpoints include single, repeated, and chronic dose toxicity, genotoxicity, carcinogenicity, immune sensitization, irritation, and blood compatibility [126]. The concern that the regulatory authorities (FDA) have regarding drug product leachables is directly related to the particular dosage form and the route of administration [8]. Of the highest concern are the inhalation drug products [orally inhaled and nasal drug products (OINDPs)] [124, 127, 128], which include metered-dose inhalers (MDIs), dry powder inhalers (DPIs), inhalation solutions and sprays, and nasal sprays. The FDA considers leachables to be a safety concern for OINDPs. Leachables are also of concern for injections and injectable suspensions, sterile powders and powders for injection, ophthalmic solutions and suspensions, and transdermal ointments and patches. Other dosage forms such as liquid and solid oral are of less concern [8].

There are many challenges (mainly scientific) for pharmaceutical development programs and teams regarding leachables and extractables in terms of analysis and questions such as: At what levels are drug product leachables of safety concern? What safety qualification processes can be applied to leachables? How do you develop control strategies (including specifications and acceptance criteria) for leachables? Can you control drug product leachables by controlling potential leachables (i.e., extratables)? [128]. A report has shown that The Product Quality Research Institute (PQRI) Leachables and Extractables Working Group proposed a complete pharmaceutical development process for OINDPs related to leachables and extractables [129] based on its own comprehensive scientific research and on some earlier proposals from the International Pharmaceutical Aerosol Consortium for Regulation and Science [130]. The PQRI recommendations also discuss selection criteria for OINDP container closure components which are designed to exclude potential leachables of known safety concern and minimize both the numbers and levels of other potential leachables. It is believed that these recommendations move beyond quality by testing paradigm and suggest a process by which quality is designed into OINDP container closure system components (quality-by-design paradigm) [128]. Extractable and leachable testing studies are recommended even if the containers or closures meet compendial suitability tests.

Aside from the toxicological risks posed by leachables and extractables, another source of drug product quality problems associated with leachables is stability because leachables could react chemically and may interact with drug product. Some of the more common chemically reactive leachables include transistion metals, radical initiators or propagators, organoperoxides, and reactive nucleophiles or electrophiles such as amines and aldehydes [131]. The principal sources of metal leachables include glass packages and product-contacting equipment in the manufacturing process, especially unpassivated stainless steel equipment. Metal leachables can often cause degradation via oxidation in which the metal catalyzes the formation of a short-lived peroxy radical, which further reacts with the drug substance [132]. Transition metals such as Fe^{n+} and Mn^{n+} are usually involved and the oxidation occurs via a Fenton catalytic cycle [133]. Degradation was observed for a developmental intravenous dosage form where metal ions leaching out of glass vials and stainless steel catalyzed single-electron oxidation. Interestingly, the antioxidants in the formulation (thioglycerol, ascorbic acid, or sodium bisulfite) were involved in the Fenton reactions leading to oxidation [134]. For a photosensitive product, the use of amber glass vials resulted in other stability problems: The higher levels of leachables from amber vials enhanced the metal-catalyzed drug oxidation [135]. Metal leachables in liquid formulations may result in the formation of insoluble complexes with the pharmaceutical active substance or other formulation ingredients. It has been reported that accelerated stability testing at higher temperatures might not be indicative of such problems since these precipitates tend to form and grow at lower temperatures in a nonlinear fashion [136]. Aluminum ions coming from USP type I glass and some plastic packages such as LDPE and rubber closures are the most prevalent source for the formation of particulates. The Al^{3+} leaches out and accumulates in solution in levels from 45 ppb up to 6 ppm, depending on factors such as the presence of buffers, the solution pH, and autoclaving [137].

Organoperoxide radicals can be present in plastic packaging materials as a result of residual free radicals from dissociation of the peroxides formed during polymerization or melt processing of the plastic or from the long-lived radicals formed in the polymer as a result of gamma-induced radiolysis of the polymer chain. Studies on the migration of radiolysis products from plastics sterilized by gamma radiation or electron beam showed that smaller molecular weight fragments are formed by radiolysis of the plastic additives and residual oligomers [138, 139]. Very reactive species such as aldehydes have been reported to react directly with drug products possessing a nucleophilic site such as a primary or a secondary amine. Formaldehyde can be formed in minute amounts as a by-product of longer chain alcohol oxidation and from other sources such as acrylates. A sterile formulation containing an anti-stroke developmental product packaged in glass vials with rubber stoppers exhibited a formadehyde-adduct degradate in levels above 2% after 13 weeks at 30°C. The formaldehyde source was found to be in the rubber stopper (formed from a reinforcing agent used in the stopper) [140]. Strategies to mitigate the stability risk of leachables include a careful examination of mode of sterilization and packaging selection. Use of silica-coated glass vials, use of nitrogen head space in the package, use of plastics, and inclusion of a chelating agents will reduce the adverse effects of leachables on stability.

Protein aggregation has recently attracted a lot of attention from the FDA due to a number of incidents in which stability problems were caused by container

leachables/extractables or changes in excipients, as suggested in the case of Eprex (epoetin alfa, Johnson & Johnson, New Brunswisk, NJ). Since aggregation can induce immunogenicity reactions with potentially severe consequences, the FDA is requ iring manufactures to pay more attention [141]. Evaluation of packaging materials for potential extractables and leachables is critical to guarantee the integrity of the drug product and assure compliance with the *Code of Federal Regulations* (CFR), Title 21, Part 211.65, which states that equipment should be constructed so that contact surfaces that contact components, in-process materials, or drug products should not be reactive, additive, or absorptive so as to alter the safety, quality, or purity of the drug product beyond official or other established requirements [142]. The U.S. Food, Drug and Cosmetic (FD&C) Act also states that a drug device should be deemed to be adulterated if its container is composed in whole or in part of any poisonous or deleterious substances which may render the components injurious to health [143]. A review of regulatory and scientific considerations on testing for extractables and leachables has been provided [144]. To facilitate the evaluation of extractables (to detect, identify, and quantify organic extractables) mass spectometry was used recently. Using the Agilent G1888 Network Headspace/680N GC/5975 inert Mass Selective Detector (MSD) system, several primary packaging materials for pharmaceuticals were evaluated. Data on two of the materials (HDPE bottle and a soft elastomer liner from the inside of the screw cap) were presented. A multiple head space extraction technique was used and the highest attainable amount of extractables that could ever be concentrated in the drug product was calculated. It was found that the analytical results obtained from the inert 5975 head space gas chromatogrophy/mass spectrometry (GC/MS) provided excellent sensitivity [145].

The effect of ammonium sulfate treatment on cerium oxide glass vials was assessed following exposure to ionizing radiation. The bulk chemical composition of irradiated cerium oxide glass remains unchanged despite a temporary browning effect. Stability against alkali leachables of the internal silica matrix was enhanced with ammonium sulfate treatment. With the exception of alumina (Al_2O_3 and Na_2O), irradiation sterilization has a limited effect on altering the surface chemistry of ammonium sulfate–treated cerium oxide glass [146].

7.3.4.6 Biotechnological Products

Despite the efforts at delivering products of biotechnology (peptides and proteins) through novel drug delivery systems, injection still remains the main mode of delivery. The unit dose for many injectable biotechnological products is the single-dose vial and occasionally prefilled syringes. The product is often provided either as a solution or as a lypholized cake to be reconstituted and injected using syringes. Packaging represents the first line of defense for all formulated drug products, protecting the product from the outside world and vice versa. At the same time, the package must be fully compatible with the product [147]. Thus the requirements for product purity, activity, and shelf life dictate a high standard for injectable drug packaging.

Packaging conditions have effects on the stability of protein products. Peptide and protein drugs are high-molecular-weight compounds with unique physicochemical properties. They are extremely sensitive to their microenvironment: heat, light, pH, chemical contaminants, and so on. Trace amounts of metals, plasticizers, and

other materials from packaging may deactivate or denature therapeutic peptide and proteins. Further, peptides and proteins have a tendency to adsorb on to the surface of container closure systems, thereby removing virtually all active material from the drug formulation. Even when the drug desorbs back into the solution, the interaction could result in loss of potency. Lyophilized biopharmaceutical products can be affected by moisture if the seal is not adequate to prevent ingress of moisture into the container. Thus packaging can make and break final formulation of lyophilized product. Vials that are not designed specifically for lyophilization (with convex rather than flat bottoms) can make the lyophilization process less efficient. Rubber closures can also hinder freeze drying if they do not permit adequate venting during sublimation. Stopper rubbers adsorb and desorb moisture at different rates and under storage conditions stoppers that were not properly dehydrated can release water into lyophilized product.

Another source of instability of biopharmaceutical products traceable to packaging is silicone oil, which is commonly used to lubricate elastomeric stoppers during fill/finish to facilitate insertion of the stopper into the vial. Silicone oil is known to inactivate protein through nucleation of protein around oil droplets. The problem is being circumvented by using a fluoroelastomer coating on stoppers to provide the needed lubricity in addition to chemical innertness, barrier protection, and safety [148]. The fluoroelastomer films reduce adsorption of drug on to the stopper, provide lubricity for proper vial sealing, and also reduce the possibility of extractables migrating from the rubber stopper into the product. The prefilled syringes are becoming very popular in the injectable markets. Some of the challenges to overcome are the compatibility and stability issues that arise when dealing with biotechnology formulations. Biotechnological products can react with the oily form of silicone, which is used as a lubricant to coat the sliding components of the syringe. It is believed that the propensity for silicone to react with the formulation is a function of the concentration of the silicone in the syringe and its chemical activity. The chemical activity is determined by the number of terminal hydroxyl groups, which is greater the shorter the silicone polymer chain length. It has been reported that baking on the silicone (which involves heating the silicone-coated syringe to a specific temperature for an appropriate time) results in longer chains that are more closely adhered to the surfaces they coat. Thus the concentration of silicone in the syringe and its chemical reactivity are both reduced and the product's stability is increased [149]. Another benefit of baked-on silicone is that it reduces the frequency of the "break-loose" effect, which occurs during storage when the rubber closure inside the syringe barrel expands outward so that eventually it displaces the low-friction silicone coating and comes into direct contact with the inner glass surface. Another challenge is the prevention of undesirable pH change that sometimes occurs in liquids stored in prefilled syringes. The shift in pH occurs because the USP type I glass used in prefilled syringe manufacture is a borosilicate which must be subjected to various temperature changes during the glass tube production process. During storage, sodium ions are released into the product and increase the concentration of hydroxide ion. This problem is solved by spraying ammonium sulfate into the glass barrel before the tempering process in the formation of syringes begins.

Factor VIII (FVIII) is an essential coagulation factor in the blood which serves as a cofactor in the complex blood-clotting cascade. A deficiency in FVIII is the

cause of hemophilia (type A), a hereditary life-treatning bleeding disorder [150]. The effect of the container on FVIII stability was shown to be temperature dependent. At 4–8°C, 2 of the 15 constituted FVIII concentrates showed better stability in plastic containers and 2 concentrates showed better stability in glass containers; at 20–23°C most concentrates showed better stability in plastic contaners; and at 37°C all concentrates showed equal or better stability in plastic containers [151]. It was concluded that stability studies comparing different types of containers should be conducted at the product storage temperature. Due to limited diffusion of molecules in a solid state, containers would not be expected to play a critical role in storing lyophilized FVIII products unless the air permeation through the container and/or container stoppers is significantly different. This is because air has been shown to accelerate the inactivation of recombinant FVIII SQ not only in solution but also in a lyophilized state [152].

It is known that interaction of proteins with the surfaces of their containers is a potentially significant problem in biotechnology. The amphiphatic nature of protein molecules results in their adsorption to a wide variety of surfaces and can result in both their loss and destabilization. Reports have shown that when adsorption of a protein/peptide drug to container occurs, the drug molecule exchanges solution interactions for surface interactions where the free energy of exchange is negative [153]. Similarly, studies have shown that some surfactants are able to reduce/eliminate protein/peptide drug adsorption to glass and PP. In the presence of surfactants, where the surfactant–surface interaction is greater than the surface–protein/peptide interaction, drug adsorption is reduced or eliminated. For protein/peptide adsorption onto glass, where an electrostatic interaction (interaction between the positively charged peptide/protein and the negatively charged glass surface) predominates, only the most hydrophobic surfactants (polysorbate 20 and benzakonium chloride) were significantly effective to reduce adsorption to surfaces as demonstrated with salmon calcitonin and bovine serum albumin (BSA). The nonionic surfactant polysorbate 20 proved to be the most effective in eliminating adsorption to both plastic and glass surfaces. The predominat mechanism of protein/peptide adsorptrion to PP (plastic surface) was by a hydrophobic/dehydration mechanism [154]. The effect of Poloxamer 407 (Pluronic F-127), a nonionic surfactant, on the adsorption of granulocyte colony-stimulating factor (G-CSF) to PVC was assessed. It was found that Poloxamer 407 at a concentration of 0.05% w/w may show promise as a solvent additive with which to minimize G-CSF adsorption to PVC [155].

The amount of surface adsorption of a number of proteins ranging in molecular mass from 6.5 to 670 kDa and isoelectric point (pI) from 4.3 to 10.5 to several commonly used container surfaces (glass vials: either untreated, siliconized, sulfur treated or Purcoat treated; plastic vials: polyester + 0.3%, polyester 5 × 0, PP, and nylon). A 5-mL volume of protein solution was added to each vial, yielding a surface-to-volume ratio of 2.4 cm^2/mL. No correlation was found between the amount adsorbed and the molecular mass or isoelectric point, although glass surfaces appeared to bind more protein under the experimental conditions examined [156].

Patients receiving total parenteral nutrient solutions frequently require exogenous insulin to fully use the administered glucose. Consequently a study was conducted to determine the percentage of insulin adsorbed to the glass and PVC when added to a nutrient solution infusion system. The following parameters on insulin

availability from parenteral nutrient solutions were investigated: sample time for infusion, insulin concentration, amino acid or polypeptide source, electrolytes and vitamins, inline filters, glass and PVC infusion containers, and human albumin. Results showed that basic solutions of amino acids and protein hydrolysates in dextrose with 30 units of insulin failed to deliver approximately 44–47% of the added insulin. Varying the concentration of insulin had a small but statistically significant effect on the degree of insulin loss. The use of inline filters and PVC bags caused an even greater loss of insulin. The addition of albumin or electrolytes and vitamins decreased the insulin loss [157].

7.3.4.7 A Look into the Future of the Effects of Packaging on Stability of Drug Products

New Drug Delivery Systems Reports have shown that the advent of new drug delivery systems for various routes of drug administration (e.g., oral, nasal, pulmonary, transdermal, needle free) and the development of new biotechnological drugs have resulted in the need for enhanced protection against such factors as moisture, light, oxygen, and mechanical forces as well as making packaging play a more integral role in drug delivery [158]. Novel drug delivery systems have necessitated the emergence of specialized packaging needs because not all of them can be packaged in bottles or standard blisters. More often than not, the packaging must be unit dose and must also become an integral part of the drug delivery technology, as drug product packaging and packaging design contribute significantly to the stability, shelf life, and performance of the drug and the drug delivery system. In fact, the old equation showing the relationship between a new chemical entity (NCE), drug delivery (DD), and drug product (DP) (i.e., NCE + DD = DP) is now being replaced by NCE + DD + packaging = DP. Table 1 gives the considerations needed when designing packaging for new drug delivery systems [158].

One of the stability problems associated with transdermal drug delivery devices is the degradation of the contents of the devices: drugs, permeation enhancers, matrix materials, and other components incorporated in the devices. Degradation undesirably breaks down the components as well as causes discoloration and formation of odors within the pouched system. Devices that are susceptible to degradation cannot be stored for a reasonable amount of time, thus causing practical problems in their distribution. A solution to this problem involves the incorporation of an antioxidant such as BHT into the drug formulation of the transdermal drug delivery device [159]. Also a desiccant has been incorporated within the sealed pouch of the transdermal drug delivery device. For example, the Climara transdermal estradiol system is packaged and sold within a sealed pouch containing a water scavenger to protect against hydrolysis of estradiol [160].

The effects of extreme temperatures on drug delivery of two albuterol sulfate hydrofluoroalkane (HFA) MDIs were evaluated. Three Proventil HFA and three Ventolin HFA MDIs were stored at room temperature and served as controls, while three of each product were placed in the trunk of a vehicle in Tuscon, Arizona. The temperature of the vehicle was monitored for six months. Product performance for each of the MDIs was evaluated at room temperature. An additional study was performed to investigate the performance of the two products when actuated at 4, 22, 47, and 60°C. Within one week of being placed in the test vehicle, all MDI

TABLE 1 Packaging Needs and Considerations for New Drug Delivery Technologies

Delivery Route	Company Names and Systems	Characteristics	Nature	Packaging Needs and Description
Oral	CIMA, OralSolv RP Scherer, Zydis Biovail, Flashdose EthyPharm, Flashtab Yamanouchi, wowTab Elan, FASTMelt Eurand, Ziplets	Fast-dissolving tablets or orally disintegrating dosage Forms	Hygroscopic (polysaccharide or protein-based, taste masked, fragile	Protection from mechanical forces: stiff packaging Peelable opening Ultrahigh moisture barrier, e.g., Aclar or foil to maintain shelf life stability for 2–3 years
Pulmonary	Inhale/Inhance™ Aradigm/AERx® Alkermes/AIR® BatellePharma Therapeutics/ EHD pulmonary delivery	Inhalable delivery deep into lungs, either powder or liquid	Can be fine powder with high surface area or aqueous/ nonaqueous formulation	Medium–high barrier protection Mechanical needs depend on delivery device design Sterile barrier Chemical inertness, especially for high liquid doses Clear barrier offers QA check for both producer and end user
Transdermal	3M/Latitude™ Noven/DOT Matrix™ Alza/D-Trans®	Sustained delivery/ absorption through skin	Depending on drug/formulation	Protection of active from environmental aggrevates Clear barrier offers aesthetics Pouching as primary/ secondary package Barrier property on carrier web critical Chemical inertness
Transmucosal delivery	Cephalon, Actiq Atrix Labs, BEMA CIMA Labs, Dravescent	Sustained delivery via mucosal layer	Moisture senstivity	Ultrahigh barrier moisture protection Clear material offers QA check Aesthetics

canisters developed physical distortions. The Proventil HFA MDIs bulged at the base of the canister, while the Ventolin HFA MDIs bulged around the valve. These distortions were thought to be the result of an increase in vapor pressure brought about by the initial high temperatures. After exposure to extreme environmental temperatures, an increase in propellant-leak rate was observed with Proventil HFA and Ventolin HFA MDIs, but little to no change was found in particle size, dose per

actuation, respirable mass, and nonrespirable mass. Despite the two formulations' tolerance to extreme temperatures, drug delivery was affected when the MDIs were tested at specific temperatures outside of the recommended usage [161]. One of the alternatives to the pressurized metered-dose inhaler (pMDI) is the breath-actuated DPI. Stable and predictable therapeutic responses require a consistent dose delivery from an inhaler throughout its life and consistency of doses from one inhaler to another. Recognizing this, specifications for inhaler dose uniformity have been defined by regulatory agencies, including the European Pharmacopoeia (EP) [162] and the FDA [163]. DPI inhaler design, especially the geometry of the mouth piece, is critical for patients to produce an airflow sufficient to lift the drug from the dose chamber, break up the agglomerates in a turbulent air stream, and deliver a dose to the lungs as therapeutically effective fine particles [164, 165]). The airflow generated by inhalation directly determines particle velocity and hence the ease with which particles are deagglomerated. The materials used in the construction of DPIs and the characteristics of the formulation affect electrostatic charge accumulation. Some formulations as well as inhaler materials accumulate and retain electrostatic charge more strongly than others, and this will affect both drug retention within these inhalers as well as delivered aerosol behavior [166].

Pulmozyme®, recombinant deoxyrucleic acid (DNA)–derived human DNase I (rhDNase) has been formulated for local delivery to the lung by inhalation. rhDNase was filled originally in glass vials for clinical studies. A direct stability comparison study was made between rhDNase in glass vials and rhDNase filled into plastic ampoules using the blow-fill seal technology of Alp, which uses low-density polyethylene resins which are gas permeable. The problem of gas permeation was addressed by packing the plastic ampoules in a gas-impermeable foil pouch which can be filled with nitrogen. The rate of deamindation was similar for rhDNase stored at 2–8°C in foiled and unfoiled ampoules but substantially different from protein stored in glass vials. This difference in deamidation rate was attributed to the 0.5-unit difference in pH of rhDNase in glass (due to leaching of ions, possibly sodium, from the glass surface) versus plastic ampoules. This result provided the rationale for the choice of container closure component for rhDNase aerosols [167].

Improvements in the design of pMDIs has facilitated the extension of pMDIs to administering macromolecules such as peptides and proteins. The belief is that the HFA environment within the pMDI is inert and essentially moisture free, thereby providing good stability for macromolecules. Further, advances in valve and actuator technology in the pMDI help ensure a consistent and efficient delivery, an important consideration based on the cost and potency of biotechnological drug substances [168]. The 3M company has made improvement in container closure systems (CCSs) for biopharmaceuticals. One improvement reduces the potentially problematic deposition of the active drug within the CCS by introducing can coating. Further 3M is using a novel semipermeable membrane system component to improve the dosing reproducibility of suspension formulations [169]. The company is also developing new fast-fill, fast-empty valve designs to improve dose-to-dose uniformity as well as to avoid the need for patients to prime the system if only used intermittently [170–172].

Methods for Prediction of Effects of Packaging on Stability of Drug Products

Drug stability studies often involve multiple batches to ensure that a product will

consistently remain within specifications for its entire expiration date. The studies usually involve the same drug products in similar packages or in multiple strengths. The belief now is that application of sound statistical design principles can reduce the amount of testing required. The principles stated in the FDA 1987 publication Guideline for Submitting Documentation for the Stability of Human Drugs and Biologics have been extended to setting expiration dating periods for more complex situations [173]. PP may be used as a substitute for glass in primary packaging for various drug products. However, the diffusion of water through the PP plastic wall often builds up the water content with time during the long-term storage. It is often the shelf life limiting parameter for chemically stable aqueous solutions in PP bottles. The water diffusion rates of the widely distributed X-ray contrast agent VisipaquesTM in PP bottles and magnetic resonance contrast agent OmniscanTM in PP syringes were evaluated with the objective of developing a mathematical method of estimating the rate constant for water diffusion through the PP wall as a function of the following variables: temperature, humidity, surface area, wall thickness, concentration of the active ingredient, and fill volume. The effect of the variables was estimated by partial least-squares regression. The predictive ability of the cross-validated models was good for the two active agents. The models were used to predict the shelf life for the relevant combinations of temperature and humidity for the four climatic zones. The method presents an opportunity to measure the effect of the variables influencing drug stability [174].

A multivariate model has been developed to identify factors influencing stability, to estimate shelf life, to select new batches for further stability testing, and to evaluate changes in new batches. The model was capable of predicting the degradation rate constant as a function of storage temperature, pH, product concentration, and container volume [175]. Other investigators have reported on the applications of statistical analysis in stability studies of drug products. The time dependency of product change has been examined by ordinary least-squares regression and variance analysis for testing the possibility of pooling batches to increase the precision in shelf life estimates [176]. The Bayesian approach has been used to quantify the uncertainty in predicting shelf life from the Arrhenius equation as a function of stability consisting of various error distributions [177]. Monte Carlo simulation has been used to test the power of the analysis of variance (ANOVA) on matrixing data and single data to estimate shelf life. The simulation showed that the large amount of data from a matrixing design gave more precise shelf life estimates [178].

Desiccant is frequently included in the packaging of moisture-sensitive products in order to maintain low relative humidity inside the package and hence protect the product from moisture. Sorption–desorption moisture transfer (SDMT) models have been successfully applied to predict moisture transfer between a solid product and a desiccant inside a closed package. Further, theoretical simulations extended the use of the SDMT model to take into account the moisture permeation properties of the package [179]. The SDMT model was also used to predict the effect of desiccant quantity, tablet quantity, and tablet initial moisture content on the relative humidity inside high-density polyethylene (HDPE) bottles containing a moisture-sensitive drug product, roxifiban tablets. Desiccant quantity, tablet weight, and initial moisture content before packaging were found to have an effect on stability. The use of theoretical calculations utilizing the SDMT model was shown to be useful in the understanding of packaging requirements for the product. Calculated relative

humidity data corroborated experimental findings regarding the effect of the variables studied on tablet stability [180]. It is known that hard gelatin capsule brittleness is a function of moisture content. A study has been carried out to indicate that the brittleness of empty capsules occurred at humidities below 40%. Mexitil-loaded capsules exhibited a similar profile. The SDMT model was employed to estimate the final relative humidity for the Mexitil/gelatin capsule system and results were presented to demonstrate the general applicability of the SDMT model for predicting the incidence of brittleness problems and for the formulation to ensure the absence of brittleness [181].

The sorption of two weak acids (warfarin and thiopentone) and two weak bases (chlorpromazine and diltiazem) into PVC infusion bags was described by a constant partition model. PVC–water partition coefficients were obtained using three different methods: equilibrium values for sorption into PVC bags, the sorption versus pH relationship, and partition into PVC strips. The data were compared with similar values derived from a liquid–liquid partition system and different organic solvents (octanol, dichloromethane, carbon tetrachloride, and hexane). Octanol is the preferred solvent, and it is suggested that octanol–water partition data can be used to predict sorption behavior [182].

Reports have shown that changes in the hardness of tablets composed of lactose and cornstarch were due to variation of the moisture content. Further it was shown that the hardness of the tablets in moisture-semipermeable packages (such as strip packs and press-through packs with or without an overwrap film) could be predicted by an iterative calculation procedure through a mathematical model based on the physicochemical properties of the tablets and the moisture permeabilities of the packaging materials [183]. In another study by the same research group, moisture and temperature were used to predict the shelf-life of packaged tablets. Cognizance was taken of the flunctuations of temperature and relative humidity during prolonged storage. A sugar-coated tablet with a core containing ascorbic acid whose color is affected by both moisture content and ambient temperature was investigated. It was found that the color change of ascorbic acid core of the sugar-coated tablet was dependent on its moisture content and the ambient temperature and that the color change of the tablet in moisture-semipermeable packages could be predicted by an iterative calculation procedure using a mathematical model based on the kinetics of the color change and the moisture permeabilities of the packaging materials [184].

It is believed that a container/formulation couple is compatible if the magnitude of ingredient loss is within acceptable limits over the entire shelf life of the product. In this connection an approach for assessing the interaction of drug with PVC plastic infusion bags was developed. The approach correlates the partition coefficients and dissociation constant (when appropriate) of the solute, the physical dimensions of the container, and the solution pH with single parameters that dictate the shape of the sorption profile. To determine the equilibrium sorption level of PVC containers, the fractional binding of a solute was correlated with its hexane–water and octanol–water partition coefficients. Calculations based on single partition coefficients are believed to be less effective in terms of mimicking the behavior of PVC. To determine the sorption profile (fractional binding with time), the partition coefficients are related to the fractional binding at a particular time through a single parameter referred to as the sorption number. Equilibrium fractional binding and

sorption profiles for various drugs stored in PVC containers are generated with the models and agree well with reported behavior [185]. The relationship between sorption number (a parameter defining initial solute uptake by PVC infusion bags) and solute octanol–water partition coefficient was investigated by following the time course of the sorption of drugs by PVC infusion bags which was approximated using a diffusion model in which the plastic was assumed to act as an infinite sink. The model was found to be suitable for estimation of storage times relevant to clinical usage and enabled the magnitude of the uptake in a specific time to be described by a single parameter referred to as the sorption number. This parameter was defined by the plastic infusion solution partition coefficient, the diffusion coefficient in the plastic, the fraction un-ionized in the solution, the volume of the infusion solution, and the surface area of the plastic. The sorption number can be extrapolated to allow prediction of the effects of time, plastic surface area, solution volume, and solution pH on fractional solute loss. A reasonable correlation was established between the logarithm of this parameter and the logarithm of the octanol–water partition coefficient of various solutes. The model allows the fraction of a solute remaining in a plastic infusion bag at a given storage time to be estimated from the octanol–water partition coefficient of the solute and other readily available data [186].

REFERENCES

1. U.S. Pharmacopeia/National Formulary (2005), *The Official Compendia of Standards*, USP 28/NF23, U.S. Pharmacopeial Convention, Rockuille, Maryland.
2. International Conference on Harmonization (ICH) Steering Committee (1994), ICH harmonized tripartite guideline, Stability testing of new drug substances and products, ICH, Geneva.
3. International Conference on Harmonization (ICH) Steering Committee (1998), Draft ICH harmonized tripartite guideline, Stability testing of new drug substances and products, ICH, Geneva.
4. Rhodes, C. T. (2000), Reasons for stability testing, in Carstensen, J. T., and Rhodes, C.T. Eds., *Drug Stability, Principles and Practices*, Marcel Dekker, New York, p. 11.
5. Center for Drugs and Biologics, U.S. Food and Drug Administration (FDA), Department of Health and Human Services (1987), Guideline for submitting documentation for the stability of human drugs and biologics, CDB, Washington, DC.
6. Yoshioka, S., and Stella, V. J. (2000), *Stability of Drugs and Dosage Forms*, Kluwer Academic, New York.
7. Guillory, J. K., and Poust, R. I. (1996), Chemical kinetics and drug stability, in Banker, G. S., and Rhodes, C. T., Eds., *Modern Pharmaceutics*, 3rd ed., Marcel Dekker, New York, p. 179.
8. Center for Drug Evaluation and Research and Center for Biologics Evaluation and Research, U.S. Food and Drug Administration (FDA), Department of Health and Human Services (1999), Guidance for industry: Container closure systems for packaging human drugs and biologics, FDA, Washington, DC.
9. Florence, A. T., and Attwood, D. (2006), *Physicochemical Principles of Pharmacy*, 4th ed., Pharmaceutical Press, London, p. 94.
10. Cartensen, J. T., Serenson, E. G., and Vance, J. J. (1964), Use of Hammett graphs in stability programs, *J. Pharm. Sci*., 53, 1547–1548.

11. Moorhatch, P., and Ciou, W. L. (1974), Interactions between drugs and plastic intravenous fluid bags. II. Leaching of chemicals from bags containing various solvent media, *Am. J. Hosp. Pharm*., 31, 149–152.
12. Venkataramanan, R., Burckart, G. J., Ptachcinski, R. J., Blaha, R., Logue, L. W., Bahnson, A., Giam, C., and Brady, J. E. (1986), Leaching of diethyl phthalate from polyvinyl chloride bags into intravenous cyclosporine solution, *Am. J. Hosp. Pharm*., 43, 2800–2802.
13. Scheiffer, G. W., Palermo, P. J., and Pollard-Walker, S. (1984), Simultaneous determination of methyl, ethyl, propyl, and butyl 4-hydroxybenzoates and 4-hydroxybenzoic acid in liquid antacid formulations by gas chromatography, *J. Pharm. Sci*., 73, 128.
14. Ullmann, E., Thoma, K., and Zelfel, G. (1963), The stability of sodium penicillin G in the presence of ionic surfactants, organic gel formers, and preservatives, *Pharm. Acta. Helv*., 38, 577–586.
15. Testa, B., and Etter, J. C. (1975), Hydrolysis of pilocarpine in carbopol hydrogels, *Can. J. Pharm. Sci*., 10, 16–20.
16. Amidon, G. L., and Middleton, K. R., (1988), Accelerated physical stability testing and long-term predictions of changes in the crushing strength of tablets stored in blister packages, *Int. J. Pharm*., 45, 79–89.
17. Taborsky-Urdinola, C. J., Gray, V. A., and Grady, L. T. (1981), Effects of packaging and storage on the dissolution of model prednisone tablets, *Am. J. Hosp. Pharm*., 38, 1322–1327.
18. Gouda, M. W., Moustafa, M. A., and Molokhia, A. M. (1980), Effect of storage conditions on erythromycin tablets marketed in Saudi Arabia, *Int. J. Pharm*., 5, 345–347.
19. Barrett, D., and Fell, J. T. (1975), Effect of aging on physical properties of phenylbutazone tablets, *J. Pharm. Sci*., 64, 335–337.
20. Khalil, S. A., Ali, L. M., and Abdel-Khalek, M. M. (1974), Effects of aging and relative humidity on drug release. *Pharmazie*, 29, 36–37.
21. Georgarakis, M., Htzipantou, P., and Kountourelis, J. E. (1988), Effect of particle size, content in lubricant, mixing time, and storage relative humidity on drug release from hard gelatin ampicillin capsules, *Drug Dev. Ind. Pharm*., 14, 915–923.
22. Dahl, T. C., Sue, I. T., and Yum, A. (1991), The effect of pancreatin on the dissolution performance of gelatin-coated tablets exposed to high-humidity conditions, *Pharm. Res*., 8, 412–414.
23. Kornblum, S. S., and Sciarrone, B. J. (1964), Decarboxylation of p-aminosalicylic acid in the solid state, *J. Pharm. Sci*., 53, 935.
24. Vromans, H., and van Laarhoven, J. A. H. (1992), A study on water permeation through rubber closures of injection vials, *Int. J. Pharm*., 79(1–3), 301–308.
25. Lamy-Freud, M. T., Ferreira, V. F. N., Faljoni-Alario, A., and Schreiter, S. (1993), Effect of aggregation on the kinetic of autoxidation of the polyene antibiotic amphotericin B, *J. Pharm. Sci*., 82, 162–166.
26. Smith, G. B., DiMichele, L., Colwell, L. F., et al. (1993), Autooxidation of simvastin, *Tetrahedron*, 49, 4447–4462.
27. Khan, M. M. T., and Martel, A. E. (1967), Metal ion and metal chelate catalyzed oxidation of ascorbic acid by molecular oxygen. I. Cupric and ferric ion catalyzed oxidation, *J. Am. Chem. Soc*., 89, 4176–4185.
28. Jensen, J., Cornett, C., Olsen, C. E., Tjornelund, J., and Hansen, S. H. (1992), Identification of major degradation products of 5-aminosalicylic acid form in aqueous solutions and in pharmaceuticals, *Int. J. Pharm*., 88, 177–187.
29. Yeh, S., and Lach, J. L. (1961), Stability of morphine in aqueous solution III. Kinetics of morphine degradation in aqueous solution, *J. Pharm Sci*., 50, 35–42.

30. Pitman, I. H., Higuchi, T., Alton, M., and Wiley, R. (1972), Deutrium isotope effects on degradation of hydrocortisone in aqueous solution. *J. Pharm. Sci*., 61, 818–820.
31. Boccardi, G., Deleuze, C., Gachon, M., Palmisano, G., and Vergnaud, J. P. (1992), Autoxidation of tetrazepam in tablets: Prediction of degradation impurities from oxidative behavior in solution, *J. Pharm. Sci*., 81, 183–185.
32. Greehill, J. V., and McLelland, M. A. (1990), Photodecomposition of drugs, *Prog. Med. Chem*., 27, 51–121.
33. International Conference on Harmonization (ICH) (1996), Harmonized tripartite guideline: on stability testing: Photostability testing of new drug substances and products, Q1B, 1996 ICH, Geneva.
34. Matsuda, Y., Inouye, H., and Nakanishi, R. (1978), Stabilization of sulfisomidine tablets by use of film coating containing UV absorber: Protection of coloration and photolytic degradation from exaggerated light, *J. Pharm. Sci*., 67, 196–201.
35. Frank, M. J., Johnson, J. B., and Rubin, S. H. (1976), Spectrophotometric determination for sodium nitroprusside and its photodegradation products, *J. Pharm. Sci*., 65, 44.
36. Asker, A. F., and Larose, M. (1987), Influence of uric acid on photostability of sulfathiazole sodium solutions, *Drug Dev. Ind. Pharm*., 13, 2239.
37. Asker, A. F., Canady, D., and Cobb, C. (1985), Influence of DL-methionine on the photostability of ascorbic acid solutions, *Drug Dev. Ind. Pharm*., 11, 2109.
38. Vandenbossche, G. M., deMuynk, C., Colardyn, F., and Remon, J. P. (1993), Light stability of molsidomine in infusion fluids, *J. Pharm. Pharmacol*., 45, 486.
39. Akimoto, K., Kurosaka, K., Nakagawa, H., and Sugimoto, I. (1988), A new approach to evaluating photo-stability of nifedipine and its derivatives in solution by actinometry, *Chem Pharm. Bull*., 36, 1483–1490.
40. Thoma, K., and Klimek, R. (1991), Photostabilization of drugs in dosage forms without protection from packaging materials, *Int. J. Pharm*., 67, 169–175.
41. Matsuda, Y., and Minamida, Y. (1976), Stability of solid dosage forms: II. Coloration and photolytic degradation of sulfisomide tablets by exaggerated ultraviolet irradiation, *Chem. Pharm. Bull*., 24, 2229–2236.
42. Murthy, K. S., Reisch, R. G., and Fawzi, M. B. (1989), Dissolution stability of hard-shell capsule products: Part I: The effects of exaggerated storage conditions, *Pharm. Technol*., 13(3), 72–86.
43. Teraoka, R., Konishi, Y., and Matsuda, Y. (2001), Photochemical and oxidative degradation of the solid-state tretinoin tocoferil, *Chem. Pharm. Bull*., 49(4), 368–372.
44. Matsuda, Y., Itooka, T., and Mitsuhashi, Y. (1980), Photostability of indomethacin in model gelatin capsules: effects of film thickness and concentration of titanium dioxide on the coloration and photolytic degradation, *Chem. Pharm. Bull*., 28, 2665–2671.
45. Desai, D. S., Abdelnasser, M. A., Rubitski, B. A., and Varia, S. A. (1994), Photostabilization of uncoated tablets of sorivudine and nifedipine by incorporation of synthetic iron oxides, *Int. J. Pharm*., 103, 69–76.
46. Carstensen, J. T., Johnson, J. B., Spera, D. C., and Frank, M. J. (1968), Equilibrium phenomena in solid dosage forms, *J. Pharm. Sci.*, 57, 23
47. Garrett, E. R., and Carper, R. F. (1955), Prediction of stability in pharmaceutical preparations I. Color stability in a liquid multisulfa preparations. *J. Am. Pharm. Assoc. Sci. Ed*., 44, 515–518.
48. Garrett. E. R. (1956), Prediction of stability in pharmaceutical preparations II. Vitamin stability in liquid multivitamin preparations. *J. Am. Pharm. Assoc. Sci. Ed*., 45, 171–178.

49. Garrett, E. R. (1956), Prediction of stability in pharmaceutical preparations III. Comparison of vitamin stabilities in different multivitamin preparations. *J. Am. Pharm. Assoc. Sci. Ed*., 45, 470–473.
50. Powell, M. F. (1986), Enhanced stability of codeine sulfate: Effect of pH, buffer, and temperature on the degradation of codeine in aqueous solution, *J. Pharm. Sci*., 75, 901.
51. Carney, C. F. (1987), Solution stability of ciclosidomine, *J. Pharm. Sci*., 76, 393.
52. Magalhales, N. S. S., Cave, G., Seiler, M., and Benita, S. (1991), The Stability and in vitro release kinetics of clofibride emulsion, *Int. J. Pharm*., 76, 225–237.
53. Shi, L., DeHaven, P. A., and Burke, C. J. (2005), Biopharmaceutical stability studies: stable vaccine dosage form development for commerical use, *Am. Pharm. Rev*., 8(6), 86–92.
54. Tydeman, M. S., and Kirkwood, T. B. L. (1984), Design and analysis of accelerated degradation tests for the stability of biological standards. I. Properties of maximum likelihood estimators, *J. Biol. Stand*., 12, 195–206.
55. Flynn, G. L. (1996), Cutaneous and transdermal delivery: Processes and systems of delivery, in Banker, G. S., and Rhodes, C. T., Eds., *Modern Pharmaceutics*, 3rd ed., Marcel Dekker, New York, p. 239.
56. Carstensen, J. T. (1974), Stability of solids and solid dosage forms, *J. Pharm. Sci*., 63(1), 1–14.
57. Horhota, S. T., Burgio, J., Lonski, L., and Rhodes, C. T. (1976), Effect of storage at specified temperature and humidity on properties of three directly compressible tablet formulations, *J. Pharm. Sci*., 65, 1746–1749.
58. Alam, A. S., and Parrott. E. L. (1971), Effect of aging on some physical properties of hydrochlorothiazide tablets, *J. Pharm. Sci*., 60, 263–266.
59. Carstensen, J. T., and Kothari, R. (1983), Solid-state decomposition of alkoxyfuroix acids in the presence of microcrystalline cellulose, *J. Pharm. Sci*., 72, 1149.
60. Ansel, H. C., Allen, L. V., and Popovich, N. G. (1999), *Pharmaceutical Dosage Forms and Drug Delivery Systems*. 7th ed., Lippincott Williams and Wilkins, Baltimore, MD, p. 98.
61. Hecht, G., Roehs, R. R., Lang, J. C., Rodheaver, D. P., and Chowhan, M. A. (1996), Design and evaluation of ophthalmic pharmaceutical products, in Banker, G. S., and Rhodes, C. T., Eds., *Modern Pharmaceutics*, 3rd ed., Marcel Dekker, New York, p. 489.
62. Lee, J. Y. (1992), Sterilization control and validation for topical ointments, *Pharm. Tech*., 16, 104–110.
63. Ibrahim, Y. K. E., and Olurinola, P. R. (1991), Comparitive microbiological contamination levels in wet granulation and direct compression methods of tablet production, *Pharm. Acta Helv*., 66, 298.
64. Carstensen, J. T. (1996), Preformulation, in Banker, G. S., and Rhodes, C. T., Eds., *Modern Pharmaceutics*, 3rd ed., Marcel Dekker, New York, p. 213.
65. Guillory, J. K., and Higuchi, T. (1962), Solid state stability of some vitamin a compounds, *J. Pharm. Sci*., 51, 100–105.
66. Pikal, M. J., Lukes, A. L., Lang, J. E., and Gaines, K. (1978), Quantitative crystalline determinations for b–lactam antibiotics by solution calorimetry: Correlation with stability, *J. Pharm. Sci*., 67, 767–773.
67. Krahn, F. U., and Mieck, J. B. (1989), Effect of type and extent of crystalline order on chemical and physical stability of carbamazepine, *Int. J. Pharm*., 53, 25–34.
68. Matsuda, Y., Akazawa, R., Teraoka, R., and Totsuka, M. (1994), Pharmaceutical evaluation of carbamazepine modifications: Comparative study of photostability of carbamazepine polymorphs by using fourier-transformed reflection-absorption infrared spectrocopy and colorimetric measurement, *J. Pharm. Pharmacol*., 46, 162–167.

69. DeVilliers, M. M., van der Watt, J. G., and Lotter, A. P. (1992), Kinetic study of the solid-state photolytic degradation of two polymorphic forms of furosemide, *Int. J. Pharm*., 88, 275–283.
70. Nakagawa, Y., Itai, S., Yoshida, T., and Nagai, T. (1992), Physicochemical properties and stability in the acidic solution of a new macrolide antibiotic, clarithromycin, In comparison with erythromycin, *Chem. Pharm. Bull*., 40, 725–728.
71. Pikal, M., and Lukes, A. L. (1976), Kundsen vapor pressure measurements on pure materials and solutions dispersed in porous media: Molded nitroglycerin tablets, *J. Pharm. Sci*., 65, 1269.
72. Fusari, S. A. (1973), Nitroglycerin sublingual tablets II: preparation and stability of a new stabilized sublingual molded nitroglycerin tablet, *J. Pharm. Sci*., 62, 2012.
73. Finholt, P., Jurgensen, G., and Kristiansen, H. (1965), Catalytic effect of buffers on degradation of penicillin G in aqueous solution, *J. Pharm. Sci*., 54, 387–393.
74. Tsuji, A., Nakashima, E., Deguchi, Y., Nishide, K., Shimizu, T., Horiuchi, S., Ishikawa, K, and Yamana, T. (1981), Degradation kinetics and mechanism of aminocephalosporins in aqueous solution: Cefadroxil, *J. Pharm. Sci*., 70, 1120–1128.
75. Zia, H., Teharan, M., and Zargarbashi. R. (1974), Kinetics of carbenicillin degradation in aqueous solutions, *Can. J. Pharm. Sci*., 9, 112–117.
76. Pramar, Y., and Gupta, V. D. (1991), Preformulation studies of spironolactone: Effect of pH, two buffer species, ionic strength, and temperature on stability, *J. Pharm. Sci*., 80, 551–553.
77. Allen, A. E., and Das Gupta, V. (1974), Stability of hydrocortisone in polyethyleneglycol ontiment base, *J. Pharm. Sci*., 63, 107–109.
78. Das Gupta, V. (1978), Effect of vehicles and other active ingredients on stability of hydrocrtisone, *J. Pharm. Sci*., 67, 299–302.
79. Jun, H. W., Whitworth, C. W., and Luzzi, L. A. (1972), Decomposition of aspirin in polyethyleneglycols, *J. Pharm. Sci*., 61, 1160–1162.
80. Whitworth, C. W., Luzzi, L. A., Thompson, B. B., and Jun, H. W. (1973), Stability of aspirin in liquid and semi-solid bases. II. Effect of fatty additives on stability in a polyethyleneglycol base, *J. Pharm. Sci*., 62, 1372–1374.
81. Ekman, R., Liponkoski, L., and Kahela, P. (1982), Formation of indomethacin esters in polyethylene glycol suppositories, *Acta Pharm. Suec*., 19, 241–246.
82. Castello, R. A., and Mattocks, A. M. (1962), Discoloration of tablets containing amines and lactose, *J. Pharm. Sci*., 51, 106–108.
83. Tonnesen, H. H. (2001), Formulation and stability testing of photolabile drugs, *Int. J. Pharm*., 225, 1–14.
84. Baugh, R., Calvert, R. T., and Fell, J. T. (1977), Stability of phenylbutazone in the presence of pharmaceutical colors, *J. Pharm. Sci*., 66, 733–735.
85. Kornblum, S. S., and Sciarrone, B. J. (1964), Decarboxylation of p-aminosalicylic acid in the solid state, *J. Pharm. Sci*., 53, 935.
86. Liebe, D. C. (1996), Packaging of pharmaceutical dosage forms, in Banker, G. S., and Rhodes, C. T., Eds., *Modern Pharmaceutics*, 3rd ed., Marcel Dekker, New York, p. 681.
87. Dean, D. A. (2000), Packaging, package evaluation, stability and shelf-life, in Carstensen, J. T., and Rhodes, C. T., Eds., *Drug Stability, Principles and Practices*, Marcel Dekker, New York, p. 483.
88. *Code of Federal Regulations*, Food and drugs, Title 21, Part 211, Current good manufacturing practice for finished pharmaceuticals, U.S. Government Printing Office, Washington, DC, rev. Apr. 1, 2001.

89. Croce, C. P., Fischer, A., and Thomas, R. H. (1986), Packaging materials science, in, Lachman, L., Lieberman, H. A., and King, J. L., Eds., *The Theory and Practice of Industrial Pharmacy*, 3rd ed., Lea & Febiger, Philadelphia, p.711.
90. Sciarra, J. J. (1996), Pharmaceutical aerosols, in Banker, G. S., and Rhodes, C. T. Eds., *Modern Pharmaceutics*, 3rd ed., Marcel Dekker, New York, p. 547.
91. Lusina, M., Cindric, T., Tomaic, J., Peko, M., Pozaic, L., and Musulin, N. (2005), Stability study of losartan/hydrochlorothiazide tablets, *Int. J. Pharm.*, 291, 127–137.
92. Allinson, J. G., Dansereau, R. J., and Sakr. A. (2001), The effects of packaging on the stability of a moisture sensitive compound, *Int. J. Pharm.*, 221, 49–56.
93. Mahajan, R., Templeton, A., Harman, A., Reed, R. A., and Chern, R. T. (2005), The effect of innert atmospheric packaging on oxidative degradation in formulated granules, *Pharm. Res.*, 22(1), 128–140.
94. Al-Zein, H., Riad, L. E., and Abd-Elbary, A. (1999), Effect of packaging and storage on the stability of carbamazepine tablets. *Drug Dev. Ind. Pharm.*, 25(2), 223–227.
95. Veillard, M., Bentejac, R., Duchene, D., and Carstensen, J. T. (1979), Moisture transfer tests in blister package testing, *Drug Dev. Ind. Pharm*. 5, 227–244.
96. Amidon, G. E., and Middleton, K. R. (1988), Accelerated physical stability testing and long-term predictions of changes in the crushing strength of tablets stored in blister packages, *Int. J. Pharm.*, 45,79–89.
97. Khalil, S. A. H., Barakat, N. S., and Boraie, N. A. (1991), Effects of package type on in vitro release and chemical stability of amoxycillin in capsules, *Pharm. Ind.*, 53(7), 698–701.
98. Gouda, M. W., Moustafa, M. A., and Molokhia, A. M. (1980), Effect of storage conditions on erythromycin tablets marketed in saudi arabia, *Int. J. Pharm.*, 5, 345–347.
99. Pikal, M. J., Bibler, D. A., and Rutherford, B. (1977), Polymer sorption of nitroglycerin and stability of molded nitroglycerin tablets in unit-dose packaging, *J. Pharm. Sci.*, 66(9), 1293–1297.
100. Barakat, N. S. (2006), Etodolac-liquid-filled dispersion into hard gelatin capsules: An approach to improve dissolution and stability of etodolac formulation, *Drug Dev. Ind. Pharm.*, 32, 865–876.
101. Santoro, M. I. R. M., Oliveira, D. A. G. D. C. E., Kedor-Hackmann, E. R., and Singh, A. K. (2005), The effect of packaging materials on the stability of sunscreen emulsions, *Int. J. Pharm.*, 297, 197–203.
102. Geary, T. G., Akkod, M. A., and Jensen, J. B. (1983), Characteristics of cheloroquine binding to glass and plastic, *Am. J. Trop. Med. Hyg*. 32, 19–23.
103. Yahya, M. A., McElnay, J. C., and D'Arcy, P. F. (1985), Binding of chloroquine to glass, *Int. J. Pharm.*, 25, 217–223.
104. Stanaszek, W. F., and Pan, I. H. (1978), Comparison of drug stability in glass versus plastic containers: Analysis of prefilled syringe admixtures, *Proc. Okla. Acad. Sci.*, 58, 102–105.
105. Arsene, M., Favetta, P., Favier, B., and Bureau, J. (2002), Comparison of ceftazidime degradation in glass bottles and plastic bags, *J. Clin. Pharm. Ther.*, 27(3), 202–209.
106. Crane, I. M., Mulhern, M. G., and Nema, S. (2003), Stability of reconstituted parecoxib for injection with commonly used diluents. *J. of Clin. Pharm. Thera.*, 28, 363–369.
107. Wong, M., Marion, R., Reed, K., and Wang, Y. (2006), Sorption of unoprostone isopropyl to packaging materials, *Int J. Pharm*. 307, 163–167.
108. Driscoll, D. F., Ling, P. R., and Bistrian, B. R. (2007), Physical stability of 20% lipid injectable emulsions via simulated syringe infusion: effects of glass versus plastic product packaging, *J. Parenteral Enteral Nutr.*, 31(2), 148–153.

109. Yuen, P. H., Denman, S. L., Sokoloski, T. D., and Burkman, A. M. (1979), Loss of nitroglycerin from aqueous solution into plastic intravenous delivery systems. *J. Pharm. Sci*., 68, 1163–1166.
110. Baaske, D. M., Amann, A. H., Wagenknecht, D. M., Carter, J. E., Hoyt, H. J., and Stoll, R. G. (1980), Nitroglycerin compatibility with intravenous fluid filters, containers and administration sets, *Am. J. Hosp. Pharm*., 37, 201–205.
111. Cossum, P. A., Roberts, M. S., Galbraith, A. J., and Boyd, G. W. (1978), Loss of nitroglycerin from intravenous infusion sets, *Lancet*., 2, 34–350.
112. Crouthamel, W. G., Dorsch, B., and Shangraw, R. (1978), Loss of nitroglycerin from plastic intravenous bags, *New Engl. J. Med*., 299, 262.
113. Sokoloski, T. D., Wu, C. C., and Burkman, A. M. (1980), Rapid adsorptive loss of nitroglycerin from aqueous solution to plastic, *Int. J. Pharm*., 6, 63–76.
114. Malick, A. W., Amann, A. H., Baaske, D. M., and Stoll, R. G. (1981), Loss of nitroglycerin from solutions to intravenous plastic containers: A theoretical treatment. *J. Pharm. Sci*., 70, 798–800.
115. Yuen, P. H., Denman, S. L., Sokoloski, T. D., and Burkman, A. M. (1979), Loss of nitroglycerin from aqueous solution into plastic intravenous delivery systems, *J. Pharm. Sci*., 68(9), 1193–1166.
116. Illum, L., and Bubdgaard, H. (1982), Sorption of drugs by plastic infusion bags, *Int. J. Pharm*., 10, 339–351.
117. Pikal, M. J., and Lang, J. E. (1978), Rubber closures as a source of haze in freeze-dried parenterals: Test methodology for closure evaluation, *J. Parent. Drug. Assoc*., 32, 162–173.
118. Jahnke, R. W. O., Kreuter, J., and Ross. G. (1991), Content/container interactions: The phenomenon of haze formation on reconstitution of solids for parenteral use, *Int. J. Pharm*., 77, 47–55.
119. Parker, W. A., and MacCara, M. E. (1980), Compatibility of diazepam with intravenous fluid containers and administration sets, *Am. J. Hosp. Pharm*., 37, 496–500.
120. Morris, M. E. (1978), Comaptibility and stability of diazepam injection following dilution with intravenous fluids, *Am. J. Hosp. Pharm*., 35, 669–672.
121. Parker, W. A., Morris, M. E., and Shearer, C. A. (1979), Incompatibility of diazepam injection in plastic intravenous bags, *J. Am. Hosp. Pharm*., 36, 505–507.
122. Van Amerongen, G. J. (1964), Diffusion in elastomers, *Rubber Chem. Technol*., 37, 1065–1152.
123. Vromans, H. and van Laarhoven, J. A. H. (1992), A study on water permeation through rubber closures of injection vials, *Int. J. Pharm*., 79, 301–308.
124. U.S. Food and Drug Administration (FDA) (1998, Oct.), Guidance for industry: Metered dose inhaler and dry powder inhaler drug products, FDA Washington, DC.
125. Castner, J., Anderson, J., and Benites, P. (2007), Strategy for development and characterization of HPLC methods to investigate extractables and leachables, *Am. Pharm. Rev*., 10(3), 10.
126. Northup, S. J. (2005), Assesing the biological safety of extractable and leachable chemicals in pharmaceutical and medicial products, *Am. Pharm. Rev*., 8(4), 38–43.
127. U.S. Food and Drug Administration (FDA) (2002, July), Nasal spray and inhalation solution, suspension, and spray drug products.: Chemistry, manufacturing, and controls documentation, Guidance for industry. FDA, Washington, DC, 1–45.
128. Norwood. D. L. (2007), Understanding the challenges of extractables and leachables for the pharmaceutical industry—Safety and requlatory enviroment for pharmaceuticals, *Am. Pharm. Rev*., 10(2), 32.

129. U.S. Food and Drug Administration (FDA), Extra 6: Safety thresholds and best practices for extractables and leachables in orally inhaled and nasal drug products, PQRI Leachables and Extratables Working Group, available: http://www.pqri.org/pdfs/LE_Recommendations_to_FDA_09-26-06.pdf.
130. IPAC-RS (1991, Mar.) Extra 7: Leachables and extractables testing: points to consider, available: http://www.ipacrs.com/leachables.html.
131. Allan, L., and Wang, Q. (2007), Impact of package on the stability of pharmaceutical products, *Am. Pharm. Rev*., 10(4), 38–44.
132. Waterman, K. C., Adami, R. C., Alsante, K. M., Hong, J., Landis, M. S., Lombardo, F., and Roberts, C. J. (2002), Stabilization of pharmaceuticals to oxidative degradation, *Pharm. Dev. Tech*., 7(1), 1–32.
133. Reed, R. A., Harmon, P., Manas, D., Wasylaschuk, W., Galli, C., Biddell, R., Bergquist, P. A., Hunke, W., Templeton, A. C., and Ip, D. (2003), The role of excipients and package components in the photostability of liquid formulations, *PDA J. Pharm. Sci. Technol*., 57(5), 351–368.
134. Hong, J., Lee, E., Carter, J. C., Masse, J. A., and Oksanen, D. A. (2004), Antioxidant-accelerated oxidative degradation: A case study of transistion metal ion catalyzed oxidation in formulation, *Pharm. Dev. Tech*., 9(2), 171–179.
135. Quarry, M. A., Sebastian, D. S., and Diana, F. (2002), Investigation of 4,5-epoxymorphian degradation during analysis by HPLC, *J. Pharm. Biomed. Anal*. 30, 99–104.
136. Nakamura, K., Yokohama, S., Sawada, M. T., and Sonobe, T. (2003), A new stressed test to predict the foreign matter formation of minodronic acid in solution, *Int. J. Pharm*., 251, 99–106.
137. Bohere, D., Nascimento, P. C., Binotto, R., and Becker, E. (2003), Influence of the glass packing on the contamination of pharmaceutical products by aluminium. Part III. Interaction of the container-chemicals during heating for sterilization, *J. Trace Elem. Med. Biol*., 17(2), 107–115.
138. Shen, F. W., and McKellop, H. A. (2002), Interaction of oxidation and crosslinking in gamma-irradiated ultrahigh molecular-weight polyethylene, *J. Biomed. Mater. Res*., 61(3), 430–439.
139. Welle, F. (2005), Migration of radiolysis products from radiation-sterilized plastics, *Pharm. Ind*., 67(8), 970–972.
140. Nassar, M. N., Nesarker, V. V., Lozano, R., Yande, H., and Palaniswany, V. (2005), Degradation of a lyophilized formulation of BMS-204352: Identification of degradants and the role of elastomeric closure. *Pharm. Dev. Tech*., 10(2), 227–232.
141. Glaser, V. (2005), Addressing stability of biological drugs: forced degradation studies predict effects on bioproducts in drug development and manufacture, *GEN Genetic eng. news*, 25(6), 1–5.
142. *Code of Federal Regulations*, Food and drugs, Title 21, Part 211.65, U.S. Government Printing Office, Washington, DC, rev. Apr. 1, 2004.
143. Federal Food, Drug and Cosmetic Act as amended through Dec. 21, 2000, Chapter V, Drugs and devices, Section 501(a)(3), U.S. Government Printing Office, Washington DC, 2001.
144. Taborsky, C. J., and Sheinin, E. B. (2006), A critical approach to the evaluation of packaging components and the regulatory and scientific considerations in developing a testing strategy, *Am. Pharm. Rev*., 9(4), 10–15.
145. Gudat, A. E., and Firor. R. L. (2006, Sept.), The determination of extractables and leachables in pharmaceutical packaging materials using headspace GC/MS, Application Note, Agilent Technology.

146. Janimak, J. J., and Marteleur, M. (2004), On the stability of cerium oxide glass for terminal radiation sterilization, *Rad. Phys. Chem*. 71, 195–198.
147. DeGrazio, F. L. (2005), Parenteral packaging concerns for drugs: Early understanding of drug to package compatibility lowers the cost in the long run. *GEN: Genetic Eng. News (Bioprocess. Channel)*, 25(21), 2.
148. DeGrazio, F. L. (2005), Parenteral packaging concerns for drugs: Early understanding of drug to package compatibility lowers the cost in the long run. *GEN: Genetic Eng. News (Bioprocess. Channel)*, 25(21), 2.
149. Romacker, M. (2005), Prefilled syringes: Why new developments are important in injectable delivery today, in Roessling, G., Ed., *Prefilled Sringes: Innovations That Meet the Growing Demand*, ONdrugDelivery, Sussex, United Kingdom.
150. Wang, W., Wang, Y. J., and Kelner, D. N. (2003), Coagulation factor VIII: Strucuture and stability, *Int. J. Pharm*., 259, 1–15.
151. Schulman, S., Gitel, S., and Martinowitz, U. (1994), Stability of factor VIII concentrates after reconstitution, *Am. J. Hematol*., 45, 217–223.
152. Osterberg, T., Fatouros, A., Neidhardt, E., Warne, N., and Mikaelsson, M. (2001), B-domain-deleted recombinant factor VIII formulation and stability, *Semin. Hematol*., 38, 40–43.
153. Andrade, J. D., and Hlady, V. (1986), Protein adsorption and materials biocompatibility: A tutorial review and suggested hypothesis, *Adv. Polym. Sci*., 79, 1–63.
154. Duncan, M. R., Lee, J. M., and Warchol, M. P. (1995), Influence of surfactants upon protein/peptide adsorption to glass and polypropylene, *Int. J. Pharm*., 120, 179–188.
155. Wang, P. L., Udeani, G. O. and Johnston, T. P. (1995), Inhibition of granulocyte colony stimulating factor (c-csf) adsorption to polyvinyl chloride using a nonionic surfactant, *Int. J. Pharm*., 114, 177–184.
156. Burke, C. J., Steadman, B. L., Volkin, D. B., Tsai, P. K., Bruner, M. W., and Middaugh, C. R. (1992), The adsorption of proteins to pharmaceutical container surfaces, *Int. J. Pharm*., 86, 89–93.
157. Weber, S. S., Wood, W. A., and Jackson, E. A. (1977), Availability of insulin from parenteral nutrient solutions, *Am. J. Hosp. Pharm*., 34, 353–357.
158. Weeren, R. V., and Gibboni, D. J., Barrier packaging as an integral part of drug delivery, available: http://www.drugdeliverytech.com/cgi-bin/articles=52 (accessed June 1, 2007).
159. U. S. Patent 5,028,431: Franz, T. J., Shah, K. R., and Kydonieus, A. Article for the Delivery to Animal Tissue of a Pharmacological Active. July 2, 1991; U. Patent 5,242,433: Smith, J. A., and Murphy, B., Packaging System with In-Tandem Applicator Pads for Topical Drug Delivery. Decemeber 7, 1992.
160. U. S. Patent 5,698,217: Wilking, S. L. Transdermal Drug Delivery Device Containing a Dessicant. December 16, 1997.
161. Hoye, W. L., Mogalian, E. M., and Myrdal, P. B. (2005), The effects of extreme temperatures on drug delivery of albuterol sulfate hydrofluoroalkane inhalation products, *Am. J. Health-Syst. Pharm*., 62(21), 2271–2277.
162. European Pharmacopoeia (EP) (2001), Preparations for inhalation, EP supplement 2001: 2.9.18, EP, Strasbourg, France.
163. U.S. Food and Drug Administration (FDA) (1998), Guidance for industry: Metered dose inhaler (MDI) and dry powder inhaler (DPI) drug products, FDA CDER, Washington, DC.
164. Petersson, G., and Wiren, J. E. (1980), The brochodilator response from inhaled terbutaline is influenced by the mass of small particles: A study on a dry powder inhaler (Turbuhaler), *Eur. Respir*. 2, 253–256.

165. Newman, S.P., and Busse, W. W. (2002), Evolution of dry powder inhaler design, formulation and performance, *Respir. Med*., 96, 293–304.

166. Carter, P. A., Rowley, G., Fletcher, E. J., and Sylianopoulos, V. (1998), Measurement of electrostatic charge decay in pharmaceutical powdersand polymer materials used in dry powder inhaler devices, *Drug Dev. Ind. Pharm*., 24, 1083–1088.

167. Shire, S. J. (1996), Stability, characterization and formulation of recombinant human deoxyribonuclease (Pulmozyme®, Dornase Alpha), in Pearlman, R., and Wang, Y. J. Eds., *Formulation, Characterization and Stability of Protein Drugs*, Plenum Press, New York, pp. 393–426.

168. Arnum, P. V. (2007), 3M drug delivery systems advances inhalation and transdermal drug delivery, Pharm. Technol. Online, available http://www.pharmtech.com, Jan. 31.

169. Jinks, P., and Marsden, S. (1999), The development and performance of a fluoropolymer lined can for suspension metered dose inhaler product, in Fradley, G., Ed., Proceedings of Drug Delivery to the Lungsa X, The Aerosol Society, Portshead, United Kingdom, pp. 177–180.

170. Wilby, M. (2005), Increasing dose consistency of pMDis, *Drug Deliv. Technol*., 5(9), 59–65.

171. Wilby, M. (2006), Novel valve designs to eliminate loss of prime, in Dalby, X. R. N. et al., Eds., Proceedings of Respiratory Drug Delviery, Vol. 2, Davis Healthcare, Boca Raton, FL, pp. 373–376.

172. Jinks, P., and Hunt, K. (2006), Improving suspension MDI dose consistency in patient use by incorporation of a novel semi-permemable system component, in Van Arnum, P., Ed., Proceedings of Drug Delivery to the Lungs XVII, The Aerosol Society, Portshead, United Kingdom, pp. 172–175.

173. Fairweather, W. R., Lin, T. Y. D., and Roswitha, K. (1995), Regulatory, design, and analysis aspects of complex stability studies, *J. Pharm. Sci*., 84(11), 1322–1326.

174. Dyrstad, K., Veggeland, J., and Thomassen, C. (1999), A multivariate method to predict the water vapour diffusion rate through polypropylene packaging, *Int. J. Pharm*., 188, 105–109.

175. Dyrstad, K., Thomassen, C., and Eivindvik, K. (1999), An opportunistic stability strategy: simulation with real data, *Int. J. Pharm*., 188, 97–104.

176. Ruberg, S. J., and Hsu, J. C. (1991), Multiple comparison procedures for pooling batches in stability studies, *Technometrics*, 34, 465–472.

177. Su, X. Y., Po, A. L. W., and Yoshioka, S. (1994), A bayesian approach to arrhenius prediction of shelf life, *Pharm. Res*., 11, 1462–1466.

178. Yoshika, S., Aso, T., and Kojima, K. (1996), Statistical evaluation of shelf-life of pharmaceutical products estimated matrixing, *Drug Stab*. 1, 147–151.

179. Kontny, M. J., Koppenol, S., and Graham, E. T. (1992), Use of the sorption-desorption moisture transfer model to assess the utility of a desiccant in a solid product, *Int. J. Pharm*., 84, 261–271.

180. Badway, S. I. F., Gawronski, A. J., and Alvarez, F. J. (2001), Application of sorption-desorption moisture transfer modeling to the study of chemical stability of a moisture sensitive drug product in different packaging configurations, *Int. J. Pharm*., 223(1–2), 1–13.

181. Kontny, M. J., and Mulski, C. A. (1998), Gelatin capsule brittleness as a function of relative humidity at room temperature, *Int. J. Pharm*., 54(1), 79–85.

182. Illum, L., Bundgaard, H., and Davis, S. S. (1983), A constant partition model for examining the sorption of drugs by plastic infusion bags, *Int. J. Pharm*., 17, 183–192.

183. Nakabayashi, K., Shimamoto, T., and Mima, H. (1980), Stability of packaged solid dosage forms. I. shelf-life prediction for packaged tablets liable to moisutre damage, *Pharm. Chem. Bull.*, 28, 1090–1098.
184. Nakabayashi, K., Shimamoto, T., and Mima, H. (1980), Stability of packaged solid dosage forms. II. Shelf-life prediction for packaged sugar-coated tablets liable to moisture and heat damage, *Pharm. Chem. Bull.*, 28, 1099–1106.
185. Jenke, D. R. (1993), Modeling of solute sorption by polyvinyl chloride plastic infusion bags, *J. Pharm. Sci.*, 82(11), 1134–1139.
186. Roberts, M. S., Kowaluk, E. A., and Polack, A. E. (1991), Prediction of solute sorption by polyvinyl chloride plastic infusion bags, *J. Pharm. Sci.*, 80(5), 449–455.

7.4

PHARMACEUTICAL PRODUCT STABILITY

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7.4.1 INTRODUCTION

Pharmaceutical product stability may be defined as the capability of a particular formulation to remain within its physical, chemical, microbiological, therapeutic,

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and toxicological specifications while in a specific container closure system. A collection of valid data on the drug in its specific container leads to assurances that the product will be stable for the assigned shelf life.

Often, stability of a drug has been referred to as the time from the date of manufacture and packaging until its chemical or biological activity is not less than a predetermined level of labeled potency without the physical characteristics changing appreciably. For most drugs, 90% of labeled potency is generally recognized as the minimum acceptable potency.

There are many factors that can affect the stability of a pharmaceutical product. These include the stability of the active drug(s), interactions between active and inactive ingredients, the dosage form, manufacturing process, the container system, and environment for shipping, handling, and storage.

The U.S. Pharmacopeia 29/National Formulary 24 (USP29/NF24) defines stability as the extent to which a product retains within specified limits and throughout its period of storage and use (i.e., shelf life) the same properties and characteristics that it possessed at the time of its manufacture [1]. The USP29/NF24 further identifies five generally recognized types of stability:

- Chemical degradation—Each active ingredient retains its chemical integrity and labeled potency within specified limits.
- Physical—The original physical properties, including appearance, palatability, uniformity, dissolution, and suspendability, may be affected.
- Microbiological—Sterility or resistance to microbial growth is retained according to the specified requirements. Antimicrobial agents that are present retain effectiveness within the specified limits.
- Therapeutic—The therapeutic effect remains unchanged.
- Toxicological—No significant increase in toxicity occurs.

Credible pharmaceutical product expiration dates are obtained by rigorous, scientifically designed studies using reliable, meaningful, and specific stability-indicating assays, appropriate statistical concepts, and computers to analyze the resulting data [2]. A comprehensive review of all aspects of pharmaceutical product stability has been published by Lintner [3] and more recently by Connors et al. [4].

7.4.2 KINETIC EQUATIONS AND SHELF LIFE

Consider the reaction

$$aA + bB \rightarrow pP + qQ \tag{1}$$

where A and B are the reactants, P and Q are the products, and *a, b, p, q* are the stoichiometric coefficients describing the reaction. The rate of change of the concentration C of any of the species can be expressed by

$$-\frac{dC_A}{dt}, \quad -\frac{dC_B}{dt}, \quad \frac{dC_P}{dt}, \quad \frac{dC_Q}{dt}$$

The reactants decrease in concentration relative to time—hence the negative sign. On the other hand, the products increase over time and are preceded by a positive sign. The rates of disappearance of A and B and the rates of appearance of P and Q are interrelated by equations that take into account the stoichiometry of the reaction:

$$-\frac{1}{a}\frac{dC_{\mathrm{A}}}{dt}=-\frac{1}{b}\frac{dC_{\mathrm{B}}}{dt}=\frac{1}{p}\frac{dC_{\mathrm{P}}}{dt}=\frac{1}{q}\frac{dC_{\mathrm{Q}}}{dt} \tag{2}$$

7.4.2.1 Rate Expression

The rate expression is a mathematical description of the rate of the reaction at any time t in terms of the concentrations(s) of the molecular species present at that time. By simplifying Equation (1) to

$$\mathrm{A}+\mathrm{B}\rightarrow \text{products} \tag{3}$$

the rate expression can be written as

$$-\frac{dC_{\mathrm{A}}}{dt}=-\frac{dC_{\mathrm{B}}}{dt}\propto C_{\mathrm{A}(t)}^{a}C_{\mathrm{B}(t)}^{b} \tag{4}$$

Equation (4) in essence states that the rate of change of the concentration of A at time t is equal to that of B and that each of these changes at time t is proportional to the product of the concentrations of the reactants raised to the respective powers. Note that $C_{\mathrm{A}(t)}$ and $C_{\mathrm{B}(t)}$ are time-dependent variables. As the reaction proceeds, both $C_{\mathrm{A}(t)}$ and $C_{\mathrm{B}(t)}$ will decrease. For simplicity, these concentrations can be denoted by C_{A} and C_{B}, respectively:

$$-\frac{dC_{\mathrm{A}}}{dt}=-\frac{dC_{\mathrm{B}}}{dt}=kC_{\mathrm{A}}^{a}C_{\mathrm{B}}^{b} \tag{5}$$

where k is a proportionality constant, commonly referred to as the reaction rate constant or the specific rate constant. The format for rate expressions generally involves concentration terms of only the reactants and very rarely those of the products. The latter occurs only when the products participate in the reaction once it has been initiated.

The order of the reaction, n, can be defined as $n = a + b$. Extended to the general case, the order of a reaction is the numerical sum of the exponents of the concentration terms in the rate expression. Thus, if $a = b = 1$, the reaction described above is said to be second order overall but first order relative to A and first order relative to B. In principle, the numerical value of a or b can be integral or fractional.

At times the rate of reaction is apparently independent of the concentration of one of the reactants, even though this reactant is consumed during the reaction. For example, in the reaction between an ester and water (hydrolysis) in a predominantly aqueous environment, the theoretical rate expression for the ester can be written in terms of the concentrations of the ester (C_{E}) and water (C_{W}):

$$-\frac{dC_E}{dt} = kC_E C_W \quad (6)$$

If the initial concentration of the ester is less than or equal to 0.5 M, complete hydrolysis of the ester will bring about a corresponding decrease in the concentration of water of 0.5 M or less. With the initial concentration of water being about 55 M for an aqueous solution, the relative loss of water through a reaction is insignificantly small, enabling C_W to be considered a constant throughout the entire course of the reaction. Therefore,

$$-\frac{dC_E}{dt} = k\pi C_E \quad (7)$$

where $k_\pi = kC_w$. The reaction appears to be first order relative to the ester and zero order relative to water. The overall reaction is known as a pseudo-first-order reaction with k_π the pseudo-first-order constant.

Pseudo-first-order kinetics are observed whenever the concentration of one of the reactants is maintained constant, either by a substantial excess in initial concentration or by rapid replenishment of one of the reactants. If one of the reactants is the hydrogen ion or the hydroxide ion, its concentration, though probably small when compared with that of the drug, can be kept constant throughout the reaction by using buffers in the solution. The concentration of an unstable drug in solution can be maintained invariant by utilization of a suspension, which provides excess solid in equilibrium with the drug in solution.

7.4.2.2 Order Determination

Reaction orders can be determined by several methods:

Substitution Method The accumulated data from a kinetic study can be substituted in the integrated form of the various equations that describe reaction orders. When the k values of one of the iterations remain constant, the reaction is considered to be of that order.

Graphic Method A plot of the data can be used to ascertain the order. If a plot of concentration versus time yields a straight line, the reaction is zero order. A straight line from the plot of $\log(a - x)$ versus time is first order and second order if the plot of $1/(a - x)^2$ versus time is a straight line (where the initial concentrations are equal).

Half-Life Method For a zero-order reaction the half-life ($t_{1/2}$) is proportional to the initial concentration. The half-life for a first-order reaction is independent of the initial concentration while a second-order reaction is proportional to 1/initial concentration.

7.4.2.3 Predicting Shelf Life

The shelf life of a formulation is currently based on rigorous physical–chemical laws and statistical concepts to obtain reliable estimates. McMinn and Lintner have

developed an information processing system for handling product stability data [5]. This system saves the time of formulators in analyzing and interpreting their product stability data. For products such as those of vitamins, for example, where large overages are required, the statistical portions of this advanced technique aid the manufacturer to tailor the formula composition to obtain the desired and most economical expiration dating.

This system stores both physical and chemical data and retrieves the information in three different formats (one of which was designed specifically for submitting to regulatory agencies). It analyzes single-temperature data through analysis of covariance and regression. Multiple-temperature data are analyzed either weighted or unweighted using the Arrhenius relationship. This method provides estimates of the shelf life of the preparation with appropriate confidence intervals, preprints the assay request cards that are used to record the results of the respective assay procedures and to enter the data into the system, and produces a 5-year master stability schedule as well as periodic 14-day schedules of upcoming assays.

Analysis of stability data obtained at a single temperature is based on a linear (zero-order) model

$$Y_{mn} = \beta_m X_{mn} + \alpha_m + \varepsilon_{mn} \tag{8}$$

where Y_{mn} is the percentage of label of the nth stability assay of the mth lot, X_{mn} is the time in months at which Y_{mn} was observed, β_m and α_m are the slope and intercept, respectively, of the regression line of the mth lot, and ε_{mn} is a random error associated with Y_{mn}. The random errors are assumed to be identically and independently distributed normal variables with a zero mean and a common variance σ^2.

A summary of the regression analysis for each individual lot and for the combination of these lots plus a summary of the analyses of covariance and deviation from regression is prepared by the computer.

Since the stability data from individual lots are pooled, these data are examined for validity by the F test. The mean square of the regression coefficient (slope) is divided by the mean square of the deviation within lots, and similarly, the adjusted mean (y intercept) is divided by the common mean square to give the respective F ratios. The latter values then are compared with the critical 5% F values. When the calculated F values are smaller than the critical F values, the data may be combined and the pooled data analyzed.

7.4.2.4 Arrhenius Equation and Accelerated Stability Testing

The purpose of stability testing is to assess the effects of temperature, humidity, light, and other environmental factors on the quality of a drug substance or product. These data sets are used to establish storage conditions, retest periods, and shelf loss and to justify overages included in products for stability reasons. The most useful equation relating temperature and reaction rate is the Arrhenius equation

$$\frac{d \ln k}{dT} = \frac{E_a}{RT^2} \tag{9}$$

This can be rewritten as

$$k = Ae^{-E_a/(RT)} \tag{10}$$

$$\ln\left(\frac{k_1}{k_2}\right) = \frac{E_a}{R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right) \tag{11}$$

where E_a is a constant and the subscripts 1 and 2 denote the two different temperature conditions. A plot of $\ln k$ as a function of $1/T$, referred to as the Arrhenius plot, is linear according to Equation (10) if E_a is independent of temperature. This observation makes it possible to conduct kinetic experiments at elevated temperatures and obtain estimates of rate constants at lower temperatures by extrapolation of the Arrhenius plot. This procedure, commonly known as accelerated stability testing, is most useful when the reaction at ambient temperature is too slow to be monitored conveniently and when E_a is relatively high. As an example, in a reaction with an E_s of 25 kcal/mol with an increase from 25 to 45°C, there is about a 14-fold increase in the reaction rate constant. In contrast, a rate increase of just threefold is obtained for the same elevation in temperature when E_a is 10 kcal/mol. The slope of the Arrhenius plot for a reaction yields the magnitude of E_a. Hydrolysis reactions typically have an E_a of 10–30 kcal/mol while oxidation and photolysis reactions have smaller energies of activation [6].

The elevated temperatures most commonly used are 40, 50, and 60°C in conjunction with ambient humidity. Occasionally, higher temperatures are used. The samples stored at the highest temperatures are examined weekly for physical and chemical changes. If a substantial change is seen, samples stored at lower temperatures are examined. If there is no change after 30 days at 60°C, the stability prognosis is excellent. Corroborative evidence must be obtained by monitoring the samples stored at lower temperatures for longer durations.

An underlying assumption of the Arrhenius equation is that the reaction mechanism does not change as a function of temperature. Since accelerated stability testing of pharmaceutical products normally employs a narrow range of temperature, it is often difficult to detect nonlinearity in the Arrhenius plot from experimental data, even though such nonlinearity is expected from the reaction mechanism [7].

Non-Arrhenius behavior has been observed in pharmaceutical systems [8]. This may be attributed to the possible evaporation of solvent, multiple reaction pathways, and the change in physical form of the formulation when the temperature of the reaction is changed [9]. Nonlinearity in Arrhenius plots frequently is observed in following the temperature dependence of protein degradation. Degradation mechanisms in proteins often change with temperature [10]. The aggregation of interleukin 1β (IL-1β) in aqueous solution follows apparent first-order behavior at 60°C to 30% of drug remaining. At or below 55°C the aggregation deviates from apparent first order and becomes biphasic [11].

Considerable interest has been generated in the use of accelerated stability testing based on a singe condition of elevated temperature and humidity. For abbreviated new drug applications (ANDAs) U.S. Food and Drug Administration (FDA) stability guidelines suggest that a tentative expiration date of 24 months may be granted for a drug product if satisfactory stability results can be documented under a stressed condition of 40°C and 75% relative humidity [12]. The simplicity of such

a guideline is attractive because a substantial saving in time can be obtained in advancing a drug product to the marketplace [7].

7.4.3 DEGRADATION PATHWAYS OF PHARMACEUTICALS

Many degradation pathways are similar to reactions described for organic compounds in organic chemistry texts. The decomposition of a drug is likely to be mediated by reaction with water, oxygen, or light. The routes most pharmaceuticals degrade include hydrolysis, oxidation, photolysis, and racemization. Chemical degradation occurs when a new chemical entity is formed as a process of the degradation process. Physical degradation occurs when drug loss does not produce distinctly different chemical products [13]. Degradation reactions of a variety of mechanisms can often be avoided by decreasing storage temperature and/or buffering pH to a stability optimum.

7.4.4 CHEMICAL DEGREDATION

7.4.4.1 Solvolysis

Solvolysis is the decomposition of the active drug with the solvent that is present. When water is the solvent, the process is called hydrolysis. The most common solvolysis reactions involve liable carbonyl compounds such as esters and β-lactams (see Table 1). Aspirin is hydrolyzed to acetic acid and salicylic acid in the presence of moisture, but in a dry environment this hydrolysis is negligible. The most significant catalysts of hydrolysis are adverse pH and specific compounds (e.g., dextrose and copper or other metal ions).The rate of hydrolysis is pH and temperature dependent. There is a generalized rule of thumb that with each 10°C increase in temperature, the rate of the reaction increases exponentially. The actual factor of rate increase depends on the activation energy of the particular reaction, with the activation energy being a function of the specific reactive bond and the drug formulation.

Hydrolysis leads to a decrease in active drug and an increase of decomposition products. The effect of this change on reaction rate depends on the order of the

TABLE 1 Liable Functional Groups to Hydrolysis

Functional Group	Examples
Esters	Aspirin, procaine, alkaloids, estrone sulfate, dexmethasone sodium phosphate
Lactams	Penicillins, cephalosporins
Amides	Chloramphenicol, thiacinamide
Lactones	Pilocarpine
Oximes	Steroid oximes
Imides	Gluthethimide
Malonic ureas	Barbiturates
Nitrogen mustards	Melphalan
Azo methines	Benzodiazepines

reaction. For zero-order reactions, the decomposition rate is independent of the drug concentration. Zero-order hydrolysis shows a lesser percentage of decomposition for higher concentration solutions that those of lower concentration. Unlike zero-order reactions, hydrolysis following first-order kinetics shows a rate of change which is directly proportional to the concentration of the reacting ingredient. Therefore, changes in active ingredient concentration do not have an influence on the percentage concentration.

Structural modifications of the drug may be employed to retard hydrolysis. The most frequently encountered hydrolysis reaction involves the ester. Substituents can have an effect on these reaction rates. Hansch and Taft [14] and Hammett [15] provide excellent reviews of the topic. Additionally, a compound may be stabilized by reducing its solubility. This can be accomplished by the addition of lipophilic substituents to side chains or aromatic rings. Often less soluble salts or esters have been employed to aid in product stability.

The rate of hydrolysis is impacted by the ionic strength of the total concentration of dissolved electrolytes. In general, the rate constant of hydrolysis is directly proportional to the ionic strength of ions of like charge (drug cation and excipient cation) and inversely proportional to the ionic strength with oppositely charged ions.

7.4.4.2 Oxidation

Oxidation is a primary cause of instability and usually involves the addition of oxygen or the removal of hydrogen. Auto-oxidations are oxidative degradations usually mediated through reactions with atmospheric oxygen. Most auto-oxidations are free-radical reactions. A drug's susceptibility to auto-oxidation can be determined by investigating its stability in a high-oxygen-tension atmosphere, usually 40% oxygen. Results are compared against those under inert or ambient temperature [16]. A listing of several functional groups that are subject to oxidation can be found in Table 2.

Oxidations are catalyzed by acids, bases, pH values that are higher than the optimum, polyvalent metal ions, peroxides, hydroperoxides, and exposure to oxygen and ultraviolet (UV) illumination. These reactions may necessitate the use of antioxidant chemicals, inert atmospheres, and opaque packaging. Chelating agents

TABLE 2 Functional Groups Subject to Oxidation

Functional Group	Examples
Phenols	Catecholamines, morphine
Conjugated dienes	Vitamin A
Thioethers	Chlorpromazine
Nitrites	Amyl nitrite
Aldehydes	Paraldehyde, flavors
Amines	Clozapine
Carboxylic acids	Fatty acids
Thiols	Dimerceprol
Ethers	Diethylether

TABLE 3 Common Antioxidants

Aqueous Systems	Oil-Based Systems	Chelating Agents
Sodium sulfite	Ascorbyl palmitate	Ethylenediamine tetraaceticacid (EDTA)
Sodium metabisulfite	Hydroquinone	Dihydroethylglycine
Sodium bisulfite	Propyl gallate	Citric acid
Sodium thiosulfate	Nordihydroguaiaretic acid	Tartaric acid
Ascorbic acid	Butylated hydroxytoluene	Gluconic acid
	Butylated hydroxyanisole	Saccharic acids
	α-Tocopherol	

added to water sequester heavy metals. Parenteral formulations should not come into contact with heavy metal ions during their manufacture, packaging, or storage [17].

Antioxidants are very effective in stabilizing products undergoing a free-radical mediated chain reaction. These products possess lower oxidation potentials than the active drug. Ideally, antioxidants are stable over a wide pH range and remain soluble in the oxidized form, colorless, and nontoxic. A listing of commonly used antioxidants can be found in Table 3.

Visual identification of oxidation products is frequently attainable due to the introduction of conjugation, but these changes may not be visible in certain concentrations.

7.4.4.3 Photolysis

When molecules absorb energy, they proceed to a higher energy state where they release the energy in a chemical reaction to attain their ground energy state. When the energy of activation that is absorbed by the compound comes from a light source, the decomposition reaction is called photolytic. The activated species can release the energy either as light of a different frequency (fluorescence) or by decomposition (photolysis). Exposure to room light or sunlight can lead to drug degradation by causing photo-oxidation and photolysis of covalent bonds. In susceptible compounds, usually those with π electrons, photochemical energy creates free-radical intermediates, which can perpetuate chain reactions.

In general, drugs that absorb UV light below 280 nm undergo decomposition in sunlight while compounds that absorb above 400 nm have the potential to degrade in both sunlight and room light. Photo-induced reactions are common in steroids [18]. The photodegradation of sodium nitroprusside in aqueous solution remains a classic example of photolytic decomposition [19].

7.4.4.4 Dehydration

Dehydrations are chemical reactions that involve the loss of water. The acid-catalyzed dehydration of tetracycline yields the toxic epianhydrotetracycline [20]. The physical dehydration of theophylline hydrate and ampicillin trihydrate leads to a change of the crystalline structure of the drug [21].

7.4.4.5 Racemization

Racemization is the process of changing from an optically active compound into a racemic mixture. Pfeiffer provided one of the earliest discussions on the importance of stereospecificity in drug action [22].

The most widely known drugs that undergo racemization are tetracycline, epinephrine [23], pilocarpine [24], and ergotamine. In tetracycline, the reaction occurs rapidly when the dissolved drug is exposed to a pH greater than 3, resulting in a steric rearrangement of the dimethylamino group [25].

Generally, racemization follows a first-order reaction rate and is dependent on temperature, solvent system, catalysts, and the presence or lack of light. Resonance stabilization through substituents adjacent to the asymmetric center tends to accelerate racemization.

7.4.5 PHYSICAL DEGRADATION

7.4.5.1 Polymorphism

Many pharmaceutical solids can exist in different physical forms. Polymorphism is often characterized as the ability of a drug substance to exist as two or more crystalline phases that have different arrangements and/or conformations of the molecules in the crystal lattice [26]. Polymorphic forms of a drug substance can have different chemical and physical properties, including melting point, chemical reactivity, apparent solubility, dissolution rate, optical and mechanical properties, vapor pressure, and density. These properties can have a direct effect on the ability to process and/or manufacture the drug substance and the drug product as well as on drug product stability, dissolution, and bioavailability. Thus, polymorphism can affect the quality, safety, and efficacy of the drug product [27]. Amorphous solids consist of disordered arrangements of molecules and do not possess a distinguishable crystal lattice. Solvates are crystalline solid adducts containing either stoichiometric or nonstoichiometric amounts of a solvent incorporated within the crystal structure. If the incorporated solvent is water, the solvates are also commonly known as hydrates. Drug exposure to changes in temperature, pressure, humidity, and pulverization during granulation, milling, and compression may lead to polymorphic phase conversion. The extent of conversion generally depends on the relative stability of the polymorphs, kinetic barriers for phase conversion, and applied stress [28]. Sulfonamides, barbiturates, and steroids are known for their propensity to form polymorphs [29].

7.4.5.2 Vaporization

Several drugs and adjuvants possess high vapor pressures at room temperature that their vaporization exists as a route of drug loss. Low-molecular-weight alcohols and "aromatics" used for flavoring and aroma may be lost through vaporization. Nitroglycerine is the most frequently cited example of loss on vaporization. The FDA issued a special regulation governing the types of containers that may be used for

dispensing nitroglycerine tablets [30]. The addition of macromolecules such as microcrystalline cellulose allows for the preparation of a stabilized nitroglycerine sublingual tablet [31].

7.4.6 MICROBIAL DEGRADATION

Microorganisms could present a risk of infection or degradation of medicinal products. These organisms may proliferate during normal storage conditions or during patient use, especially in multidose preparations. Preparations containing water bear the greatest risk of contamination. These products include solutions, suspensions, and emulsions as well as sterile multidose injections and ophthalmic preparations.

Antimicrobial preservatives are used to prevent or inhibit the growth of microorganisms. Factors influencing the level of observable efficacy include the chemical structure of the preservative, the physical and chemical characteristics of the pharmaceutical product, the concentration of the preservative, and the type and load of initial contamination. At no time should preservatives be used as an alternative to good manufacturing practice.

7.4.7 STABILITY GUIDELINES AND REGULATIONS

FDA guidelines for stability testing are outlined in the *Code of Federal Regulations* (CFR), Part 21, Section 211.166, under Current Good Manufacturing Practice for Finished Pharmaceuticals. The guidelines state that there must be a testing program designed to assess the stability characteristics of drug products. The results of such stability testing are to be used in determining appropriate storage conditions and expiration dates. This section of the *Federal Register* provides guidelines for sample size and test intervals, storage conditions for samples, and specific test methods [32].

Section 211.166 guidelines also require an adequate number of batches of each drug product tested for expiration date assignment. Accelerated studies, combined with basic stability information on the components, drug products, and container closure system may be used to support tentative expiration dates provided full shelf life studies are not available and are being conducted. Where data from accelerated studies are used to project a tentative expiration date that is beyond a date supported by actual shelf life studies, there must be stability studies conducted, including drug product testing at appropriate intervals, until the tentative expiration date is verified or the appropriate expiration date determined.

Additional guidelines outline the need for reserve samples [33], expiration dating [34], and laboratory recordkeeping [35].

7.4.8 ICH QUALITY GUIDELINES

The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) is a project that brings together the regulatory authorities of Europe, Japan, and the United States and

TABLE 4 ICH Document Codes and Guidelines for Stability

ICH Code	Title	Description
Q1A(R2)	Stability Testing of New Drug Substances and Products	Stability testing protocols including temperature, humidity, and trial duration
Q1B	Stability Testing: Photostability Testing of New Drug Substances and Products	Basic testing protocol required to evaluate light sensitivity and stability of new drugs and products
Q1C	Stability Testing for New Dosage Forms	Extends main stability guideline for new formulations of already approved medicines and defines circumstances under which reduced stability data can be accepted
Q1D	Bracketing and Matrixing Designs for Stability Testing of New Drug Substances and Products	General principles for reduced stability testing and provides examples of bracketing and matrixing designs
Q1E	Evaluation of Stability Data	Explains possible situations where extrapolation of retest periods/shelf lives beyond real-time data may be appropriate; provides examples of statistical approaches to stability data analysis
Q1F	Stability Data Package for Registration Applications in Climatic Zones III and IV	Besides proposing acceptable storage conditions for long-term and accelerated studies, gives guidance on data to cover situations of elevated temperature and/or extremes of humidity; referenced literature provides information on classification of countries according to climatic zones

experts from the pharmaceutical industry in the three regions to discuss scientific and technical aspects of product registration. The purpose is to make recommendations on ways to achieve agreement in the interpretation and application of technical guidelines and requirements for product registration to reduce the need to duplicate the testing carried out during the research and development of new medicines. The objective of such harmonization is a more economical use of human, animal, and material resources and the elimination of unnecessary delay in the global development and availability of new medicines while maintaining safeguards on quality, safety and efficacy, and regulatory obligations to protect public health [36].

The guidelines provide a breath of recommendations from stability testing protocols, including temperature, humidity, and trial duration [Q1A(R2)]; basic testing protocols required to evaluate the light sensitivity and stability of new drugs and products (Q1B); and stability testing for new formulations of already approved medicines (Q1C). A description of the various ICH guidelines for stability can be found in Table 4.

The ICH also provides guidelines in analytical validation [Q2(R1), impurities (Q3 series), pharmacopeias (Q4 series), quality of biotechnological products (Q5

series), specifications (Q6 series), good manufacturing practice (Q7), pharmaceutical development (Q8), and risk management (Q9)].

REFERENCES

1. U.S. Pharmacopeia (USP), Stability considerations in dispensing practice, USP 29/NF 24, USP, Rockville, MD, p. 3029.
2. Guillory, P., and Poust, R. (2002), Chemical kinetics and drug stability, in Banker and Rhodes, Eds., *Drugs in the Pharmaceutical Sciences*, Vol. 121, Marcel Dekker, New York, pp. 139–166.
3. Lintner, C. C. (1973), *Quality Control in the Pharmaceutical Industry*, Vol. 2, Academic, New York, p. 141.
4. Connors, K. A., Amidon, G. L., and Stella, J. V. (1986), *Chemical Stability of Pharmaceuticals*, 2nd ed., Wiley, New York.
5. McMinn, C. S., and Lintner, C. J. (1973, May), paper presented at the American Pharmaceutical Association Academy of Pharmaceutical Sciences Meeting, Pharmaceutical Technical Section, Chicago, IL.
6. Lachman, L., DeLuca, P., and Akers, J. J. (1986), Kinetic principles and stability testing, in Lachman, L., Lieberman, H. A., and Kanig, J. L., Eds., *The Theory and Practice of Industrial Pharmacy*, 3rd ed., Lea & Febiger, Philadelphia, p. 766.
7. Fung, H.-L., and King, S.-Y. (1983), in Pharmaceutical Technology Conference ’83 Proceedings, Aster Publishing, Springfield, OR.
8. Pikal, M. J., Lukes, A. L., and Lang, J. E. (1977), *J. Pharm. Sci.*, 66, 1312.
9. Woolfe, J., and Worthington, H. E. C. (1974), *Drug Dev. Commun.*, 1, 185.
10. Wang, W. (1999), Instability, stabilization, and dormulation of liquid protein pharmaceuticals, *International Journal of Pharmaceutics*, 185, 129–188.
11. Gu, L.C., Erdos, E. A., Chang, H.-S. (1991), *Pharm. Res*. 8, 485.
12. (a) Center for Drugs and Biologics, U.S. Food and Drug Administration (FDA) (1987, Feb.), Guideline for submitting documentation for the stability of human drugs and Biologics, FDA, Rockville, MD. (b) Center for Drug Evaluation and Research and Center for Biologics Evaluation and Research, U.S. Department of Health and Human Services, FDA (1998, June), Guidance for industry: Stability testing of drug substances and drug products: Draft guidance, FDA, Rockville, MD.
13. U.S. Pharmacopeia (USP), Pharmaceutical stability, USP 29/ NF 24, USP, Rockville, MD. p. 2994.
14. Hansch, A. L., and Taft, R. E. (1991), A survey of Hammett substituent constants and resonance and field parameters, *Chem. Rev.*, 91, 165.
15. Hammett, L. P. (1970), *Physical Organic Chemistry*, 2nd ed., McGraw-Hill, New York.
16. Johnson, D. M., and Gu, L. C. (1998), Autooxidation and antioxidants, in Swarbrick, J., and Boylan, J. C. Eds., *Encyclopedia of Pharmaceutical Technology*, Marcel Dekker, New York, pp. 415–449.
17. Vadas, E. B. (2000), Stability of pharmaceutical products, in *Remington: The Science and Practice of Pharmacy*, 20th ed, Lippincott Williams and Wilkins, Baltimore, MD, p. 989.
18. Ogata, M., Noro, Y., Yamada, M., Tahara, T., and Nishimura, T. (2000), Photo-degradation products of methylprednisolone sulphanate in aqueous solution—Evidence of a bicyclo[3.1.0]hex-3-en-2-one intermediate, *J. Pharm. Sci.*, 87(1), 91–95.
19. Hauser, U., Oestreich, V., and Rohrweck, H. D. (1977), On optical dispersion in transparent molecular systems, *Zeits. Phys. A*, 280, pp. 17–25.

20. Yuen, P. H., and Sokoloski, T. D., (1977), Kinetics of concomitant degradation of tetracycline to epitetracycline, anhydrotetracycline, and epianhydrotetracycline in acid phosphate solution, *J. Pharm. Sci.*, 66(11), 1648–1650.
21. Shefter, E., Fung, H.-L., and Mok, O. (1973), Dehydration of crystalline theophylline monohydrate and ampicillin trihydrate, *J. Pharm. Sci.*, 62, 791.
22. Pfeiffer, C. C. (1956), Optical isomerism and pharmacological action, a generalization, *Sci.*, 123, 3210, 29–31.
23. Schroeter, L. C., and Higuchi, T. (1958), Racemization of epinephrine, *J. Am. Pharm. Assoc. (Baltimore)*, 47, 6, 426, 30.
24. Nunes, M. A., and Brochmann- Hanssen, E. (1974), Hydrolysis and epimerization kinetics of pilocarpine in aqueous solution, *J. Pharm. Sci.*, 63, 716.
25. Sheberstova, N. V., Perel'son, M. E., and Kuzovkov, A. D. (1975), Study of the epimerization of tetracycline by the NMR method, *Chem. Nat. Comp.*, 10(1), 61–65.
26. Haleblian, J., and McCrone, W. (1969), Pharmaceutical applications of polymorphism, *J. Pharm. Sci.*, 58, 911.
27. U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER), U.S. Food and Drug Administration (FDA) (2004, Dec.), Guidance for industry ANDAs: Pharmaceutical solid polymorphism chemistry, manufacturing, and controls information, FDA, Rockville, MD.
28. Vippagunta, S. R., Brittain, H. G., and Grant, D. J. W. (2001), Crystalline solids, *Adv. Drug Deliv. Rev.*, 48, 3–26.
29. Kuhnert-Brandstatter, M. (1971), Thermomicroscopy, in *The Analysis of Pharmaceuticals*, Pergamon, Oxford, pp. 37–42.
30. *Federal Register*, 37, 15959 (1972).
31. Fung, H.-L., Yap, S. K., and Rhodes, C. T. (1974), Development of a stable sublingual nitroglycerin tablet. I. Interaction of nitroglycerin with selected macro-molecules, *J. Pharm. Sci.*, 63, 1810.
32. *Code of Federal Regulations*, Title 21, Food and drugs, Part 211, Current good manufacturing practice for finished pharmaceuticals, Subpart I, Laboratory controls, Section 211.166, Stability testing.
33. *Code of Federal Regulations*, Title 21, Food and drugs, Part 211, Current good manufacturing practice for finished pharmaceuticals, Subpart I, Laboratory controls, Section 211.170, Reserve samples.
34. *Code of Federal Regulations*, Title 21, Food and drugs, Part 211, Current good manufacturing practice for finished pharmaceuticals, Subpart G, Packaging and labeling control, Section 211.137, Expiration dating.
35. *Code of Federal Regulations*, Title 21, Food and drugs, Part 211, Current good manufacturing practice for finished pharmaceuticals, Subpart J, Records and reports, Section 211.194, Laboratory records.
36. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, available: http://www.ich.org/cache/compo/363-272-1.html.

7.5

ALTERNATIVE ACCELERATED METHODS FOR STUDYING DRUG STABILITY: VARIABLE-PARAMETER KINETICS

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7.5.1 INTRODUCTION

Studies on drug stability have a central role in physicochemical profiling [1–6]. It is important to know (i) how long the substrate maintains its chemical identity in various environments carrying out its therapeutic action and (ii) the pathway followed when it degrades. Both sets of information are vitally important issues in the pharmaceutical industry. The first is necessary to decide whether to continue to investigate the molecule of interest or not: An unstable drug can reduce its efficiency

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to a little percentage and can generate products with undesired effects; the second is necessary to understand whether it could be possible to find a remedy or not, for example synthesizing a new drug with a similar structure and reduced reactivity or devising a suitable carrier able to stabilize the molecule and deliver it to the desired target.

Temperature, pH, ionic strength, concentration of a metal ion, and other environmental parameters influence a chemical reaction and varying their values can signify drastic changes, depending on the case, on the rate, mechanism, or direction of the reaction. For this reason quantitative studies on the effects of physical parameters on reactivity often take a very long time in this kind of research.

The usual way consists of isolating and characterizing the reaction of interest and then determining the rate constant under first- or pseudo-first-order conditions for different values of the involved parameters. The result is a polydimensional representation of the specific rate as a function of these parameters that can be of great use for practical applications and, particularly, constitutes the experimental structure on which to base the investigation directed to clarify the reaction mechanism. In fact, the dependence of k_{obs} on parameters is a consequence of their influence on the rate-determining step and fitting kinetic data to suitable mathematic models gives information that enables one to formulate a reaction scheme, discriminate between kinetically equivalent mechanisms, and determine the values of elementary kinetic constants [1–4, 7, 8].

Many examples are present in the scientific literature underlining the effort in producing kinetic data [9–11]. The Edwards historical study that started the investigation on the mechanism of the hydrolysis of aspirin required hundreds of kinetic experiments [12, 13]. Several examples are reported by Carstensen [1] in his review on the subject where, beside the large space dedicated to the determination of the pH–rate profile, the effect of temperature, ionic strength, buffer concentration, and dielectic constant on the stability of drugs was treated.

Physicochemical profiling concerns the determination of some basic molecular properties of pharmaceutical interest that can support the successive clinical studies and can help a rapid identification and elimination of compounds with unsuitable physicochemical and pharmacokinetic properties. New accelerated methods for physicochemical profiling have assumed particular importance in recent years because of the great amount of new chemical entities coming from modern synthetic strategies. Thousands of compounds have to be profiled each year and traditional technologies cause a bottleneck in drug development. Methods have been developed for high-throughput physicochemical profiling for solubility, permeability, pK_a, lipophilicity, stability, and integrity, and a great interest from pharmaceutical industries and instrument maker groups is reserved for this subject [5, 6].

Among physicochemical properties, the stability of drug candidates is receiving increasing attention. Unfortunately, its evaluation very often requires a lot of experimental time and profiling of many molecules would be nearly impossible without new, computer-aided methods. Any effort useful to facilitate this first part of pharmaceutical investigation is appreciated because it can be converted to a considerable lowering of the research cost.

Here a panoramic picture of the effort directed to accelerating the characterization of the reactivity in solution of molecular candidates is shown. In particular,

variable-parameter kinetics (VPaK) is considered, a potential new way to save time and chemicals while studying drug stability.

7.5.2 THEORY

Consider a molecule A degrading in products,

$$\mathrm{A} \rightarrow \mathrm{P} \tag{1}$$

the rate law, operating under first- or pseudo-first-order conditions, is given by Equation (2), where C is the molar concentration of the substrate and $k_{\mathrm{obs}}(\mathrm{Par}_1, \mathrm{Par}_2, \ldots)$ is the observed rate constant function of various parameters:

$$-\frac{dC}{dt} = k_{\mathrm{obs}}(\mathrm{Par}_1, \mathrm{Par}_2, \ldots)C \tag{2}$$

In the usual kinetic experiments all these parameters must be rigorously constant so that the experimental C–t data, that is, the kinetic profile obtained following the change in concentration with time, can be easily fitted to the differential form of the first-order equation (2) or to its integrated, exponential [Equation (3)] or logarithmic [Equation (4)], forms to obtain the optimized values of k_{obs}:

$$C = C_0 \exp(-k_{\mathrm{obs}} t) \tag{3}$$

$$\ln C = -k_{\mathrm{obs}} t + \ln C_0 \tag{4}$$

The dependence of k_{obs} on one parameter (temperature T, pressure P, pH, ionic strength I, etc.), that is, the $k_{\mathrm{obs}}(\mathrm{Par}_i)$ profile, can be obtained by carrying out several experiments for different values of that parameter. This yields a set of k_{obs}–Par_i data and, having an analytical model describing this dependence, by a second step fitting treatment, the terms regulating such dependence can be obtained. For example, for the parameter temperature (T) the dependence function can be the Eyring equation (5) and the terms regulating the dependence, the entropy of activation $\Delta S^{\ddagger}$ and the enthalpy of activation $\Delta \mathrm{H}^{\ddagger}$ (k = Boltzmann's constant, h = Planck's constant, R = gas constant) [1, 7, 8]:

$$k_{\mathrm{obs}} = \frac{kT}{h} \exp\left(\frac{\Delta S^{\ddagger}}{R}\right) \exp\left(-\frac{\Delta H^{\ddagger}}{RT}\right) \tag{5}$$

The Eyring equation is usually used in the logarithmic form (6) to have a linear plot of kinetic data. The intercept gives the $\Delta S^{\ddagger}$ value and the slope gives the $\Delta H^{\ddagger}$ value:

$$\ln \frac{k_{\mathrm{obs}}}{T} = \ln \frac{k}{h} + \frac{\Delta S^{\ddagger}}{R} - \frac{\Delta H^{\ddagger}}{RT} \tag{6}$$

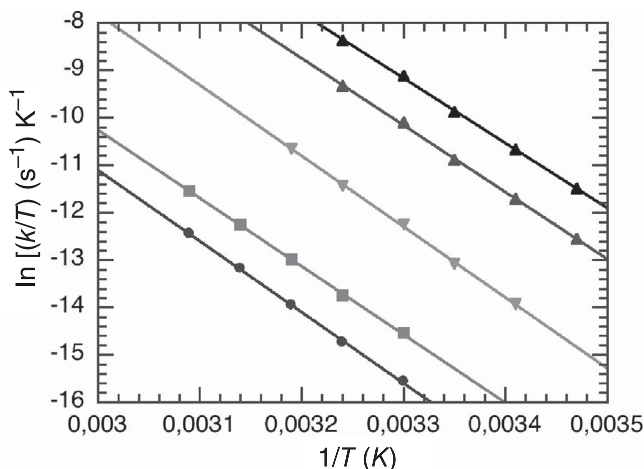

FIGURE 1 Eyring plots (solid lines) for reactions of homologous series of five molecules obtained with kinetic experiments carried out at constant temperature (plain markers) (simulated data from ref. 14).

Figure 1 shows an example of this kind of study where 25 constant-temperature kinetic (CTK) experiments were necessary to find the activation parameters for the decomposition of five molecules. When the parameter is a concentration of a species present in the reaction environment (H^+, OH^-, Me^{n+}, a nucleophile, a catalyst, etc.), the dependence function can be, for example, of the type shown in the equation

$$k_{obs} = k_{OH}[OH^-] \tag{7}$$

and the fit of k_{obs}–$[OH^-]$ data, obtained in several experiments carried out at constant $[OH^-]$, gives the term k_{OH} (Figure 2). These dependence functions, where concentrations are concerned, can be of great complexity but they are very important information for the formulation of the reaction mechanism [1, 3, 7, 8].

When the parameter is the ionic strength the dependence function is given by the Brönsted–Bjerrum equation (8) [1, 7, 8, 15], where k_0 is the rate constant at zero ionic strength, a is a constant (for water at 25°C, a = 0.50925 $L^{1/2}/mol^{1/2}$ [16]) and Z_AZ_B is the product of charges of the reacting species taking part in the rate-determining step:

$$k_{obs} = k_0 \times 10^{2aZ_AZ_B\frac{\sqrt{I}}{1+\sqrt{I}}} \tag{8}$$

Even in this case, traditionally, a logarithmic form is used [Equation (9)] to treat kinetic data. The intercept of the linear plot (Figure 3) gives the value of k_0 and the slope the value of Z_AZ_B, and this is very useful information for ionic reactions to discriminate between kinetically equivalent mechanisms:

$$\log k_{obs} = \log k_0 + 2aZ_AZ_B\frac{\sqrt{I}}{1+\sqrt{I}} \tag{9}$$

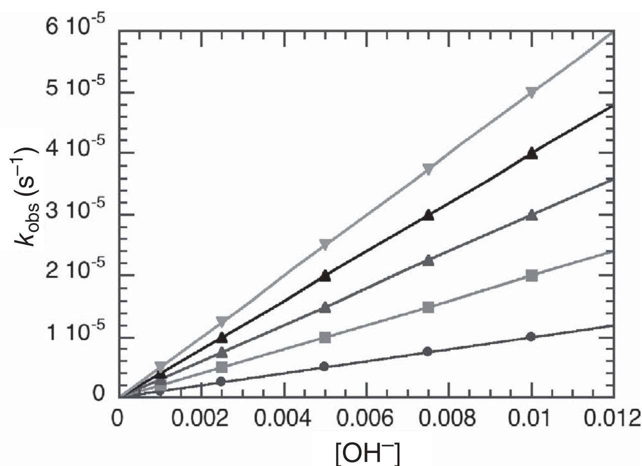

FIGURE 2 $k_{obs}([OH^-])$ profiles (solid lines) obtained for homologous series of five molecules by 25 kinetic experiments (plain markers) carried out at constant concentration of OH^- (simulated data).

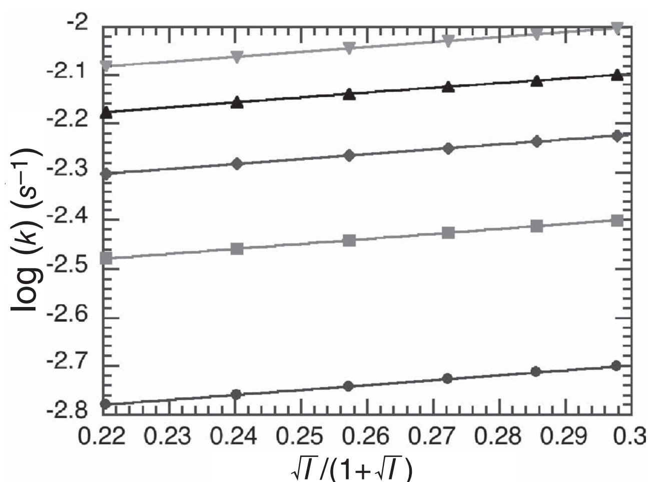

FIGURE 3 Brönsted–Bjerrum plots (solid lines) obtained by constant-ionic-strength kinetic experiments (plain markers) for homologous series of five compounds having the same mechanism (same values of $Z_A Z_B = +1$) and different intrinsic reactivity (different values of k_0) (simulated data).

Constant-parameter kinetic (CPaK) experiments are very time consuming. Nowadays, the use of personal computers and new-generation analytical instruments enables a step forward to be made in collecting kinetic data.

VPaK [17] can be defined as that part of chemical kinetics concerning experiments carried out while varying the value of an environmental parameter in a controlled way. The aim is to obtain, in a single run, the dependence of the observed rate constant on that parameter in a time 10–100 times less compared to that usually

spent using traditional methods. This is possible by fitting the kinetic profile obtained during the reaction to a suitable mathematic model analytically describing it.

VPaK can be considered a generalization of nonisothermal kinetics [18, 19], largely applied in thermal analysis [20–25] for solid-state systems. Several examples of variable-temperature experiments concerning solution chemistry were reported in the past. Different approaches were used depending on the different scientific background of the authors (inorganic, organic, organometallic, and pharmaceutical) and on the instruments available at the time [14, 26–51]. Few examples of variable-concentration kinetic (VCK) experiments were reported [31, 35, 52, 53]. Just one example of variable-ionic-strength kinetic experiment has so far been reported [54]. Here a simple approach is described making a reference to important similar studies.

The mathematical form describing a VPaK experiment is given by the equation,

$$-\frac{dC}{dt} = \{k_{\text{obs}}[\text{Par}_i(t)]\}_{\text{Par}\neq\text{Par}_i} C \tag{10}$$

where C is the concentration of the monitored reacting species. In this equation k_{obs} is not a constant but depends on a parameter (Par_i) varying with time. Then $k_{\text{obs}}[\text{Par}_i(t)]$ is a function of a function: $k_{\text{obs}}(\text{Par}_i)$ is the dependence function (D) describing the dependence of the rate constant on the parameter i and $\text{Par}_i(t)$ is the modulating function (M) showing the way the parameter changes with time while the other parameters are maintained constant. The experimental kinetic profile contains in each point information about the value of the rate constant at that time, as can be seen writing Equation (10) in the form of Equation (11). The ratio of the derivative of the concentration on the concentration itself gives k_{obs}. Then, the $k_{\text{obs}}(\text{Par}_i)$ profile can be obtained in a single kinetic run:

$$-\frac{1}{C}\frac{dC}{dt} = \{k_{\text{obs}}[\text{Par}_i(t)]\}_{\text{Par}\neq\text{Par}_i} \tag{11}$$

Furthermore, knowing the mathematical form of the dependence function, a single step fitting to Equation (10), or its integrated form, gives the terms regulating such dependence. For example, in variable-temperature kinetic (VTK) experiments the dependence function, as stated above, is the Eyring equation and the terms are the activation parameters $\Delta S^{\ddagger}$ and $\Delta H^{\ddagger}$. If the temperature changes in a linear way, that is, $M\colon T = T_0 + \alpha t$, where T_0 is the initial temperature and α is the temperature gradient, the model changes to the equation

$$-\frac{dC}{dt} = \frac{k(T_0+\alpha t)}{h}\exp\left(\frac{\Delta S^{\ddagger}}{R}\right)\exp\left(-\frac{\Delta H^{\ddagger}}{R(T_0+\alpha t)}\right)C \tag{12}$$

or to the relative integral form

$$C = C_0 \exp\left[-\int_0^t \frac{k(T_0+\alpha t)}{h}\exp\left(\frac{\Delta S^{\ddagger}}{R}\right)\exp\left(-\frac{\Delta H^{\ddagger}}{R(T_0+\alpha t)}\right)dt\right] \tag{13}$$

The fit of C–t experimental data, that is, the kinetic profile obtained in a single VTK run, to one of these equations gives the optimized values of $\Delta S^{\ddagger}$ and $\Delta H^{\ddagger}$.

For an experiment concerning the investigation of the dependence on the concentration of a catalyst Y, [Y], the molar concentration of the catalyst could be varied in a linear way, that is, M: $[\mathrm{Y}] = \beta t$, where β is the concentration gradient. If the dependence function is known, for example, D: $k_{\mathrm{obs}} = k_{\mathrm{Y}}[\mathrm{Y}]$, the mathematical model will assume the forms

$$-\frac{dC}{dt} = k_{\mathrm{Y}}\beta tC \tag{14}$$

$$C = C_0 \exp\left(-\frac{1}{2}k_{\mathrm{Y}}\beta t^2\right) \tag{15}$$

A single fit of the C–t experimental data to one of these equations gives the optimized value of the term k_{Y}.

When the dependence function is not known, Equation (16) has to be used:

$$-\frac{1}{C}\frac{dC}{dt} = k_{\mathrm{obs}}\{[\mathrm{Y}](t)\} \tag{16}$$

and the $k_{\mathrm{obs}}([\mathrm{Y}])$ profile can be obtained by dividing the derivative of the kinetic profile to the profile itself. This method is particularly important when no kind of information is available on the dependence of the rate constant on concentrations of species present in the reaction environment and taking part in the rate-determining step. The VCK experiment, in this case, gives the empirical relation that enables a hypothesis on the dependence function to be formulated.

7.5.3 EXPERIMENTAL

Some basic points have to be discussed to conveniently carry out VPaK experiments:

1. Computer simulation
2. Devices to obtain variable-parameter conditions
3. Analytical instruments
4. Software for processing experimental data

All these points involve in some way the use of a personal computer. It is important to underline that it is the availability of fast and inexpensive computers that enables, nowadays, VPaK to be applied, providing a real saving of time and acceptable confidence in the obtained data.

7.5.3.1 Computer Simulation

Computer simulation can be very useful in the phase of projecting a VPaK experiment. VPaK profiles are often difficult to imagine compared to CPaK ones where

the rate always decreases exponentially with time [Equation (3)]. In VPaK the reaction rate is given at each time t by the product of two factors: $k_{obs}[Par_i(t)]$ and C. The concentration always decreases with time but $k_{obs}[Par_i(t)]$ can vary in many ways depending on the form of both the dependence function and the modulating function. The search for good experimental parameters could signify a waste of precious time. Using a personal computer it is possible in a few minutes to have an acceptable profile, just obtaining some preliminary data from tests that are always carried out before a kinetic study [55]. Any software able to plot a function and, when necessary, to evaluate numerically a derivative or an integral can be used. We found the MicroMath SCIENTIST program [56] to be very versatile, very easy to use, and enabling us to use the differential form of the rate equation (10) without the necessity of integrating it. With this tool, giving as input the dependence function and the modulating function, the kinetic profile can be obtained immediately for every value of the experimental parameters. Figure 4 shows a typical list of a SCIENTIST program where the modulating function, dependence function, and first-order differential equation are indicated by arrows for a VTK simulation.

Figure 5 shows a profile obtained imposing as experimental data the values T_0 = 298 K and α = 0.0033 K/s. It is also possible to obtain in a single simulation several profiles relative to different values of terms conditioning them. Figure 6, for example, shows a multiparametric simulation of VCK profiles for a reaction of basic hydrolysis with dependence function $k_{obs} = k_{OH}[OH^-]$ and a modulating function $[OH^-] = \alpha t$, with $10^{-3} \times \alpha M\ s^{-1}$ = 1, 2.5, 4, 5.5, 7.

7.5.3.2 Devices to Obtain Variable-Parameter Conditions

A device is necessary to obtain VPaK conditions inside the reaction vessel. The parameter must change homogeneously and coherently with the modulating function and without altering any other factors important for the reaction or its analytical monitoring.

The parameter can change in a vessel being part of the analytical instrument, for example, an ultraviolet–visible (UV–Vis) spectrophotometric cell [39, 41, 45, 14, 47, 48], an infrared (IR) cell [42, 46], or a fluorometer cell [45, 51], or a polarimetric tube [27, 49]. It can change in a reactor vessel where the analytical signal can be read in some way, for example using an optical fiber cell for spectrophotometry [52–54] or a conductometric cell [16, 34, 40]. Another possibility is to transport the solution from the reaction vessel to the analytical instrument by a peristaltic pump [38]. When altenative ways are not practicable, samples can be taken at suitable time intervals and analyzed apart [29, 31, 35, 39, 43, 50].

Any system satisfying the conditions cited above can be useful as VPaK devices. For VTK programmable thermostatic baths (with the possibility of external circulation) and temperature programmers using the Peltier effect are very convenient. It is always necessary to homogenize the temperature and the composition of the solution with good stirring, monitoring the temperature by measuring it inside the reaction vessel, possibly memorizing it in a computer. A linear increasing temperature is sufficient and it is enough easy to realize, but other modulating functions, for various reasons, have been tested [33, 35, 47]. However, for future particular applications, the use of a computer with suitable software can easily generate $T(t)$ profiles having various shapes.

```
// VTK_SIM

IndVars: t

DepVars: C, V, K,TE

Params: ΔH, ΔS, C0, kb, h, R, TEo, α

→  TE = TEo + α*t

→  K = (kb*TE/h)*exp(ΔS /R)*exp(-ΔH /(R*TE)

→  C' = -K*C

   V = -C'

   // Parameter values

   ΔH = 118000

   ΔS = 61

→  TEo = 298

→  α = 0.0033

   C0 = 1e-3

   kb = 1.3806e-16

   h = 6.6262e-27

   R = 8.314

   // Initial conditions

   t = 0

   C = C0
```

FIGURE 4 List of SCIENTIST model used to simulate VTK experiment. The first three arrows show, respectively, the modulating function, dependence function, and first-order kinetic equation. The last two arrows show experimental parameters T_0 and α.

The thermal expansion of the reaction solution and vessel, because of their influence on the concentration of the reacting species, can be a problem for coherence to the kinetic model. The best way to solve it is to limit the range of temperature to 20–25°C; otherwise corrections are required that make the procedure less simple. Furthermore, a limited thermal excursion assures one about the constancy of the activation parameters during the experiments.

For VCK autoburettes releasing concentrated solution of species having some effect on the reaction are usually used. The concentration gradient α (in molars per second) inside the vessel is given by the relation

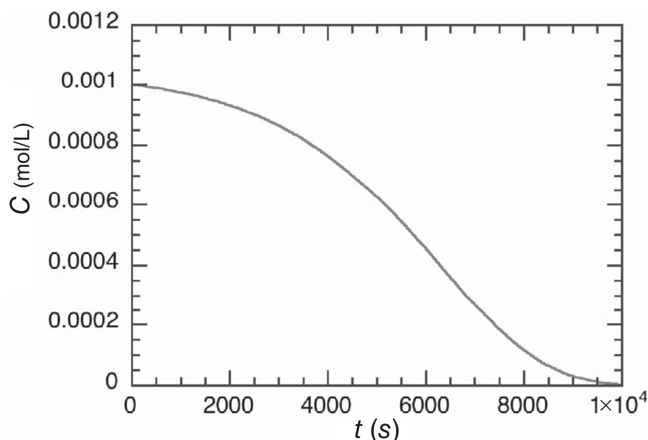

FIGURE 5 Simulated kinetic profile for VTK experiment obtained by SCIENTIST model in Figure 4.

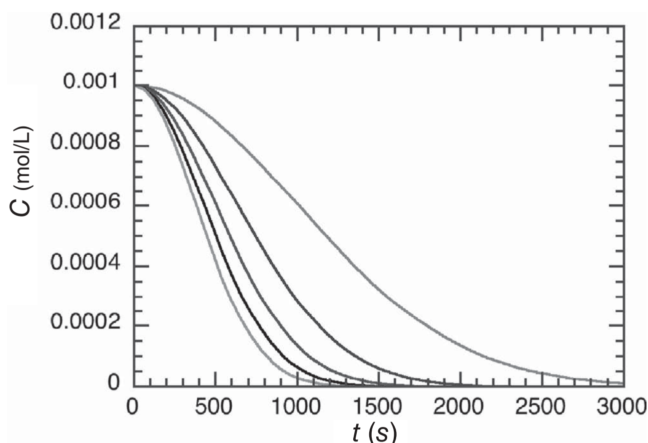

FIGURE 6 Simulated VCK profiles obtained by SCIENTIST multiparametric model. *D*: $k_{obs} = k_{OH}[OH^-]$, *M*: $[OH^-] = \alpha t$. $10^{-3} \times \alpha M\ s^{-1}$ = 1, 2.5, 4, 5.5, 7.

$$\alpha = \frac{gM}{V_0} \tag{17}$$

where g (in liters per second) is the releasing rate, M (moles per liter) the molar concentration of the added solution, and V_0 (liters) the initial reaction volume. The chemical homogeneity is assured by good stirring. A problem can arise when the added percentage volume is large and cannot be neglected in the mathematical model. Computer simulation can help to find suitable conditions [55]. Some attempts have been made to introduce a correction in the kinetic equation [31]. The species released into the reaction environment must influence the reaction exclusively in the way described by the dependence function.

Equation (10) is generally valid for any parameter provided that a suitable apparatus is available to generate the relative experimental conditions. Some cases are very difficult to manage. For example, to our knowledge, variable-pressure kinetic experiments have so far not been reported.

7.5.3.3 Analytical Instruments

Theoretically, any analytical instrument useful to follow a reaction in a traditional constant-parameter kinetic experiment can be used for VPaK experiments. Obviously, some practical applications can be problematic. For example, while it is very easy to change the temperature inside a UV–Vis spectrophotometric cell, changing the concentration of a species inside a nuclear magnetic resonance (NMR) tube can be very complicated.

Following a physical property of the reacting species instead of their concentration leads to a series of consequences. First, while Equations (2), (3), and (4) used in CPaK experiments change respectively to the equations

$$-\frac{d(\lambda-\lambda_\infty)}{dt}=k_{obs}(\mathrm{Par}_1,\ \mathrm{Par}_2,\ldots)(\lambda-\lambda_\infty) \tag{18}$$

$$\lambda-\lambda_\infty=(\lambda_0-\lambda_\infty)\exp(-k_{obs}t) \tag{19}$$

$$\ln(\lambda-\lambda_\infty)=-k_{obs}t+\ln(\lambda_0-\lambda_\infty) \tag{20}$$

Equation (10) and its integral form change respectively to the equations

$$-\frac{d(\lambda-\lambda_\infty)}{dt}=\{k_{obs}[\mathrm{Par}_i(t)]\}_{\mathrm{Par}\neq\mathrm{Par}_1}(\lambda-\lambda_\infty) \tag{21}$$

$$\lambda=(\lambda_0-\lambda_\infty)\exp\left(-\int_0^t k_{obs}[\mathrm{Par}_i(t)]dt\right)+\lambda_\infty \tag{22}$$

where λ is a physical quantity related to the compounds involved in the reaction at time t, λ_0 and λ_∞ its values, respectively, at the start and at the end of the reaction. The parameter λ can be the absorbance, the conductance, the optical rotation, the area of an NMR peak, and so on.

The more the precision of the instrument and the more the points for the time unit in the acquired profile, the better the result of the fitting of experimental data. For this reason instruments with a low measure error and connectable to a computer for the automatic and continous aquisition of data are very much prefered. The UV–Vis spectrophotometer is by far the most used instrument in chemical kinetics. It has a good sensitivity and a good control of the temperature. It is connected or easily connectable to a computer and is available nearly everywhere. The absorbance has a very low dependence on the temperature so that, in the used temperature range, its variation can be neglected during the VTK experiments.

For VCK experiments the usual 10-mm cuvette may be not suitable as a reaction vessel so an external reactor can be used where the absorbance can be read by an

UV–Vis sensor or using a flux cell. A problem can arise by adding a reagent absorbing in the same range where the reacting species or products absorb. When this is unavoidable, a correction of the kinetic profile can be made by a blank scan [53].

Unfortunately not all the molecules absorb so that other physical properties and other analytical instruments have to be used.

For reactions involving change in optical rotation, a professional polarimeter is a good solution. It has high sensitivity and can be connected to a computer for the automatic acquisition of the analytical data. The changing and monitoring of temperature can be easily done [49]. The effect of temperture on optical rotation is not large. Examples of VCK experiments using this instrument have not been reported, but they could be realized, for example, using an external reactor connected to a flux cell.

Infrared [42, 46], fluorimeters [45, 51], and conductometers [16, 34, 40] have been used with success. Gas–liquid chromatography (GLC) and high-performance liquid chromatography (HPLC) are very much used in pharmaceutical studies, for both analytical investigations and kinetic studies. Unfortunately they are not the ideal for VPaK experiments. The kinetic profile obtained is made by a few points (each one requiring a time-consuming cromatographic analysis) and the fit to the model can lead to a large error in the evaluation of the terms. Nevertheless, examples are reported and the results are acceptable [38, 39, 43, 50]. If large numbers of routine measurments would require it, suitable automation in the analysis [38] and software for the fast processing of the computer acquired chromatograms could be devised.

7.5.3.4 Software for Processing Experimental Data

When a VPaK experimental profile has been produced and stored on a computer, suitable software is needed for its quick processing. While in the past the treatment of constant-temperature kinetic data was easy to do, even without computers, in VPaK the use of a computer is of fundamental importance for both the complexity of the mathematical model, which does not require time-consuming calculations, and confidence in the obtained results. Algorithms are necessary to fit the experimental data to Equation (21) or (22) in their particular form depending on the investigated parameter and the used analytical instrument. Algorithms are also necessary for the evaluation of the derivative and sometimes the integral [e.g., in Equation (11) it is not possible to solve the integral in terms of elementay functions so it must be evaluated numerically]. Any language can be used for a personalized, home-made program, but application programs are commercially available to facilitate the work. The MicroMath SCIENTIST, cited for simulation for the search of the experimental conditions, is an example. The same models used to simulate VPaK profiles are used to fit the obtained data with the difference that the terms given for the simulation are in this case the input values of the terms to be optimized. SCIENTIST uses a Powell modified Marquadt for the fitting and the (default) Episode method to solve the differential equation [57].

The modulating function is always known because the value of the parameter is imposed by a program and/or monitored by a sensor. The dependence function is sometimes known and sometimes not. For example, for the parameter temperature the dependence function is always known (Arrhenius equation, Eyring equation). For the parameter concentration the dependence function can be known in studies

where a mechanism has already been proposed or guessed and a series of similar substrates have to be quantitatively investigated in detail. It can be unknown for new studies. In these cases Equation (21) can be written in the form

$$-\frac{1}{\lambda-\lambda_\infty}\frac{d\lambda}{dt}=\{k_{\mathrm{obs}}[\mathrm{Par}_i(t)]\}_{\mathrm{Par}\neq\mathrm{Par}_i} \tag{23}$$

to underline that the main calculation required is for the evaluation of the derivative of the kinetic profile. The ratio of the derivative on $\lambda - \lambda_\infty$, that is, the profile minus its value at the end of the reaction, gives at each point the value of k_{obs}.

Some examples concerning pharmaceutical systems will be discussed.

7.5.4 EXAMPLES OF VARIABLE-PARAMETER KINETIC EXPERIMENTS

7.5.4.1 Variable-Temperature Kinetic Experiments

Figure 7 shows a kinetic profile relative to a VTK experiment concerning the racemization of (–)-adrenaline in acidic aqueous solution [49]. It was obtained polarimetrically following the optical rotation α. The modulating function used was $T = T_0 + \gamma t$, where $T_0 = 309.6\,\mathrm{K}$ and $\gamma = 0.001694\,\mathrm{K/s}$, realized by a circulation of water coming from a programmed thermostatted bath. The temperature was read inside the polarimetric cell by a platinum resistor. Temperature and absorbance were automatically acquired by a computer connected to the instruments. The typical sigmoidal shape comes from the relation $r = k_{\mathrm{obs}}[T(t)]C$, that is, the reaction rate is given by the product of two terms: $k_{\mathrm{obs}}[T(t)]$, which always increases for the increasing temperature, and C, which decreases continously during the reaction. For this reason the reaction is accelerated in its first part but after the inflection point the low concentration of C is overwhelming and the reaction decelerates until the rate

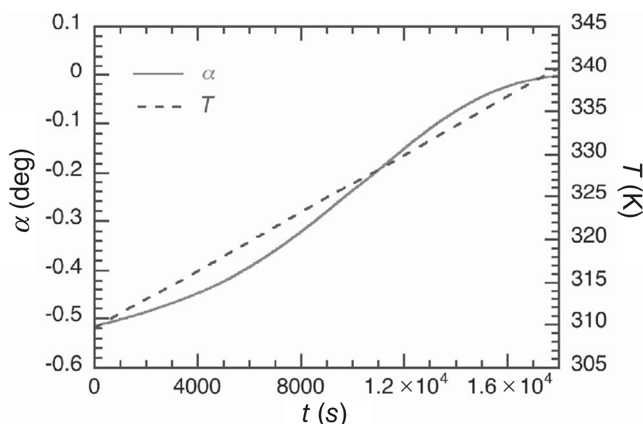

FIGURE 7 Change in optical rotation (solid line) during racemization of (–)-adrenaline in aqueous solution (1 *M* HCl) carried out under variable-temperature conditions. *M:* $T(\mathrm{K}) = 309.6 + 0.001694t$ (dashed line).

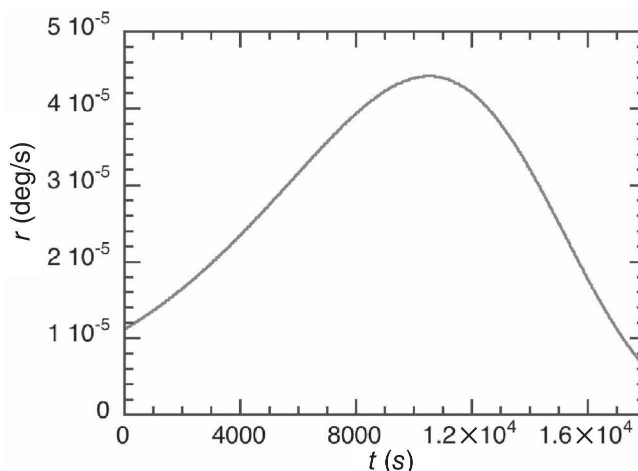

FIGURE 8 Change in reaction rate during racemization of (–)-adrenaline as obtained using Savitzky–Golay method [58] by derivative of VTK profile reported in Figure 7.

gets to zero at the end of the reaction (Figure 8). A direct fitting of this profile to Equation (24), with α_0, α_∞, $\Delta S^\ddagger$, and $\Delta H^\ddagger$ the parameters to be optimized, gave the activation parameters ($\Delta S^\ddagger$ = –27 ± 1 J/K·mol, $\Delta H^\ddagger$ = 95 ± 1 kJ/mol, R^2 = 0.99999). Their values were identical to those obtained by five traditional VTK experiments:

$$\alpha = (\alpha_0 - \alpha_\infty)\exp\left\{-\frac{k}{h}\exp\left[\frac{\Delta S^\ddagger}{R}\right]\int_0^t (T_0 + \gamma t) \exp\left[-\frac{\Delta H^\ddagger}{R(T_0 + \gamma t)}\right]dt\right\} + \alpha_\infty \quad (24)$$

Figure 9 shows the profile relative to another VTK experiment carried out spectrophotometrically [45]. The reaction followed was the hydrolysis of aspirin [12, 13, 59, 60] at pH 4.50. The temperature was controlled by a cell compartment thermostatted by a Peltier temperature programmer and measured by a platinum resistor inserted into the spectrophotometric cell. Magnetic stirring was assured by a suitable device. Lots of data points were memorized by a computer connected to the analytical instrument and easy processings, both differential and integral, were carried out. Values of activation parameters were in agreement with each other and in agreement with those obtained by comparative constant-temperature kinetic experiments carried out in the same conditions ($\Delta S^\ddagger$ = –115 ± 1 J/K · mol, $\Delta H^\ddagger$ = 69 ± 1 kJ/mol, R^2 = 0.99999).

The sigmoidal profile in Figure 10 is relative to the hydrolysis of aspirin carried out at pH 7.00 under variable-temperature conditions. In this case a fluorometer was used as an analytical instrument irradiating the salicylic acid at 310 nm and recording the fluorescence signal at 404 nm [45, 61]. A programmable thermostatted bath was used to apply a linear increasing temperature (T_0 = 323.05 K, α = 1.631 × 10^{-3} K/s) in the thermostatted fluorometric cell compartment. The temperature was

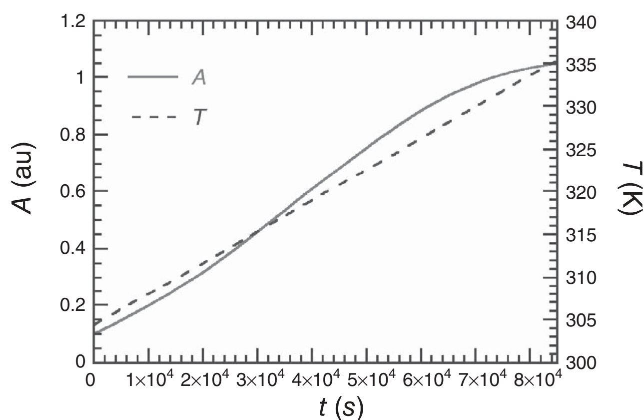

FIGURE 9 VTK profile (solid line) obtained spectrophotometrically (λ = 298.5 nm) for hydrolysis of aspirin in water (pH = 4.50). *M:* $T(\mathrm{K}) = 304.36 + 3.647 \times 10^{-4}t$ (dashed line).

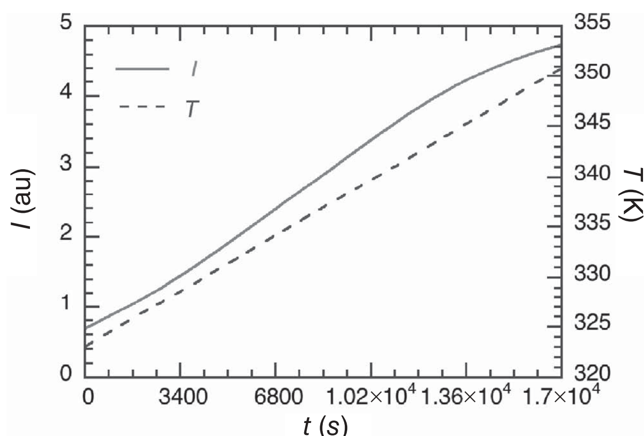

FIGURE 10 Change in fluorescence intensity (solid line; 404 nm) during hydrolysis of aspirin (pH 7.00) in variable-temperature kinetic experiment. *M:* $T(\mathrm{K}) = 323.05 + 1.631 \times 10^{-3}t$ (dashed line).

read inside the cell and monitored and stored by a computer together with the fluorescence intensity.

A fluorometer is more sensitive than a spectrophotometer and enables a very low concentration of substrate to be used. This can be convenient in the first stage of pharmaceutical studies. Unfortunately the dependence of fluorescence intensity on temperature cannot be avoided. A blank scan at the end of the reaction enables a temperature-independent signal [45] useful for VTK processing to be obtained. The results were consistent ($\Delta S^{\ddagger} = -109 \pm 1$ J/K · mol, $\Delta H^{\ddagger} = 71 \pm 1$ kJ/mol, R^2 = 0.99999) with those obtained spectrophotometrically under both CTK and VTK conditions.

Polarimeters, spectrophotometers, and fluorimeters connected to the computer can store, during a VPaK experiment, hundreds or, when necessary, even thousands

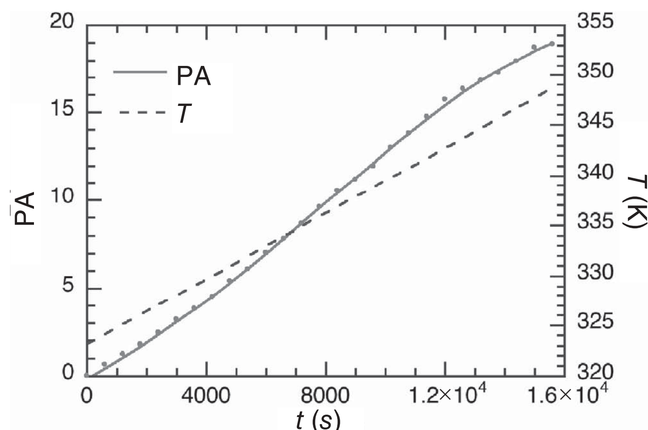

FIGURE 11 VTK profile obtained by HPLC peak area (PA) of salicylic acid (plain circles) during hydrolysis of aspirin at pH 7.00. *M:* $T(\mathrm{K}) = 323.2 + 1.631 \times 10^{-3}t$ (dashed line). Solid line is relative theoretical curve.

of analytical points. This increases the fitting of experimental data to the mathematical model or the evaluation of the derivative of the kinetic profile.

Figure 11 shows a VTK profile obtained by HPLC analysis concerning the hydrolysis of aspirin at pH 7.00 and at a linear increasing temperature, with $T_0 = 323.2\,\mathrm{K}$ and $\alpha = 1.631 \times 10^{-3}\,\mathrm{K/s}$ [50]. The experiment was carried out in a reaction vessel immersed in a programmable thermostatic bath. Samples of the reaction mixture were taken at suitable time intervals and injected into the liquid chromatography (LC) column. Few points were collected (27) to build it up because of the time required for the chromatographic separation. Nevertheless, the fit to the relative mathematical model gave values of activation parameters similar to those obtained spectrophotometrically, although with greater statistical error ($\Delta S^{\ddagger} = -102 \pm 8\,\mathrm{J/K \cdot mol}$, $\Delta H^{\ddagger} = 73 \pm 2\,\mathrm{kJ/mol}$, $R^2 = 0.9997$).

7.5.4.2 Variable-Concentration Kinetic Experiments

The dependence on the concentration of species present in the reaction environment is certainly the most important in the context of mechanistic studies. A species can influence the reaction by taking part in it as a reagent or as a catalyst or simply by altering in some way the physicochemical character of the reaction environment: for example, a nucleophile in a nucleophilic substitution, an ionic metal in a reaction catalyzed by it, or a salt altering the ionic strength or molecules of solvent altering the dielectric constant. In the first case the dependence function is the heart of the rate law. It indicates the species taking part in the rate-determining step and gives an idea of the way they interact with each other [1, 3, 7, 8]. For this reason the search for its form and for the values of the terms inside it requires a great deal of attention.

A study on the nucleophilic substitution on the square planar complex *trans*-$[Pt(PEt_3)_2Cl_2]$, a substrate similar to cisplatinum and other compounds largely used as antitumoral agents [53, 62–65], has been carried out spectrophotometrically while changing the concentration of the nucleophile with time. The reaction vessel was

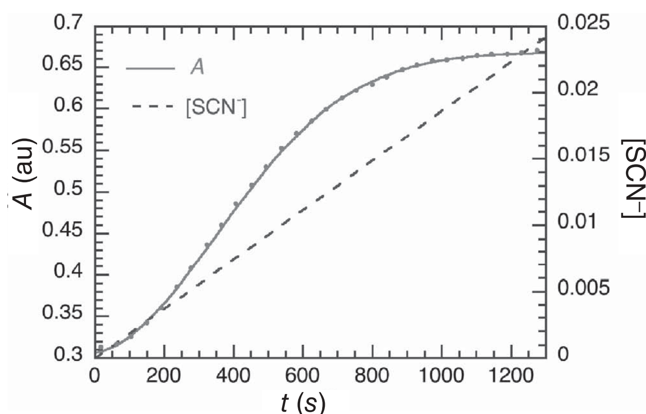

FIGURE 12 VCK profile obtained spectrophotometrically (plain cirlce, selected points) and theoretical curve (solid line) (280 nm) for reaction *trans*-$[Pt(PEt_3)_2Cl_2] + 2\ SCN^- \rightarrow$ *trans*-$[Pt(PEt_3)_2(SCN)_2] + 2\ Cl^-$ in methanol. *M*: $[SCN^-] = 0.048t\ M$ (dashed line); $T = 303.2$ K.

immersed in a thermostatted bath, an autoburette released a suitable concentrated solution of nucleophile (thiourea, iodide, bromide, thiocianide), the absorbance was read by an optical fiber cell, and good stirring was assured by an immersion stirrer.

Pseudo-first-order conditions were assured. In classic constant-concentration kinetic (CCK) experiments this means the use of a reactant in a high enough concentration, compared to that of the substrate, to neglect its consumption during the reaction. In this way the simple first-order kinetic equation can be used for the data treatment. In VCK experiments these conditions have to be considered in a different way: The concentration of the species must vary with time in a way that deviation from the modulating function, caused by its consumption, can be neglected. Concentrated solutions able to create a suitable excess of reagent in the reaction environment can be easily prepared. In particular cases, when this is not possible, the consumption of the species can be inserted into the modulating function [53].

Figure 12 shows the variable-concentration kinetic profile obtained for the reaction of the platinum complex with SCN^-. The modulating function was $[SCN^-] = \alpha t$, with $\alpha = 0.048$ M/s. Even in this case the reaction is accelerated in the first part of the kinetics, but this is caused by the increasing concentration of nucleophile. The dependence function for this reaction is given as

$$k_{obs} = k_S + k_Y[Y] \tag{25}$$

where k_S is a solvolytic constant and k_Y is the direct attack constant for a generic nucleophile Y [62, 63]. Fitting the profile to the model

$$-\frac{dA}{dt} = (k_S + k_Y \alpha t)(A - A_\infty) \tag{26}$$

gave the optimized values of k_S and k_Y (respectively, $0.92 \times 10^{-4}\,s^{-1}$ and $0.351\,M^{-1}\cdot s^{-1}$)

```
    // VCK_SIM
    IndVars: t
    DepVars: A, V, K,Y
    Params: KS, KY, A0, AI
    α = g*M/V0
→   Y =  α*t
→   K = KS + KY*Y
→   A' = -K*(A-AI)
    V = -A'
    // Parameter values
    KS = 0.0001
    KY = 0.3
→   g = 0.59
→   M = 0.63
→   V0 = 20000
    A0 = 0.3
    AI = 0.7
    // Initial conditions
    t = 0
    A = A0
```

FIGURE 13 SCIENTIST list of model used for processing of VCK profile in Figure 11. The first three arrows show, respectively, the modulating function, dependence function, and first-order kinetic equation. The last three arrows show the experimental parameters *g, M*, and V_0.

Figure 13 shows the SCIENTIST list of the model used for the fitting procedure of the kinetic profile, where KS, KY, A0, and AI are input values to be optimized.

The reaction of the same substrate with thiourea in variable-concentration conditions was also followed conductometrically with good results [16]. The reaction was carried out in a thermostatted reaction vessel. An autoburette was used to add a concentrated solution of thiourea. The conductance was read by means of a conductometric cell and acquired by a computer.

Among the studies on the dependence of k_{obs} on the concentration of species present in the reaction environment, in the pharmaceutical field a particular space is dedicated to pH–rate profiles, that is, the dependence on $[H^+]$ and/or $[OH^-]$. They can give a lot of information on the reaction mechanism and on the way to confront the instability [1, 3, 4]. Very often these studies require lots of kinetic experiments because the hydrogen ion concentration varies over 14 orders of magnitude. To

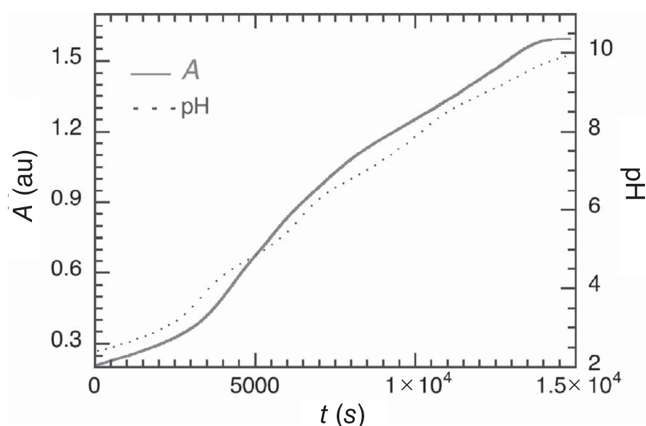

FIGURE 14 Change in absorbance (solid line) during a VpHK experiment concerning hydrolysis of aspirin at $T = 342.5\,\text{K}$, $\lambda = 298.5\,\text{nm}$. The dotted line shows the variation of pH. M: not known analytically.

delineate the pH–rate profile of aspirin, for example, about 50 kinetic experiments are necessary. Considering different temperatures, comparative molecules, and other various effects, a study on homologous series can be very hard to carry out.

With VCK experiments, a single run can be enough to obtain the pH–rate profile of a reacting substrate.

Figure 14 shows the variable-pH kinetic (VpHK) profile obtained spectrophotometrically for the reaction of hydrolysis of aspirin with pH varying in the range 2–10 at $T = 342.5\,\text{K}$. The variable-concentration conditions were realized by adding a concentrated solution of NaOH (0.6 M) to the thermostatted reaction vessel containing the aqueous solution of acetylsalicylic acid and a buffer composed of acetic acid (0.01 M), fosforic acid (0.01 M), and boric acid (0.01 M). In this way an almost linear increase of pH was generated. The absorbance was read by an optical fiber cell and stored in a computer. The pH was monitored by a pH sensor connected to a computer.

This profile is more complex than the others seen before. This is caused by the fact that, in the reaction rate equation, given by the product of k_{obs} and the concentration of substrate, $k_{obs}[\text{pH}(t)]$ varies with the pH in an irregular way because of the particular shape of the pH–rate profile. Figure 15 shows the derivative of the pH–rate profile as obtained by the Savitzky–Golay method [58]. It gives an idea of the variation of the reaction rate during the VpHK experiment. There is an acceleration in the first part, caused by the increase of k_{obs} with the pH, followed by a deceleration for the stabilization of k_{obs} and the continuous decrease of the substrate concentration. Then, the rate increases again for the increase of k_{obs} for higher values of pH and after decreases again for concentration approaching zero.

The ratio of the derivative of the profile to $A - A_\infty$ gave, according to Equation (27), the entire pH–rate profile in a single scan (Figure 15).

For such large ranges of pH, the dependence function can be very complex. For example, in the case of aspirin, changing the pH, four different mechanisms of reaction operate and the global rate constant requires several terms [3]. Nevertheless, once the pH–rate profile has been obtained and a reaction mechanism formulated,

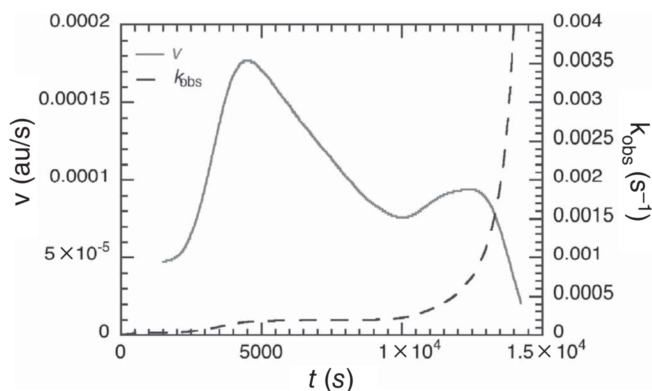

FIGURE 15 Change in derivative of VpHK profile reported in Figure 14 (solid line) and k_{obs} [pH(t)] profile as obtained by Equation (27) (dashed line).

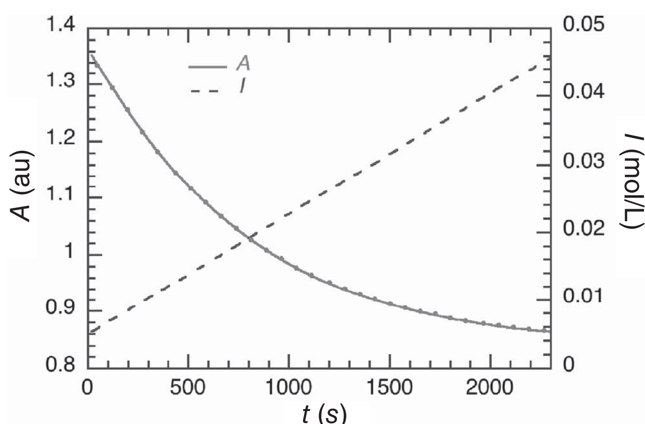

FIGURE 16 Change in absorbance (plain circle, selected points) during VIK experiment concerning reaction of indomethacine with NaOH 0.005 M and $T = 299.0 \pm 0.1$ K. The dashed line shows the increase in ionic strength. The solid line is relative to the theoretical model.

a dependence function can be inserted into Equation (27) and a global fit can be done for the evaluation of the values of the elementary constants:

$$-\frac{d(A - A_\infty)}{dt} = \{k_{obs}[[H^+](t)]\}(A - A_\infty) \quad (27)$$

7.5.4.3 Variable-Ionic-Strength Kinetic Experiments

Figure 16 shows the kinetic profile for a variable-ionic-strength kinetic (VIK) experiment concerning the hydrolysis of indomethacin [54, 66, 67] at NaOH 0.005 M and $T = 299.0 \pm 0.1$ K at a varying ionic strength $I = I_0 + \alpha t$ with $I_0 = 0.005$ M, due to the concentration of NaOH, and $\alpha = 1.77 \times 10^{-5}$ M/s, calculated by the equation $\alpha = gM/V_0$. The experimental apparatus was similar to that used for the VpHK experiment. In this case a concentrated solution of LiCl (3 M) was released by the auto-

burette into the reaction vessel. Hundreds of absorbance data points were acquired. A fit of the experimental data to the mathematical model (28) was performed using the MicroMath SCIENTIST program with A_0, A_∞, k_0, and $Z_A Z_B$ the parameters to be optimized. The fitting was excellent ($R^2 = 0.99999$) and the results were in good agreement with those obtained in the traditional way [$k_0 = (1.02 \pm 0.04)\ 10^{-4}\ s^{-1}$, $Z_A Z_B = 1.01 \pm 0.04$]:

$$-\frac{dA}{dt} = k_0 \times 10^{1.04 Z_A Z_B \frac{\sqrt{I}}{1+\sqrt{I}}} (A - A_\infty) \tag{28}$$

The differential method was also applied for processing the experimental data. The result was in good agreement.

7.5.5 CONCLUSIONS

Stability is an essential property of the drug product. A fast screening of new molecular entities and low-time-consuming detailed studies on selected candidates can avoid delay and consequent high cost in the first part of pharmaceutical investigation.

Potentially, VPaK provides a powerful new method for collecting kinetic data. It is a new way to look at kinetic experiments. Instead of obtaining a single value of the observed rate constant, with a single run, it is possible to obtain the entire dependence of the rate constant on a physical parameter. Usually, in mechanistic studies, what one looks for is the intimate way of interaction of the reagents in the rate-determining step. The nature of the reagent and products are well known because of the very good analytical instuments available. Kinetic experiments are just routine operations but, so far, they are of fundamental importance because kinetics is the only way to look at this aspect of the reactivity of the substrate.

Without fear of spending too much time it is possible to obtain a full panoramic picture of the chemical behavior of a long series of homologous compounds. Plain mechanistic studies can be carried out using easily accessible instruments and software. Furthermore, kinetic data are obtained using a single sample, avoiding the inhomogeity characterizing traditional CPaK experimental data, loss of time in preparing more samples, and often, but not of secondary importance, using a lower quantity of compound.

REFERENCES

1. Carstensen, J. T. (2000), Solution Kinetics; Kinetic pH profiles; Oxidation in solution; Catalysis, Complexation, and Photolysis, in Carstensen, J. T. and Rhodes, C. T., Eds., *Drug Stability, Principle and Practice*, 3rd ed., Marcel Dekker, New York, Chapters 2–5, pp. 19–143.
2. Connors, K. A., Amidon, G. L., and Stella, V. J. (1986), *Chemical Stability of Pharmaceuticals: A Handbook for Pharmacists*, 2nd ed., Wiley-Interscience, New York.
3. Loudon, G. M. (1991), Mechanistic interpretation of pH–rate profiles, *J. Chem. Ed.*, 68, 973–984.
4. Jenks, W. P. (1969), *Catalysis in Chemistry and Enzymology*, McGraw Hill, New York.

5. Kerns, E. H. (2001), High throughput physicochemical profiling for drug discovery, *J. Pharm. Sci*., 90, 1838–1858.
6. Avdeef, A., and Testa, B. (2002), Physicochemical profiling in drug reserch: A brief survey of the state-of-the-art of experimental techniques, *Cell. Mol. Life Sci*., 59, 1681–1689.
7. Moore J. W., and Pearson R. G. (1981), *Kinetics and Mechanism*, Wiley, New York.
8. Wilkins R. G. (1991), *Kinetics and Mechanism of Reactions of Transition Metal Complexes*, VCH, Weinheim.
9. Kresge, A. J. (1987), Unusual reactivity of prostacyclin: Rational drug design through physical organic chemistry, *Acc. Chem. Res*., 20, 364–370.
10. Page, M. I., and Webster, P. (1990), The hydrolysis of azetidinyl amidinium salts. Part 1. The unimportance of strain release in the four-membered ring, *J. Chem. Soc. Perkin Trans*., 2, 805–811.
11. Lajis, N. H., Noor, H. M., and Khan, M. N. (1995), Kinetic and mechanism of the alkaline hydrolysis of securinine, *J. Pharm. Sci*., 84, 126–130.
12. Edwards, L. J. (1950), The hydrolysis of aspirin. A determination of the thermodynamic dissociation constant and a study of the reaction kinetics by ultraviolet spectrophotometry, *Trans. Faraday Soc*., 46, 723–735.
13. Edwards, L. J. (1952), The hydrolysis of aspirin. Part 2, *Trans. Faraday Soc*., 48, 696–699.
14. Romeo, R., and Alibrandi, G. (1997), Structure-reactivity correlations for the dissociative uncatalyzed isomerization of monoalkylbis(phosphine)platinum(II) solvento complexes, *Inorg. Chem*., 36, 4822–4830.
15. Carstensen, J. T. (1970), Kinetic salt effect in pharmaceutical investigation, *J. Pharm. Sci*., 59, 1140–1143.
16. Koryta, J., Dvorak J., and Kavan, L. (1993), *Principles of Electrochemistry*, Wiley, Chichester.
17. Alibrandi, G. (1994), Variable-concentration kinetics, *J. Chem. Soc. Chem. Commun*., 23, 2709–2710.
18. Koch, E. (1977), *Non-Isothermal Reaction Analysis*, Academic, London.
19. Brown, M. E., and Phillpotts, C. A. R. (1978), Non-isothermal kinetics, *J. Chem. Ed*., 55, 556–560.
20. Kissinger, H. E. (1957), Reaction kinetics in differential thermal analysis, *Anal. Chem*., 29, 1702–1706.
21. Flynn, J. H. (1969), in Schwenker, R. V., and Garn, P. D., Eds., *Thermal Analysis*, Vol. 2, Academic, New York, p. 1111.
22. Daniels, T. (1973), *Thermal Analysis*, Kogan Page, London.
23. Dollimore, D. (1996), Thermal analysis, *Anal. Chem*., 68, 63R–71R.
24. Ozawa, T. (2000), Thermal analysis—Review and prospect, *Thermoch. Acta*, 335, 35–42.
25. Glass, B. D., Vovàk, Cs., and Brown M. E. (2004), The thermal and photostability of solid pharmaceuticals. A review, *J. Therm. Anal. Calorim*., 77, 1013–1036.
26. Rogers, A. R. (1963), An accelerated stage test with programmed temperature rise, *J. Pharm. Pharmacol*., 15, 101T–105T.
27. Ahlberg, P., and Wold, S. (1970), Evaluation of activation parameters for a first order reaction from one kinetic experiment. Theory, numerical methods and computer program, *Acta Chem. Scand*., 24, 618–632.
28. Ahlberg, P. (1970), Determination of activation parameters in one kinetic experiment. *Acta Chem. Scand*., 24, 1883–1893.
29. Madsen, B. W., and Anderson, R. A. (1974), Integral approach to nonisothermal estimation of activation energy, *J. Pharm. Sci*., 63, 777–781.

30. Edel, B., and Baltzer, M. O. (1980), Nonisothermal kinetics with programmed temperature steps, *J. Pharm. Sci.*, 69, 287–290.
31. Tucker, I. G., and Owen, W. R. (1982), High information kinetic studies: Non-isothermal programmed acid concentration kinetics, *Int. J. Pharm.*, 10, 323–337.
32. Gonzalez, J. L., and Salvador, F. (1982), Kinetics of reactions in solution: Method for the treatment of data from non-isothermal chemical kinetic experiments, *React. Kinet. Catal. Lett.*, 21(1–2), 167–171.
33. Gonzalez, J. L., and Salvador, F. (1984), Comparative studies of non-isothermal methods in linear and non-linear temperature variation, *React. Kinet. Catal. Lett.*, 25(1–2), 125–130.
34. Mason, T. J., and Lorimer, J. P. (1983), A method for the determination of the activation energy for a reaction from a single kinetic run, *Comp. Chem.*, 7(4), 159–163.
35. Li Wan Po, A., Elias, A. N., and Irwin, W. J. (1983), Non-isothermal and non-isopH kinetics in formulation studies, *Acta Pharm. Suec.*, 20, 277–286.
36. Ortiz Uribe, M. I., Romero Salvador, A., and Irabien Gulias, A. (1985), Kinetic analysis for liquid-phase reactions from programmed temperature data. I. Simple analysis of potential kinetic laws, *Thermochem. Acta*, 94, 323–331.
37. Kipp, J. E. (1985), Non-isothermal kinetics—Comparison of two methods of data treatment, *Int. J. Pharm.*, 26, 339–354.
38. Kipp, J. E., Jensen, M. M., Kronholm, K., and McHalsky, M. (1986), Automated liquid chromatography for non-isothermal kinetic studies, *Int. J. Pharm.*, 34, 1–8.
39. Bunce, N. J., Forber, C. L., and McInnes, C. (1988), Single-step methods for calculating activation parameters from raw kinetic data, *J. Chem. Soc. Perkin Trans.*, II, 363–368.
40. Schoenemann, E., Hahn, H., and Bracht, A. (1991), Determination of kinetic parameters from non-isothermal conductivity measurements by an integral method, *Thermochim. Acta*, 185(1), 171–176.
41. Alibrandi, G. (1994), Non-isothermal spectrophotometric kinetics applied to inorganic reactions, *Inorg. Chim. Acta*, 221, 31–34.
42. Zhang, S., and Brown, T. L. (1995), Application of non-isothermal approach to the kinetics of organometallic reactions: The substitution of (η^5-pentamethylcyclopentadienyl) dicarbonylrhodium(I), *Inorg. Chim. Acta*, 240, 427–433.
43. Junnarkar, G. H., and Stavchansky, S. (1995), Isothermal and nonisothermal decomposition of famotidine in aqueous-solution, *Pharm. Res.*, 12(4), 599–604.
44. Lee, M. L., and Stavchansky, S. (1995), Isothermal and nonisothermal decomposition of thymopentin and its analogs in aqueous-solution, *Pharm. Res.*, 15(11), 599–604.
45. Alibrandi, G., Micali, M., Trusso, S., and Villari, A. (1996), Hydrolysis of aspirin studied by spectrophotometric and fluorometric variable-temperature kinetics, *J. Pharm. Sci.*, 85, 1105–1108.
46. Maeder, M., Molloy, K. J., and Schumacher, M. M. (1997), Analysis of non-isothermal kinetic measurements, *Anal. Chim. Acta*, 337, 73–81.
47. Hodgson, S. C., Ngeh, L. N., Orbell, J. D., and Bigger, S. W. (1998), A student experiment in non-isothermal chemical kinetics, *J. Chem. Ed.*, 75(9), 1150–1153.
48. Ficarra, R., Villari, A., Micali, N., Tommasini, S., Calabrò, M. L., Di Bella, M. R., Melardi, S., Agresta, M. F., Coppolino, S., and Stancanelli, R. (1999), Stability study of piroxicam and cinnoxicam in solid pharmaceuticals, *J. Pharm. Biomed. Anal.*, 20, 283–288.
49. Alibrandi, G., Coppolino, S., D'Aliberti, S., Ficarra, P., Micali, N., and Villari, A. (2002), Temperature-rate profiles by polarimetric variable-temperature kinetic experiments to study racemization reactions, *J. Pharm. Biomed. Anal.*, 29, 1025–1029.

50. Alibrandi, G., Coppolino, S., D'Aliberti, S., Ficarra, R., Micali, N., and Villari, A. (2003), Fast drug stability determination by LC variable-parameter kinetic experiments, *J. Pharm. Biomed. Anal.*, 32, 1073–1079.
51. Mood, A. R. H., Haghighi, S., and Gholami, M. R. (2004), Fluorometric variable-temperature kinetic investigations of the transesterification reaction of procaine with aliphatic alcohols, *J. Pharm. Pharm. Sci.*, 7(1), 88–91.
52. Alibrandi, G., Coppolino, S., Micali, N., and Villari, A. (2001), Variable-pH kinetics: An easy determination of pH-rate profile, *J. Pharm. Sci.*, 90, 270–274.
53. Alibrandi, G., D'Aliberti, S., and Tresoldi, G. (2003), Spectrophotometric variable-concentration kinetic experiments applied to inorganic reactions, *Int. J. Chem. Kinet.*, 35, 497–502.
54. Alibrandi, G., Coppolino, S., D'Aliberti, S., Ficarra, P., Micali, N., and Villari, A. (2003), Variable-ionic strength kinetic experiments to study drug stability, *J. Pharm. Sci.*, 92, 1730–1733.
55. Alibrandi, G., D'Aliberti, S., and Pedicini, R. (2001), Computer simulation of variable-parameter kinetic experiments, *Chem. Ed.*, 6, 185–191.
56. MicroMath Scientific Software, Salt Lake City, UT.
57. Press, W. H., Flannery, B. P., Teukolsky, S. A., and Vetterling, W. T. (1986), *Numerical Recipes*, Cambridge University Press, Cambridge.
58. Savitzky, A., and Golay, M. J. E. (1964), Smoothing and differentiation of data by simplified least squares procedures, *Anal. Chem.*, 36, 1627–1639.
59. Garrett, E. R. (1957), The kinetics of solvolysis of acyl esters of salycilic acid, *J. Am. Chem. Soc.*, 79, 3401–3408.
60. Fersht, A. R., and Kirby, A. J. (1967), The hydrolysis of aspirin. Intramolecular general base catalysis of ester hydrolysis, *J. Am. Chem. Soc.*, 89, 4857–4863.
61. Miles, C. I., and Schenk, G. H. (1970), Fluorescence of acetylsalicylic acid in solution and its measurement in presence of salicylic acid, *Anal. Chem.*, 42, 656–659.
62. Tobe, M. L. (1987), Substitution reactions, in Wilkinson, G., Gillard, R. D., and McCleverty, J. A., Eds., *Comprehensive Coordination Chemistry*, Vol. 1, Pergamon, Oxford, Chapter 7.1, pp. 281–329.
63. Belluco, U., Cattalini, L., Basolo, F., Pearson, R. G., and Turco, A. (1965), Nucleophilic constants and substrate discrimination factors for substitution reactions of platinum(II) complexes, *J. Am. Chem. Soc.*, 87, 241–246.
64. Sherman, S. E., and Lippard, S. J. (1987), Structure aspect of platinum anticancer drug interactions with DNA, *Chem. Rev.*, 87, 1153–1181.
65. Nicolini, M., Ed. (1988), *Platinum and Other Metal Coordination Compounds in Cancer Chemiotherapy*, Martinus Nijoff Publishing, Boston, MA.
66. Hajratwala, B. R., and Dawson, J. E. (1977), Kinetics of indomethacin degradation I: Presence of alkali, *J. Pharm. Sci.*, 66, 27–29.
67. Cipiciani, A., Ebert, C., Linda, P., Rubessa, F., and Savelli, G. (1983), Kinetics and mechanism of the basic hydrolysis of indomethacin and related compounds: A reevaluation, *J. Pharm. Sci.*, 72, 1075–1076.

SECTION 8

VALIDATION

8.1

ANALYTICAL METHOD VALIDATION: PRINCIPLES AND PRACTICES

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8.1.1 INTRODUCTION

Validation of an analytical procedure is the process by which it is established, by laboratory studies, that the performance characteristics of the procedure meet the

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requirements for its intended use. All analytical methods that are intended to be used for analyzing any clinical samples will need to be validated. Validation of analytical methods is an essential but time-consuming activity for most analytical development laboratories. It is therefore important to understand the requirements of method validation in more detail and the options that are available to allow for optimal utilization of analytical resources in a development laboratory.

8.1.2 WHY VALIDATE ANALYTICAL PROCEDURES

There are many reasons for the need to validate analytical procedures. Among them are regulatory requirements, good science, and quality control requirements. The *Code of Federal Regulations* (CFR) 311.165c explicitly states that "the accuracy, sensitivity, specificity, and reproducibility of test methods employed by the firm shall be established and documented." Of course, as scientists, we would want to apply good science to demonstrate that the analytical method used had demonstrated accuracy, sensitivity, specificity, and reproducibility. Finally management of the quality control unit would definitely want to ensure that the analytical methods that the department uses to release its products are properly validated for its intended use so the product will be safe for human use.

8.1.3 CURRENT GOOD MANUFACTURING PRACTICES IN TWENTY-FIRST CENTURY

The overarching philosophy in current good manufacturing practices (cGMPs) of the twenty-first century and in robust modern quality systems is *quality should be built into the product, and testing alone cannot be relied on to ensure product quality.* From the analytical perspective, this will mean that analytical methods used to test these products should have quality attributes built into them. To have quality attributes built into the analytical method will require that fundamental quality attributes be applied by the bench-level scientist. This is a paradigm shift that requires the bench-level scientist to have the scientific and technical understanding, product knowledge, process knowledge, and/or risk assessment abilities to appropriately execute the quality functions of analytical method validation. It will require (1) the appropriate training of the bench-level scientist to understand the principles involved with method validation and able to validate an analytical method and understand the principles involved with the method validation, (2) proper documentation and understanding and interpreting data, and (3) cross-functional understanding of the effect of their activities on the product and the customer (the patient). It is the responsibility of management to verify that skills gained from the training are implemented in day-to-day performance.

8.1.4 CYCLE OF ANALYTICAL METHODS

The analytical method validation activity is not a one-time study. This is illustrated and summarized in the life cycle of an analytical procedure in Figure 1. An analytical

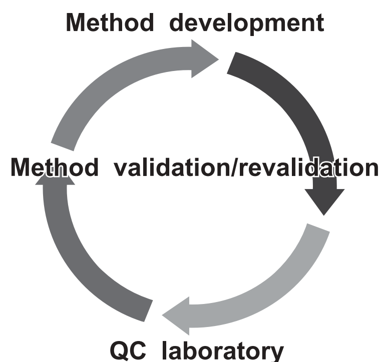

FIGURE 1 Life cycle of analytical method.

method will be developed and validated for use to analyze samples during the early development of an active pharmaceutical ingredient (API) or drug product. As drug development progresses from phase 1 to commercialization, the analytical method will follow a similar progression. The final method will be validated for its intended use for the market image drug product and transferred to the quality control laboratory for the launch of the drug product. However, if there are any changes in the manufacturing process that have the potential to change the analytical profile of the drug substance and drug product, this validated method may need to be revalidated to ensure that it is still suitable to analyze the API or drug product for its intended purpose.

8.1.5 ANALYTICAL METHOD VALIDATION CHARACTERISTICS

Typical analytical performance characteristics that should be considered in the validation of the types of procedures described in this chapter are listed below. Each validation characteristic is defined to ensure consistency in usage of terminology and interpretation:

- Accuracy
- Precision
  - Repeatability
  - Intermediate precision
- Specificity
- Detection limit
- Quantitation limit
- Linearity
- Range
- Robustness

8.1.5.1 Accuracy

The International Convention on Harmonization (ICH) defines the accuracy of an analytical procedure as the closeness of agreement between the values that are accepted either as conventional true values or an accepted reference value and the value found. For drug substance, accuracy may be defined by the application of the analytical procedure to an analyte of known purity (e.g., a reference standard). For the drug product, accuracy will be determined by application of the analytical procedure to synthetic mixtures of the drug product components to which known amounts of analyte have been added within the range of the procedure. The ICH document also recommends assessing a minimum of nine determinations over a minimum of three concentration levels covering the specified range (e.g., three concentrations/three replicates).

Accuracy is usually reported as percent recovery by the assay (using the proposed analytical procedure) of known added amount of analyte in the sample or as the difference between the mean and the accepted true value together with the confidence intervals. The range for the accuracy limit should be within the linear range. Typical accuracy of the recovery of the drug substance is expected to be about 99–101%. Typical accuracy of the recovery of the drug product is expected to be about 98–102%. Values of accuracy of recovery data beyond this range need to be investigated as appropriate.

8.1.5.2 Method Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple samples of the same homogeneous sample under prescribed conditions. Precision is usually investigated at three levels: repeatability, intermediate precision, and reproducibility. For simple formulation it is important that precision be determined using authentic homogeneous samples. A justification will be required if a homogeneous sample is not possible and artificially prepared samples or sample solutions are used.

Repeatability Repeatability is a measure of the precision under the same operating conditions over a short interval of time, that is, under normal operating conditions of the analytical method with the same equipment. It is sometimes referred to as intra-assay precision.

The ICH recommends that repeatability be assessed using a minimum of nine determinations covering the specified range for the procedure (e.g., three concentrations/three replicates as in the accuracy experiment) or using a minimum of six determinations at 100% of the test concentration. Reporting of the standard deviation, relative standard deviation (coefficient of variation), and confidence interval is required. The assay values are independent analyses of samples that have been carried through the complete analytical procedure from sample preparation to final test result. Table 1 provides an example set of repeatability data.

Intermediate Precision Intermediate precision is defined as the variation within the same laboratory. The extent to which intermediate precision needs to be estab-

TABLE 1 Repeatability Data

Replicate	Percentage of Labeled Claim
1	100.6
2	102.1
3	100.5
4	99.4
5	101.4
6	101.1
Mean	100.9
Percentage relative standard deviation (%RSD)	0.90

lished depends on the circumstances under which the procedure is intended to be used. Typical parameters that are investigated include day-to-day variation, analyst variation, and equipment variation. Depending on the extent of the study, the use of experimental design is encouraged. Experimental design will minimize the number of experiments that need to be performed. It is important to note that ICH allows exemption from doing intermediate precision when reproducibility is proven. It is expected that the intermediate precision should show variability that is in the same range or less than repeatability variation. ICH recommends the reporting of standard deviation, relative standard deviation (coefficient of variation), and confidence interval of the data.

Reproducibility Reproducibility measures the precision between laboratories. This parameter is considered in the standardization of an analytical procedure (e.g., inclusion of procedures in pharmacopeias and method transfer between different laboratories).

To validate this characteristic, similar studies need to be performed at different laboratories using the same homogeneous sample lot and the same experimental design. In the case of method transfer between two laboratories, different approaches may be taken to achieve the successful transfer of the procedure. The most common approach is the direct-method transfer from the originating laboratory to the receiving laboratory. The originating laboratory is defined as the laboratory that has developed and validated the analytical method or a laboratory that has previously been certified to perform the procedure and will participate in the method transfer studies. The receiving laboratory is defined as the laboratory to which the analytical procedure will be transferred and that will participate in the method transfer studies. In the direct-method transfer, it is recommended that a protocol be initiated with details of the experiments to be performed and acceptance criteria (in terms of the difference between the means of the two laboratories) for passing the method transfer. Table 2 provides examples of a set of method transfer data between two laboratories.

8.1.5.3 Specificity

The ICH defines specificity as the ability to assess unequivocally an analyte in the presence of components that may be expected to be present. In many publications,

TABLE 2 Results from Method Transfer between Two Laboratories

	Runs	Average Percent
Originating laboratory	12	100.7
Receiving laboratory	4	100.2

selectivity and specificity are often used interchangeably. However, there are debates over the use of specificity over selectivity and some authorities, for example, the International Union of Pure and Applied Chemistry (IUPAC), have preferred the term *selectivity*, reserving *specificity* for those procedures that are completely selective. For pharmaceutical application, the above definition of ICH will be used.

For identity test, compounds of closely related structures which are likely to be present should be discriminated from each other. This could be confirmed by obtaining positive results (by comparison with a known reference material) from samples containing the analyte, coupled with negative results from samples which do not contain the analyte. Furthermore, the identification test may be applied to material structurally similar or closely related to the analyte to confirm that a positive response is not obtained. The choice of such potentially interfering materials should be based on sound scientific judgment with a consideration of the interferences that could occur.

The specificity for an assay and impurity tests should be approached from two angles:

1. *When Impurities Are Available* The specificity of an assay method is determined by comparing test results from an analysis of sample containing the impurities, degradation products, or placebo ingredients with those obtained from an analysis of samples without the impurities, degradation products, or placebo ingredients. For a stability-indicating assay method, degradation peaks need to be resolved from the drug substance. However, these impurities do not need to be resolved from each other.

   For the impurity test, the determination should be established by spiking drug substance or drug product with the appropriate levels of impurities and demonstrating the separation of these impurities individually and/or from other components in the sample matrix. Representative chromatograms should be used.
2. *If Impurities Are Not Available.* Specificity may be demonstrated by comparing the test results of samples containing impurities or degradation products to a second well-characterized procedure or other validated analytical procedure (orthogonal method). This should include samples stored under relevant stress conditions (light, heat, humidity, acid/base hydrolysis and oxidation). For the assay method, the two results should be compared; for impurity tests, the impurity profiles should be compared. Peak homogeneity tests should be performed using PDA or mass spectrometry to show that the analyte chromatographic peak is not attributable to more than one component. Figure 2 illustrates the selectivity of a method to resolve known degradation peaks from the parent peak.

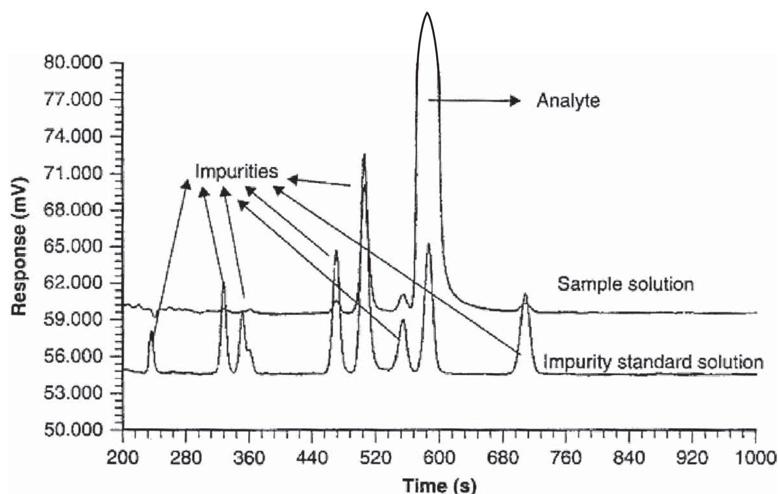

FIGURE 2 Overlay chromatogram of impurity solution with sample solution.

8.1.5.4 Detection Limit

The detection limit (DL) is a characteristic for the limit test only. It is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated under the stated experimental conditions. The detection is usually expressed as the concentration of the analyte in the sample, for example, percentage, parts per million (ppm), or parts per billion (ppb).

There are several approaches to establish the DL. Visual evaluation may be used for noninstrumental (e.g., solution color) and instrumental methods. In this case, the DL is determined by the analysis of a series of samples with known concentrations and establishing the minimum level at which the analyte can be reliably detected. Presentation of relevant chromatograms or other relevant data is sufficient for justification of the DL.

For instrumental procedures that exhibit background noise, it is common to compare measured signals from samples with known low concentrations of analyte with those of the blank samples. The minimum concentration at which the analyte can reliably be detected is established using an acceptable signal-to-noise ratio of 2:1 or 3:1. Presentation of relevant chromatograms is sufficient for justification of the DL.

Another approach estimates the DL from the standard deviation of the response and the slope of the calibration curve. The standard deviation can be determined either from the standard deviation of multiple blank samples or from the standard deviation of the *y* intercepts of the regression lines done in the range of the DL. This estimate will need to be subsequently validated by the independent analysis of a suitable number of samples near or at the DL:

$$\mathrm{DL} = \frac{3\sigma}{S}$$

where σ is the standard deviation of the response and S is the slope of the calibration curve.

8.1.5.5 Quantitation Limit

The quantitation Limit (QL) is a characteristic of quantitative assays for low levels of compounds in sample matrices, such as impurities in bulk drug substances and degradation products in finished pharmaceuticals. QL is defined as the concentration of related substance in the sample that will give a signal-to-noise ratio of 10:1. The QL of a method is affected by both the detector sensitivity and the accuracy of sample preparation at the low concentration of the impurities. In practice, QL should be lower than the corresponding ICH report limit.

ICH recommends three approaches to the estimation of QL. The first approach is to evaluate it by visual evaluation and may be used for noninstrumental methods and instrumental methods. QL is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantitated with acceptable accuracy and precision.

The second approach determines the signal-to-noise ratio by comparing measured signals from samples with known low concentrations of anlayte with those of blank samples. QL is the minimum concentration at which the analyte can be reliably quantified at the signal-to-noise ratio of 10:1.

The third approach estimates QL by the equation

$$\mathrm{QL} = \frac{10\sigma}{S}$$

The slope S may be estimated from the calibration curve of the analyte. The value of σ may be estimated by (1) calculating the standard deviation of the responses obtained from the measurement of the analytical background response of an appropriate number of blank samples or (2) calculating the residual standard deviation of the regression line from the calibration curve using samples containing the analyte in the range of the QL.

Whatever approach is applied, the QL should be subsequently validated by the analysis of a suitable number of samples prepared at the QL and determining the precision and accuracy at this level.

8.1.5.6 Linearity

ICH defines linearity of an analytical procedure as the ability (within a given range) to obtain test results of variable data (e.g., absorbance and area under the curve) which are directly proportional to the concentration (amount of analyte) in the sample. The data variables that can be used for quantitation of the analyte are the peak areas, peak heights, or the ratio of peak areas (heights) of analyte to the internal standard peak. Quantitation of the analyte depends on it obeying Beer's law for the spectroscopic method over a concentration range. Therefore, the working sample concentration and samples tested for accuracy should be in the linear range.

There are two general approaches for determining the linearity of the method. The first approach is to weigh different amounts of standard directly to prepare

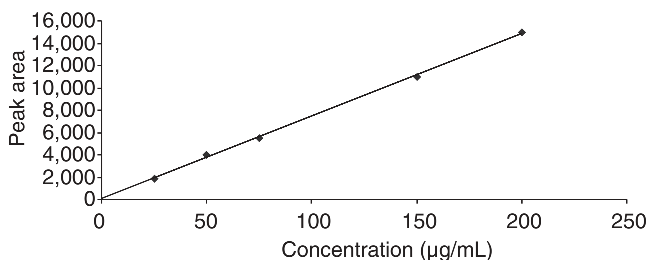

FIGURE 3 Linearity plot of peak-area response versus concentration.

linearity solutions at different concentrations. However, it is not suitable to prepare solution at very low concentration, as the weighing error will be relatively high.

Another approach is to prepare a stock solution of high concentration. Linearity is then demonstrated directly by dilution of the standard stock solution. This is more popular and the recommended approach. Linearity is best evaluated by visual inspection of a plot of the signals as a function of analyte concentration. Subsequently, the variable data are generally used to calculate a regression line by the least-squares method. At least five concentration levels should be used. Under normal circumstances, linearity is acceptable with a coefficient of determination (r^2) of ≥0.997. The slope, residual sum of squares, and *y* intercept should also be reported as required by ICH.

The slope of the regression line will provide an idea of the sensitivity of the regression, and hence the method that is being validated. The *y* intercept will provide an estimate of the variability of the method. For example, the ratios percent of the *y* intercept with the variable data at nominal concentration are sometimes used to estimate the method variability.

For the determination of potency assay of a drug substance or a drug product, the usual range of linearity should be ±20% of the target or nominal concentration. For the determination of content uniformity, it should be ±30% of the target or nominal concentration. Figure 3 illustrates the linearity of a sample set of data.

8.1.5.7 Range

The range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy, and linearity. The range is normally expressed in the same units as test results (e.g., percent, parts per million) obtained by the analytical procedure.

For the assay of drug substance or finished drug product, it is normally recommended to have a range of 80–120% of the nominal concentration.

For content uniformity, a normal range would cover 70–130% of the nominal concentration, unless a wider and more appropriate range (e.g., metered-dose inhalers) is justified.

For dissolution testing, a normal range is ±20% over the specified range. If the acceptance criterion for a controlled-release product covers a region from 20% after

1 h, and up to 90% after 24 h, the validated range would be 0–110% of the label claim. In this case, the lowest appropriate quantifiable concentration of analyte will be used as the lowest limit as 0% is not appropriate.

8.1.5.8 Robustness

Robustness of an analytical procedure is a measure of the analytical method to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

The evaluation of robustness is normally considered during the development phase and depends on the type of procedure under study. Experimental design (e.g., fractional factorial design or Plackett–Burman design) is common and useful to investigate multiple parameters simultaneously. The result will help to identify critical parameters that will affect the performance of the method. Common method parameters that can affect the analytical procedure should be considered based on the analytical technique and properties of the samples:

1. Sample preparation
   a. Extraction time
   b. Sample solvent (pH ± 0.05 unit, percent organic ± 2% absolute)
   c. Membrane filters
   d. Sample and standard stability
2. High-performance liquid chromatography (HPLC) conditions
   a. Mobile-phase composition (pH ± 0.05 unit, percent organic ± 2% absolute)
   b. Column used (equivalent columns, lots and/or suppliers, age)
   c. Temperature
   d. Flow rate
3. Gas chromatography (GC) conditions
   a. Column used (lots and/or suppliers, age)
   b. Temperature
   c. Flow rate

When the results are affected by some critical experimental parameters, a precautionary statement should be included in the analytical procedure to ensure that this parameter is tightly controlled between experiments. For example, if percent ionpairing of mobile phase affects the results significantly, the analytical procedure should explicitly be written with a precautionary statement for aqueous component, for example, 40% aqueous 20 m*M* octanesulfonic acid ± 2% absolute.

Other robustness considerations for ruggedness of the analytical procedure during validation include the following:

(a) *Sample Extraction* Mechanical shaking is preferred over sonication as the latter is affected by a number of factors, for example, water level in bath and position of sample.

(b) *Dilution of Sample and Solvent.* Minimize the number of dilution steps to reduce introduction of error. Dilution solvent should be as similar to mobile phase as possible.

8.1.6 PROCESS OF ANALYTICAL METHOD VALIDATION

The typical process that is followed in an analytical method validation is chronologically listed below:

1. Planning and deciding on the method validation experiments
2. Writing and approval of method validation protocol
3. Execution of the method validation protocol
4. Analysis of the method validation data
5. Reporting the analytical method validation
6. Finalizing the analytical method procedure

The method validation experiments should be well planned and laid out to ensure efficient use of time and resources during execution of the method validation. The best way to ensure a well-planned validation study is to write a method validation protocol that will be reviewed and signed by the appropriate person (e.g., laboratory management and quality assurance).

The validation parameters that will be evaluated will depend on the type of method to be validated. Analytical methods that are commonly validated can be classified into three main categories: identification, testing for impurities, and assay. Table 3 lists the ICH recommendations for each of these methods.

Execution of the method validation protocol should be carefully planned to optimize the resources and time required to complete the full validation study. For example, in the validation of an assay method, linearity and accuracy may be validated at the same time as both experiments can use the same standard solutions. A normal validation protocol should contain the following contents at a minimum:

(a) Objective of the protocol
(b) Validation parameters that will be evaluated
(c) Acceptance criteria for all the validation parameters evaluated
(d) Details of the experiments to be performed
(e) Draft analytical procedure

The data from the method validation data should be analyzed as the data are obtained and processed to ensure a smooth flow of information. If an experimental error is detected, it should be resolved as soon as possible to reduce any impact it may have on later experiments. Analysis of the data includes visual examination of the numerical values of the data and chromatograms followed by statistical treatment of the data if required.

TABLE 3 Validation Parameters

Type of Analytical Procedure Characteristics	Identification	Testing for Impurities		Assay – Dissolution (Measurement Only) – Content/ Potency
		Quantitation	Limit	
Accuracy	–	+	–	+
Precision	–			
Repeatability	–	+	–	+
Intermediate precision		+[a]	–	+[a]
Specificity[b]	+	+	+	+
Detection limit	–	–[c]	+	–
Quantitation limit	–	+	–	–
Linearity	–	+	–	+
Range	–	+	–	+

Note: –, characteristic not normally evaluated; +, characteristic normally evaluated.[a]In cases where reproducibility has been performed, intermediate precision is not needed.[b]Lack of specificity of one analytical procedure could be compensated by other supporting analytical procedure(s).[c]May be needed in some cases.

Upon completion of all the experiments, all the data will be compiled into a detailed validation report that will conclude the success or failure of the validation exercise. Depending on the company's strategy a summary of the validation data may also be generated. Successful execution of the validation will lead to a final analytical procedure that can be used by the laboratory to support future analytical work for the drug substance or drug product.

8.1.7 INFORMATION REQUIRED IN ANALYTICAL PROCEDURE

The minimal information that should be included in a final analytical procedure are as follows:

(a) Rationale of the analytical procedure and description of the capability of the method. Revision of analytical procedure should include the advantages offered by the new revision.

(b) Proposed analytical procedure. This section should contain a complete description of the analytical procedure in sufficient detail to enable another analytical scientist to replicate it. The write-up should include all important operational parameters and specific instructions, for example, preparation of reagents, system suitability tests, precautions, and explicit formulas for calculation of the test results.

(c) List of permitted impurities and its levels in an impurity assay.

(d) Validation data. Either a detailed set or summary set of validation data is included

(e) Revision history.
(f) Signature of author, reviewer, management, and quality assurance.

8.1.8 PHASE-APPROPRIATE METHOD VALIDATION

The original intent of the cGMPs was to describe standards and activities designed to ensure the strength, identity, safety, purity, and quality of pharmaceutical products introduced into commerce. However, the GMPs are silent on explicit guidances for the development phase of pharmaceuticals in several areas.

Regulatory bodies recognize that knowledge of the drug product and its analytical methods will evolve through the course of development. This is stated explicitly in ICH Q7A: Changes are expected during development, and every change in product, specifications, or test procedures should be recorded adequately. It is therefore reasonable to expect that changes in testing, processing, packaging, and so on, will occur as more is learned about the molecule. However, even with the changes, the need for ensuring the safety of subjects in clinical testing should not be compromised.

According to the ICH guidance, the objective of method validation is to demonstrate that analytical procedures "are suitable for their intended purpose." Therefore the method's purpose should be linked to the clinical studies and the pharmaceutical purpose of the product being studied.

The purpose in the early phase of drug development is to deliver a known dose that is bioavailable for clinical studies. As product development continues, increasing emphasis is placed on identifying a stable, robust formulation from which multiple, bioequivalent lots can be manufactured and ultimately scaled up, transferred, and controlled for commercial manufacture.

The development and validation of analytical methods should follow a similar progression. The purpose of analytical methods in early stages of development is to ensure potency, to understand the impurity and degradation product profile, and to help understand key drug characteristics. As development continues, the method should be stability indicating and capable of measuring the effect of key manufacturing parameters to ensure consistency of the drug substance and drug product.

Analytical methods used to determine purity and potency of an experimental API that is very early in development will need a less rigorous method validation exercise than would be required for a quality control laboratory method at the manufacturing site. An early phase project may have only a limited number of lots to be tested and the testing may be performed in only one laboratory by a limited number of analysts. The ability of the laboratory to "control" the method and its use is relatively high, particularly if laboratory leadership is clear in its expectations for the performance of the work.

The environment in which a method is used changes significantly when the method is transferred to a quality control laboratory at the manufacturing site. The method may be replicated in several laboratories, multiple analysts may use it, and the method may be one of many methods used in the laboratory daily. The developing laboratory must therefore be aware of the needs of the receiving

TABLE 4 Assay Method Validation in Early Phase for Drug Substance and Drug Product

	Drug Substance	Drug Product
Accuracy	Inferred from precision, linearity, and specificity	Recovery at 100% for each strength (bracket for multiple strengths)
Repeatability	Three sample preparation at 100% nominal	Three sample preparations at 100% nominal
Intermediate precision	To be completed at later stages of development	To be completed at later stages of development
Specificity	Resolution from most likely impurities	Resolution from impurities and excipients
Quantitation/detection limit	Not required	Not required
Linearity	Minimum three levels from 80 to 120%	Minimum three levels from 80 to 120%
Range	Defined in linearity	Defined in linearity
Robustness	Solution stability	Solution stability

laboratories, for example, quality control laboratory, and regulatory expectations for the successful validation of a method to be used in support of a commercial product.

An example of the minimum requirement for potency assay of the drug substance and drug product is tabulated in Table 4. Note that the postponement of intermediate precision is aligned with previous discussion that the use of early phase analytical method resides mainly in one laboratory and is used only by a very limited number of analysts. Each individual company's phased method validation procedures and processes will vary, but the overall philosophy is the same. The extent of and expectations from early phase method validation are lower than the requirements in the later stages of development. The validation exercise becomes larger and more detailed and collects a larger body of data to ensure that the method is robust and appropriate for use at the commercial site.

However, certain fundamental concepts of cGMPs must be applied regardless of the details of the phased appropriate method validation strategy used. Examples are (1) proper documentation, (2) change control, (3) deviations, (4) equipment and utilities qualification, and (5) proper training.

A detailed method validation report may not be necessary until submission of the final market application. However, summary reports should be available to facilitate efficient data retrieval and fulfill requests from regulatory agencies for the information when required.

8.1.9 METHOD VERIFICATION

The U.S. Food and Drug Administration (FDA) regulation 21 CFR 211.194(a)(2) specifically states that users of analytical methods in the U.S. Pharmacopeia/National Formulary (USP/NF) are not required to validate the accuracy and reliability of these methods but merely verify their suitability under actual conditions of use. USP

TABLE 5 Validation and Verification Requirements for HPLC Assay of Final Dosage Forms

Performance Characteristics	Validation	Verification
Accuracy	Yes	Maybe
Precision	Yes	Yes
Specificity	Yes	Yes
Limit of detection (LOD)	No	No
Limit of quantification (LOQ)	No	No
Linearity	Yes	No
Range	Yes	No

has issued a guidance for verification in general chapter ⟨1226⟩. This proposal provides general information to laboratories on the verification of compendial procedures that are being performed for the first time to yield acceptable results utilizing the laboratories' personnel, equipment, and reagents.

Verification consists of assessing selected analytical validation characteristics described earlier to generate appropriate, relevant data rather than repeating the validation process for commercial products. The guidance in this general chapter is applicable to applications such as titrations, chromatographic procedures (related compounds, assay, and limit tests), and spectroscopic tests. However, general tests (e.g., water, heavy metals, residue on ignition) do not typically require verification.

Table 5 summarizes the comparison of the validation requirements with the verification requirements of the HPLC assay of an example final dosage form. ICH requires the validation of accuracy, precision, specificity, linearity, and range. Generally, verification will only require a minimal of precision and specificity validation. The accuracy requirements will be dependent on the specific situation of the final dosage form.

8.1.10 METHOD REVALIDATION

There are various circumstances under which a method needs to be revalidated. Some of the common situations are described below:

1. During the optimization of the drug substance synthetic process, significant changes were introduced into the process. To ensure that the analytical method will still be able to analyze the potentially different profile of the API, revalidation may be necessary.
2. If a new impurity is found that makes the method deficient in its specificity, this method will need to be modified or redeveloped and revalidated to ensure that it will be able to perform its intended function.
3. A change in the excipient composition may change the product impurity profile. This change may make the method deficient in its specificity for the assay or impurity tests and may require redevelopment and revalidation.

4. Changes in equipment or suppliers of critical supplies of the API or final drug product will have the potential to change their degradation profile and may require the method to be redeveloped and revalidated.

8.1.11 CONCLUSION

This chapter summarizes the validation parameters that are required according to the requirements of ICH Q2R(1). The paradigm shift under cGMP in the twenty-first century that requires the bench-level scientist to have the scientific and technical understanding, product knowledge, process knowledge, and/or risk assessment abilities to appropriately execute the quality functions of analytical method validation is presented in detail. The method validation process and the minimum requirements to be included in a regulatory method are also discussed. An overview of phase-appropriate method validation, method verification, and method revalidation are presented to stimulate ideas and the thought process to follow when such situations are encountered.

FURTHER READING

1. International Conference on Harmonization (ICH) (2005, Nov.), Harmonised tripartite guideline Q2(R1), Validation of analytical procedures: Text and methodology.
2. International Conference on Harmonization (ICH) (1999, Oct.), Harmonised tripartite guideline Q6A, Specifications: Test procedures and acceptance criteria for new drug substances and new drug products: Chemical substances.
3. International Conference on Harmonization (ICH) (2000, Nov.), Harmonised tripartite guideline Q7A GMP for active pharmaceutical ingredient.
4. International Conference on Harmonization (ICH) (2006, Sept.), Guidance for industry: Quality systems approach to pharmaceutical cGMP.
5. Code of Federal Regulations (CFR), Part 211, Current good manufacturing practice for finished pharmaceuticals.
6. Chan, C. C., et al., (2004), *Analytical Method Validation and Instrument Performance Verification*, J Wiley, Hoboken, NJ.
7. U.S. Pharmacopeia (USP), General Chapter ⟨1225⟩, Validation of compendial procedures, USP, Rockville, MD.
8. U.S. Pharmacopeia (USP), General Chapter ⟨1226⟩, Verification of compendial procedures, USP, Rockville, MD.

8.2

ANALYTICAL METHOD VALIDATION AND QUALITY ASSURANCE

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8.2.1 INTRODUCTION

Credibility of analytical data has never caught the public's eye more than today. Rather than on the used techniques and methodologies themselves, attention is nowadays paid to the quality and reliability of the final results. This is influenced by

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a higher demand for regulatory compliance, a higher consciousness of the customer—the client wants to know the level of confidence of the reported result—and under impulse of new, more exigent European and international standards such as the International Organization for Standardization/International Electrotechnical Commission (ISO/IEC) 17025 norm for laboratory accreditation. The underlying key principle is comparability of results between laboratories and on a wider, international basis. In order for results to be comparable, they must be reported with a statement of measurement uncertainty (MU) and they must be traceable to common primary references. Methods must be validated to show that they actually measure what they are intended to measure—that they are fit for their specific purpose.

Because validation and quality assurance (QA) apply for a specific analytical method, it is important to approach each method on a case-by-case basis. An analytical method is a complex, multistep process starting with sampling and ending with the generation of a result. Although every method has its specific scope, application, and analytical requirement, the basic principles of validation and QA are the same regardless the type of method or sector of application. The information in this chapter is mainly taken from analytical chemistry, but it applies to other sectors as well. The validation of analytical methods, the establishment of traceability of results, and the assessment of MU should be done in a uniform, harmonized way, conforming with internationally recognized standards from institutions such as Eurachem, the International Union of Pure and Applied Chemistry (IUPAC), or ISO.

It is important to issue a common understanding on the topics of method validation, traceability, and uncertainty of measurements. Here, the interrelationships between method validation, traceability, and MU of results will be elucidated. Throughout the landscape of guidelines and standards, the most relevant information is selected, compiled, and summarized. Great importance is attached to the different method performance parameters and their definitions, ways of expression, and approaches for practical assessment. We discuss the role of method validation within QA as well as the topics of standardization, internal and external quality control (IQC and EQC, respectively), and accreditation and the links between these different aspects.

This chapter provides a general, complete, and up-to-date overview of the topic of quality of analytical measurements in the wide sense of the word. It is useful for the completely inexperienced scientist as well as those involved in this topic for a long time who have lost their way in the labyrinth and are looking for more explanation on a particular aspect or deeper insight and knowledge.

8.2.2 TRACEABILITY AND MEASUREMENT UNCERTAINTY

8.2.2.1 Introduction: Quality of Analytical Results

Innumerable types of analytical methods exist in the fields of analytical and bioanalytical chemistry, biochemistry, biology, clinical biology, and pharmacology and related application domains such as forensic, toxicological, environmental, agricultural, and food analyses. Regardless of the type of method, scope, and application, laboratories must be able to produce reliable data when performing analytical tests for a client or for regulatory purposes. Together with the fast development of ana-

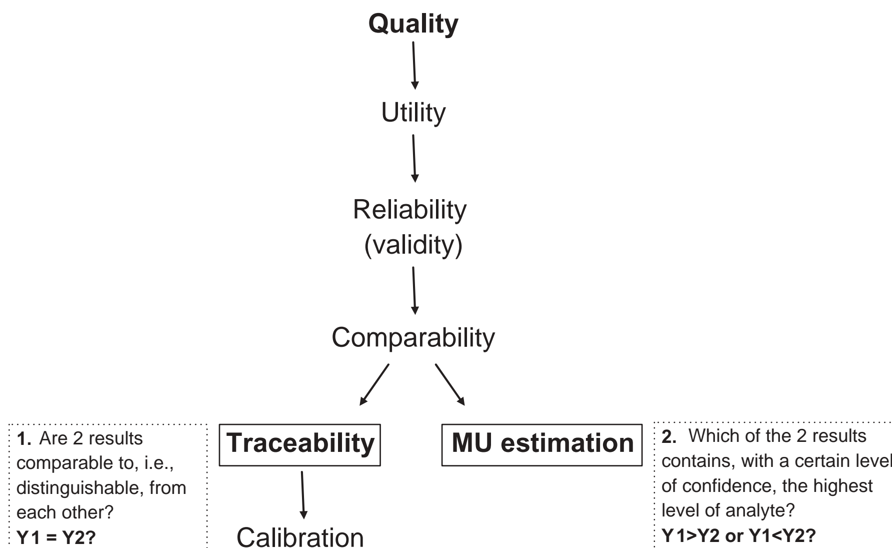

FIGURE 1 Relationship between quality, traceability, and MU of results [3].

lytical methodologies, great importance is nowadays attached to the "quality" of the measurement data. *Quality* of analytical measurement data encompasses two essential criteria: *utility* and *reliability* (Figure 1) [1]. Utility means that analytical results must allow reliable decision making. A key aspect of reliability or *validity* of results is that they are *comparable*, whatever their origin. Comparability between results in the strict sense is provided by *traceability* to appropriate standards. Traceability to common reference standards underlies the possibility of making a comparison (i.e., a distinction) between different results. If results are to be compared also in terms of their quantities or levels of analyte, additional information on the analytical result is needed: *measurement uncertainty*. Uncertainty of results arises from the combination of all uncertainties of the reference values (to which the results are traceable) and all additional uncertainties associated with the measurement procedure. Measurement uncertainty and traceability are related concepts both defining the quality of analytical data (Figure 1) [2, 3].

Quality of results reflects *adequacy* (or *inadequacy*) of a method in terms of the extent to which the method fulfills its requirements or is fit for its particular analytical purpose (see below). Quality is always a relative notion, referring to the requirements fixed beforehand on the basis of national or international regulations or customer needs [1, 4]. The need for reliability of analytical data is stressed by the fact that measurement results will be used and may form the basis for decision making. Unreliable results bring along a high risk for incorrect decisions and may lead to higher costs, health risks, illegal practices and so on. Imagine, for instance, the consequences if results are false positives or if the uncertainty is much larger than reported [1, 5, 6].

8.2.2.2 Role of Method Validation in Traceability and MU

An analysis is a complex multistage investigation of the property values of materials, being the identity and the concentration of a specific component in a specific sample

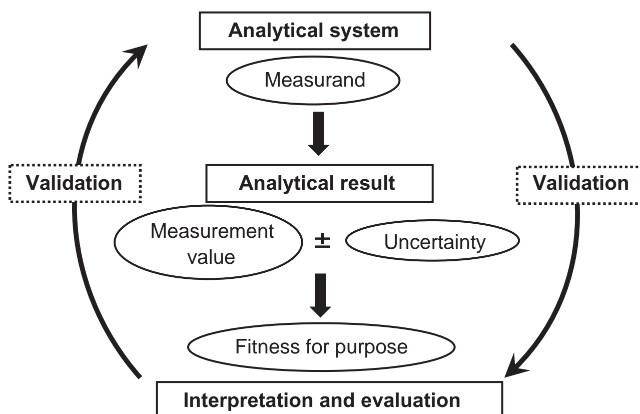

FIGURE 2 Role of method validation in quality of analytical measurements. Validation is the process to demonstrate the fitness for purpose of the analytical system [4, 8, 14, 15].

material [2, 7]. Van Zoonen et al. [1] presented a chemical analysis as a cyclic process in which the final objective is the generation of chemical information. This integral process starts with defining the basic analytical problem (specifying the analytical requirement) and ends with evaluating and reporting the analytical result. Ideally, the last step provides an answer to the starting problem, stated by a client or based on regulatory requirements.

The process of providing an answer to a particular analytical problem is presented in Figure 2. The analytical system—which is "a defined method protocol, applicable to a specified type of test material and to a defined concentration rate of the analyte"—must be "fit for a particular analytical purpose" [4]. This analytical purpose reflects the achievement of analytical results with an acceptable standard of accuracy. Without a statement of uncertainty, a result cannot be interpreted and, as such, has no value [8]. A result must be expressed with its expanded uncertainty, which in general represents a 95% confidence interval around the result. The probability that the mean measurement value is included in the expanded uncertainty is 95%, provided that it is an unbiased value which is made traceable to an internationally recognized reference or standard. In this way, the establishment of traceability and the calculation of MU are linked to each other. Before MU is estimated, it must be demonstrated that the result is traceable to a reference or standard which is assumed to represent the truth [9, 10].

Traceability and MU both form parts of the purpose of an analytical method. Validation plays an important role here, in the sense that it "confirms the fitness-for-purpose of a particular analytical method" [4]. The ISO definition of validation is "confirmation by examination and provision of objective evidence that the particular requirements of a specified intended use are fulfilled" [7]. Validation is the tool used to demonstrate that a specific analytical method actually measures what it is intended to measure and thus is suitable for its intended purpose [2, 11]. In Section 8.2.3, the classical method validation approach is described based on the evaluation of a number of method performance parameters. Summarized, the criteria-based validation process consists of precision and bias studies, a check for

specificity/selectivity, a linearity check, robustness studies, and eventually, based on the practical requirements of the method, an assessment of the limits of detection and/or quantification. The objective of validation is to verify that the measurement conditions and the equation used to calculate the final result include all the influences that will affect the final result. Validation measures the different effects throughout the whole analytical system which have an influence on the result and ensures that no other effects have to be taken into account. A specificity test ensures that the method responds to the specific analyte of interest only, and not to other interferents or contaminants. A linearity check verifies that the supposed relationship between the signal and units used for the analyte may actually be used. A bias study is a certified reference material check and as such demonstrates that the method is not significantly biased, and precision and robustness studies cover the effects of variability in conditions, operators, equipment, and time.

The roles of method validation in the achievement of reliable results are (1) to include all possible effects or factors of influence on the final result, (2) to make them traceable to stated references [reference methods, reference materials, or International System of Units (SI)], and (3) to know the uncertainties associated with each of these effects and with the references. Validation is thus a tool to establish traceability to these references [2, 4]. In this context, it is important to see the difference between traceability and accuracy. A method which is accurate, in terms of "true" (i.e., approximating the true value), is always traceable to what is considered to be the true value. The opposite however is not correct. A method that is traceable to a stated reference is not necessarely true (accurate). Errors can still occur in this method, depending on the reference [12].

Analytical method validation forms the first level of QA in the laboratory. Analytical quality assurance (AQA) is the complete set of measures a laboratory must undertake to ensure that it is able to achieve high-quality data continuously. Besides the use of validation and/or standardized methods, these measures are effective IQC procedures (use of reference materials, control charts, etc.), with participation in proficiency testing schemes and accreditation to an international standard, normally ISO/IEC 17025 [4]. Method validation and the different aspects of QA form the subject of Section 8.2.3.

8.2.2.3 Guidelines on Traceability and Uncertainty of Results

Table 1 shows an overview of prominent guiding institutions and their guidelines on traceability, MU, and related topics. In Europe, a leading role is played by Eurachem, a working group on analytical chemistry centralized at and originating from the United Kingdom's LGC (Laboratory of the Government's Chemists). Basic references are CITAC/Eurachem guides on quality in analytical chemistry [2] and traceability in chemical measurement [3] and a Eurachem guide on MU [13, 14]. Eurachem also published guides on related topics such as reference materials [7] and method validation [15].

On the international level, relevant standards are available from the IUPAC, ISO, and AOAC International [4, 8, 16, 17] and from the Codex Alimentarius' working group CCMAS [18–21]. Other helping guides have been published by the EAL [22] and ILAC [23] (see Table 1 for explanation of abbreviations).

TABLE 1 Overview of European and International Guiding Institutions and Regulatory Bodies with Guidelines and Standards on Traceability, MU and Related Topics

Body	Full Name	Guidance On	References
Eurachem/CITAC	A Focus for Analytical Chemistry in Europe/ Cooperation on International Traceability in Analytical Chemistry	Traceability, MU, reference materials, validation	2, 3 13, 14, 7, 15
IUPAC, ISO, and AOAC International	International Union of Pure and Applied Chemistry, International Organisation for Standardization, and Association of Official Analytical Chemists	MU	4, 8, 16, 17
FAO/WHO: Codex/CCMAS	Food and Agricultural Organization /World Health Organisation: Codex Committee on Methods of Analysis and Sampling	MU	18–21
EAL	European Cooperation for Accreditation	MU	22
ILAC	International Laboratory Accreditation Cooperation	MU	23

8.2.2.4 Concept of Traceability

Definitions Traceability is a relatively new term, gaining more and more attention in analytical measurement sciences. Traceability can be assigned to different aspects related to a measurement: traceability of a result, method, procedure, laboratory, product, material, equipment, and son on. As such, there is no single definition of traceability. Before exploring the different concepts of traceability, we can look to a more general, extended meaning. According to Valcarcél and Rios [24], the basic meaning of traceability integrates (1) the establishment of one or more relationships to well-stated references or standards and (2) the documented "history" of a product or a system. These two parts of the basic meaning can be found again when defining traceability as a property or a characteristic of different analytical facets. The different traceability concepts are shown in Figure 3.

The most obvious approach for traceability is a "property of the result of a measurement or the value of a standard whereby it can be related to stated references, usually national or international standards, through an unbroken chain of comparisons all having stated uncertainties." [2] The different elements and the practical use of this basic definition will be explained further below under Traceability in Practice.

Traceability of a result is related to traceability of a method, which in turn is linked to traceability of standards and traceability of the equipment used in the analytical procedure (Figure 3). A method is called traceable when it produces results (with their uncertainties) which are characterized by a defined traceability to well-stated references [24]. Walsh [25] defines traceable methods as "validated official or standard methods or validated methods which contain uncertainty state-

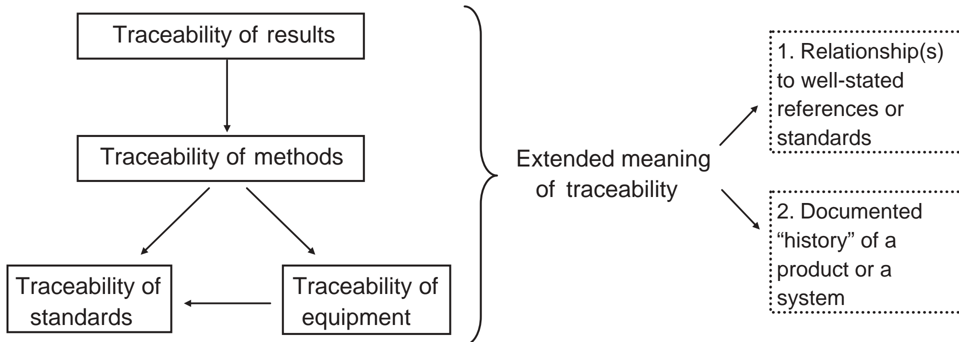

FIGURE 3 Different concepts and extended meaning of traceability [24].

ments and which are embedded into a quality system and anchored to a common reference point." Traceability among standards is considered the most relevant basis for traceability of results [26], as shown in Figure 3. Traceability of equipment is defined as "the detailed, timely, and customised recording of installation, malfunctioning and repairs, periodic calibration and corrections (if needed), hours of use, samples processed, standard used, etc., in such a way that all questions (what?, how?, who?, etc.) should have a detailed answer in the pertinent documents" [24]. Calibration is the set of operations used to establish the relationship between values shown by a measuring instrument and the values of measurement standards. By doing so, the results of measurements are related to and thus made traceable to values of standards or references. In practice, calibration is performed by measuring samples with known amounts of analyte, such as certified reference materials, and monitoring the measurement response [2, 3]. These definitions confirm the links between traceability of equipment, standards, and results (Figure 3).

Traceability in Practice The practical establishment of traceability is based on a step-by-step implementation of the definition. The ISO definition of traceability, originating from metrology (see above), can be translated into three basic steps:

1. Establish one or more links to well-stated references,
2. through an unbroken chain of comparisons and
3. estimate all uncertainties associated with those comparisons [12, 24, 27].

This definition is very much in line with the more practical definition described by Eurachem/CITAC [3]. Their procedure for traceability consists of the following steps [3]:

1. Specifying the measurand, scope of measurement, and required uncertainty
2. Choosing the method of measurement
3. Validating the method of measurement
4. Identifying/quantifying all influences which will affect the result
5. Choosing appropriate references
6. Estimating the uncertainty components associated with all influences and references.

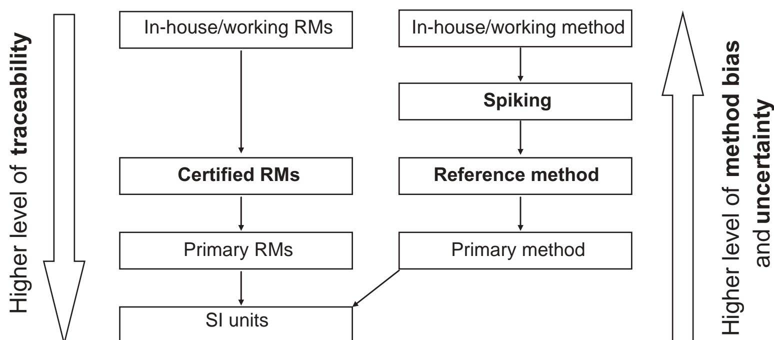

FIGURE 4 Traceability chain and relationship between traceability and uncertainty of measurements. The three possibilities for establishing traceability referred to in Figure 5 are indicated in bold [25, 28].

The key principle in both approaches is that relationships to stated references are established and that this is done through an unbroken chain of comparisons. Practically, this means that the analytical procedure is first described as a chain or a flow diagram (step 2 in ISO definition; steps 1 and 2 in Eurachem/CITAC definition). The word *unbroken* means that there is no loss of information when considering the different steps in the analytical procedure leading to the measurement result. Each step in the procedure then needs to be linked to a reference method, a reference material, or a SI unit (step 1 in ISO definition; step 5 in Eurachem/CITAC definition) [12, 24, 27]. Figure 4 depicts the successive classes of stated references (materials or methods) in a so-called traceability chain. Establishing traceability through a traceability chain brings along a certain level of uncertainty, called *calibration uncertainty* or *traceability uncertainty* (see also below) [3, 7, 28]. This brings us then to the third key element when applying the traceability definitions: the stated uncertainties. Each step in the traceability chain, with the uncertainty components of all the stated references, will contribute to the measurement result and thus to the uncertainty associated with it. Uncertainty components must thus be estimated at each step in the analytical process (step 3 in ISO definition; step 6 in Eurachem/CITAC definition). As described above, validation is a tool to identify all possible effects or factors within the analytical procedure which can influence the final result. As such, steps 3 and 4 in the Eurachem/CITAC definition are additional steps which can be very helpful in the establishment of traceability [3].

Examples of how traceability is established in practice can be found in the literature. In 2004, Trends in Analytical Chemistry published a special issue on challenges for achieving traceability of environmental measurements (volume 23, 2004). This issue contains a lot of up-to-date information and practical examples in the particular domain of environmental analysis. Many authors have reported on the most important and most difficult step in establishing traceability: the selection of stated references or standards. For different stages in the traceability chain shown in Figure 4, descriptions and examples are given by Quevauviller and Donard [27], Charlet and Marschal [29], and Segura et al. [30]. Pan [28], Förstner [31], and Theocharopoulos et al. [32] applied the ISO definition for establishing traceability in different types of environmental methods of analysis. A similar approach was followed by

Sabé and Rauret [33] and Drolc et al. [34], however based upon the Eurachem/CITAC guide on traceability [3]. In their examples, all authors take into account specific steps or influences in the analytical procedure which can lead to a "broken" chain of comparisons, such as sampling and sample treatment or preparation steps. Some authors have reported on uncertainties associated with sampling in particular [35, 36].

8.2.2.5 Concept of MU

Measurement uncertainty is the most important criterium in both method validation and IQC. It is defined as "a parameter, associated with the result of a measurement, that characterizes the dispersion of the values that could reasonably be attributed to the measurand" [14]. The measurand refers to the particular quantity or the concentration of the analyte being measured. The parameter can be a standard deviation or the width of a confidence interval [14, 37]. This confidence interval represents the interval on the measurement scale within which the true value lies with a specified probability, given that all sources of error are taken into account [37]. Within this interval, the result is regarded as being accurate, that is, precise and true [11].

It cannot be overemphasized that MU is different from error. The error of an individual analytical result, the difference between the result and the true value of the measurand, is always a single value [38]. Part of the value of a known error, the systematic error, can be used to correct a result. This means that after correction the result of an analysis may be very close to the true value. However, the uncertainty of the measurement may still be very large because there is doubt or limited knowledge about how close the result is to the value. Uncertainty is expressed as a range and applies to an analytical procedure and a specific sample type but to different determinations and thus measurement results. The value of the uncertainty cannot be used to correct a measurement result.

The error of an analytical result is related to the (in)accuracy of an analytical method and consists of a systematic component and a random component [14]. Precision and bias studies form the basis for evaluation of the accuracy of an analytical method [18]. The accuracy of results only relates to the fitness for purpose of an analytical system assessed by method validation. Reliability of results however has to do with more than method validation alone. MU is more than just a single-figure expression of accuracy. It covers all sources of errors which are relevant for all analyte concentration levels. MU is a key indicator of both fitness for purpose and reliability of results, binding together the ideas of fitness for purpose and quality control (QC) and thus covering the whole QA system [4, 37].

The MU of an analytical procedure is thus derived from but is different from the error of a single analytical result. The deviation of the measurement result from the true value comprises a number of systematic and random errors, as shown in Figure 5. Each of these error components adds its own uncertainty to the total uncertainty budget of the analytical procedure. Therefore, the different error components are referred to as *sources of uncertainty*. Depending on the sources of uncertainty taken into account and thus on the conditions of the measurement, the overall MU will be different and another definition of MU will apply. This means that there is no single, straighforward definition of MU. It is rather a concept the interpretation of

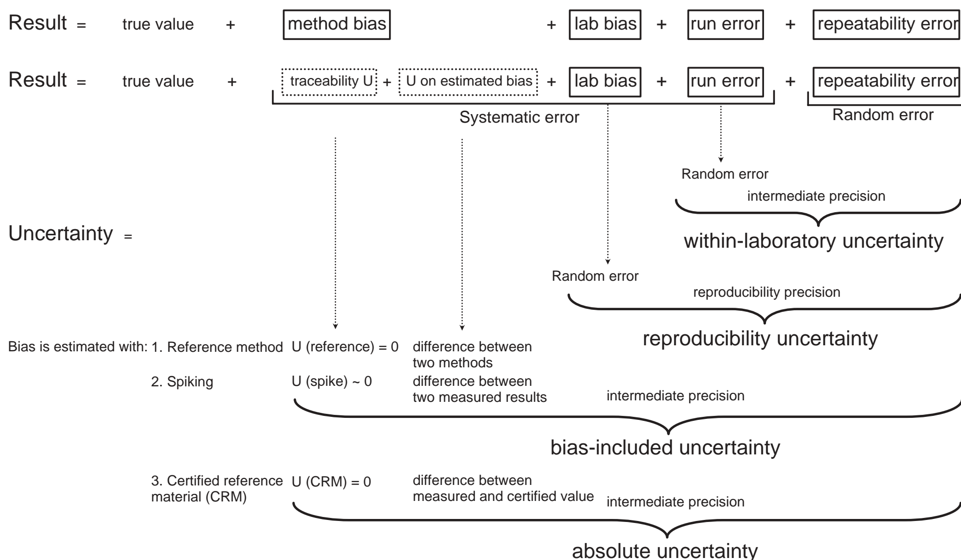

FIGURE 5 Composition of error of analytical result related to MU. Different operational definitions of MU [10]. At the bottom on the left are the three possibilities for establishing traceability (see also Figure 4).

which changes according to the measurement conditions and to the reference to which the result is traceable [10]. The different definitions of MU form the subject of the next topic.

8.2.2.6 Different Operational Definitions of MU

As illustrated in Figure 5, the error of an analytical result for a specified analyte concentration is composed of different error components, forming together the "ladder of errors":

- The *method bias* is a systematic error associated with the method as such.
- The *laboratory bias* is either a systematic error—if the laboratory is considered on its own—or a random error—if the laboratory is considered as one of a group, as is the case in interlaboratory studies.
- The *run error* is seen as a systematic error for one run and as a random variation over several runs performed intralaboratory.
- The *repeatability error* is a random error due to the replicate measurements performed within a single run [10].

As the considered error only applies for a specified concentration of analyte isolated from a specified type of sample or matrix, *sampling errors* and *matrix variation effects* are not included here [4].

The more traditional distinction of error components is between *random errors* and *systematic errors*. In this classical approach, random errors are generally referred to as "precision" (repeatability, intermediate precision, and reproducibility), while systematic errors are typically attributed to the uncertainty on the bias estimate and

calibration uncertainty. To this classification, other uncertainty contributions are added, such as sampling effects, matrix effects, and uncertainties associated with certain assumptions which underlie the measurement method and/or the calculation equation [2].

As mentioned above, each of these error components is a potential source of uncertainty. Depending on the conditions under which the analysis is performed, different sources of uncertainty contribute to the overall uncertainty. Hund et al. [10] introduced different operational definitions of uncertainty, according to the number and type of uncertainty sources considered (Figure 5):

- *Within-laboratory uncertainty* is derived from intermediate precision and includes only the repeatability error and the run error.
- *Reproducibility uncertainty* is derived from reproducibility precision (interlaboratory tests) and counts for the repeatability error, run, and laboratory effects.
- *Bias-included uncertainty* and *absolute uncertainty* additionally take into account the method bias. Method bias is the most important source of uncertainty because it refers to a reference or a standard to which the method is considered to be traceable. If the working method is not a primary method—which is traceable to SI units (see Figure 4)—the method is always compared to another, reference method or is applied on appropriate certified reference materials. This reference or standard is to be considered when the uncertainty associated with the method bias is estimated. In addition to the uncertainty associated with this reference or standard, there is the uncertainty on the estimated bias (Figure 5). The different possibilities of bias estimation—and thus of traceability—are depicted in Figure 5. If the method is compared to a reference method, the uncertainty associated with this reference method is considered negligible and the bias is estimated as the difference between the two methods (case 1 in Figure 5). If there is no method to compare with, bias can be estimated by spiking samples and assessing the difference between the spiked sample and the measured sample. In this case also, the uncertainty on the spike will approximate zero (case 2) and the only method bias is the difference between the measured sample and the spiked sample. Absolute uncertainty can only be estimated if certified reference materials are used (case 3). Only in this case, full traceability to SI units can be guaranteed [10].

8.2.2.7 Approaches to Establish MU

In general, to estimate the overall uncertainty on a particular result, it is necessary to know (1) all uncertainties arising from the measurement procedure itself and (2) all uncertainties associated with the references or standards to which the analytical results are made traceable [3]. Different approaches exist for the estimation of overall MU, as reviewed by several authors [9, 10, 39] and as summarized in Table 2.

The most well-known and traditional approach is based on identifying, quantifying, and combining all individual contributions to uncertainty. In this "bottom-up approach," the overall uncertainty is derived from the uncertainties of the individual components. The component-by-component assessment of MU was originally

TABLE 2 Approaches for Estimating MU

Name of Approach	Basic Principle	Strengths	Weaknesses	Reference
Bottom-up, error budget, error propagation or component-by-component approach	Identification, quantification, and combination of all sources of uncertainly	Holistic: all important sources of error should be included	Complex, expensive, time consuming	Eurachem [13], ISO [16]
Fitness-for-purpose approach	Establishment of a fitness function $u = f(c)$ based mainly on precision and bias studies	Simple, MU can be assessed for different concentrations	Some sources of uncertainly may be overlooked	Codex Alimentarius/ CCMAS [18–21]
Top-down approach	Based on data obtained from interlaboratory studies (precision)	MU can be assessed for different concentrations	Some sources of uncertainly may be overlooked; only if data on collaborative studies are available	Analytical Methods Committee [37]
Validation-based approach	Based on inter- or intralaboratory validation studies (precision, trueness, robustness)	An extension of validation work, no extra work needed	Some sources of uncertainly may be overlooked	Eurachem [14], Barwick and Ellison [47]
Robustness-based approach	Based on robustness tests as intralaboratory simulations of interlaboratory studies	Simple, time-efficient approach	Some sources of uncertainly may be overlooked; method must first show to be robust	Hund et al. [39]

developed for physical measurements and adopted by Eurachem for chemical measurements [13]. Because of its complexity, however, this methodology requires significant costs in time and effort and has never found widespread applications.

A simplified approach to assess MU is the *fitness-for-purpose approach*, defining a single parameter called the fitness function. This fitness function has the form of an algebraic expression $u = f(c)$ and describes the relationship between the MU and the concentration of the analyte. For example, $u = 0.05c$ means that the MU is 5% of the concentration. Calculation of the MU will hereby rely on data obtained by evaluating individual method performance characteristics, mainly on repeatability and reproducibility precision, and preferably also on bias [21, 40, 41]. This approach can more or less be seen as a simplification of the step-by-step protocol for testing the MU, as described by Eurachem [14].

Although MU comprises more than systematic and random errors, it can be estimated from method validation data. Data from method performance studies can give all or nearly all information required to evaluate the uncertainty [2, 4, 18, 37]. This includes the use of data from in-house and collaborative validation studies (typically precision data), proficiency testing schemes (typically bias data), or whichever QA data relevant for uncertainty. If such data are available and used to estimate the uncertainty, it is not necessary to estimate MU using the component-by-component approach [18, 19]. In particular, validation studies and QC measures are considered as highly relevant sources for MU estimation [42, 43]. Table 2 describes three methodologies for MU assessment which are based on validation data. In the Analytical Methods Committees top-down approach [37], the laboratory is seen from a higher level, as a member of a population of groups. As a consequence, systematic errors within one laboratory become random errors and the estimated uncertainty is the reproducibility uncertainty (Figure 5). Examples of MU estimation studies using data from collaborative ring trials are worked out by Dehouck et al. [44] and Maroto et al. [45, 46]. The other two approaches mentioned in Table 2 [14, 39, 47] make use of different method performance parameters. All three validation-based methodologies can be seen as more simple, time- and cost-efficient extensions of validation. However, important to note is that not all sources of uncertainty are covered by method performance data. Some sources that may need particular consideration in addition to the available data are sampling, pretreatment, method bias, variation in conditions, and changes in the sample matrix [14, 18, 41].

Many of those principles for MU estimation have been applied in a case study for toluene in groundwater performed by Armishaw [48]. His idea is that for a routine method which has been validated previously and which is performed in a laboratory where QC measures are in place, it is possible to estimate MU in a working afternoon. The key is to extract all relevant information from already available data: validation studies (bias/recovery and precision data), instrument calibration data [reference material (RM) uncertainties], information from regularly performed QC measures (replicate analyses and control samples), and the method procedure itself (sampling, homogeneity of samples, etc.). After identification of the components contributing to the uncertainty budget and assignment of the relevant sources of information, standard uncertainties were quantified and combined to form the combined standard uncertainty. The results for toluene in water were reported as $x \pm U$, where U is the expanded uncertainty, obtained by multiplying

the combined standard uncertainty u_c with a coverage factor of 2. In addition to this bottom-up MU estimation (approach 1), Armishaw reported the expanded uncertainty as a function of the concentration of toluene (approach 2). Finally, the authors compared this experimentally assessed MU with calculated MU values, based on (1) a within-laboratory reproducibility estimate, (2) proficiency test data, and (3) the models of Horwitz [49] and Thompson and Lowthian [50]. These models allow to calculate the percent relative standard deviation (RSD) values as a function of the analyte concentration. To obtain expanded uncertainty values, Armishaw multiplied the predicted standard deviation (SD) values from the models by 2 [48]. All three MU calculations are variants of validation-based approaches (approaches 3 and 4, Table 2).

8.2.2.8 Importance of Traceability and MU

The underlying motivation to establish traceability and MU is the need to make decisions based on the obtained analytical results or to be in compliance with regulatory limits (for quantitative determinations) [51]. MU is an essential feature of analytical results for different reasons. First, customers want to have an idea about the dispersion of results and about the comparability of results between different laboratories [43]. Any result must be accompanied by a statement of MU, so that the user of the result knows what level of confidence is associated with it [52]. The concepts of *comparability* and *reliability* of results have been discussed briefly in Section 8.2.1 and are presented in Figure 1. Second, MU is necessary because it demonstrates *traceability*. Before MU can be evaluated, traceability to stated references or standards must be established. Moser et al. [43] claim that traceability is proven by the appropriate use of reference materials or standards and by a full uncertainty budget. This brings us then to a third reason for MU estimation, which is the requirement to *know* the method, to understand the underlying principles and mechanisms of the measurement procedure. Lack of profound knowledge on the method itself will lead to certain unknown uncertainty contributions not being taken into account and thus to gaps in the uncertainty budget. MU can only be estimated if the method is well understood [43, 53].

Measurement uncertainty is increasingly gaining attention, in particular in the framework of accreditation. The new accreditation standard ISO/IEC 17025 [17], which has been in force from December 2002 on, contains clear requirements on the estimation of MU and when and how it should be stated in test reports. ISO/IEC 17025 requires MU to be reported when required by the client and when relevant to the application and interpretation of the measurement results in the framework of certain specifications or decision limits. The MU should be readily available and reported together with the result as $X \pm U$, where U is the expanded uncertainty [17, 47, 51, 54]. Also Eurachem and CCMAS within the Codex Alimentarius deal with MU as a separate issue [14, 18–20]. Some even claim that MU will become the main unifying principle of analytical data quality [37].

8.2.2.9 Summary

Rather than on the used techniques and methodologies themselves, attention is nowadays paid to the quality and reliability of the final results. This is influenced by

a higher demand for regulatory compliance, a higher consciousness of the customer—the client wants to know the level of confidence of the reported result—and under impulse of new, more exigent European and international standards such as the ISO/IEC 17025 norm for laboratory accreditation. On the basis of quality and reliability of analytical data lies comparability of results between laboratories. In order for results to be comparable, they must be reported with a statement of MU and they must be traceable to common primary references. Methods must be validated to show that they actually measure what they are intended to measure—that they are fit for their specific purpose.

An analytical method is a complex, multistep process, starting with sampling and ending with the generation of a result. Although every method has its specific scope, application, and analytical requirement, the basic principles of QA are the same regardless of the type of method or sector of application. The information in this chapter is mainly taken from the analytical chemistry, but it applies to other sectors as well. The validation of analytical methods, the establishment of traceability of results, and the assessment of MU should be done in a uniform, harmonized way, conforming with internationally recognized standards from institutions such as Eurachem, IUPAC, or ISO.

This update on analytical quality issues a common understanding on the topics of method validation, traceability, and MU of measurements. The interrelationships between method validation and traceability and MU of results have been elucidated. Throughout the landscape of guidelines and standards, the most relevant information was selected, compiled, and summarized. Different approaches are discussed for establishing traceability and assessing MU of analytical methods in general. The importance of both concepts and the link with method validation and analytical quality assurance are highlighted.

8.2.3 METHOD VALIDATION AND QUALITY ASSURANCE

8.2.3.1 Role of Method Validation in AQA

The terms validation and QA are widely used. However, a lot of analysts and laboratories do not know the exact meaning—neither the difference nor the relationship between the two terms. Validating a method is investigating whether the analytical purpose of the method is achieved, which is obtaining analytical results with an acceptable uncertainty level [4]. Analytical method validation forms the first level of QA in the laboratory (Figure 6). AQA is the complete set of measures a laboratory must undertake to ensure that it is able to achieve high-quality data continuously. Besides the use of validation and/or standardized methods, these measures are effective IQC procedures (use of reference materials, control charts, etc.), participation in proficiency-testing schemes, and accreditation to an international standard, normally ISO/IEC 17025 [2, 4, 6].

The different levels in Figure 6 represent the different measures a laboratory must undertake in order to ensure that it is qualified and competent to perform analytical measurements which satisfy their agreed requirements. A laboratory must be capable of providing analytical data of the required quality. The “agreed requirement” of an analytical method and the “required quality” of an analytical result refer to the fitness for purpose of the method [4, 8, 15].

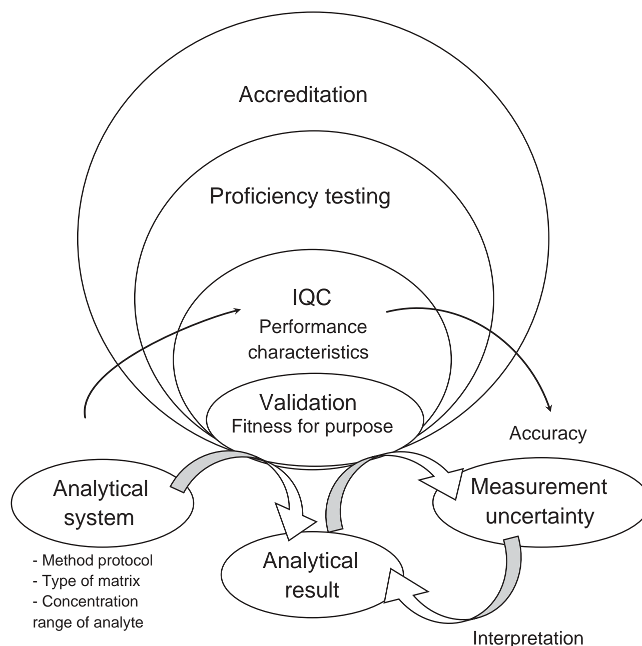

FIGURE 6 Different levels of QA measurements for analytical chemistry and food laboratories [4, 8, 15].

The ISO definition of validation is "confirmation by examination and provision of objective evidence that the particular requirements of a specified intended use are fulfilled" [15]. Method validation is needed to "confirm the fitness for purpose of a particular analytical method," that is, to demonstrate that "a defined method protocol, applicable to a specified type of test material and to a defined concentration rate of the analyte"—the whole is called the "analytical system"—"is fit for a particular analytical purpose" [4]. This analytical purpose reflects the achievement of analytical results with an acceptable standard of accuracy. An analytical result must always be accompanied by an uncertainty statement, which determines the interpretation of the result (Figure 6). In other words, the interpretation and use of any measurement fully depend on the uncertainty (at a stated level of confidence) associated with it [8]. Validation is thus the tool used to demonstrate that a specific analytical method actually measures what it is intended to measure and thus is suitable for its intended purpose [11, 55, 56].

Validation is required in the first place for any new method. As the definition says, validation always concerns a particular analytical system. This means that for a particular type of material and a particular operating range of concentrations, the method must be able to solve a particular analytical problem [4]. As a consequence, *revalidation* is needed whenever any component of the analytical system is changed or if there are indications that the established method does not perform adequately anymore [15, 56, 57].

Method validation is closely related to method development. When a new method is being developed, some parameters are being evaluated already during the *development stage* while in fact this forms a part of the *validation stage* [15]. On the other hand, a validation study may indicate that a change in the method protocol is necessary, which may then require revalidation [58].

Before any method validation is started, the scope of validation must be fixed, comprising both the *analytical system* and the *analytical requirement*. A description of the analytical system includes the purpose and type of method, the type and concentration range of analyte(s) being measured, the types of material or matrices for which the method is applied, and a method protocol. On the basis of a good analysis lies a clear specification of the analytical requirement. The latter reflects the minimum fitness-for-purpose criteria or the different performance criteria the method must meet in order to solve the particular problem. For example, a minimum precision (RSD, see below) of 5% may be required or a limit of detection (LOD) of 0.1% (w/w) [2, 4, 15, 58]. The established criteria for performance characteristics form the basis of the final acceptability of analytical data and of the validated method [58].

Validation of a new analytical method is typically done at two levels. The first is the level of prevalidation, aiming at fixing the scope of the validation. The second level is an extensive, "full" validation performed through a collaborative trial or interlaboratory study. The objective of full validation, involving a minimum number of laboratories, is to demonstrate that the method performs as was stated after the prevalidation.

8.2.3.2 Guidelines and Guidance on AQA

As shown in Figure 6, using validated methods is the first level of QA required within a system of IQC. The latter is needed for participance in proficiency-testing schemes (PTSs), which in turn form a prerequisite for accreditation [4].

For the different levels of QA presented in Figure 6, guidelines and requirements are well described in detail by several regulatory bodies, standardization agencies, and working groups or committees. Just as for traceability and MU (Section 8.2.2), relevant guidelines are given in Table 3. Eurachem guides are published on quality in the laboratory in general [2], method validation [15], and proficiency testing [59]. A guideline on proficiency testing from the joint Eurachem–Eurolab–EA group is in addition available [60]. On the European level there is also the CEN, working through different technical committees and working groups on standardization of analytical methods for all sectors [61].

On the international level, we distinguish IUPAC, ISO, and the AOAC International. All three bodies develop validation and standardization frameworks for analytical chemistry. AOAC International introduced the AOAC Peer Verified Methods Program [62]. Different harmonized guidelines and protocols are developed by the IUPAC, ISO, and AOAC International together [4, 8, 63–67], in addition to a number of ISO standards [68–71]. The FDA, USP, and ICH developed guidelines specific for pharmaceutical and biotechnological methods [55, 72–74].

The international Codex Alimentarius Commission within the United Nations FAO/WHO Food Standards Program has a CCMAS. CCMAS works out criteria

TABLE 3 Overview of European and International Regulatory Bodies and Their Guidelines and Standards on Different Aspects of Analytical QA

Body	Full Name	Guidance On	References
Eurachem/CITAC and EA	A Focus for Analytical Chemistry in Europe/Cooperation of International Traceability in Analytical Chemistry and European Cooperation for Accreditation	Method validation, Proficiency testing, QA, accreditation	2, 15, 59–60
CEN	European Committee for Normalization	Standardization	61
IUPAC, ISO, and AOAC International	International Union of Pure and Applied Chemistry, International Organisation for Standardization, Association of Official Chemists	Method validation, Standardization, internal quality control, proficiency testing, accreditation	4, 8, 62–71
FDA, USP, and ICH	U.S. Food and Drug Administration, U.S. Pharmacopeia, International Conferences on Harmonization	Method validation	55, 72–74
FAO/WHO: Codex/ CCMAS	Food and Agricultural Organization/World Health Organisation: Codex Committee on Methods of Analysis and Sampling	Method validation	21, 75–78
ILAC	International Laboratory Accreditation Cooperation	Proficiency testing, accreditation	79–81

for evaluating the acceptability of methods of analysis as well as guidelines on single-laboratory and interlaboratory validation of methods [75–78]. For single-laboratory validation, CCMAS defends the harmonized IUPAC guidelines [4]. On the international level, also ILAC provides guidelines on proficiency testing [79] and accreditation [80, 81] (Table 3).

8.2.3.3 Approaches for Evaluating Acceptable Methods of Analysis

The purpose of an analytical method is the deliverance of a qualitative and/or quantitative result with an acceptable uncertainty level. Therefore, theoretically, “validation” boils down to “measuring uncertainty”. In practice, method validation is done by evaluating a series of method performance characteristics, such as precision, trueness, selectivity/specificity, linearity, operating range, recovery, LOD, limit of quantification (LOQ), sensitivity, ruggedness/robustness, and applicability. Calibration and traceability have been mentioned also as performance characteristics of a method [2, 4]. To these performance parameters, MU can be added, although MU is a key indicator for both fitness for purpose of a method and constant reliability of analytical results achieved in a laboratory (IQC). MU is a comprehensive parameter covering all sources of error and thus more than method validation alone.

In practice, data from method validation and collaborative studies form the basis for but are only a part of MU estimation. MU is thus more than just a "method performance parameter," as described extensively in Section 8.2.2. Over the years, the concept of MU has won attention in all analytical areas and this has led to two different approaches currently accepted and used for analytical method validation.

The traditional *criteria approach* is to identify specific performance parameters and to assign numeric values to these. These numeric values represent cutoff or threshold values the method parameters must meet in order for the method to be acceptable. The alternative approach is focused on fitness for purpose and MU. In this *fitness-for-purpose approach*, the overall MU is estimated as a function of the analyte concentration (see Section 8.2.2).

Generally, the criteria approach is used for rational methods, that is, methods where the measurement result can be obtained independently of the method used. Opposite to rational methods, there are empirical methods in which the measured value depends on the method used. For empirical methods, the criteria approach cannot simply be applied. Instead, precision data from collaborative studies are normally used as a basis for MU estimation and validation [75, 76].

Validation is needed to demonstrate that the analytical method complies with established criteria for different performance characteristics [82]. When these different characteristics are being evaluated individually, this is generally done for the analytical method as such—where the input is the purified or isolated analyte and the output is the analytical result. However, MU covers the whole analytical procedure, starting from the original sample lot. The assessment of MU (see Section 8.2.2) is in line with the so-called modular validation approach. Modular validation refers to the "modularity" of an analytical procedure divided up into several sequential steps needed to analyze the material. These may be sample preparation, analyte extraction, and analyte determination (Figure 7). Each step in the procedure can be seen as an analytical system and can thus be validated separately and combined

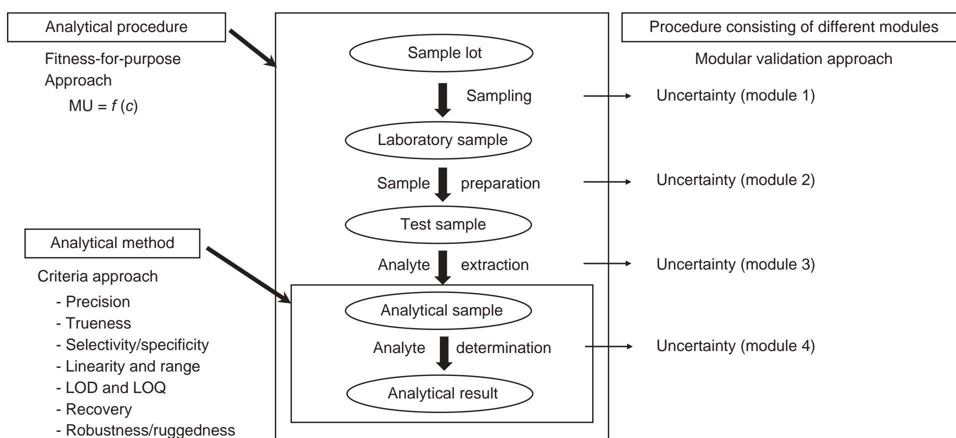

FIGURE 7 Schematic representation of "analytical method" in "analytical procedure" and different approaches for validation: $f(c)$ = function of concentration.

later on with other "modules" in a flexible way. Modular validation is thus a stepwise validation of a whole procedure, taking into consideration all possible difficulties or uncertainty factors at each level in the procedure. The concept of modular validation originates from the domain of predictive microbiology and is now being proposed for methods of analysis for genetically modified organisms (GMOs) [83]. The relationship between the three validation approaches described above is shown in Figure 7.

8.2.3.4 Method Performance Characteristics and Criteria Approach

Extent of Validation Depends on Type of Method On the one hand, the extent of validation and the choice of performance parameters to be evaluated depend on the status and experience of the analytical method. On the other hand, the validation plan is determined by the analytical requirement(s) as defined on the basis of customer needs or as laid down in regulations. When the method has been fully validated according to an international protocol [63, 68] before, the laboratory does not need to conduct extensive in-house validation studies. It must only verify that it can achieve the same performance characteristics as outlined in the collaborative study. As a minimum, precision, bias, linearity, and ruggedness studies should be undertaken. Similar limited validation is required in cases where it concerns a fully validated method which is applied to a new matrix, a well-established but noncollaboratively studied method, and a scientifically published method with characteristics given. More profound validation is needed for methods published as such in the literature, without any characteristic given, and for methods developed in-house [84].

Which performance criteria have to be evaluated depends also on the purpose of the method. Different ICH/USP guidelines are set up for (1) identification tests, (2) impurity tests, and (3) assay tests. An identification test ensures the identity of an analyte in a sample by comparison to a known reference material. An impurity test is intended to confirm the identity of (limit impurity test) or to accurately quantify (quantitative impurity test) an impurity, defined as an entity which may normally not be present. An assay test finally implies the major component or active ingredient in a sample and quantifies the drug substance as such as a whole or the drug substance in a drug product.

For an assay test, where the major component or active ingredient is supposed to be present at high levels, other criteria should be investigated than for an impurity test. The same is valid for quantitative tests versus identification and limit impurity tests (Table 4) [55, 56, 72, 85].

The literature gives a wide range of practical guidelines for the evaluation of method performance characteristics [58]. Besides the diversity of approaches, also the terminology and way of reporting results vary widely. Differences may occur depending on the purpose and the application field of the method, and validation studies may become more difficult as the complexity of the analysis increases [86]. In what follows, terms and formulas are taken from the accepted IUPAC nomenclature for the presentation of results of chemical analysis [66]. For each validation parameter, definitions, ways of expression, determination guidelines, and acceptance criteria are reported in Table 5.

TABLE 4 Criteria to Establish for Different Categories of Methods of Analysis

Method Performance Parameter	Identification Test	Impurity Test: Limit Impurity Test	Impurity Test: Quantitative Impurity Test	Assay Test
Precision	–[a]	–	+	+
Trueness	–	–[a]	+	+
Specificity	+	+	+	+
LOD	–[a]	+	–	–
LOQ	–[a]	–	+	–
Linearity	–[a]	–	+	+
Range	–[a]	–[a]	+	+
Ruggedness	+	+	+	+

Source: From ref. 56.

[a]May be performed.

Accuracy Precision and bias studies, which form a part of the MU estimate, are the most important validation criteria.

Precision measures are divided into (1) repeatability precision measures s or SD (s_r or SD_r) and RSD (RSD_r), (2) intralaboratory reproducibility precision or intermediate-precision measures SD and RSD, and (3) interlaboratory reproducibility precision s or SD (s_R or SD_R) and RSD (RSD_R) [66].

Besides standard deviations and coefficients of variation, repeatability/reproducibility values or limits (r, R) are additional parameters of high value in the assessment of precision (see formulas in Table 5). These criteria mean that the absolute variation between two independent results—obtained within the same laboratory respectively between different laboratories—may only exceed the values of r and R in a maximum 5% of the cases [2]. Another measure for the precision is the confidence interval, in which all measurements fall with a certain propability or confidence level $1 - \alpha$ (α is often 0.05 giving a probability here of 95%) [66].

Calculated repeatability, intermediate precision, and reproducibility values can be compared with those of existing methods. If there are no methods with which to compare the precision parameters, theoretical relative reproducibility and repeatability standard deviations can be calculated from the Horwitz equation and the Horrat value (Table 5). Horwitz RSD values are reported in Table 6. Higher variability is expected as the analyte levels approach the detection limit (see below). Next to the Horwitz equation, the AOAC's Peer Verified Program proposes its own levels of acceptability of %RSD as a function of analyte concentration level [56, 72].

Precision data can be documented in bar charts or control charts such as Shewhart control charts (see the discussion of internal quality control in Section 8.2.3.5). Bar charts plot %RSD values with their corresponding confidence interval. Control charts plot the individual measurement results and the means of sets of measurements with their confidence level (or with horizontal lines representing "limits," see below) as a function of the measurement number and the run number, respectively [15, 55, 56, 58, 72, 85].

TABLE 5 Summary of Method Performance Parameters

Parameter	Definition	Expression	Requirements	Practical Assessment
Accuracy	Closeness of agreement between test result and accepted reference value			
1. Precision	Closeness of agreement between independent test results obtained under stipulated conditions	• Standard deviation s or SD • Relative standard deviation s_{rel} or RSD • % coefficient of variation %CV or % • RSD = $100 \times SD/\bar{x}$ • repeatability limit $r = 2.83 \times SD_r$ and reproducibility limit $R = 2.83 \times SD_R$ • confidence interval CI = $\bar{x} \pm C$, where $C = \frac{s \times t_{p,\nu}}{\sqrt{n}}$	• RSD_r = 0.5 at 0.6 times theoretical values determined by Horwitz function = 2 exp (1–0.5log/C) with C = analyte concentration in decimal fraction • RSD_R = 0.5 at 2 times theoretical values determined by Horwitz function • Horrat value = RSD_R (trial)/RSD_R(Horwitz) ≤ 2 • RSD_r and RSD_R values according to AOAC peer-verified program	• Minimum of three concentration levels over full range of analytical method (~at LOD, in middle, high) • Minimum of three repeats per concentration level • Calculate repeatability precision SD_r, RSD_r, $r = 2.8 \times SD_r$, C, CI • Calculate intermediate precision SD_{int}, RSD_{int}, $r = 2.8 \times SD_{int}$, C, CI • Calculate reproducibility precision SD_R, RSD_R, $r = 2.8 \times SD_R$, C, CI • Document in bar chart or control chart
1.1. Repeatability precision (intrarun precision)	*Precision under conditions where independent test results are obtained with same method on identical test items in same laboratory by same operator using same equipment within short intervals of time*			
1.2. Intermediate precision (interrun precision)	*Precision under conditions where independent test results are obtained with same method on identical test items in same laboratory but by different analysts using different equipment over extended period of time*			
1.3. Reproducibility precision (interlaboratory precision)	*Precision under conditions where test results are obtained with same method on identical test items in different laboratories with different operators using different equipment*			

2. Trueness	Closeness of agreement between expectation of test result (expected mean value) and accepted reference value (true value)	*If CRMs are used*: Bias = % error: difference between measured value and true value Z score: difference between measured value and certified reference value % $z = \frac{X_{\text{found}} - X_{\text{certified}}}{\sqrt{\frac{\text{SD}_{\text{found}}}{n_{\text{found}}} + \left(\frac{\text{SD}_{\text{certified}}}{n_{\text{certified}}}\right)^2}} = \frac{X_{\text{found}} - X_{\text{certified}}}{\sqrt{\frac{\text{SD}_{\text{found}}}{n_{\text{found}}} + \left(\frac{\text{CI}}{2}\right)^2}}$ *If no CRMs are available*: Percent recovery of known, spiked amount of analyte	*General*: compare results with second, validated reference method *If CRMs are used*: Z score ≤ |2| *If spiking method is used*: Percent recovery according to USP/ICH guidelines (depending on analyte concentration level)	• Minimum of three concentration levels over full range of analytical method (~at LOD, in middle, high) • Minimum of three at 10 repeats per concentration level • *Use of CRMs*: calculate bias (% error) and/or Z score • *No CRMs*: spike typical matrix and blank sample with known amount of analyte; calculate %recovery = 100 × [$\text{spike}_{\text{matrix sample}}/\text{spike}_{\text{blanco}}$]
Recovery	Fraction of analyte added to test sample (fortified or spiked) prior to analysis, which is measured by method	Percent recovery of known, spiked amount of analyte	Percent recovery according to USP/ICH guidelines (depending on analyte concentration level)	• Minimum of six repeats of matrix blanks or samples unfortified and fortified with analyte at different concentrations • Calculate %recovery = 100 × [$\text{spike}_{\text{matrix sample}}/\text{spike}_{\text{blanco}}$]

TABLE 5 ***Continued***

Parameter	Definition	Expression	Requirements	Practical Assessment
Specificity	Ability of method to determine accurately and specifically analyte of interest in presence of other components in sample matrix (that may be expected to be present in sample matrix) under stated conditions of test (specificity = 100% selectivity)	*Note*: cannot be expressed, must be demonstrated; depends on type and purpose of method *For identification tests*: Percent correct classification of • Non-analyte-containing samples as negatives • Analyte-containing samples as positives Ability to discriminate with compounds of closely related structures (negative results) *For quantitative tests*: Percent recovery of samples, spiked with possible interferants	*For identification tests*: Percent correct classification~100 *For quantitative tests*: Percent recovery according to USP/ICH guidelines (depending on analyte concentration level)	*Identification tests*: • Determine percentage of false positives for minimum number of blank samples • Determine percentage of false negatives for minimum number of positive samples (RMs) • Test minimum number of structurally similar or closely related samples which must be negative *Impurity and assay tests*: • Spike different samples with possible interferents and calculate percentage of recovery • If no interfering compounds are available, compare with second method

Limit of detection (LOD)	Lowest concentration or amount of analyte: • that can be reliably distinguished from zero or • that can be detected/ measured with reasonable statistical certainty	• *Note*: LOD is expressed as concentration or amount of analyte, which is derived from measured signal • Lowest signal: $x_L = x_{bl} + 3s_{bl}$ • Lowest concentration/ amount: LOD = $q_L(c_L) = x_L/S = 3s_{bl}/S$ • *Note*: signal of three times SD_{blanco} corresponds to %RSD of 33%		• Measure, each once, a minimum of either 10 independent sample blanks ($x_L = x_{bl} + 3s_{bl}$) or 10 independent sample blanks fortified at lowest acceptable concentration ($x_L = 3s_x$) • Choose number of low concentration levels near the expected LOD • Set up calibration curve: signal $x = A$ concentration $+ B$ and thus $x_L = A$LOD • Determine slope A and calculate LOD = $x_L/A = [x_{bl} + 3s_{x,bl}]/A$
Limit of quantitation (LOQ)	Lowest concentration or amount of analyte that can be determined quantitatively with acceptable level of repeatability, precision, and trueness	• *Note*: LOQ is expressed as concentration or amount of analyte, which is derived from measured signal • Lowest signal: $x_L = x_{bl} + 10s_{bl}$ • Lowest concentration/ amount: LOQ = $q_L(c_L) = x_L/S = 10s_{bl}/S$ • *Note*: a signal of 10 times SD_{blanco} corresponds to % RSD of 10%		• Measure minimum of 10 independent sample blanks each once ($x_L = x_{bl} + 10s_{bl}$) • Choose number of low concentration levels near calculated LOD • Set up calibration curve: signal $x = A$ concentration $+ B$ and thus $x_L = A$LOQ • Determine slope A and calculate LOQ = $x_L/A = [x_{bl} + 10s_{bl}]/A$

TABLE 5 ***Continued***

Parameter	Definition	Expression	Requirements	Practical Assessment
Linearity Linear range = working range = linearity limits	Ability of method to obtain test results proportional to concentration of analyte (within given range) Range of concentrations/ amounts of analyte over which • method gives test results proportional to concentration of analyte or • linear calibration model can be applied with known confidence level	• *Note*: *linearity* cannot be expressed, must be demonstrated • *Range*: a range of concentrations between lower and upper linearity limits	*For assay tests*: range = 80–120% of analyte level *For impurity tests*: range = LOQ 50–150% or 0–150% according to USP/ICH and IUPAC guidelines Lower linearity limit = LOQ	• First rough estimation of linear range: measure blank + minimum of six independent concentration levels, set up calibration curve and estimate linear range visually • Choose CRMs or spiked samples of minimum of six different concentrations within estimated linear range • Calculate residual Y values from calibration curve and plot them as funcion of concentration • If randomly distributed → linearity; if *systematic* trends → non-linearity • Calculate relative signals = signal/concentration and plot them as function of concentration • If horizontal line → linearity; linearity limits correspond to 95% and 105% relative signal values →*range*

Ruggedness	The (intralaboratory tested) behavior of analytical process when small changes in environmental and/or operating conditions are made (generally used term)	*Ruggedness*: measure for variability (reproducibility of results obtained under variety of conditions); expressed as %RSD (interlaboratory)		• Evaluate effect of different small changes in paramters (in days, instruments, analysts, reagents, material, amount of sample material used, etc.) individually • Calculate precision data
Robustness	Measure of capacity of analytical procedure to remain unaffected by small but deliberate variations in method performance parameters, which provides an indication of its reliability during normal usage (term used by USP/ICH only)			
Sensitivity	Change in response of measuring instrument divided by corresponding change in stimulus	Slope of calibration curve (arbitrary)		

Source: From refs. 4, 15, 21, 55–56, 72, 75, and 76.

Note: Definitions, ways of expression, requirements or acceptance criteria, and guidelines for practical assessment (for more details, see text).

$t_{p,v}$ is the Student factor corresponding to the confidence level $1 - \alpha$ and v degrees of freedom. The symbol p represents the percentile or percentage point of the t distribution. For one-sided intervals, $p = 1 - \alpha$; for two-sided intervals, $p = 1 - \alpha/2$. Values of t can be found in the IUPAC nomenclature ($t = 2.776$ for $n = 5$ and $t = 3.182$ for $n = 4$ at $p = 0.95$) [67].

X is the mean determined value and n is the number of measurements for which the SD was calculated. If SD data of the certified reference materials are not available, 95% confidence limits may be used as an estimate of CRM SD (see second form of formula for z score) [21].

x_{bl} is the mean of the blank measurements, s_{bl} is the SD on the blank measurements and S is the sensitivity of the method or the slope of the calibration function. The calibration function is the relationship between the measured response x_L and the concentration c_L or amount q_L[56, 72, 95–96].

TABLE 6 Horwitz Function

Analyte Percent	Analyte Ratio	Unit	Horwitz %RSD	AOAC PVM
100	1	100%	2	1.3
10	1×10^{-1}	10%	2.8	2.8
1	1×10^{-2}	1%	4	2.7
0.1	1×10^{-3}	0.10%	5.7	3.7
0.01	1×10^{-4}	100 ppm	8	5.3
0.001	1×10^{-5}	10 ppm	11.3	7.3
0.0001	1×10^{-6}	1 ppm	16	11
0.00001	1×10^{-7}	100 ppb	22.6	15
0.000001	1×10^{-8}	10 ppb	32	21
0.0000001	1×10^{-9}	1 ppb	45.3	30

Note: This function if given as an empirical relationship between the precision of an analytical method and the concentration of the analyte regardless of the nature of the analyte, matrix, and method used. Acceptable RSD_R and RSD_r values according to [75] and to AOAC International [56, 62]; % RSD = percentage relative standard deviation, PVM = peer-verified methods program.

Precision relates to the random error of a measurement system (see Figure 8) and is a component of MU (see also Section 8.2.2 and Figure) [2].

Trueness is expressed in terms of *bias* or percentages of error. Bias is the difference between the mean value determined for the analyte of interest and the accepted true value or known level actually present [87]. It represents the systematic deviation of the measured result from the true result. Method trueness is an indicator also for utility and applicability of that method with real samples [88].

Different sources of systematic errors contribute to the overall bias (Figure 8). Thompson and Wood [8] describe *persistant bias* as the bias affecting all data of the analytical system over longer periods of time and being relatively small but continuously present. Different components contribute to the persistant bias, such as laboratory bias, method bias, and the matrix variation effect. Next to persistant bias, the larger *run effect* is the bias of the analytical system during a particular run [4, 8, 15].

One or more of these bias components are encountered when analyzing RMs. In general, RMs are divided into certified RMs (CRMs, either pure substances/solutions or matrix CRMs) and (noncertified) laboratory RMs (LRMs), also called QC samples [89]. CRMs can address all aspects of bias (method, laboratory, and run bias); they are defined with a statement of uncertainty and traceable to international standards. Therefore, CRMs are considered useful tools to achieve traceability in analytical measurements, to calibrat equipment and methods (in certain cases), to monitor laboratory performance, to validate methods, and to allow comparison of methods [4, 15, 30]. However, the use of CRMs does not necessarely guarantee trueness of the results. The best way to assess bias practically is by replicate analysis of samples with known concentrations such as reference materials (see also Section 8.2.2). The ideal reference material is a matrix CRM, as this is very similar to the samples of interest (the latter is called *matrix matching*). A correct result obtained with a matrix CRM, however, does not guarantee that the results of unknown samples with other matrix compositions will be correct [4, 89].

The usefulness of CRMs for validation (in particular for trueness assessment) and traceability purposes has been debated for years. This is illustrated by the enor-

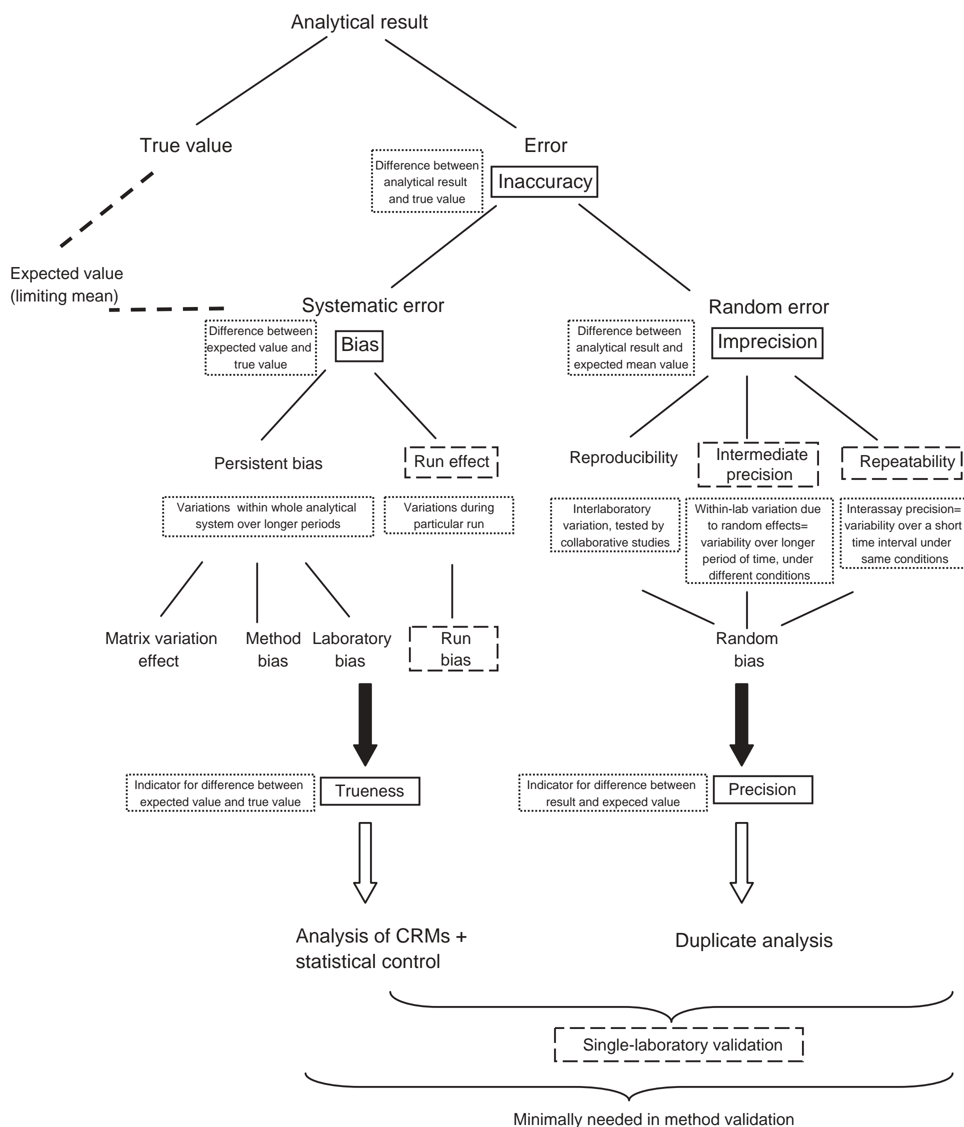

FIGURE 8 Composition of error of analytical result related to accuracy of analytical method [4, 8].

mous number of papers published on this topic. We mention here only some interesting references [89–91]. Examples of the use of pure substance RMs, matrix CRMs, or LRMs can be found in the special issues of *Accreditation and Quality Assurance* (volume 9, 2004) and *Analytical and Bioanalytical Chemistry* (volume 278, 2004) on biological and environmental reference materials and the special issue of *Trends in Analytical Chemistry* (volume 23, 2004) on challenges for achieving traceability of environmental measurements.

If no such (certified) reference materials are available, a blank sample matrix of interest can be "spiked" with a known amount of a pure and stable in-house material, called the *spike* or *surrogate*. Recovery is then calculated as the percentage of

TABLE 7 Acceptable recovery percentages as a function of the analyte concentration [56]

Analyte Percent	Analyte Ratio	Unit	Mean Recovery (%)
100	1	100%	98–102
10	1×10^{-1}	10%	98–102
1	1×10^{-2}	1%	97–103
0.1	1×10^{-3}	0.10%	95–105
0.01	1×10^{-4}	100 ppm	90–107
0.001	1×10^{-5}	10 ppm	80–110
0.0001	1×10^{-6}	1 ppm	80–110
0.00001	1×10^{-7}	100 ppb	80–110
0.000001	1×10^{-8}	10 ppb	60–115
0.0000001	1×10^{-9}	1 ppb	40–120

the measured spike of the matrix sample relative to the measured spike of the blank control or the amount of spike added to the sample. The smaller the recovery percent, the larger the bias which is affecting the method and thus the lower the trueness [4, 56, 72, 92, 93]. An indication of trueness can also be obtained by comparing the method with a second, well-characterized reference method under the condition that the precision of the established reference method is known. Results from the two methods, performed on the same sample or set of samples, are compared. The samples may be CRMs, in-house standards, or just typical samples [15]. For a comparison between the three ways of establishing bias, see also Section 8.2.2 on analytical quality. It should be clear here that the use of recovery estimates and comparing methods are alternative ways which encompass serious limitations. They can give an idea about data comparability, but trueness cannot be assured [89].

Trueness or exactness of an analytical method can be documented in a control chart. Either the difference between the mean and true value of an analyzed (C)RM together with confidence limits or the percentage recovery of the known, added amount can be plotted [56, 62]. Here, again, special caution should be taken concerning the used reference. Control charts may be useful to achieve trueness only if a CRM, which is in principle traceable to SI units, is used. All other types of references only allow traceability to a "consensus" value, which however is assumed not to be necessarely equal to the "true" value [89]. The expected trueness or recovery percent values depend on the analyte concentration. Therefore, trueness should be estimated for at least three different concentrations. If recovery is measured, values should be compared to acceptable recovery rates as outlined by the AOAC Peer Verified Methods Program (Table 7) [56, 62]. Besides bias and percent recovery, another measure for the trueness is the z score (Table 5). It is important to note that a considerable component of the overall MU will be attributed to MU on the bias of a system, including uncertainties on reference materials (Figures 5 and 8) [2].

Recovery is often treated as a separate validation parameter (Table 5). Analytical methods intend to estimate the true value of the analyte concentration with an uncertainty that is fit for the intended purpose. However, in such analytical methods, the analyte is transferred from the complex matrix to a simpler solution whereby there is a loss of analyte. As a consequence, the measured value will be lower than the true concentration present in the original matrix. Therefore, assessing the effi-

ciency of the method in detecting all of the analyte present is a part of the validation process. Eurachem, IUPAC, ISO, and AOAC International state that recovery values should always be established as a part of method validation. Recovery or spiking studies should be performed for different types of matrices, several examples of each matrix type, and each matrix type at different levels of analyte concentration [2, 4, 15].

Specificity and Selectivity Specificity and selectivity both give an idea of the reliability of the analytical method (for definitions see Table 5). Some authors give different definitions for both terms while for others they are identical. The term *specific* generally refers to a method that produces a response for a single analyte only, while the term *selective* is used for a method producing responses for different chemical entities or analytes which can be distinguished from each other. A method is called selective if the response is distinguished from all other responses. In this case, the method is perfectly able to accurately measure an analyte in the presence of interferences [56, 94]. According to Eurachem, specificity and selectivity principally reflect the same characteristic and are related very closely to each other in such a way that specificity means 100% selectivity. In other words, a method can only be specific if it is 100% selective. Another related term is *confirmation of identity*, which is the proof that "the measurement signal, which has been attributed to the analyte, is only due to the analyte and not to the presence of something chemically or physically similar or arising as coincidence" [15]. A method must first show high specificity before true quantification can be performed [87].

There is no single expression form for specificity. It is rather something which must be demonstrated. The way in which this is done depends on the objective and the type of analytical method (see also below). For identification tests, the goal is to ensure the identity of an analyte. Specificity is here the ability to discriminate between compounds of closely related structures which can be present.

For impurity tests (limit impurity test, quantitative impurity test) and assay tests, the accent lies on the ability to determine or discriminate for the analyte in the presence of other interferants. Selectivity can be assessed by spiking samples with possible interferants (e.g., degradation products) [55, 56, 72].

Limit of Detection There is no analytical term or parameter for which there exists a larger variety in terminology and formulations than for the limits of detection and quantification. *Limit of detection* or *detection limit* is the terminology most widely used as accepted by Eurachem. ISO uses *minimum detectable net concentration* while IUPAC prefers *minimum detectable (true) value* [15]. All official organizations however refer to the same definition: the lowest amount of an analyte in a sample which can be detected but not necessarily quantified as an exact value. In general, the LOD is expressed as a concentration c_L or a quantity q_L, derived from the smallest signal x_L which can be detected with reasonable certainty for a given analytical procedure. The lowest signal x_L is the signal which lies k times SD_{blank} above the mean blank value, whereby k is a numerical factor chosen according to the level of confidence required [56, 72, 95–97].

The larger the value of k, the larger the confidence level. Eurachem and IUPAC recommend a value of 3 for k, meaning that the chance that a signal more than $3s$ above the sample blank value is originating from the blank is less than 1%. The

detection limit is thus the concentration or amount corresponding to a measurement level (response, signal) three s_{bl} units above the value for zero analyte (Table 5). At the concentration or amount level of 3 times the s_{bl}, the RSD or coefficient of variation (CV) on the measured signal is 33% (measure for uncertainty) [2, 4, 15, 75, 95, 98]. According to USP/ICH, the detection limit corresponds to that signal where the *signal-to-noise ratio* is 2:1 or 3:1 [72, 85].

It is not true—as is often thought—that detection or quantification is impossible below the determination limit, but at these lower levels, the uncertainty of the detection/quantification measurement is higher than the actual value itself [21]. In this context, Huber [56] defines the limit of detection also as the point at which a measured value is larger than the uncertainty associated with it. According to Krull and Swartz [88], the LOD is a concentration point where only the qualitative identification is possible but no accurate and precise quantification.

For qualitative methods, the LOD is defined as the threshold concentration at which the test becomes unreliable. A series of blank samples, spiked with different concentrations of the analyte, are each analyzed at least 10 times. The threshold, or *cutoff*, concentration is determined visually based on a response curve, plotting the percentage of positive results versus the concentration. In this respect, the LOD is also defined as the concentration at which 95% of the experiments give a clearly positive signal [15].

The LOD may not be confused with the sensitivity of the method. The latter is the capability of the method to discriminate small differences in concentration or mass of the test analyte and is equal to the slope of the calibration curve (see below) [56].

Limit of Quantification For the *limit of quantification*, or *limit of determination*, definitions and formulas are very similar to those of LOD, except that for LOQ, k is taken to be 5, 6, or even 10 [2, 4, 15, 56, 72, 96]. A value of 10 for k means that the %RSD at the limit of quantification is 10%. The LOQ thus corresponds to that concentration or amount of analyte quantifiable with a variation coefficient not higher than 10% [98]. The LOQ is always higher than the LOD and is often taken as a fixed multiple (typically 2) of the detection limit [4]. Also, the determination limit is referred to as the signal 10 times above the noise or background signal, corresponding to a signal-to-noise ratio of 10:1 [72, 85].

In practice, the LOQ can be calculated in an analogous way as for the LOD, as indicated in Table 5. An alternative way of practically assessing the LOD and LOQ is the following. In a first step, 10 independent sample blanks are measured each once, the blank standard deviation s_{bl} is calculated, and the lowest signals corresponding to both the LOD and the LOQ are calculated as $x_{LOD} = x_{bl} + 3s_{bl}$ and $x_{LOQ} = x_{bl} + 10s_{bl}$, respectively. In a second step, sample blanks are spiked with various analyte concentrations (e.g., 6) close to the LOD. Per concentration, 10 independent replicates are measured and the standard deviation of the measured signals calculated. These standard deviations s (or the %RSD) are then plotted against the concentration. LOD and LOQ values are those concentrations of analyte corresponding to %RSD values of 33 and 10%, respectively [15, 21].

As is said for the LOD, neither is it true that at and below the LOQ quantification becomes impossible. Quantification is possible; however it becomes unreliable as the uncertainty associated with it at these lower levels is higher than the measure-

ment value itself. Quantification becomes reliable as soon as the MU is lower than the value measured [21].

Decision Limit and Detection Capability: For Specific Sectors Only In the context of analytical method validation, the terms *decision limit* (CC_α) and *detection capability* (CC_β) as well as *minimum required performance limits* (MRPLs) are often used and need some clarification. These terms are applicable for the measurement of organic residues, contaminants, and chemical elements in live animals and animal products, as regulated within the European Union (EU) by directives 96/23/EC [99], 2002/657/EC [82], and 2003/181/EC [100]. The Commission distinguishes group A substances for which no *permitted limit* (*maximum residue level*, MRL) has been established and group B substances having a fixed permitted limit.

The decision limit CC_α is the limit at and above which it can be concluded with an error probability of α that a sample is *noncompliant*. If a permitted limit (PL) has been established for a substance (group B or the regulated compounds), the result of a sample is noncompliant if the decision limit is exceeded ($CC_\alpha = x_{PL} + 1.64s_{MRL}$). If no permitted limit has been established (group A), the decision limit is the lowest concentration level at which the method can discriminate with a statistical certainty of 1-α that the particular analyte is present ($CC_\alpha = x_{bl} + 2.33s_{sample}$). The detection capability CC_β is the smallest content of the substance that may be detected, identified, and/or quantified in a sample with an error probability of β ($CC_\beta = CC_\alpha + 1.65s_{sample}$).

Minimum required performance limits have been established for substances for which no permitted limit has been fixed and in particular for those substances whose use is not authorized or even prohibited within the EU (group A). A MRPL is the minimum content of an analyte in a sample which at least has to be detected and confirmed. A few MRPLs for residues of certain veterinary drugs have been published so far in directive 2003/181/EC.

For group A substances (no PL established), CC_α and CC_β are comparable with LOD and LOQ, respectively, as their concentrations correspond to measured signals laying *y* times above the blank signal. For substances having a PL (group B), CC_α and CC_β are not related to LOD and LOQ but are expressed in relation to this PL. It is important to note that these terms apply specifically for inspection of animals and fresh meat for the presence of residues of veterinary drugs and specific contaminants and are therefore different from LOD and LOQ [82, 99–102].

Linearity and Range For assessment of the linearity of an analytical method, linear regression calculations do not suffice. In addition, residual values should be calculated (Table 5). The latter represent the differences between the actual *y* value and the *y* value predicted from the regression curve for each *x* value. If residual values, calculated by simple linear regression, are randomly distributed about the regression line, linearity is confirmed, while systematic trends indicate nonlinearity. If such a trend or pattern is observed, this suggests that the data are best treated by weighted regression. For either simple or weighted linear regression, linearity supposes that the intercept is not significantly different from zero [4, 15, 21, 75].

An alternative approach to establish linearity is to divide the response by the respective concentrations and to plot these "relative responses" as a function of the concentration on a log scale. The obtained line should be horizontal over the full

linear range, with a positive deviation at low concentrations and a negative deviation at higher concentrations. By drawing parallel horizontal lines, corresponding to, for example, 95 and 105% of the horizontal relative response line, the intersection points can be derived at which the method becomes nonlinear [56].

It is important that a linear curve is repeatable from day to day. However, linear ranges may be different for different matrices. The reason for this is a possible effect of interferences inherent to the matrix. A test for general matrix effects can be performed by means of "standard additions" or the method of analyte additions. For a set of samples, obtained by adding different concentrations of analyte to a certain matrix, the slope of the calibration curve is compared with the slope of the usual calibration function. A lack of significance (curves are parallel) means that there is no matrix effect [21, 75].

Ruggedness and Robustness Although the terms *ruggedness* and *robustness* are often treated as the same and used interchangeably, separate definitions exist for both terms, as indicated in Table 5.

To have an idea about the ruggedness, Eurachem recommends to introduce deliberate variations to the method, such as different days, analysts, instruments, and reagents and variations in sample preparation or sample material used. Changes should be made separately and the effect evaluated of each set of experimental conditions on the precision and trueness [4, 15, 85]. To examine the effects of different factors, a "factorial design" methodology can be applied, as described by von Holst et al. [103]. By combining changes in conditions and performing a set of experiments, one can determine which factors have a significant or even critical influence on the analytical results. In ICH/USP guidelines, ruggedness is not defined separately but treated under the same denominator as reproducibility precision: It is "the degree of reproducibility of the results obtained under a variety of conditions, expressed as %RSD" [56].

Robustness is a term introduced by USP/ICH [88]. Although Eurachem has included the term robustness in its official list of definitions, the term is not used by official organizations other than USP/ICH. According to Eurachem, both parameters do present the same and are thus synonyms [15, 72, 85].

Sensitivity The sensitivity of a method is the gradient of the response curve. In practical terms, sensitivity refers to the slope of the calibration curve. Sensitivity is often used together with detection and quantification limits. Indeed, the slope of the calibration curve is used for the calculation of limits of detection and quantification. A method is called sensitive if a small change in concentration or amount of analyte causes a large change in the measured signal [4, 15, 21]. Sensitivity is not always mentioned as a validation parameter in official guidelines. According to Thompson et al. [4], it is not useful in validation because it is usually arbitrary, depending on instrument settings. USP/ICH does not mention sensitivity at all.

8.2.3.5 Analytical Quality Assurance

Quality assurance is the complete organizational infrastructure that forms the basis for all reliable analytical measurements [8]. It stands for all the planned and systematic activities and measures implemented within the quality system [2, 104]. A quality system has a quality plan, which emphasizes the implementation of good

laboratory practices (GLPs). GLPs are comparable to the good manufacturing practices (GMPs) and the larger HACCPs (hazard analysis critical control points) quality systems of food production factories. Attention goes to all aspects of quality management in the laboratory organization, including staff training, the maintenance and calibration of all equipment used, the laboratory environment, safety measures, the system of sample identification, recordkeeping, and storage—the latter may be simplified by the use of a laboratory information management system (LIMS), the use of validated and standardized methods, and the documentation of these methods and of all information concerning the followed procedures (standard operating procedures, or SOPs).

Quality assurance embraces both QC and "quality assessment." QC is defined as the mechanism or the practical activities undertaken to control errors, while quality assessment is the mechanism to verify that the system is operating within acceptable limits. Quality assessment and control measures are in place to ensure that the measurement process is stable and under control [2, 8].

Within QC, internal and external quality control are distinguished. In general, QA comprises the following topics as also schematized in Figure 6.

Use of Validated Methods: In-House Versus Interlaboratory Validation Wherever possible or practically achievable, a laboratory should use methods which have been "fully validated" through a collaborative trial, also called interlaboratory study or method performance study. Validation in collaborative studies is required for any new analytical method before it can be published as a standard method (see below). However, single-laboratory validation is a valuable source of data usable to demonstrate the fitness for purpose of an analytical method. In-house validation is of particular interest in cases where it is inconvenient or impossible for a laboratory to enter into or to organize itself a collaborative study [4, 5].

On the one hand, even if an in-house validated method shows good performance and reliable accuracy, such a method cannot be adopted as a standard method. In-house validated methods need to be compared between at least eight laboratories in a collaborative trial. On the other hand, a collaborative study should not be conducted with an unoptimized method [58]. Interlaboratory studies are restricted to precision and trueness while other important performance characteristics such as specificity and LOD are not addressed [105]. For these reasons, single-laboratory validation and interlaboratory validation studies do not exclude each other but must be seen as two necessary and complementary stages in a process, presented in Figure 9. The added value of single-laboratory validation is that it simplifies the next step—interlaboratory validation—and thereby minimizes the gap between internally (validated or not) developed methods and the status of interlaboratory validation. By optimizing the method first within the laboratory, as a kind of preliminary work, an enormous amount of collaborators' time and money is saved [58].

The importance of conducting such a single-laboratory preliminary validation step is increasingly highlighted by international standardization agencies. The IUPAC and AOAC International include a preliminary work paragraph in their guidelines for collaborative studies, stating that within-laboratory testing data are required on precision, bias, recovery, and applicability. Additionally, a clear description of the method, including statements on the purpose of the method, the type of method, and the probable use of the method, is required within this preliminary work [62, 63]. It is however not only in the harmonized guidelines for collaborative

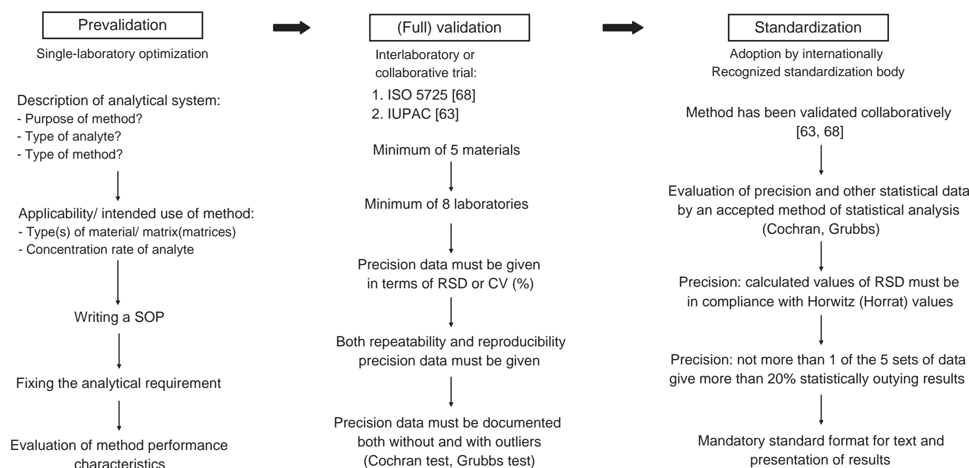

FIGURE 9 Hierarchy of, relationship between, and objectives and requirements for prevalidation [106], validation [62, 63, 68], and standardization of analytical methods [62, 63, 67, 68, 75, 84]: RSD = relative standard deviation, CV = coefficient of variation, SOP = standard operating procedure.

trials (see below) that the link between a single-laboratory prevalidation step and the collaborative trial is emphasized. Separate guidelines for single-laboratory validation of methods of analysis have recently been published by the IUPAC, ISO, and AOAC International [4]. The IUPAC guidelines have also been considered and accepted by the CCMAS [77]. In addition, specific, individual working groups or scientists are presenting their own framework for pre-, single-laboratory validation of methods of analysis. The latter do not concern official, published guidelines but can be found on the Internet [see, e.g., 58, 85] or are distributed through national or international specific working groups. The objectives of a single-laboratory or in-house validation process are depicted in Figure 9. Depending on the type of method (Table 4), data can be obtained for all criteria except for the reproducibility (interlaboratory) precision. However, it is this *among-laboratories variability* or reproducibility which is the dominating error component in analytical measurement and which underlies the need for interlaboratory validation [106].

Interlaboratory or collaborative validation studies can be organized by any laboratory, institute, or organization but should preferably be conducted according to one of the following recognized protocols: (1) ISO 5725 on Accuracy (Trueness and Precision) of Measurement Methods and Results [68] or (2) IUPAC Protocol for the Design, Conduct and Interpretation of Method-Performance Studies [63]. The latter revised, harmonized guidelines have been adopted also by AOAC International as the guidelines for the AOAC Official Methods Program [62]. The main requirements for collaborative studies outlined in these guidelines are shown in Figure 9.

Precision plays a central role in collaborative studies. Wood [84] defines a collaborative trial as "a procedure whereby the precision of a method of analysis may be assessed and quantified." Precision is the objective of interlaboratory validation studies, and not trueness or whichever other method performance parameter. Evalu-

ation of the acceptability of precision data is important for the standardization of methods (see below).

Use of Standardized Methods The first level of AQA is the use of validated or standardized methods. The terms *validated* and *standardized* here refer to the fact that the method performance characteristics have been evaluated and have proven to meet certain requirements. At least, precision data are documented, giving an idea of the uncertainty and thus of the error of the analytical result. In both validated and standardized methods, the performance of the method is known.

Validated methods can be developed by the laboratory itself or by a standardization organization after interlaboratory studies. Standardized methods are developed by organizations such as the AOAC International, ISO, USP (see Table 3), U.S. Environmental Protection Agency (EPA), American Society for Testing and Materials (ASTM), or Food Standards Association (FSA) [56]. Here exactly lies the difference between a validated and a standardized method: An analytical method can only be standardized after it has been validated through interlaboratory comparisons. The main prerequisite for a standards organization is that a method has been adequately studied and its precision shown to meet a required standard, as summarized in Figure 9. The format of a standard method as outlined in ISO Guide 78/2 [58] is shown in Table 8 [2]. A specific IUPAC protocol [67] describes in detail how to present AQA data such as the performance characteristics.

TABLE 8 Mandatory Text Format for Standardized Methods

1. Scope	States briefly what method determines
2. Definitions	Precise definition of analyte or parameter determination by method
3. Fields of application	Type of materal(s)/matrix(ces) to which method is applicable
4. Principle	Basic steps involved in procedure
5. Apparatus	Specific apparatuses required for determination are listed
6. Reagents	Analytical reagent-grade reagents needed for determination are listed
7. Sampling	
8. Procedure	Divided into numbered paragraphs or subclauses, includes a preparation-of-test-sample step and reference to QA procedures
9. Calculation and expression of results	Indication of how final results are calculated and units in which results are to be expressed
10. Notes	Additional information as to procedure; may be in form of notes, placed here or in body of text
Annex	Includes all information on analytical quality control, such a precision clauses (repeatability and reproducibility data), table of statistical data outlining accuracy (trueness and precision) of method
References	Reference to published report on collaborative study which was carried out prior to standardization of method

Source: According to ISO Guide 78/2 [2, 58].

Effective Internal Quality Control (IQC) In the IUPAC harmonized guidelines for IQC, Thompson and Wood [8] define IQC as a "set of procedures undertaken by the laboratory staff for the continuous monitoring of operation and the results of measurements in order to decide whether results are reliable enough to be released". IQC guarantees that methods of analysis are fit for their intended purpose, meaning the continuous achievement of analytical results with the required standard of accuracy. The objective of IQC is in fact the elongation of method validation: to continuously check the accuracy of analytical data obtained from day to day in the laboratory. In this respect, both systematic errors, leading to bias, as well as random errors, leading to imprecision, are monitored. In order to be able to monitor these errors, they should remain constant. Within the laboratory, such constant conditions are typically achieved in one analytical run. The word *internal* in IQC implicates that repeatability conditions are achieved. Thus monitoring the precision as the objective of IQC concerns not reproducibility or interlaboratory precision but only repeatability or intralaboratory precision. In fact, the monitoring of accuracy of an analytical method in IQC can be translated into the monitoring of the analytical system [8].

Two aspects are important for IQC: (1) the analysis of *control materials* such as reference materials or spiked samples to monitor trueness and (2) replication of analysis to monitor precision. Of high value in IQC are also blank samples and blind samples. Both IQC aspects form a part of statistical control, a tool for monitoring the accuracy of an analytical system. In a control chart, such as a *Shewhart control chart*, measured values of repeated analyses of a reference material are plotted against the run number. Based on the data in a control chart, a method is defined either as an *analytical system under control* or as an *analytical system out of control*. This interpretation is possible by drawing horizontal lines on the chart: $\bar{x}$(mean value), $\bar{x} + s$ (SD) and $\bar{x} - s$, $\bar{x} + 2s$ (upper warning limit) and $\bar{x} - 2s$ (lower warning limit), and $\bar{x} + 3s$ (upper action or control limit) and $\bar{x} - 3s$ (lower action or control limit). An analytical system is under control if no more than 5% of the measured values exceed the warning limits [2, 6, 85].

Participance in Proficiency Testing Schemes Proficiency testing (PT) is the periodic assessment of the competency or the analytical performance of individual participating laboratories [23]. An independent coordinator distributes individual test portions of a typical uniform test material. The participating laboratories analyze the materials by their method of choice and return the results to the coordinator. Test results obtained by different laboratories are subsequently compared with each other and the performance of each participant evaluated based on a single competency score [64, 107]. International harmonized protocols exist for the organization of PT schemes [59, 60, 64, 69, 79].

Participation in proficiency tests is not a prerequisite or an absolute substitute for IQC measures or vice versa. However, participance in proficiency tests is meaningless without a well-developed IQC system. IQC underlies participance in PT schemes, while IQC and participance in PT schemes are both important substitutes of AQA (Figure 6). It is shown that laboratories with the strongest QC procedures score significantly better in PT schemes [8, 50]. Participance in PT can to a certain extent improve the laboratory's performance; however unsatisfactory performance in schemes (up to 30% of all participants) has been reported. This means that there

TABLE 9 Differences between Method Performance Studies and Proficiency-Testing Schemes

Characteristic	Collaborative/Interlaboratory (Method Performance) studies	Proficiency-Testing Schemes
Main Objective	*Validation* of new methods	*Competency check* of analytical laboratories
Application	New methods, required for full validation and standardization, first prerequisite for IQC and QA	Routinely used (validation and/or standardization methods), recommended within IQC and QA system
Results aimed at	*Precision*: multiple results, both repeatability and reproducibility; >%RSD is compared to theoretical Horwitz Horrat values	*Trueness*: Single result per test material, > calculation of Z score as measure for bias
Method and protocol used	Single prescribed method for which SOP must strictly be followed	Multiplicity of methods; participants have free choice of (validated and/or standardized) method
Test materials	Minimum of five different materials, no stipulations for homogeneity and stability of test samples	No minimum, per round often less than five test samples; homogeneity and stability of materials must be assured
Participating laboratories	Minimum of eight, assumed to be equally competent	No minimum, variety in participants is possible throughout one scheme (different rounds); not assumed to have equal competency (will be tested)

Source: From refs. 8, 78, and 107.

is no correlation between good analytical performance and participation in PT [108]. However, PT has a significant educational function as it helps a laboratory to demonstrate competency to an accreditation body or another third party [60].

The terms *PT schemes* and *collaborative trials* are often confused with each other, as in both QA measures, a number of different laboratories are involved. However, there is a clear distinction between both. The mean differences with respect to the objective and application, the results, used method, test materials, and participating laboratories are summarized in Table 9. It is important to note also that the results obtained from PT schemes, as well as those from collaborative performance studies, can be used for assessing the MU (see Section 8.2.2).

External Quality Control and Accreditation Participation in PT schemes is an objective means of evaluating the reliability of the data produced by a laboratory. Another form of external assessment of the laboratory performance is the physical inspection of the laboratory to ensure that it complies with externally imposed standards. Accreditation of the laboratory indicates that it is applying the required

QA principles. The "golden standard" ISO/IEC 17025 [17], which is the revised version of ISO Guide 25 [70], describes the general requirements for the competence of calibration and testing laboratories. In Europe, the accreditation criteria have been formalized in European standard EN45001 [109]. Participation in PT schemes forms the basis for accreditation, because PT is a powerful tool for a laboratory to demonstrate its competency. Accreditation guides use the information obtained by PT schemes [6, 17, 60, 64].

Accreditation is a formal recognition that a laboratory is competent to carry out specific (types of) calibrations or tests [2]. After the use of validated and standardized methods, the introduction and use of appropriate IQC procedures and the participation in PT schemes, accreditation to ISO/IEC 17025 is the fourth basic principle related to laboratory QA in general [4]. Guidelines on the implementation of ISO/IEC 17025, including the estimation of MU (see also Section 8.2.2), are published in the literature and by official accreditation bodies such as Eurachem, CITAC, EA, Eurolab, and ILAC (see Table 1) [2, 60, 80, 81, 110]. It is worthwile to mention that accreditation, just like participance in PT schemes, does not necessarily indicate good performance of the laboratory [108].

8.2.4 SUMMARY

Together with the fast development of analytical methodologies, great importance is nowadays attached to the quality of the measurement data. Besides the necessary reporting of any result with its MU and traceability of the results to stated standards or references (Section 8.2.2), a third crucial aspect of analytical methods of whichever type is their status of validation. It is internationally recognized that validation is necessary in analytical laboratories. However, less is known about *what* is validation and *what* should be validated, *why* validation is important, *when* and by *whom* validation is performed, and finally, *how* it is carried out practically. This Chapter has tried to answer these questions.

Method validation is defined in detail and different approaches to evaluate the acceptability of analytical methods are described. Great importance is attached to the different method performance parameters, their definitions, ways of expression, and approaches for practical assessment. Validation of analytical methodologies is placed in the broader context of QA. The topics of standardization, internal and external quality control, and accreditation are discussed as well as the links between these different aspects. Because validation and QA apply for a specific analytical method, it is important to approach each method on a case-by-case basis. An analytical method is a complex, multistep process, starting with sampling and ending with the generation of a result. Although every method has its specific scope, application, and analytical requirement, the basic principles of validation and QA are the same, regardless of the type of method or sector of application. The information in this work is mainly taken from analytical chemistry, but it applies to other sectors as well.

Section 8.2.3 on quality in the analytical laboratory provides a good, complete, and up-to-date collation of relevant information in the fields of analytical method validation and QA. It is useful for the completely inexperienced scientist as well as for those involved in this topic for a long time but somewhere having lost the way

in the labyrinth, looking for more explanation on a particular aspect, or longing for deeper insight and knowledge.

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REFERENCES

1. van Zoonen, P., Hoogerbrugge, R., Gort, S. M., Van de Wiel, H. J., and Van't Klooster, H. A. (1999), Some practical examples of method validation in the analytical laboratory, *Trends Anal. Chem.*, 18, 584–593.
2. CITAC/Eurachem Guide (2002), *Guide to Quality in Analytical Chemistry—An Aid to Accreditation*, available: http://www.eurachem.org.
3. Eurachem/CITAC Guide (2003), *Traceability in Chemical Measurement. A Guide to Achieving Comparable Results in Chemical Measurement*, Joint Eurachem/CITAC Working Group on Measurement Uncertainty and Traceability, available: http://www.eurachem.org.
4. Thompson, M., Ellison, S., and Wood, R. (2002), Harmonised guidelines for single-laboratory validation of methods of analysis, IUPAC Technical Report, *Pure Appl. Chem.*, 74, 835–855.
5. Battaglia, R. (1996), Quality assurance in a food analytical laboratory—The introduction of EN 45001 in the food analytical laboratories of a retail company, *Accred. Qual. Assur.*, 1, 256–261.
6. Mesley, R. J., Pocklington, W. D., and Walker, R. F. (1991), Analytical quality assurance: A review, *Analyst*, 116, 975–990.
7. Eurachem Guide EEE/RM/062rev3 (2002), *The Selection and Use of Reference Materials.* A Basic Guide for Laboratories and Accreditation Bodies, available: http://www.eurachem.org.
8. Thompson, M., and Wood, R. (1995), Harmonised guidelines for internal quality control in analytical chemistry laboratories, *Pure Appl. Chem.*, 67, 649–656.
9. Maroto, A., Boqué, R., Riu, J., and Rius, F. X. (1999), Evaluating uncertainty in routine analysis, *Trends Anal. Chem.*, 18, 577–584.
10. Hund, E., Massar, D. L., and Smeyers-Verbeke, J. (2001), Operational definitions of uncertainty, *Trends Anal. Chem.*, 20, 394–406.
11. Fleming, J., Albus, H., Neidhart, B., and Wegschieder, W. (1996), Glossary of analytical terms (II), *Accred. Qual. Assur.*, 1, 87–88.
12. Quevauviller, Ph. (2004), Traceability of environmental chemical measurements, *Trends Anal. Chem.*, 23, 171–176.
13. Eurachem/CITAC Guide (1995), in Ellison, S. L. R., Rosslein, M., and Williams, A., Eds., *Quantifying Uncertainty in Analytical Measurement*, 1st ed., available: http://www.eurachem.org.

14. Eurachem/CITAC Guide (2000), in Ellison, S. L. R., Rosslein, M., and Williams, A., Eds., *Quantifying Uncertainty in Analytical Measurement*, 2nd ed., available: http://www.eurachem.org.
15. Eurachem Guide (1998), *The Fitness for Purpose of Analytical Methods. A Laboratory Guide to Method Validation and Related Topics*, LGC, Teddington, available: http://www.eurachem.org.
16. International Organization for Standardization (ISO) GUM (1995), *Guide to the Expression of Uncertainty in Measurement*, ISO, Geneva.
17. International Organization for Standardization (ISO)/IEC 17025 (1999), *General Requirements for the Competence of Calibration and Testing Laboratories*, ISO, Geneva.
18. CX/MAS 01/8 (2001), Codex Alimentarius Commission, Codex Committee on Methods of Analysis and Sampling (FAO/WHO), Measurement uncertainty. Relationship between the analytical result, the measurement uncertainty and the specification in Codex standards, agenda item 4a of the 23rd session, Budapest, Hungary, Feb. 26–Mar. 2, 2001.
19. CX/MAS 02/6 (2002), Codex Alimentarius Commission, Codex Committee on Methods of Analysis and Sampling (FAO/WHO), Proposed draft guidelines on measurement uncertainty, agenda item 5 of the 24th session, Budapest, Hungary, Nov. 18–22, 2002.
20. CX/MAS 02/13 (2002), Codex Alimentarius Commission, Codex Committee on Methods of Analysis and Sampling (FAO/WHO), The use of analytical results: Sampling, relationship between the analytical results, the measurement uncertainty, recovery factors and the provisions in Codex standards, agenda item 9 of the 24th session, Budapest, Hungary, Nov. 18–22, 2002.
21. CX/MAS 02/4 (2002), Codex Alimentarius Commission, Codex Committee on Methods of Analysis and Sampling (FAO/WHO), Proposed draft guidelines for evaluating acceptable methods of analysis, agenda item 4a of the 24th session, Budapest, Hungary, Nov. 18–22, 2002.
22. European Cooperation for Accreditation of Laboratories (EAL) (1996), *The Expression of Uncertainty in Quantitative Testing*, EAL-G23, The Netherlands.
23. International Laboratory Accreditation Cooperation (ILAC) (2002), *Introducing the Concept of Uncertainty of Measurement in Testing in Association with the Application of the Standard ISO/IEC 17025*, ILAC-G17, ILAC Technical Accreditation Issues Committee, available: www.ilac.org.
24. Valcarcél, M., and Rios, A. (1999), Traceability in chemical measurements for the end users, *Trends Anal. Chem.*, 18, 570–576.
25. Walsh, M. C. (1999), Moving from official to traceable methods, *Trends Anal. Chem.*, 18, 616–623.
26. Valcarcél, M., and Rios, A. (1997), Is traceability an exclusive property of analytical results? An extended approach to traceability in chemical measurement, *Fresen. J. Anal. Chem.*, 359, 473–475.
27. Quevauviller, Ph., and Donard, O. F. X. (2001), Stated references for ensuring traceability of chemical measurements for long-term environmental monitoring, *Trends Anal. Chem.*, 20, 600–613.
28. Pan, X. R. (1996), The traceability scheme in chemical measurement, *Accred. Qual. Assur.*, 1, 181–185.
29. Charlet, P., and Marschal, A. (2004), Improvement in the traceability of environmental analysis by the relevant use of certified pure solutions and a matrix certified reference material, *Trends Anal. Chem.*, 23, 178–184.

30. Segura, M., Camara, C., Madrid, C., Rebollo, C., Azcarate, J., Kramer, G. N., Gawlik, B. M., Lamberty, A., and Quevauviller, Ph. (2004), Certified reference materials (CRMs) for quality control of trace-element determinations in wastewaters, *Trends Anal. Chem.*, 23, 194–202.
31. Förstner, U. (2004), Traceability of sediment analysis, *Trends Anal. Chem.*, 23, 217–236.
32. Theocharopoulos, S. P., Mitsios, I. K., and Arvanitoyannis, J. (2004), Traceability of environmental soil measurements, *Trends Anal. Chem.*, 23, 237–251.
33. Sabé, R., and Rauret, G. (2004), Challenges for achieving traceability of analytical measurements of heavy metals in environmental samples by isotopic dilution mass spectrometry, *Trends Anal. Chem.*, 23, 273–280.
34. Drolc, A., Ros, M., and Cotman, M. (2004), Establishment of traceability of ammonium nitrogen determination in wastewater, *Anal. Bioanal. Chem.*, 378, 1243–1250.
35. Thompson, M. (1998), Uncertainty of sampling in chemical analysis, *Accred. Qual. Assur.*, 3, 117–121.
36. Roy, S., and Fouillac, A.-M. (2004), Uncertainties related to sampling and their impact on the chemical analysis of groundwater, *Trends Anal. Chem.*, 23, 185–193.
37. Analytical Methods Committee (1995), Uncertainty of measurement: Implications of its use in analytical science, *Analyst*, 120, 2303–2308.
38. Fleming, J., Albus, H., Neidhart, B., and Wegschieder, W. (1997), Glossary of analytical terms (VIII), *Accred. Qual. Assur.*, 2, 160–161.
39. Hund, E., Massart, D. L., and Smeyers-Verbeke, J. (2003), Comparison of different approaches to estimate the uncertainty of a liquid chromatographic assay, *Anal. Chim. Acta.*, 480, 39–52.
40. Eurachem/EA Guide 04/10 (2002), *Accreditation for Microbiological Laboratories*, available: http://www.eurachem.org.
41. Küppers, S. (1998), Is the estimation of measurement uncertainty a viable alternative to validation? *Accred. Qual. Assur.*, 3, 412–415
42. Ellison, S. L. R., and Barwick, V. J. (1998), Using validation data for ISO measurement uncertainty estimation. Part 1. Principles of an approach using cause and effect analysis, *Analyst*, 123, 1387–1392.
43. Moser, J., Wegscheider, W., and Sperka-Gottlieb, C. (2001), Quantifying the measurement uncertainty of results from environmental analytical methods, *Fresen. J. Anal. Chem.*, 370, 679–689.
44. Dehouck, P., Vander Heyden, Y., Smeyers-Verbeke, J., Massart, D. L., Crommen, P. H., Marini, R. D., Smeets, O. S., Decristoforo, G., Van de Wauw, W., De Beer, J., Quaglia, M. G., Stella, C., Veuthey, J.-L., Estevenon, O., Van Schepdael, A., Roets, E., and Hoogmartens, J. (2003), Determination of uncertainty in analytical measurements from collaborative study results on the analysis of a phenoxymethylpenicillin sample, *Anal. Chim. Acta*, 481, 261–272.
45. Maroto, A., Riu, J., Boqué, R., and Rius, F. X. (1999). Estimating uncertainties of analytical results using information from the validation process, *Anal. Chim. Acta*, 391, 173–185.
46. Maroto, A., Boqué, R., Riu, J., and Rius, F. X. (2001), Estimation of measurement uncertainty by using regression techniques and spiked samples, *Anal. Chim. Acta*, 446, 133–145.
47. Barwick, V. J., and Ellison, S. L. R. (2000, Jan.), *VAM Project 3.2.1*: *Development and Harmonisation of Measurement Uncertainty Principles. Part d. Protocol for Uncertainty Evaluation from Validation Data*, LGC, UK, available: http://www.vam.org.uk.

48. Armishaw, P. (2003), Estimating measurement uncertainty in an afternoon. A case study in the practical application of measurement uncertainty, *Accred. Qual. Assur.*, 8, 218–242.
49. Horwitz, W. (1982), Evaluation of analytical methods used for regulation of food and drugs, *Anal. Chem.*, 54, 67A–76A.
50. Thompson, M., and Lowthian, P. J. (1997), The Horwitz function revisited, *J. AOAC Int.*, 80, 676–679.
51. King, B. (2001), Meeting the measurement uncertainty and traceability requirements of ISO/IEC standard 17025 in chemical analysis, *Fresen. J. Anal. Chem.*, 371, 714.
52. Mueller-Harvey, I. (2003), Do we need quality assurance and quality control of analytical measurements in R&D laboratories? *Food Agric. Environ.*, 1, 9–11.
53. Rösslein, M. (2000), Evaluation of uncertainty utilising the component by component approach, *Accred. Qual. Assur.*, 5, 88–94.
54. Mueller, N. (2002), Introducing the concept of uncertainty of measurement in testing in association with the application of the standard ISO/IEC 17025, *Accred. Qual. Assur.*, 7, 79–80.
55. International Organization on Harmonization (1995), Guideline for industry: Text on validation of analytical procedures, ICH-Q2A, available: http://www.fda.gov/cder/guidance/index.htm.
56. Huber, L. (1998), Validation of analytical methods. Part 9, in Huber, L., Ed., Validation and Qualification in Analytical Laboratories, Interpharm Press, Agilent Technologies, available: http://www.labcompliance.com.
57. Wells, R. J. (1998), Validation requirements for chemical methods in quantitative analysis—Horses for courses? *Accred. Qual. Assur.*, 3, 189–193.
58. Green, M. (1996), A practical guide to analytical method validation, *Anal. Chem.*, 68, 305A–309A.
59. Eurachem Guide (2000), *Selection, Use and Interpretation of Proficiency Testing (PT) Schemes by Laboratories*, available: http://www.eurachem.org.
60. Eurachem/Eurolab/EA Guide EA-03/04 (2001, Aug.), *Use of Proficiency Testing as a Tool for Accreditation in Testing*, available: http://www.eurachem.org.
61. European Committee for Normalisation (CEN), (2006), Standardization and related activities—General Vocabulary (from ISO/IEC Guide 2:2004), EN45020:2006, www.cenorm.be.
62. Association of Official Analytical Chemists (AOAC) International (2000), Method validation programs (OMA/PVM Department), including Appendix D: Guidelines for collaborative study procedures to validate characteristics of a method of analysis, available: http://www.aoac.org/vmeth/devmethno.htm.
63. Horwitz, W. (1995), Protocol for the design, conduct and interpretation of method performance studies, *Pure Appl. Chem.*, 67, 331–343.
64. Thompson, M., and Wood, R. (1993), The international harmonized protocol for the proficiency testing of (chemical) analytical laboratories, *Pure Appl. Chem.*, 65, 2123–2144: also *J. AOAC Int*., 76, 26–940 (1993).
65. Currie, L. A. (1995), Nomenclature in evaluation of analytical methods including detection and quantification capabilities, *Pure Appl. Chem.*, 67, 1699–1723.
66. Currie, L. A., and Svehla, G. (1994), Nomenclature for the presentation of results of chemical analysis, International Union of Pure and Applied Chemistry (IUPAC), *Pure Appl. Chem.*, 66, 595–608.
67. Pocklington, W. D. (1990), Harmonized protocols for the adoption of standardized analytical methods and for the presentation of their performance characteristics,

International Union of Pure and Applied Chemistry (IUPAC), *Pure Appl. Chem.*, 62, 149–162.

68. International Organization for Standardization (ISO) Guide 5725 (1994), *Accuracy (Trueness and Precision) of Measurement Methods and Results*, ISO, Geneva.
69. International Organization for Standardization (ISO) Guide 43 (1984), *Development and Operation of Laboratory Proficiency Testing*, ISO, Geneva.
70. International Organization for Standardization (ISO) Guide 25 (1990), *General Requirements for the Competence of Calibration and Testing Laboratories*, ISO, Geneva.
71. International Organization for Standardization (ISO)/IEC 17025 (1999), *General Requirements for the Competence of Calibration and Testing Laboratories*, ISO, Geneva.
72. International Conference on Harmonization (1996), *Guidance for Industry: Validation of Analytical Procedures: Methodology*, ICH-Q2B, available: http://www.fda.gov/cder/guidance/index.htm.
73. International Conference on Harmonization (1999), *Harmonised Tripartite Guideline: Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products*, ICH-Q6B, available: http://www.fda.gov/cder/guidance/index.htm.
74. U.S. Food and Drug Administration (FDA)/CDER/CVM (2001), Guidance for industry—Bioanalytical method validation, available: http://www.fda.gov/cder/guidance/index.htm.
75. CX/MAS 01/4 (2001), Codex Alimentarius Commission, Codex Committee on Methods of Analysis and Sampling, Criteria for evaluating acceptable methods of analysis for Codex purposes, agenda item 4a of the 23rd session, Budapest, Hungary, Feb. 26–Mar. 2, 2001.
76. CX/MAS 02/5 (2002), Codex Alimentarius Commission, Codex Committee on Methods of Analysis and Sampling (FAO/WHO), Criteria for evaluating acceptable methods of analysis for Codex purposes, agenda item 4b of the 24th session, Budapest, Hungary, Nov. 18–22, 2002.
77. CX/MAS 02/11 (2002), Codex Alimentarius Commission, Codex Committee on Methods of Analysis and Sampling (FAO/WHO), Requirements for single-laboratory validation for Codex purposes, agenda item 8b of the 24th session, Budapest, Hungary, Nov. 18–22, 2002.
78. CX/MAS 02/12 (2002), Codex Alimentarius Commission, Codex Committee on Methods of Analysis and Sampling (FAO/WHO), Validation of methods through the use of results from proficiency testing schemes, agenda item 8c of the 24th session, Budapest, Hungary, Nov. 18–22, 2002.
79. International Laboratory Accredidation Cooperation (ILAC) (2000), Guidelines for the requirements for the competence of providers of proficiency testing schemes, ILAC-G13 ILAC Technical Accreditation Issues Committee, available: www.ilac.org.
80. International Laboratory Accredidation Cooperation (ILAC) (2001), Guidance for accreditation to ISO/IEC 17025, ILAC-G15, ILAC Technical Accreditation Issues Committee, available: www.ilac.org.
81. International Laboratory Accredidation Cooperation (ILAC) (2002), The scope of accreditation and consideration of methods and criteria for the assessment of the scope in testing, ILAC-G18, ILAC Technical Accreditation Issues Committee, available: www.ilac.org.
82. European Commission (EC) (2002), Commission decision 2002/657/EC implementing council directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, *Off. J. Eur. Commun.*, L 221/8, 17.8.2002.
83. Holst-Jensen, A., and Berdal, K. G. (2004), The modular analytical procedure and validation approach and the units of measurement for genetically modified materials in foods and feeds, *J. AOAC Int.*, 87, 1–9.

84. Wood, R. (1999), How to validate analytical methods, *Trends Anal. Chem.*, 18, 624–632.
85. Waters Corporation (2004), Validation Guidelines: Terminology and Definitions, available: http://www.waters.com/WatersDivision.
86. Hibbert, D. B. (1999), Method validation of modern analytical techniques, *Accred. Qual. Assur.*, 4, 352–356.
87. Fleming, J., Neidhart, B., Albus, H., and Wegscheider, W. (1996), Glossary of analytical terms (III), *Accred. Qual. Assur.*, 1, 135.
88. Krull, I. S., and Swartz, M. (1999), Analytical method development and validation for the academic researcher, *Anal. Lett.*, 32, 1067–1080.
89. Quevauviller, Ph. (2004), Traceability of environmental chemical measurements, *Trends Anal. Chem.*, 23, 171–176.
90. Holcombe, G., Lawn, R., and Sargent, M. (2004), Improvements in efficiency of production and traceability for certification of reference materials, *Accred. Qual. Assur.*, 9, 198–204.
91. Lauwaars, M., and Anklam, E. (2004), Method validation and reference materials, *Accred. Qual. Assur.*, 9, 253–258.
92. Fleming, J., Albus, H., Neidhart, B., and Wegschieder, W. (1996), Glossary of analytical terms (IV), *Accred. Qual. Assur.*, 1, 191.
93. Thompson, M., Ellison, S. L. R., Fajgelj, A., Willets, P., and Wood, R. (1999), Harmonised guidelines for the use of recovery information in analytical measurement, *Pure Appl. Chem.*, 71, 337–348.
94. Vessman, J. (1996), Selectivity or specificity? Validation of analytical methods from the perspective of an analytical chemist in the pharmaceutical industry, *J. Pharm. Biomed. Anal.*, 14, 867–869.
95. Analytical Methods Committee (1987), Recommendations for the definition, estimation and use of the detection limit, *Analyst*, 112, 199–204.
96. Fleming, J., Albus, H., Neidhart, B., and Wegschieder, W. (1997) Glossary of analytical terms (VII), *Accred. Qual. Assur.*, 2, 51–52.
97. Huber, W. (2003), Basic calculations about the limit of detection and its optimal determination, *Accred. Qual. Assur.*, 8, 213–217.
98. Kuselman, I., and Sherman, F. (1999), Assessment of limits of detection and quantification using calculation of uncertainty in a new method for water determination, *Accred. Qual. Assur.*, 4, 124–128.
99. European Commission (EC), Council directive 96/23/EC of April, 29 1996, on measures to monitor certain substances and residues thereof in live animals and animal products and repealing directives 85/258/EEC and 86/469/EEC and decisions 89/187/EEC and 91/664/EEC, *Off. J. Eur. Commun.*, L125, 0010–0032, 23.05.1996.
100. European Commission (EC), Commission decision 2003/181/EC amending decision 2002/657/EC as regards the setting of minimum required performance limits (MRPLs) for certain residues in food of animal origin, *Off. J. Eur. Commun.*, L 71/17, 0017–0018, 15.3.2003.
101. Antignac, J.-P., Le Bizec, B., Monteau, F., and Andre, F. (2003), Validation of analytical methods based on mass spectrometric detection according to the "2002/657/EC" European decision: Guideline and application, *Anal. Chim. Acta*, 483, 325–334.
102. De Wasch, K., De Brabander, H. F., Courtheyn, D., Van Hoof, N., Poelmans, S., and Noppe, H. (2003), The commission decision 2002/657/EC: Disscussion on some new analytical aspects. EURO FOOD CHEM XII, in Strategies on Safe Food, Proceedings, Vol. 1, Brugge, Belgium, Sept. 24–26, 2003, pp. 45–48.

103. von Holst, C., Müller, A., Björklund, E., and Anklam, E. (2001), In-house validation of a simplified method for the determination of PCB's in food and feedingstuffs, *Eur. Food Res. Technol.*, 213, 154–160.
104. Prichard, E., Albus, H., Neidhart, B., and Wegscheider, W. (1997), Glossary of analytical terms (IX), *Accred. Qual. Assur.*, 2, 348–349.
105. van der Voet, H., Van Rhijn, J. A., Van de Wiel, H. J. (1999), Inter-laboratory, time, and fitness-for-purpose aspects of effective validation, *Anal. Chim. Acta*, 391, 159–171.
106. Horwitz, W., and Albert, R. (1996), Reliability of the determinations of polychlorinated contaminants (bifenyls, dioxins, furans), *J. AOAC Int.*, 79, 589–621.
107. Thompson, M., and Lowthian, P. J. (1993), Effectiveness of analytical quality control is related to the subsequent performance of laboratories in proficiency tests, *Analyst*, 118, 1495–1500.
108. King, B., Boley, N., Kannan, G. (1999), The correlation of laboratory performance in proficiency testing with other QA characteristics, *Accred. Qual. Assur.*, 4, 280–291.
109. The Joint European Standard Institution (1989), *General Criteria for the Operation of Testing Laboratories*, EN45001, CEN/CENELEC, Brussels.
110. Pritzkow, J. (2003), Practical experience of the laboratories in implementing the ISO/IEC 17025, *Accred. Qual. Assur.*, 8, 25–26.

8.3

VALIDATION OF LABORATORY INSTRUMENTS

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Contents

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8.3.1 INTRODUCTION

The reliability of chemical and physical measurements is critically dependent on the suitability and performance of the instruments from which the measurements are obtained. It is a challenge for any laboratory to develop a pragmatically structured validation program for laboratory instruments of varying complexity. However, implementation of such a program is highly valuable as it provides assurances that instruments meet performance requirements and are suitable for their intended use. These assurances are required to comply with the good manufacturing practices (GMPs) and good laboratory practices (GLPs).

In recent years, tremendous efforts have been put forth by many organizations to address the validation of a wide variety of laboratory instruments of varying complexity. Instrument validation–related topics have been discussed at great length

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in meetings and conferences. Many guidance documents and reference literatures on the topic of the validation of laboratory instruments are available [1–11]. Several key concepts and a common framework for the validation of laboratory instruments have been consolidated into the form presented herein:

1. The fundamental purpose of laboratory instrument validation is to provide assurances that the instrument is suitable for its intended use. The assurance is supported by documented evidence that the system consistently performs according to predetermined specifications for its intended applications.
2. The validation of laboratory instruments is the responsibility of the user's organization and not the suppliers of the instruments. The users can partner with the suppliers to facilitate installation and validation testing.
3. Most of the instruments used in the laboratory are commercial off-the-shelf (COTS) instruments, and consequently the users have little or no input into their design. A full system development life-cycle (SDLC) approach [8], which is used to develop complex computerized systems such as Laboratory Information Management System (LIMS) or Chromatographic Data System (CDS) or custom design laboratory equipment, is not appropriate for COTS instruments. Some laboratory instruments such as a pH meter or centrifuge are fairly simple and therefore do not warrant the SDLC approach.
4. Scalable validation for laboratory instruments is being developed to provide appropriate levels of coverage for laboratory instruments of various complexities for their intended use.
5. Laboratory instruments are classified into different categories according to their complexity to enable a scalable validation approach. The expected validation deliverables and documentation requirements for each class of laboratory instrument can be defined in the validation guide for the laboratory.
6. Validation efforts should be commensurate with the complexity of the different classes of instruments as defined in the validation guide developed for the laboratory.

8.3.2 SCOPE

This chapter provides an overview of laboratory instrument validation for GMPs and GLPs. The term GxP is used as a general referral to GMP and GLP. The focus of this chapter is on the validation of COTS instruments using a scalable approach.

The validation of large-scale computerized systems such as LIMS, chromatographic data systems, and custom-designed systems (bespoke systems) are beyond the scope of this chapter. Readers are encouraged to consult *GAMP (Good Automated Manufacturing Practice) Guide for Validation of Automated System in Pharmaceutical Manufacturing*, Version 4[8] for the validation for those systems.

8.3.3 LABORATORY INSTRUMENT CLASSIFICATIONS

A practical way to provide an appropriate level of validation for a wide range of laboratory instruments is to develop a classification system to categorize the laboratory instruments according to their functional complexity, usage, and risk to data integrity. For each category or class of instruments, a set of predetermined scalable deliverables and testing is defined. These definitions will in turn be used to determine the extent and type of testing required to validate various instruments. This instrument classification system and its associated deliverables provide simple and structured guidance on deciding the validation effort required for a whole spectrum of laboratory instruments from a simple pH meter to a fully automated dissolution workstation.

An instrument classification system which is based on the complexity of the instrument is given in Table 1. There are similar laboratory instrument classifications outlined in *GAMP: Good Practice Guide: Validation of Laboratory Computerized Systems* [10], and the U.S. Pharmacopeia (USP) general chapter on analytical instrument qualification [11]. As there can be overlap between categories of instrument classifications, individual organizations should make modifications to the definitions and the types of instruments in the classification system as required, thereby customizing the classification system to their respective operation. After the laboratory instrument classification has been established, the deliverables required for the validation for each class of instruments will be defined to provide the framework for the validation process. Table 2 lists the deliverables commonly encountered in the laboratories.

TABLE 1 Instrument Classification

Category[a]	Definition	Examples[b]
A	Equipment/tools used for sample preparations	Hotplates, stirrers, shakers
B	Basic firmware instruments used to measure fundamental physical parameters such as weight, dimension, temperature, and pH	Balances, pH meters, digital thermometers, centrifuges, sonicators
C	Advanced firmware instruments that utilize spectrometry, chromatography, dissolution, etc.	Liquid chromatographs, gas chromatographs, dissolution baths, Karl Fischer titrators
D	Commercial COTS controlled by external computer Hybrid systems such as automated dissolution workstation with high-performance liquid chromatography (HPLC) or ultraviolet–visible (UV–Vis) interface	Liquid chromatographs, gas chromatographs, UV/Vis spectrophotometers, Fourier transform infrared (FTIR) spectrophotometers, near-infrared (NIR) spectrophotometers, mass spectrometers, atomic absorption spectrometers, thermal gravimetric analyzers, COTS automation workstations

[a]Categories may vary. Consult the user's organization laboratory instrument classification guide or references in the GAMP guide and USP general chapter ⟨1058⟩ [11, 12].
[b]Examples are not all inclusive.

TABLE 2 Key Instrument Validation Deliverables

	By Category			
Deliverable	A	B	C	D
Business requirements[a]	✓[b]	✓[b,c]	✓	✓
Compliance assessment (GxP criticality assessment)	✓[b]	✓[b,c]	✓	✓
Validation plan	✓[b]	✓[b]	✓[c]	✓
Electronic records/electronic signatures (ER/ES) assessment	✗	✗	✗	✓
User and functional requirements (design qualification)	✓[b]	✓[a]	✓[c]	✓
Installation qualification	✓[b]	✓[c]	✓[c]	✓
Operational qualification	✓[b]	✓[c]	✓[c]	✓
Performance qualification	✓[b]	✓[c]	✓[c]	✓
Validation report	✗	✓[c]	✓[c]	✓
Traceability matrix and configuration documents	✗	✗	✗	✓
Standard operating procedures	✓[b]	✓	✓	✓
Performance verification	✗	✓	✓	✓
Periodic review	✗	✓[b]	✓	✓
Decommissioning	✓[b]	✓	✓	✓

Note: ✓: required; ✗: not required.[a]Supplier audit may be required for some category D instruments.
[b]Optional.
[c]Simplified standard forms can be used.

8.3.4 VALIDATION PHASES

Conceptually, a life-cycle approach to laboratory instrument validation can be divided into four phases, as shown in Figure 1.

8.3.4.1 Planning and Requirements Phase

The activities in the planning phase are commenced after a business need to implement an instrument in the laboratory has been identified. Planning-phase activities include concisely documenting the user requirements and functional requirements of the instrument. Once established, the requirements are then used to evaluate the instrument candidates available from suppliers. A validation plan with deliverables that are commensurate with the class of the instrument being implemented should be developed during the planning phase.

Validation Plan A validation plan is prepared to highlight the activities and deliverables required in the implementation of the laboratory instrument. The details of the validation plan should be scaled according to the complexity of the system, its intended use, and the impact on the business. A generic validation plan can be used for similar types of laboratory instruments with similar applications. For example, one validation plan can be prepared for the HPLC. A validation plan typically includes the following sections:

- *Objectives and Scope of Project* Rationale for implementing the instrument with its key applications and to state the assumptions, exclusions, and limitations of the project.

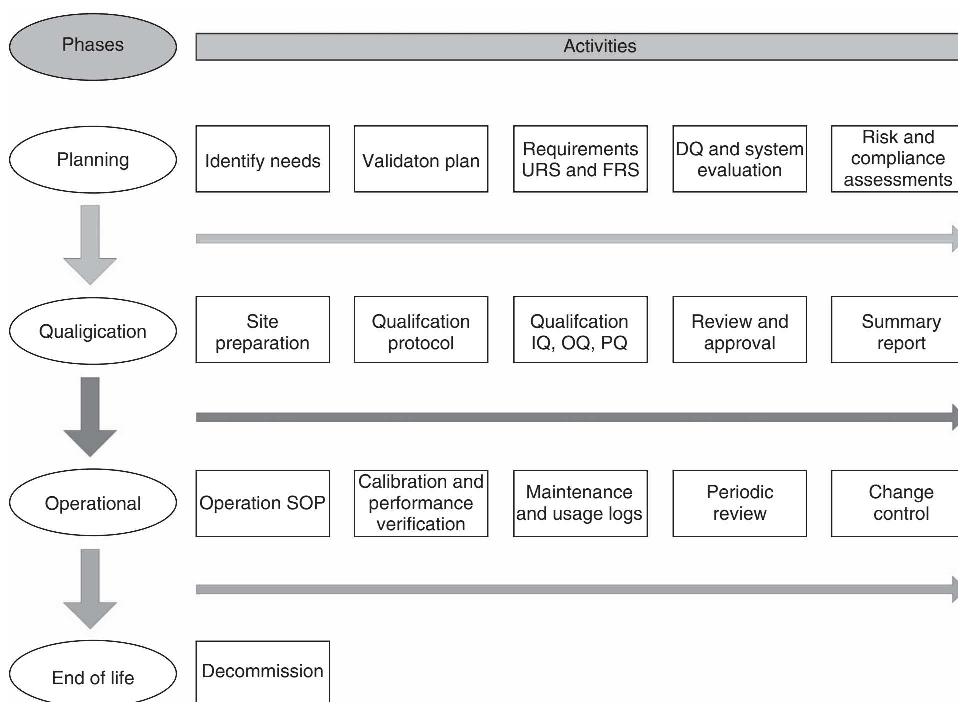

FIGURE 1 Validation phases. URS: user requirement specification; FRS: functional requirement specification; DQ, IQ, OQ, PQ: design, installation, operational, and performance qualifications; SOP: standard operating procedure.

- *System Description* Hardware, software, and system configuration (if the system is comprised of different modules or components).
- *Compliance Assessment* State whether the use of the analytical instrument is subject to GxP and regulatory requirements. Instruments subjected to GxP will need validation. A rationale should be provided to support the decision of the assessment.
- *Validation Approach and Deliverables* Outline the required documents, qualification testing, and reports to be included in the validation project. The deliverables should be based upon the complexity and the risk associated with the intended use of the system. The deliverables will highlight the acceptance criteria to demonstrate that the system has met the requirements for its intended use.
- *Roles and Responsibilities* List the group(s) or personnel that are responsible for the validation of the instrument.
- *Project Timeline and Milestones* List the projected completion date of major tasks in the validation. This information is useful in monitoring the progress of the project and making sure proper resources are available at various stages of the validation.
- *Record Management* Describe the format, numbering sequence, revision, version control, and storage of the validation documentation.

- *References of Relevant Information* List the internal and external instrument validation guidance documents, compendial reference methods, qualification testing, and related SOPs for validation.

User Requirements The user requirements outline the applications that the user wants the analytical instrument system to run and the major tasks that the instrument is required to execute in order to deliver the applications. The requirements should be clear, unambiguous, and verifiable or testable. The user requirements dictate the amount of validation that will be required. A successful validation should ultimately demonstrate that the user requirements can be met by the instrument and that the instrument can be used reliably for its intended applications. It is very important to state clearly what the instrument is supposed to do. Only the mandatory requirements should be validated. Including the "nice to have" or optional features in the user requirements will unnecessarily add more work to the validation.

For example, a simple HPLC system with an isocratic mobile-phase delivery system and a multiple-wavelength UV detector may be sufficient for simple routine product release testing. However, an HPLC system for running impurity assays or stability-indicating methods is likely to require a gradient mobile-phase delivery system and a diode array UV detector. If only the isocratic applications will be required for an HPLC system, there is no need to validate the gradient feature of the HPLC system even though the system is capable of running gradient applications. Another example is that of a dissolution bath that is solely utilized for running paddle dissolution methods. Although the bath may have the capability to run both basket and paddle methods, it is only the validation of the paddle method that should be considered necessary.

Functional Requirements The high-level user requirements establish the framework for the functional specifications which identify the system functions, mode of operation, and operation environment necessary to meet the user requirements. Again, the requirements should be verifiable or testable to demonstrate that the requirements can be met during the qualification testing.

Functional requirements usually include the following considerations:

- Functions of each system hardware component or modules: The expected operational range of each function and its performance attributes such as the accuracy, precision, and linearity required for its intended use should be defined.
- Site requirements to support the operations of the instrument: Power supply (electrical voltage and current), ventilation, gas supply, water supply, and drainage.
- Health and safety requirements: Mechanical safety for robotic movement, radiation safety for systems that involve the use of radioactive sources, and laser safety for systems that use high-power lasers.
- Computer operation system and network requirements.
- Data type (analog and/or digital output/input) and capacity (size of the data files).

TABLE 3 Typical Functional Requirements for HPLC with UV–Vis Detector

Modules	Functional Requirements
Pump	• The pump should be capable of a flow rate between 0.50 and 5.00 mL/min. • The pump should be a quaternary gradient pump and have a compositional accuracy of ±1.5% of the theoretical values for the four channels. • The pump should have relative standard deviation (RSD) of ≤2.0% for six successive readings from a calibrated flowmeter.
Autosampler	• The autosampler should have an injector capable of injecting sample volumes from 1 to 100 µL. • The injector should have a precision of ≤1.5% RSD. • The injector carryover should be ≤0.5% of peak area. • The autosampler should pick the correct vial. • The autosampler should use relays and/or contacts to communicate with the laboratory CDS. • Temperature-controlled sample racks should be capable of maintaining samples in the temperature range of 4–15 °C (±3 °C).
UV–Vis detector	• The detectors should operate with a wavelength range from 200 to 800 nm. • The detector should have a wavelength accuracy of ±2 nm. • The detector response should be linear with a correlation coefficient r^2 not less than 0.999 over the full dynamic range. • The linear range should be up to 2.0 Absorbance Unit Full Scale (AUFS).
Column compartment	• The column oven should be capable of maintaining a temperature range of 5 °C above ambient to 60 °C. • The oven should be within ±3 °C of the set temperature and the temperature precision should be ≤2.0%.

- Requirements for electronic records and electronic signatures [13–15]: System security, data integrity, traceability, and data archive/retrieval.
- Communication and control of other systems.
- System recovery from a major failure or disaster.

Typical hardware functional requirements for a gradient HPLC system with UV–Vis detection are listed in Table 3 as an example.

Design Qualification For COTS analytical instruments, the user has little or no input into the design. The design information is usually not available to the users. In this case, the design qualification demonstrates the user requirements and the functional requirements can be fulfilled by the analytical instrument being considered by comparing the requirements against the technical specifications from the suppliers. For analytical systems which comprise of multiple COTS components (e.g., a dissolution system with online HPLC analysis capability), the configuration and compatible components from different suppliers should be demonstrated in the DQ.

System Evaluation and Supplier Assessment Usually there are many choices and suppliers for common COTS laboratory instruments. The user requirements and the operational requirements will provide the basic criteria for the selection. Obviously the chosen instrument must be able to fulfill the key requirements for its intended use. Other factors concerning the instrument such as its ease of use, maintenance, and reputation of the suppliers in terms of quality, reliability, and support should be considered. From a practical point of view, a supplier audit may not be viable or necessary for commonly used COTS instruments. A supplier assessment is sometimes used to evaluate whether the supplier has a good-quality system in place to support the development and manufacturing of the instrument of interest. The need for a supplier assessment depends on the criticality and complexity of the system to be obtained.

8.3.4.2 Qualification (Testing) Phase

The terms *instrument qualification* and *instrument validation* are sometimes used indiscriminately. In this chapter, the term *qualification* refers to the site preparation and the testing employed to demonstrate that the instrument is properly installed in a suitable environment and the performance meets the predetermined specifications for its intended use. Qualification is a part of the whole validation life cycle. Validation refers to the process to provide assurance that the instrument is suitable for the intended application throughout the lifetime of the instrument. Installation qualification (IQ), operation qualification (OQ), and performance qualification (PQ) are performed to provide evidence that the user requirement specifications (URSs), functional requirement specifications (FRSs), and design qualification (DQs) have been met. The sequence of requirements setting and qualification events as well as the relationships between IQ, OQ, PQ and URS, FRS, and DQ are generally illustrated by the "V" diagram shown in Figure 2. Installation qualification demonstrates the fulfillment of the DQ. Similarly, OQ demonstrates the fulfillment of the functional requirements and PQ demonstrates the fulfillment of the user requirements.

Site Preparation Prior to the installation of the instrument in the laboratory, all the preparations that support the operation of the instrument must be ready. The

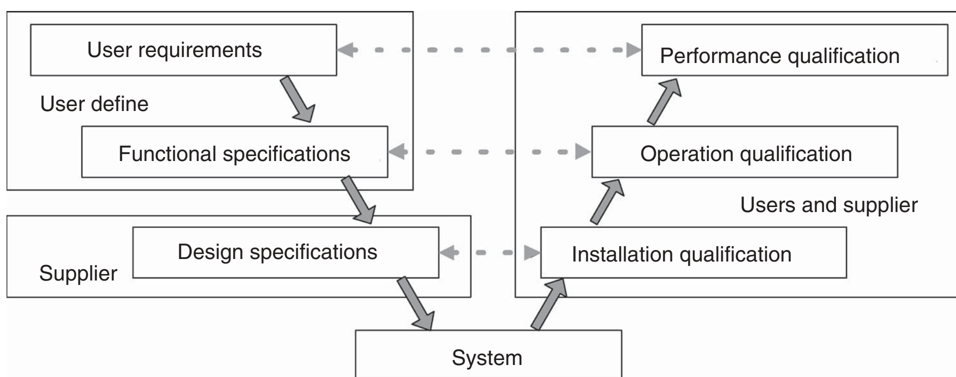

FIGURE 2 Qualification "V" diagram.

supplier usually provides a site preparation document which outlines the information of the necessary facilities required to support the operations of the instrument. The site preparation document usually includes the following information:

- Physical dimensions and weight of the instrument
- Environmental conditions for proper operations: temperature, humidity, and vibration control
- Utilities: power supply, water, gas, drainage, ventilation, network connection
- Health and safety requirements

It is a common mistake to underestimate the effort and time required for site preparation. The users should carefully study the site preparation guide to ensure that the necessary preparations to house the new instrument in the laboratory are completed prior to installation. Inadequate site preparation can cause major inconveniences and long delays in the installation process. It is a waste of time and resources to have the service engineer show up in the laboratory but not able to do anything due to incomplete site preparation.

Qualification Approaches If an analytical instrument is comprised of different functionally discrete modules, a modular approach to qualification testing that focuses on the specific operations of the individual module can be suitable for certain aspects of some operational testing (such as the flow rate precision and accuracy testing of a HPLC pump and the temperature accuracy column compartment).

When it comes to performance qualification, a holistic approach must be taken to test the analytical system with all the necessary modules working together to deliver the intended applications as specified in the user requirement (Figure 3). The proper functioning of each individual module of the analytical system does not

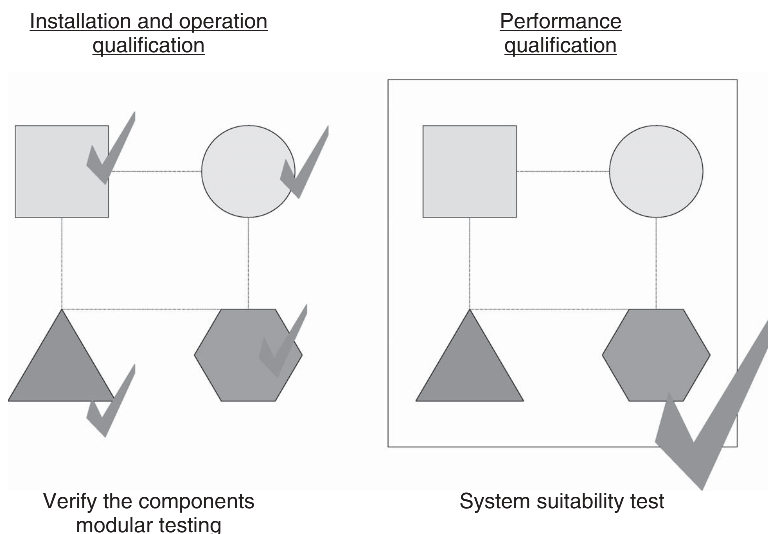

FIGURE 3 Modular versus holistic approach for qualification.

infer proper functioning of the whole system with all the modules working in concert.

Qualification Protocols For COTS instruments, the supplier often provides test protocols for installation and operation qualification. It is a common practice to purchase the qualification services from the supplier to execute the testing. The supplier should have a good knowledge of the instrument being installed and qualified, and therefore utilizing their services to perform these steps can provide significant time and resource savings. However, it is the responsibility of the user to review the test protocol for meaningful testing, that proper procedures will be used, and that reasonable acceptance criteria for the testing to ensure the objective of the testing are met. The supplier procedures and/or testing should be compatible with the procedures and practices of the user company. Acceptance criteria which are too loose do not provide enough assurance to demonstrate instrument performances. In contrast, acceptance criteria which are too tight can lead to unnecessary failures that cause a lot of effort and time to investigate and justify. The acceptance criteria should reflect both the operation requirements and the user requirements. The protocols must be approved before execution.

The test protocol typically has a general description of the system, the configurations, and the intended use. The test scripts in the test protocol provide detailed information of the testing procedures. In each test script, the following information should be provided:

- Description of the module to be tested
- Purpose and objective of the tests
- Scope and limitations
- Test procedure
- Acceptance criteria for the testing
- Sections to capture the test results
- A section to document deviation and exceptions encountered during the testing

Installation Qualification Installation qualification provides documented evidence that the instrument was received and successfully installed in accordance with the approved design requirements and properly installed in an environment suitable for its operation. Proper installation is the first step to ensure that the instrument will function properly. An improperly installed instrument is likely to cause problems during the operational qualification and performance qualification. The following are some checks for the IQ process:

IQ checks before instrument is installed:

- Verify hardware and software against shipping list.
- Check for visible damage.
- Complete site preparation check list.
- Health and safety precautions.
- Add the instrument to the instrument inventory list.

IQ checks during installation:

- Document the location of the instrument.
- Document all the hardware components, including computer, printer, analytical instrument firmware, interfaces, and network connection..
- Document of all software applications, the operating system, and the storage location of actual software package.
- Document the system configuration.

IQ checks after installation:

- System powers up properly.
- Proper initialization and homing position.
- Proper communication between modules.
- Calibration of modules if necessary.
- Software version verification.
- Proper launching of the software and compatibility with hardware.
- Back up critical files for the system settings.
- Set up log book.

Availability of reference documents:

- Site preparation guide.
- System operation manuals.
- Sale and shipping documents.
- Factory testing records if available.
- Certificate of quality compliance from the vendor.

During system installation and qualification testing, use screen capture to print the information for evidence whenever possible as it is a more efficient and complete way to document the results and observations than writing down the information on paper.

Operational Qualification Operational qualification provides documented evidence that the instrument will perform in accordance with its functional requirements throughout the representative operation range in a suitable environment. The OQ for simple instruments such as pH meters, balances, stirrers, water baths, and thermometers can simply achieved by execution of a calibration for the instrument. For moderately complex systems, OQ verifies the correct operation of the hardware and software for the instrument.

The testing of the hardware should cover the functionality of the instrument expected during normal operation. For example, the testing for an HPLC system would include the operation of the pump, the injector, and the detector [15].

Typical OQ tests for HPLC modules include:

- Pump: flow rate accuracy and gradient accuracy
- Detector: linearity of response, noise, drift, and wavelength accuracy
- Injector: precision, linearity, and carryover
- Column heater: temperature accuracy

Typical OQ tests for the UV–Vis spectrophotometers [16] include:

- Wavelength accuracy and reproducibility
- Stray light
- Resolution
- Photometric accuracy, reproducibility, and linearity
- Noise
- Baseline flatness and stability

In addition to testing the system components, a test of software functionality would be performed to test the system software operation and electronic records and electronic signatures (ERES) compliance (security, data integrity, data backup, and archive). In order to test the software functionality, a predetermined set of instructions can be entered step by step into the system. The system responses are then compared to the expected outcomes of the instruction and any problems with the execution are determined. Some vendors will provide a standard set of data which can be processed by the system to verify the data-handling capability of the system.

Electronic Records and Electronic Signatures Considerations ERES compliance testing for computerized (personal-computer-controlled) instruments is required to demonstrate the functional requirements in the following three key areas [12–14]:

1. *Security* To prevent unauthorized access.
   - Control unauthorized access to the instrument through physical, logical, and procedural controls. Physical control is normally achieved by controlling access to the site, building, laboratory, and instrument. Logical security is provided by setting up different levels of access accounts such as user, supervisor, and system administrator and distinct passwords for each account user. Dual logical access controls are usually available from the applications software or the operating system. A procedural control using SOPs can be used to assign users access.
2. *Data Integrity* To demonstrate that records are valid and trustworthy. A record is a combination of raw data and the metadata (processing parameters plus other related information necessary to reconstruct the records):
   - Traceability: Helps to reconstruct the events.
   - Use of audit trails to track the activities for the creation, modification, or deletion of a record. It provides a record of who did what, wrote what, when, and why.
   - Use of secure date and time stamp for the creation, modification, and deletion of data.
3. *Data Preservation* To preserve a complete and accurate set of records by copying electronic data to a removable or remote storage medium and to manage the data retention. The data integrity must not be compromised during the preservation process:

- Data backup and restore: Active data are periodically copied from the hard drive or the personal computer controlling the instrument to a suitable medium such as CD-ROM or DVD or to a separate location such as a controlled file server.
- Data archive and retrieval: Data that are no longer required in the day-to-day operation of the instrument are archived in a retrievable manner.

Performance Qualification Performance qualification is the process that provides documented evidence to demonstrate that the instrument has fulfilled the user requirements. Holistic testing which involves all the functional components in the system is required for the PQ testing. Performance qualification can be demonstrated by running a typical application that requires all the modules to function together as a whole system to deliver the intended application and the expected results.

If applicable, it is advantageous to execute a highly reliable test that is frequently performed by users on the particular type of instrument for the PQ. For example, the PQ test for a gradient HPLC system can be a gradient HPLC method with which the user has in-depth experience. In this case, the test results will mainly reflect the performance of the instrument and will not be affected by the uncertainty of the method used.

Test Exceptions and Data Review In case the test results fail to meet the acceptance criteria, an investigation is required to determine the cause of the failure. The failures may be caused by execution errors of the test procedure or by instrument-related problems. Based on the outcome of the investigation, corrective actions can be taken to rectify the problem. Once the problem has been resolved, retesting can be executed to confirm that the instrument operations meet the requirements. The failure investigation, impact assessment, corrective actions, and retesting must be documented in the exception log of the qualification testing. After the qualification testing has been completed, the testing process, data generated, and results must be reviewed for accuracy, completeness, and justifiable deviations. All major issues with regard to compliance have to be addressed prior to releasing the instrument for production use.

Summary Report After completion of the qualification activities, a summary report should be prepared to summarize all the qualification testing and results and deviations from the validation plan as well as include a conclusion to state whether the instrument is ready for its intended uses. Any deviations from planned activities including testing failures and requirements not met by the system should be addressed in the report. The summary report typically includes the following sections:

- Introduction
- System description
- Reference documents
- Validation activities, testing, acceptance criteria, and results
- Summary of deviations, problems, and mitigations

- Restrictions on the system (if any)
- Deliverables
- Conclusion

For simple analytical instruments, a simple table to summarize the qualification testing, acceptance criteria, results, and pass/fail decision of the tests will be sufficient since there are fewer tests that are required and the tests are usually relatively simple. For complex analytical systems, a more complex table often referred to as a traceability matrix which traces the requirements, testing, acceptance criteria, test results, and storage locations of the validation documents, test data, and other supporting documents is usually included in the summary report for easy reviewing and quick references.

After the actual qualification testing and regular performance verification testing, the documents and the related test data are the only proof that the instrument has gone through such testing and has been properly installed and maintained to support its intended applications. The document should be stored systematically in a centralized location and maintained with care in order to prevent any losses. A good recordkeeping system can be extremely useful in audit preparation and help to speed up the turnaround time for documents during an inspection.

8.3.4.3 Operational Phase

After an instrument has been qualified, it is ready for production use. The activities in the operational phase support the day-to-day use and maintain the instrument in a validated state.

Standard Operating Procedure A SOP has to be written to provide instructions for the operation, maintenance, and calibration of the new instrument. A typical SOP should include:

- A general system description
- Operation instructions
- Responsibilities of the system users and system administrators
- Calibration or performance verification requirements, acceptance criteria, frequency of testing, and the actions required if the instrument does not meet the performance verification requirements.
- Maintenance requirements
- Service, major and minor repairs, and parts replacement that will necessitate a requalification of the instrument. For example, the replacement of a UV lamp in a UV detector does not require a full requalification, whereas a replacement of circuitry board will warrant full requalification.

Maintenance Normal wear and tear as well as aging of various components may compromise the performance of the instrument or lead to operation failure. The instrument needs to be maintained in order to function consistently and reliably. A preventive maintenance program which identifies and replaces the consumable parts will likely save time and money in the long run. The usage and service records

are kept with the instrument to provide a performance history of the system. These records can provide clues to simplify troubleshooting in case of instrument failure and in addition are used to help develop a meaningful preventive maintenance schedule.

Performance Verification and Calibration In order to maintain the instrument in a validated state, regular performance verification and calibration are required to demonstrate the instrument is functioning reliably according to a predetermined set of criteria to support the applications required in the user requirements. The current GMP (cGMP) requirements also dictate the calibration of instruments at suitable intervals in accordance with an established written program. The terms *calibration* and *performance verification* are very often used interchangeably. Calibration involves measuring and adjusting the instrument response using known standards. Performance verification verifies the operation and performance characteristics of an instrument against a predetermined set of requirements. Calibration can be considered as a part of performance verification.

The frequency of performance verification testing should be based on the knowledge of the operational reliability for the type of instrument and the type of operations that the instrument will be supporting. Initial frequency can be based on the recommendation from the supplier. A good performance record can justify less frequent verification. One potential drawback for overextending the period between performance verification can be the increased amount of impact assessment on the data generated since the last performance verification in case of system failure. Running system suitability testing before the analysis cannot replace the need for regular instrument calibration. System suitability testing is method specific whereas system calibration verifies the general performance of the instrument. The system suitability test only demonstrates that the instrument is suitable for a particular method at the time of analysis. It cannot reveal marginal performance of the system. For example, the system suitability test for an HPLC assay using UV detection is not likely to detect a wavelength accuracy problem since both the standards and the samples are analyzed at the same wavelength.

Change Control Change control provides a structured mechanism for requesting, authorizing, evaluating, testing, implementing, and releasing changes to validated systems. It should be performed in accordance with approved and documented procedures. These procedures should include the following elements:

Prechange:

- Description of the proposed change
- Assessment of impact of a proposed change
- Reviewing and approval prior to execution
- Communicating changes to system users
- Management approval to implement the change

Postchange:

- Implementing the change
- Testing after the change is implemented

- Training and/or retraining system users
- Management approval to complete the change process

Typical change control forms to are shown in Figures 4 and 5.

The version of firmware may be inadvertently updated by the service engineer during service or routine maintenance without making the changes known to the system owners. The change may not have any impact on the operation of the instrument and may not be detected. However, the new firmware version is different from

Equipment make and type		Instrument identifier	
Current software		New software	
Operating system		Validation plan reference	
Description of change			
Reason for change			
Impact of change			
Validation document references			
Documentation that requires updating			
Requalification necessary (attach protocols)			
Request initiator			Date
Mangement approval			Date
QA approval			Date

FIGURE 4 Prechange control form.

Equipment (make and type)	Instrument identifier	
Test results summary		
Requalification reference	Reference	Date
Documentation reviewed	Reference	Date
Equipment records updated	Reference	Date
SOP reviewed	Reference	Date
System register updated	Reference	Date
Program validation data archived	Reference	Date
System ready for use		Date
Request initiator		Date
Management approval		Date
QA approval		Date

FIGURE 5 Postchange control form.

the version documented in the validation documents. The system owners should work with the service engineers to prevent this potential violation.

Business Continuity Planning (Disaster Recovery) A disaster recovery plan should be in place to ensure the continued operation of the laboratory in case of an adverse event that renders the instrument out of commission and hence causes interruption to the business processes which the system supports. Adverse events like the failure of the critical hardware components of the instrument and the failure of the application software do happen in the day-to-day operation of a laboratory. The disaster recovery plan should provide the necessary steps to restore the systems back to a functional state. The steps typically include instructions to reinstall the application software to the personal computer controlling the instrument, to reconfigure the instrument, and to restore the backup data to the instrument.

Periodic Review The performance of the instrument should be reviewed on a regular basis, typically once every two to three years, to ensure the instrument is still reliable and that it continues to comply with the user requirements. The review should include:

- *Operation Environment* Changes such as temperature, humidity, and vibration that may impact the operation of the instrument.
- *Change Control* System configuration changes; hardware, firmware, and software change and its impacts.
- *Records* Usage, maintenance, services, performance verification testing, and location of the records.
- *Documentation* Validation documents are current and the requirements are being met by the instrument, SOPs, operation manuals, business continuity plan, and location of the documents.
- *User Training* Adequate training for a new version of software operation.

The outcome of the review will determine whether the instrument is maintained in a validated state. In the case that the records indicated the instrument is more prone to certain types of failure, a preventive maintenance program may be desirable to avoid system failure during operation.

8.3.4.4 End of Life

The decommissioning of an instrument is the last step in the validation life cycle. When an instrument is no longer required in the laboratory and is ready for retirement, the following activities and related recordkeeping are required:

- Document in the instrument binder or logbook the reason for decommissioning and the effective date.
- Archive all related records such as the instrument binder or logbook, software, and manuals if no longer required in the laboratory.
- Ensure that all electronic records are archived following established procedures.

- Disconnect all building services from the instrument.
- Make arrangement for the removal of the instrument.
- Withdraw or amend any affected SOPs.
- Update the calibration program and the inventory list.

8.3.5 SUMMARY

The fundamental purpose of validating laboratory instruments is to provide assurance that the instrument is suitable for its intended use. The validation effort associated with a laboratory instrument should be commensurate with the complexity of the instrument, its intended use, and the impact of the data generated. A scalable validation approach to manage the whole life cycle of the instrumentation in the laboratory, from the planning stage to decommissioning, is a good business practice for smooth laboratory operation and will avoid preventable instrument failures. A systematic approach to instrument validation based on sound scientific rationales balancing the potential risks and business affordability can convey confidence to the auditors during laboratory inspections.

REFERENCES

1. Freeman, M., Leng, M., Morrison, D., and Munden, R. (1995), Position paper on the qualification of analytical equipment, *Pharm. Technol. Eur.*, November 1995, 40.
2. Huber, L. (1995), *Validation of Computerized Analytical Systems*, Interpharm Press.
3. Huber, L. (1996), Quality assurance and instrumentation, *Accred. Qual. Assur.*, 1, 24.
4. Huber, L. (1999), *Validation and Qualification in Analytical Laboratories*, Interpharm Press.
5. International Organization for Standardization (ISO)/IEC 17025 (1999), General requirements for the compliance of testing and calibration laboratories, ISO, Geneva.
6. Miller, J. M., and Crowther, J. B. (2000), *Analytical Chemistry in a GMP Environment—A Practical Guide*, Wiley Interscience, Hoboken, NJ.
7. International Conference on Harmonization (ICH) (2000), Quality guideline, Q7A, ICH, Geneva.
8. International Society for Pharmaceutical Engineering (ISPE) (2002), *GAMP (Good Automated Manufacturing Practice) Guide for Validation of Automated System in Pharmaceutical Manufacturing*, Version 4, ISPE.
9. Lam, H. (2004), Procurement qualification and calibration of laboratory instrument: An overview, in Chan et al., Eds., *Analytical Method Validation and Instrument Performance Verification*, Wiley Interscience, Hoboken, NJ, Chapter 9.
10. International Society for Pharmaceutical Engineering (ISPE) (2005), *GAMP (Good Automated Manufacturing Practice) Good Practice Guide: Validation of Laboratory Computerized Systems*, ISPE.
11. U.S. Pharmacopoeia (USP) (2006), general chapter ⟨1058⟩, Analytical instrument qualification, USP, Rockville, MD.
12. U.S. Food and Drug Administration (FDA) (2005), Guidance for industry, 21 CFR Part 11: Electronic records and electronic signatures, FDA, Rockville, MD.

13. International Society for Pharmaceutical Engineering (ISPE) (2002), *Good Practice and Compliance for Electronic Records and Signatures, Part 1, Good Electronic Records Management*, ISPE and PDA.
14. International Society for Pharmaceutical Engineering (ISPE) (2005), *GAMP (Good Automated Manufacturing Practice) Good Practice Guide: A Risk-Based Approach to Compliant Electronic Records and Signatures*, ISPE.
15. Lam, H. (2004), Performance verification of HPLC, in Chan et al., Eds., *Analytical Method Validation and Instrument Performance Verification*, Wiley Interscience, Hoboken, NJ, Chapter 11.
16. Lam, H. (2004), Performance verification of UV—vis spectrophotometers, in Chan et al., Eds., *Analytical Method Validation and Instrument Performance Verification*, Wiley Interscience, Hoboken, NJ, Chapter 10.

8.4

PHARMACEUTICAL MANUFACTURING VALIDATION PRINCIPLES

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8.4.1 INTRODUCTION

The pharmaceutical industry has been a pioneer in the development of quality and safety procedures assuring that the risk of its work is reduced to a minimum. The

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establishment in the last century of good manufacturing practices (GMPs) was a small step in the pharmaceutical work but a giant step in the reduction of risk to patients and operators and financial losses in pharmaceutical industry. Furthermore, to assure a GMP, one of the most important means is the validation procedure. Validation aims to confirm that quality is assured step by step in the process and not just at the end of the pharmaceutical procedures. Generally, an entire process is validated and every step is verified. Validation procedures are intended to ensure that all the different parts, steps, and components of a process are well defined and controlled in order to guarantee that the final product will not change over time. In general, validation is the process of checking if something satisfies a certain criterion and providing accurate and documented evidence that a process or system, when operated within established parameters, can effectively and reproducibly be performed while meeting its predetermined specifications and quality attributes. Therefore, validation is an integral part of quality assurance, involving the methodical study of systems, facilities, and processes aimed at determining whether they perform their intended functions adequately and consistently as specified. When validated the process will provide a high degree of uniform batch assurance, meeting the required specifications and therefore being formally approved. Validation does not improve processes but confirms that these have been properly developed and are under control. It is not only a requirement by the regulatory authorities but also an improvement to the pharmaceutical industry. The pharmaceutical industry benefits with the validation procedures since decreases the risk of problems and thus assure the smooth running of the processes. Also, validation procedures contribute to decreasing the risk of defect costs, the risk of regulatory noncompliance, and in process controls and end-product testing.

Validation can also be defined as documenting that any procedure, process, and activity consistently lead to the expected results, including the qualification of systems and equipment. The processes to be validated must be defined in a validation master plan (VMP) that consists of an approved written plan of objectives and actions stating how and when a company will achieve compliance with the GMP requirements regarding validation. The validation procedure must be well defined in a validation protocol, which is a written plan of actions defining how the process validation will be conducted, specifying as well who will conduct the various tasks and describing the testing parameters. The validation protocol must also contain sampling plans, testing methods, specifications of product characteristics, and equipment to be used. Moreover, it must specify the acceptance criteria and who will sign/approve/disapprove the conclusions derived from such a scientific study. The validation report is the final product of a validation procedure and must contain the results and interpretation of the tasks defined in the validation protocol.

Validation procedures must be applicable to computer systems; cleaning processes; manufacturing processes heating, ventilation, and air-conditioning systems; water systems; and analytical methods and equipment.

The process validation provides a high degree of assurance that a specific process will consistently result in a product that congregates its predetermined specifications and quality characteristics. Process validation provides documented evidence that a process is capable of reliably and repeatedly rendering a product of the required quality. Water and air systems validations establish that the system is under control

over a long period of time. Analytical validation proves that the analytical procedure is suitable for its intended purpose. Equipment validation is applicable to critical equipment whose performance may have an impact on the quality of the product guaranteeing that it works in a suitable manner. Computer validation is intended to provide a high degree of assurance that a computerized system analyzes, controls, and records data correctly and that data processing complies with predetermined specifications. Cleaning validation establishes that cleaning procedures are removing residues to predetermined levels of acceptability, taking into consideration factors such as batch size, dosing, toxicology, and equipment size.

To summarize, validation procedures are a tool to develop a product with good and reproducible characteristics, assuring quality throughout the lifetime and improving security for patients and the pharmaceutical industry.

8.4.2 SCOPE OF VALIDATION PROCESSES

The scope of validation has been widely stated and attempts to clearly define the competences of validation has been described [1]. It is now perfectly understood in both regulatory and compliance points that all laboratory processes, including facilities, equipment, analytical methods, and computer programs used in the analytical testing of pharmaceutical products, must be validated [2]. In the implementation of validation, it is fundamental to outline from the beginning the objectives throughout the validation process, which means that there should be proper preparation and planning before validation is performed as well as a specific program for validation activities.

There should be an appropriate and sufficient structured system, including organizational structure and documentation infrastructure, sufficient sufficient personnel and financial resources to perform validation tasks in a timely manner. Management and the personnel responsible for quality assurance should be involved in this discussion. Validation performance must be under the responsibility of appropriate and experience personnel that should represent different departments depending on the validation work to be performed.

Validation should be performed for new premises or equipment, utilities, or even systems, processes, and procedures at periodic intervals and when major changes have been made in accordance to written protocols. Validation can be prospective, concurrent, or retrospective, depending on when the validation is performed.

There should be a clear distinction between in-process controls and validation. In-process tests are performed during the manufacture of each batch using specifications and methods devised during the development phase. The aim is to monitor the process continuously and not exactly validate it. When a new manufacturing formula or method is adopted, steps should be taken to demonstrate its suitability for routine processing prior to the validation. The defined process using the materials and equipment specified should be shown to yield a product consistently of the required quality.

Essential validation work must therefore be identified to prove control of the critical aspects of the operations. A risk assessment approach should be used to determine the scope and extent of validation.

8.4.3 VALIDATION MASTER PLAN

Validation is intended to establish documented evidence with a high degree of probability that a process previously established will constantly perform according to the intended specified products.

According to the World Health Organization (WHO), the VMP is a high-level document that establishes an umbrella validation plan for the entire project and summarizes the manufacturer's overall philosophy and approach to be used for establishing performance adequacy. It provides information on the manufacturer's validation work program, including the manufacturer's intentions and methods used to establish the adequacy of performance of equipment, systems, and controls and the processes to be validated. The VMP is an approved document that provides the details and time scales for the validation work to be performed, including the responsibilities relating to the plan. VMPs may be defined as structured, detailed plans of work providing information about the overall manufacturer's philosophy, intentions, and approaches to be used to establish performance adequacy and how all of the validation work on a project will be controlled.

Although only a recommendation, the VMP must be, undeniably, the most significant document in any validation program. Throughout this document must clearly postulate the validation policy of each manufacturer, referring the organizational structure of all validation activities. The VMP supplies the main guidelines for the validation program, identifies the responsibilities of the personnel involved in the validation activities, identifies all aspects subject to validation, and expresses the nature and extent of testing on each point [3]. Other issues that must be mentioned in the VMP are a summary of existent facilities, systems, and equipment; a list of processes already validated and those intended to be; the planning and scheduling; the changing control; and references of existing documents prior to elaboration of the VMP. In this way, it is intended to be a retrospective, current, and prospective validation plan. All validation activities relating to critical technical operations relevant to product and process controls within a firm should be included in a VMP as well. This includes qualification of critical manufacturing and control equipment.

The VMP should be a summary document and should therefore be brief, concise, and clear. It should not repeat information documented elsewhere but should refer to existing documents such as policy documents, standard operating procedures (SOPs), and validation protocols/reports. The documentation format is illustrated in the VMP.

A VMP should be divided into chapters covering different subjects. First, an introduction should state the manufacture's validation policy, general description of the scope of those validation activities covered by the VMP, and their objectives, derivation, location, and schedule. Then, it must declare all validation activities and their organizational structure in terms of personnel responsibility for the VMP, validation protocols, validation work, report and document preparation projects, approval of the same validation protocols, reports in all stages of validation processes, and the training needs in support of validation. Other requirements of the VMP are cross references to other documents and to specific characteristics of the processes that are critical for yielding a quality product. Next, all validation activities comprised in the VMP should be summarized and compiled in a matrix format. Such a matrix should provide an overview and contain all items covered by the VMP that

are subject to validation describing the extent of validation required. It should include validation of analytical techniques which are to be used in determining the validation status of other processes or systems, validation approaches, revalidation activities, actual status, and future planning. Finally, the VMP must possess a general statement on key acceptance criteria for all validation activities. A planning section with detailed planning of subprojects must be included with an estimate of staffing, equipment, validation schedule of activities, and other specific requirements to complete the validation effort described. This time plan could be included in the above-mentioned matrix. A VMP requires regular updating. It must end with a statement of the enterprise's commitment to controlling critical changes to materials, facilities, equipment, or processes (including analytical techniques) as well as references and a glossary.

The VMP should contain references to the SOP's and information relevant to all aspects of the validation process. These actions are carried out in the GMP manufacturing process of a product and executed in accordance with written instructions if the manufacture of the product is intended to be GMP compliant. The list of relevant SOPs must be included in the VMP and should define how they must be validated. By planning and scheduling the validation procedure, the VMP defines the periodicity required to assure the validation statements. Validation of the different activities, facilities, and systems will occur in predetermined areas as defined by the VMP. The VMP should state who is responsible for preparing the VMP, preparing the protocols and SOPs, the validation work, the report and documentation preparation and control, approval of validation protocols and reports, tracking systems, and defining training needs.

The VMP helps not only the manufacturer and team members but also the inspection. It helps all members of the validation team to know their tasks, responsibilities of various groups during the validation of the equipment, and utility and validation program with respect to time, people, and money. It also helps auditors to understand the enterprise approach to validation and the setup and organization of all validation activities. Furthermore, the VMP is a living document updated and adjusted during the course of the project. Therefore, there are special changes that require revalidation, namely software changes, site and operational changes, and changes in the source of materials, the process, the equipment, production areas, and support systems.

8.4.4 VALIDATION PROTOCOLS AND REPORTS

8.4.4.1 Validation Protocols

A protocol is a written set of instructions broader in scope than a SOP. SOPs are the detailed written instructions for procedures routinely performed in the course of any of the activities associated with pharmaceutical manufacturing. A protocol describes the details of a comprehensive planned study to investigate the consistent operation of new system/equipment, a new procedure, or the acceptability of a new process before it is implemented.

Protocols include significant background information, explain the rationale behind and the aim of the study, and give a full description of the procedures to be followed. This means as well description of the site of the study; the responsible

personnel; the equipment to be used; the standards and criteria for the relevant products and processes; the type of validation, sampling, testing, and monitoring requirements; a description of how the results will be analyzed; and predetermined acceptance criteria for conclusive purposes. Validation, stability, and clinical studies are examples of written protocols for pharmaceutical manufacturers.

A validation protocol is a document that describes the item to be qualified, the tests and checks to be performed, as well as the results that are expected to be obtained. It is a file in which the records, results, and evaluation of a completed validation program are assembled. It may also contain proposals for the improvement of processes and/or equipment [1]. Validation protocols are important in ensuring that documented evidence is taken which demonstrates that an equipment item, a system, a process, or a method consistently performs at a specified level [4].

Validation protocols are required to describe the objective, methodology, and acceptance criteria for installation, operational, and performance qualifications. They are written to ensure test methods, and acceptance criteria are reviewed and approved before qualification of protocols. In practical terms, there are several stages for the production of protocols. First, an acceptable format needs to be agreed. No universal format exists for protocols, but to some extent, the type of equipment, the size of the project, and the personal preferences will dictate the protocol style. However, some norms have been established. Like other controlled documents, protocols are assigned unique reference numbers and revision numbers. They are titled and numbered on every page and have a particular place for approval signatures. Other common elements in protocols tend to be brief descriptions of the item being qualified and a clear statement of responsibilities.

Often the protocol will incorporate test sheets or sections for recording data. In this way, once the protocol has been executed, the document constitutes a record of the results and conclusion [3]. It must describe the activities to be performed in a validation, including the acceptance criteria for the approval of a manufacturing process or a part thereof for routine use [1].

A validation protocol is necessary to define the specific items and activities that will constitute any validation study. It is advisable for companies to draw up a VMP indicating the overall validation strategy for either the product range or equipment type or the entire site. The protocol must be prepared before initiation of the study and must either include or refer to the documentation required to provide information about a specific process, the parameters involved in that specific process, the personnel responsibilities, and the acceptance criteria.

The list of items to be qualified in the validation protocol must be produced and the approach agreed upon. There are several procedures to perform the validation protocols. Nevertheless, it is important that the approach agreed upon is executed to ensure internal consistency and prevent items being inadvertently forgotten. Thus, for instance, it is possible to group identical items together under one protocol or to have a protocol per item. A single or multiple protocols can be written covering installation qualification (IQ), operational qualification (OQ), and design qualification (DQ). For complex items such as large utilities, is it acceptable to address the generating system and the distribution system in separate protocols. Computer systems validation or control systems for process equipment can be documented within the mechanical validation protocol or in a separate protocol.

The protocol should be approved prior to its use. Furthermore, any changes to a protocol should be approved prior to implementation of the change. Once the approach and format are agreed upon, the protocol preparation can start. Preparing protocols requires individuals who are trained to extract information from a variety of sources and synthesize it into a coherent whole. Typically, sources of information include material requisition, specifications, data sheets, piping and instrument diagrams, manufacturer's literature, and equipment operating manuals. Significant protocols cannot be written until sufficient sources of information are available. Once the first draft is written, protocols require review. Usually, individuals from manufacturing, engineering, and quality assurance will perform the review. It is fairly normal for first-draft protocols to require considerable modification as this will be the first time that other interested parties have seen them. Rather than check everything, it is preferable that the review disciplines concentrate on their specialist areas. For example, manufacturing should evaluate if the proposed acceptance criteria are consistent with the process requirements; engineering should ensure that the list of instruments, major components, and utilities requirements are accurate; and quality assurance should ensure that compendial requirements have been met and that the protocol meets normal quality expectations.

In a large project, the coordination of protocol review, keeping track of the revision status, and the storage and retrieval of protocols are tasks that require careful planning. At some points it is probable that a number of protocols at different draft stages are circulating for review, some are being modified, new protocols are being prepared, and approved protocols are stored prior to execution. A tracking system should be implemented that can very quickly identify where a protocol is, providing a picture of the current protocol production status. The method and time allowed for review must also be agreed upon. Review methods range from traditional circulation of a single document to each individual for comment to assembling a team for a joint review to electrically accessing the document and revising or commenting on it. The method chosen must reflect the available time, resource, and technology. In some cases, the resources for protocol review are a limiting factor that can critically affect the schedule [3].

8.4.4.2 Validation Reports

A validation report is a written document that cross-references the validation protocol, summarizes the results obtained, describes any deviations observed, and draws the necessary conclusions, including recommending changes required to correct deficiencies for the qualification and validation performed [5]. In this report it is required to present both the results and conclusions and the secure approval of the study. The report should include a summary of the procedures used to clean, sample, and test as well as the physical and analytical test results or references for the same. The conclusions regarding the acceptability of the results should also be included. Other information would be the status of the procedures being validated, any recommendations based on the results, or any relevant information obtained during the study. These include, revalidation practices (if applicable), the approved conclusions, and any deviations of the protocol that might have occurred. In cases where it is unlikely that further batches of the product will be manufactured for a period

of time, it is advisable to generate temporary reports on a batch-by-batch basis until such time as the cleaning validation study has been completed [1].

Finally, the departments responsible for the qualification and validation work should approve the completed report and the conclusion of the report should state if the outcome of the qualification and/or validation was considered successful. The final review is performed by the quality assurance department, which gives the approval of the report according to the company's quality assurance system [1].

8.4.5 FACILITIES VALIDATION

8.4.5.1 Generalities

Facilities validation is related to the location, design, and construction of the plant to facilitate cleaning, maintenance, and operations in order to be appropriate for the type and stage of manufacture [6].

All facilities in a pharmaceutical industry must be designed and validated in order to assure the minimum risk of cross-contamination. Facilities validation must include the products and personnel flow, rooms design and cleaning, air and humidity systems, and water pipelines.

8.4.5.2 Design of Facilities

With regard to the design, the overall facilities should always be developed according to the most simplistic route of material flow and control of cross-contamination. Several layouts have therefore been described [7], with the aim of separating released materials from quarantined or rejected ones.

One of the most popular layouts consists of a center, or core, that has been conceived as a storage or warehouse area for raw materials, packaging components, and bulk stocks. Following this warehouse area immediately in the outer perimeter the manufacturing and packaging operations should be located to allow the flow of raw materials and components from the receiving and quarantine areas to approved storage. After materials are weighed into batch quantities, they are moved into the manufacturing area. When the manufacturing process is finished, the obtained products are placed in quarantine and then moved to bulk stock upon release. The packaging run follows when scheduled, and then the product and packaging components are delivered from the bulk stock and approved storage areas. An advantage of this layout is space conservation by virtue of having the supply areas close to the areas being supplied. Nonetheless, a significant disadvantage is the crossover of materials with the potential risk of contamination or mix-up.

An alternative layout design could be having the receiving, approved raw materials, components storage, and dispensing on one side and the manufacturing, quarantine, bulk stock, and packaging areas across a central corridor. Material flow from one area to another occurs in the same way as in the previous layout. However, in this case, flow is circular, eliminating much of the crossover described above.

To minimize contamination or mix-up, a third layout could be basic straight-line flow moving the materials along a critical path. The main advantage over the abovementioned layouts is minimal crossover of materials, thus minimizing the potential

for contamination or mix-up. The additional space required to accommodate this configuration can be pointed to as the main disadvantage.

Cross-Contamination Control Two parameters particularly useful in controlling the cross-contamination are the air-handling systems and dust collection.

Regarding the GMPs, air-handling systems are designed with the supply of outside air, moving on to the filtration systems that will be used, determining where positive and negative air pressures are required and whether to recirculate or exhaust spent 100% air, and finally to dust collection and exhaust systems.

Air filtration systems, including prefilters and particulate matter air filters, should be used when appropriate on air supplies to production areas. If air is recirculated of dust from production in areas where air contamination occurs during production, appropriate exhaust systems or other systems adequate to control contaminants should be requested.

A typical design involves one or more bag or cartridge filters located close to the area of dust generation. These coarse filtration devices should remove 95% of the dust generated from normal pharmaceutical manufacturing operations [8]. The prefiltered air is then mixed with 10–15% outside make-up air and passed through a high-efficiency particulate air (HEPA) filter and reenters the rooms through the supply plenum diffuser.

Regarding dust collection, the first area that must be addressed is the raw material sampling rooms and dispensing area. This area should be an enclosed facility with separate booths or hoods where the individual weighing or sampling can take place. These areas may be designed using horizontal laminar flow or appropriate hoods and other dust pickup devices. The supply air to these stations will therefore be HEPA filters either at the pickup stations or after the dust collector prior to returning to the general area or supply air.

The last area of dust collection must be the packaging. Some machines are designed with a self-contained vacuum system that returns the air, filtered through an absolute filter, back to the packaging area. There should also be some provisions made at the cottoning stations.

Humidity and Temperature Control Humidity and temperature control systems are also important considerations as these impair both product protection and working environment comfort. Unless otherwise stated, 45% room humidity and 21°C are usually appropriate for critical manufacturing areas. Comfortable conditions should be provided for all operations. However, temperature controls should be such that little or no variation will be caused by external ambient temperatures. Thus, comfortable working conditions are achieved and there should be no impact on the characteristics of in-process materials. Warehousing operations should have adequate ventilation, particularly in areas of high storage, either pallet racks or pallet-to-pallet storage. The ventilation could be provided by large roof fans to circulate air. In addition, some form of supplemental air heating, such as hot-air blowers, should be provided for cold areas, such as shipping or receiving docks [7].

Water Systems Control The supply of potable water in a plumbing system must be free of defects that could contribute contamination to pharmaceutical products.

Therefore, an effective water system is required. Nowadays, several techniques can be used to obtain water of high pharmaceutical quality. These include ionexchange treatment, reverse osmosis, distillation, electrodialysis, and ultrafiltration. However, there is no single optimum system for producing high-purity water, and selection of the final system is dependent on factors such as the quality of raw water, intent of its use, flow rate, and costs. In the pharmaceutical industry, the different water classes normally encountered are well water, potable water, purified water, and specially purified grades of water, such as water for injection (e.g., MilliQ water).

Water drawn directly from a well is called well water. The water may not be either chemically or microbiologically pure because it is untreated [7]. Therefore, the use of well water should be restricted to nonmanufacturing operations, such as lawn sprinklers, fire protection systems, and utilities.

Potable water is city water or private well water that has usually been subjected to some form of microbiological treatment, such as chlorine addition [7]. Potable water can be used in processing operations for cleaning and sanitation purposes. Periodic monitoring of use points should be conducted to ensure adequate residual chlorine levels and the absence of microbial contamination. Purified water is treated to attain specified levels of chemical purity and it is the type of water used in most pharmaceutical processing operations and final equipment cleaning. Purified water generally is produced by deionization or distillation, although reverse osmosis or ultrafiltration systems might be utilized if the required chemical purity could be achieved. Water softening or activated carbon filtration is frequently employed as a pretreatment process to remove calcium and magnesium ions or chlorine and organic materials. Ion exchange and demineralization through deionization is a very common method to obtain the purified water used in the pharmaceutical industry.

Deionization equipment should have proper size to allow frequent regeneration. A recirculation system should also be installed in the unit that approaches the rated flow of the deionization unit. Procedures should be written to ensure that all water treatment equipment is properly operated, monitored, maintained, and sanitized on a regular basis.

Regarding water filtration procedures first, prefilters should be provided to prevent large particulates from entering the system and microfiltration to remove bacteria. Prefilters are usually the replaceable cartridge type with porosities ranging as high as 25 µm. Microfiltration follows and is generally accomplished with 0.2-µm absolute filters, which will remove most bacteria.

After definition of the water type required, the water pipeline must be validated in order to ensure an adequate flow and purity (chemical and microbiological) of water. After the validation process, periodic verification of the pipeline and water collection points is required. This verification must be based on well-defined SOPs.

Sanitizing is best accomplished through several methods. After periods of low water usage, the system should be flushed with a supply of water that has residual chlorine. Periodic hyperchlorination and microbial control are also recommended. Microbial control can be achieved by storing the water at 80 °C. Alternatively, ultraviolet radiation can be applied.

Pest Control Each manufacture, processing, packing, or holding area of a pharmaceutical product should be maintained clean and sanitary. This means that these

areas must be free of infestation by rodents, birds, insects, and others (other than laboratory animals). Trash and organic waste matter should be held and disposed of in a timely and sanitary manner.

A pest control program should be developed in order to assure the integrity and quality of products produced and comply with existing guidelines. The program should be written to include a general statement of the purpose and the company position. Effectiveness of the program should be assured by defining the plant individual with overall responsibility for the program and how the responsibility will be carried out. In addition, the extermination staff, whether they be in-house or subcontracted personnel, should have their training and experience requirements well defined. Assistance in supporting the program may be gained from other plant personnel by their pointing out problem areas.

This program should be treated to line management personnel. Furthermore, its content regarding the list of approved pesticides to be used in the plant should also be verified periodically. Basic information should be controlled, such as the trade name of the pesticide; classification; type of action; chemical name and concentration of active ingredient; effectiveness, usefulness, area of usage, mode, and frequency of application; toxicities and any specific toxic symptoms; status of government approval; and specific restrictions and cautions [7]. The development of sheets depicting such information will serve a twofold purpose. First, these sheets are subject to approval by the plant safety organization to determine if the materials comply with the Occupational Safety and Health Administration (OSHA) requirements and the requirements of other state or local agencies. Additionally, these sheets would also facilitate compliance with the GMP regulations. Written procedures for use of suitable rodenticides, insecticides, fungicides, fumigating agents, and cleaning and sanitizing agents should be provided in order to prevent the contamination of equipment, components, drug product containers, closures, packaging, labeling materials, or drug.

All manufacturing areas should be constructed using nonporous materials on the walls and floors. Any protrusions, such as pipes and electrical boxes, should be minimized. Space should be allocated carefully to provide sufficient rooms for all operations. There should be adequate lighting and the areas should be remote from any openings to the outside. Adequate training in understanding GMPs should be given to all personnel, including outside contractors.

Scheduled inspections and preventive treatments should be documented specifying the problem encountered as well as the special service that has shown effectiveness to the treatment.

Manufacturing Rooms Manufacturing rooms must be well designed in order to ensure adequate cleaning and reduction of cross-contamination. Points of dust accumulation like 90° angles areas must be avoided in the room, dust collectors or air lines must be presented restrictly at the wall surface and only the minimum equipments must be presented in a room.

Air pressure inside the manufacturing rooms must be positive or negative depending on whether the product is a liquid or a powder, respectively, in order to avoid cross-contamination. It must be monitored by validated pressure systems.

Packaging and Labeling Control Type of construction in the packaging area is very important because it is particularly sensitive to mix-ups in either product or labeling. Individual packaging lines should have minimum separation between each line. Depending on the equipment used, more space might need to be used. Consideration should be given to the separation of lines by partitions. The partitions should be closed at floor level to prevent migration of product and be high enough to prevent any crossover of product.

One possibility might be to start packaging operations with a prestaging area large enough to contain all required components for the packaging operation. This area is separate but adjacent to or in front of the filling area. The filling area should be equipped, if possible, with air filtered through absolute filters. Dust collector from the central dust collector system should be located in the filling area to remove dust from the filling area. The air circulation in the room must enter in the filling area and then be moved to the area where cottoning, capping, or labeling takes place. Thus, maximum product protection is exercised in the filling area while the product is exposed, with lesser degrees of control being required in the other areas because the product is in containers.

SOPs should be developed for all packaging operations. These include specific procedures for line setup, approval of line before start of operations, periodic line check during operation, close out of line, line clearance at end of operation, and reconciliation of product and components.

Labeling operations must also receive special attention. The use of cut labels in the pharmaceutical industry has already disappeared. Electronic label counting and verification are the norm, with bar codes, universal product codes (UPCs) or health industry bar codes (HIBCs) being used more frequently as label identifiers. The storage of labels is very important for both security and preservation reasons. The verification of labeling at the final label application point is also becoming more popular, since the the U.S. Food and Drug Administration (FDA) has identified errors.

SOPs must be developed for label accountability with specific tolerances spelled out. Provision must be made for label security, such as locked cabinets on operating lines. Accountability sheets for other components should be provided so that reconciliation between used and finished packages can be developed. If possible, a cleaning area and shop should be set up for the cleaning of filters and parts and the disassembly and assembly of filling machines.

8.4.6 MANUFACTURING PROCESS VALIDATION

The purpose of manufacturing process validation is to ensure that the manufacturing process for the drug product is fully controlled and capable of providing a product that complies with established quality specifications consistently and repetitively. This validation process covers manufacturing operations from formulation through packaging. Process validation is being conducted in support of launch of the drug product.

Process validation also establishes documented evidence that the manufacturing process, when executed according to the VMP and pertinent process SOPs, consis-

tently manufactures a drug product that meets all of the critical specifications required for its purpose. It is designed to ensure that all of the prerequisites are in place as well as to allow technical analysis of all applicable performance tests.

Nowadays the manufacturing validation tendencies tend to focus on pharmaceutical development. Therefore, the primary responsibility of the pilot plant is to ensure that the developed product is efficiently, economically, and consistently reproducible on a large scale. Since low production costs are of higher competitive advantage, attention must be paid to the production costs. Each operation unit should therefore be optimized. To avoid excessive production times, the manufacturing instructions transferred to the production department should be clearly written, readily understood, and unambiguous. Unless the economic advantage of purchasing new equipment has been proven to be necessary, in-house equipment should be preferentially used. Nonetheless, if international companies are intended to manufacture products at several sites, alternate manufacturing equipment and procedures might also be required.

The physical properties and specifications for the manufactured formulations should match those established earlier by the product formulator, the pilot plant staff, and the quality control department. Therefore, the product manufactured on a large scale should possess the appropriate quality properties.

Additional responsibilities for pilot plant staff are those related to the evaluation of new processing equipment, which aims to find causes and solutions for problems that occasionally might arise during the production. Since the department of pharmaceutical research and its development division are responsible for developing the formulations and the manufacturing processes for the dosage forms, the experience gained in the development of the manufacturing process will be of major importance during the validation process. Such validation conducted at a pilot scale should simplify large-scale validation.

It is clear that the aim of pharmaceutical research is to achieve zero defects and zero batch rejections, and this can be verified by process validation. One must bear in mind that exhaustive finished testing of product is not a substitute for in-process controls and process validation.

The validation process will consistently deliver product that has a direct relationship to a dosage form on which clinical efficiency and safety were determined. Thus, comparisons between the manufacturing process, the raw material used, in-process control, and finished-process test results are in order. During scale up, consideration of these factors at the development stage must be carefully performed to produce quality batches. Matching equipment from laboratory to production helps to eliminate possible validation problems afterward. The physicochemical characteristics of the active drug substances should be controlled. Special attention should be paid to products in which the drug substance comprises a very small percentage of the pharmaceutical dosage forms.

The physicochemical characteristics of raw materials play an important role in content uniformity and bioavailability. Therefore, bioavailability of the drug over time must be thoroughly investigated before any significant changes are made. Once the physicochemical properties of drug substances (e.g., particle size of raw materials) can influence the availability and clinical effect of a product, the main characteristics of raw materials should be considered in a validation program. The

characteristics of raw materials can vary among manufacturers, but also from lot to lot of the same manufacturer. Control of the physicochemical characteristics of excipients is also important and must be stated in the specifications.

In order to develop a reproducible manufacturing process, attention must be given to particular instructions and screening procedures. For instance, excipients should be free of lumps and proper screening will aid raw material dispersion. Additionally, one should specify the size and design of containers and all equipment to be used.

Changes in process decisions regarding the suitability of new manufacturing equipment that is intended to be employed should be performed by management and by production personnel. Equipment changes for some products might guarantee additional dissolution and/or content uniformity studies. The degree and depth of a study are largely dependent upon the specific product. For some products with very good historical data coming from a reproducible and controlled process, particular tests can be obviated. However, if potent dosage forms are to be developed, those are usually performed (e.g., sampling the mix in tablets technology) [9]. If products with lack of historical data are to be handled, additional finished product testing should be performed. For instance, with respect to tablet production dissolution testing and content uniformity testing are usually implied in the finished product tests.

The batches utilized in this type of approach for manufacturing process validation should be presented on different scales as the manufacturing process is developed. Laboratory-scale batches are of very small size (e.g., 100–1000 times less than production scale) and are produced at the research and early development laboratory stages, which are used to support the formulation and packaging development and clinical and/or preclinical studies. In the development pharmaceutics, data validation of laboratory-scale batches can contribute to the choice of the appropriate manufacturing process (evolution and definition of critical product performance characteristics). Pilot batches correspond to at least 10% or 100,000 units for tablets (the biggest) of the production batches and are used in the development or optimization stage to support stability studies. Pilot batches are used to provide data predictive of the production batches and therefore provide the link between process development and industrial manufacture of the pharmaceutical product. Finally, production-scale batches will be produced during routine marketing of the drug product.

Traditionally a proper process validation performance must be established based on the following considerations:

- All raw materials used for the validation batches must be approved by quality control (QC).
- Drug products are validated concurrently. Samples from three consecutive batches should meet all of the predetermined specifications without unexplained failures. If any of the three batches does not comply, the validation will be repeated up to three times to try to obtain three consecutive acceptable results. If not obtained, the tests will be suspended until the manufacturing process has been reviewed.
- The validation batches must use the regular production equipment and personnel.

- Critical in-process control parameters must be specified by ranges, and full-range monitoring is performed. The limit determined to be the "worst case" will be challenge at least twice and the other limit challenged at least once to complete the full-range monitoring.
- For critical process control parameters specified by fixed points, the value ought to be challenged within an acceptable tolerance, typically ±1 unit.
- All tests must be conducted by training and experienced technical personnel and must be documented in a scientific manner using the established format of the protocol.
- Any test function that does not have results which support the parameters defined in the approved protocol must be conclusively rationalized for their nonconformance and approved; otherwise, the qualification will be considered invalid.

Samples are to be taken during and/or after each critical manufacturing step. All control parameters for the manufacturing process have to be monitored and recorded. Each sample analysis will be performed in duplicate using validated or accepted pharmacopeia methods. The sample results will be used to confirm in-process and final product quality attributes as defined by the preestablished specifications. Conformance with specifications will justify the appropriateness of the critical parameters used during the process validation.

Validation data should be generated for all products to demonstrate the adequacy of the manufacturing process. The process validation data may not always be available, however, where the manufacturing process uses a nonstandard method of manufacture. Data demonstrating the validity of that method should be submitted in the marketing authorization file.

8.4.7 ANALYTICAL METHODS

The aim of validation of an analytical procedure is to demonstrate that the method employed in any product testing, such as the identification, control of impurities, assay, dissolution, particle size, water content, or residual solvents, is validated in the most important characteristics. Identification tests, quantitative tests for impurities content, limit tests for control of impurities, and quantitative tests of the active moiety in samples of pharmaceutical product are the most common types of analytical procedures that validation addresses [1].

However, other analytical procedures, such as dissolution testing for dosage form or particle size determination for drug substance, are required for validation of analytical procedures. The revalidation of an analytical procedure is possible when, in particular circumstances, it could show changes in the synthesis of the drug substance, the composition of the finished product, or the analytical procedure. However, certain other changes may require validation as well.

Method validation should confirm that the analytical procedure employed for a specific test is suitable for its intended use. The validation of an analytical method

is the process by which it is established by laboratory studies that the performance characteristics of the method meet the requirement for the intended application. This implies that validity of a method can be demonstrated only through laboratory studies. Methods should be validated or revalidated before their introduction and routine use whenever the conditions change for which the method has been validated (e.g., an instrument with different characteristics) and the method change is outside the original scope of the method.

Depending on the use of the assay, different parameters will have to be measured during the assay validation. Validation of analytical assays is the process of establishing one or more of the following as appropriate to the type of assay: accuracy precision (repeatability, intermediate precision), linearity, range, limit of detection, limit of quantification, specificity, and robustness [1]. For physicochemical methods there are accepted defined limits for these test parameters:

1. Specificity is the ability to assess unequivocally the analyte in the presence of components, which may be expected to be present and include impurities, degradants, matrices, and so on.
2. Accuracy is the replica of agreement between the value which is accepted as either a conventional true value or an accepted reference value and the value found.
3. Precision is the degree of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions. Precision may be considered at three levels: repeatability (precision under the same operating conditions over a short interval of time), intermediate precision (precision within-laboratory variations: different days, different analysis, different equipment, etc.), and reproducibility (precision between laboratories, collaborative studies, usually applied to standardization of methodology).
4. Detection limit is the lowest amount of analyte in a sample which can be detected but not necessarily quantified as an exact value.
5. Quantitation limit is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantification limit is a parameter of quantitative assays for low levels of compounds in sample matrices and is used particularly for the determination of impurities and/or degradation products.
6. Linearity is the ability, within a given range, to obtain test results which are directly proportional to the concentration of analyte in the sample.
7. Range is the interval between the upper and lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy, and linearity.
8. Robustness is a measure of the capacity to remain unaffected by small but deliberate variations in the method parameters and provides an indication of its reliability during normal usage.

8.4.8 EQUIPMENT AND COMPUTER SYSTEMS

8.4.8.1 Equipment Systems

Generalities With the advent of the industrial revolution in the eighteenth century, machinery became essential for our lifestyle. Machinery is one of most important key factors of our society and the same occurs in the pharmaceutical industry. In the middle of the twentieth century, many procedures in the pharmaceutical industry were hand manufactured, requiring a lot of time and manpower. Packaging was one of the most rudimentary tasks performed; an image of hundreds of persons seated at a table packaging pharmaceutical products was a normal view in the middle of the last century. In the second half of the twentieth century manpower was replaced by machinery. Blister machines, automatic capsule-filling machines, and bigger compression and drying equipment became common in industry, dramatically increasing output and profits. Since then, the equipment used in the manufacture of medications has shown great improvement. According to GMPs all equipment must be located, designed, constructed, adapted, and maintained to suit the operations to be carried out. Their layout and design must minimize the risk of errors, allowing effective cleaning and maintenance in order to avoid cross-contamination, buildup of dust or dirt, and, in general, any adverse effects on the quality of products [10]. Equipment and premises must improve the product quality and safety and should never create any risk to the product. Adequate design must be taken into account when new equipment is created in order to improve its efficiency and cleanness and reduce errors or breakdowns.

Equipment should be placed under adequate environmental conditions in order to function accurately and in such a way as to prevent any risk of error or contamination. The environment must show minimal risk of causing contamination of materials or products when considered together with measures to protect the manufacture [10].

Manufacturing equipment should be designed, located, and maintained to suit its intended purpose. Repair and maintenance operations should not present any hazard to the quality of the products [10]. It should be designed so that it can be easily and thoroughly cleaned. It should be cleaned according to detailed and written procedures and stored only in a clean and dry condition. Washing and cleaning equipment should be chosen and used in order not to be a source of contamination.

Production equipment should not present any hazard to the products. The parts of the production equipment that come in contact with the product must not be reactive, additive, or absorptive to such an extent that it will affect the quality of the product and thus present any hazard [10]. Equipment should not be made of materials that contaminate the final product; therefore manufacturing equipment is usually composed of stainless steel and polymeric materials that are easily cleaned. According to this, natural materials must be avoided.

Balances and measuring equipment of an appropriate range and precision should be available for production and control operations. Measuring, weighing, recording, and control equipment should be calibrated and checked at predetermined intervals by appropriate methods. Adequate records of such tests should be maintained [10].

All equipment for which calibration is applicable must be periodically calibrated. Periodicity of calibration must take into account the type of the equipment, risk assessment, and previous results. An extremely short calibration periodicity becomes extremely expensive, whereas an extremely long calibration periodicity could result in poor verification results. Poor verification results mean that the results generated are wrong and therefore all results obtained since the previous calibration must be reviewed. In conclusion, adequate evaluation of the calibration periodicity is essential.

All equipment must be well identified with name and code and must have a single operation instruction. A good operation instruction must contain at least the following points: (i) equipment description, containing the function of the equipment, name, code, serial number, model, manufacturer, accessories, dimensions, power source, connections, and component descriptions; (ii) function, containing a description of a procedure of how to handle the equipment, and all the steps required and options; (iii) calibration procedure, described in detail and providing the calibration periodicity; (iv) maintenance, a simple and easily maintenance procedures should be available; (v) cleaning described in detail; and (vi) security procedures.

Equipment System Validation The GMP has special features regarding the equipment, setting several points for the validation, such as design qualification, installation qualification, operational qualification, and performance qualification. Each point must be given in a different document, so four documents must be generated for each equipment. Without any of these documents, the equipment cannot be considered adequate for GMP purposes. GMPs do not refer equipment validations; nonetheless, they refer these four items, and therefore, equipment can only be considered validated after the approval of these four documents.

Equipment validation is not reliable, and several special features are required instead. GMP is concerned with several procedures before the equipment is placed into the service. After entrance into service, maintenance and calibration must be made periodically. The following focuses on the procedures required for new equipment to enter into service.

Design Qualification When a pharmaceutical industry intends to purchase new equipment, it must first know and define what type of equipment and which specifications it must have. Specifications must be set taking into account specific requirements of the company or facility. All the specifications of the equipment must be reviewed. This is called design qualification. The requirements of the equipment must be defined in a specific document previous to the purchase by any pharmaceutical company. The document generated is used to justify the equipment selection from the various proposals.

Installation Qualification After equipment selection, it is necessary to assure that the equipment is installed well. The IQ document describes and validates the procedure of the equipment installation. It establishes confidence that the process equipment and ancillary systems are capable of consistently operating within established limits and tolerances [10]. The equipment manufacturer and pharmaceutical company must agree and check the IQ, which must be approved by the pharmaceutical company at the end. This document certifies that equipment was installed as specified by the manufacturer and the purchaser.

Operational Qualification The OQ document certifies that the equipment works as desired and defined by the manufacturer and the purchaser. An example is the acquisition of a new high-shear mixer/granulator where the paddle is put on rotation with a real calibrated rotation speed tester and, if the value obtained meets the specifications, the mixer paddle rotation passes the OQ test. If not, additional requalification must be performed. All the test results must be introduced and verified in the OQ report that is approved by the company at the end. The OQ document must describe several tests and related specifications to perform on the equipment in order to evaluate if it is working well, and the test to be performed must be described and approved by the manufacturer and the purchaser. Therefore, tests must be performed on the equipment, and for each one a description and signature of who performed and verified the test are required. Usually the tests are performed by the manufacturer and verified by the purchaser. These tests usually consist of evaluating if the mechanical and electric components of the equipment are working as desired.

Performance Qualification After evaluation of the way the equipment is working, it is required to perform some tests applying real situations to evaluate if the results are in agreement with those specified. It is called performance qualification (PQ) and establishes confidence through appropriate testing that the finished product obtained by a specified process meets all release requirements for functionality and safety [11].

Commonly when a pharmaceutical industry purchases a new compression machine, a PQ is conducted. First, the company must select some of its well-known products and prepare them until the compression phase as usual. The product must be compressed by the new equipment using the same compression conditions, such as compression force and tablet output. The obtained tablets must meet all the specifications of that product. The parameters specified could be aspect, hardness, thickness, diameter, average mass and uniformity of mass, friability, and tablet output. If the tablets obtained comply with specifications, the compression machine is considered reliable to obtain a product with good quality and in a reproducible manner and the equipment is considered performance qualified.

Also in the PQ a document must be created with the description of the tests to be performed and the related specifications. This document must be verified and approved before the test performance. After the tests, the results must be introduced in the document and the pharmaceutical company must approve the final version.

All the equipment qualification documents must define the equipment designation, tests to be performed, test specifications, materials, operators, reviewers, and responsibility for approval.

After approval of design qualification, installation qualification, operational qualification, and performance qualification, the equipment is considered adequate for GMP proposes and can be placed in service.

8.4.8.2 Computer Systems

Generalities Technological advances are increasing extraordinarily, with the advent of computer system processes that were unthinkable a few years ago but nowadays are easy and simple. These advances are available every day in almost

everything. Particularly in the pharmaceutical industry the last decade has seen the development of new analytical equipment, such as near Infrared, Fourier transform infrared (FTIR), or Raman spectroscopy using complex software to perform modulation or high-performance liquid chromatography (HPLC) and mass spectrophotometry, which have easier and simpler software. All this evolution in analytical equipment allows the industry to produce better and have higher profits. The computational systems revolution has reached not only analytical equipment used in pharmaceutical industry but also manufacturing equipment. Nowadays in the pharmaceutical industry almost every operation uses a computer system. Therefore, all computer systems in the pharmaceutical industry are supposed to be validated in order to assure that the results they generate are accurate and precise. In addition, wherever a computerized system replaces a manual operation, there should be no resultant decrease in product quality or quality assurance. Consideration should be given to the risk of losing aspects of the previous system which could result from reducing the involvement of operators.

A computer system is composed of software and hardware, equipment, a processor, and a user, and it is used to execute a specific procedure. Regardless of whether the computer system is developed in-house or by a contractor or purchased off the shelf, establishing documented end-user requirements is extremely important for computer systems validation. Without first establishing end-user needs and intended use, it is virtually impossible to confirm that the system can consistently meet them. Once established, it should obtain evidence that the computer system implements those needs correctly and that they are traceable to system design requirements and specifications. It is important that the end-user requirements specifications take into account predicate rules [12].

Computer System Validation All computer systems presently used in the pharmaceutical industry need to be evaluated for risk assessment. The first task in the validation of a computer system must be to determine if a potential failure in the computer system can cause a risk in product security, efficacy, and quality. This must be applicable in all pharmaceutical industry areas, related with good clinical practices (GCPs), good distribution practices (GDPs), good laboratory practices (GLPs), or GMPs. Validation of a computer system also provides evidence that all of its components are working perfectly and generating adequate results. Thus, all of its components must be validated, such as applications, processes, users, and facilities.

Risk assessment is the first critical step in the validation of a computer system. After risk assessment, the validation protocol must be created, including all the points referred in the VMP. The procedures to be executed in a computer system should be defined, in addition to the specifications and tests to be performed. These tests could be trials for IQ, OQ, and PQ. After the test procedure and data evaluation, a validation report must be available. Therefore, a very well written and periodically reviewed (e.g., every year) VMP, validation protocol, and validation report are always necessary.

The extent of validation necessary will depend on a number of factors, including the use to which the system is to be put, whether the validation is to be prospective or retrospective, and whether novel elements are incorporated. Validation should be considered as part of the complete life cycle of a computer system. This cycle

includes the stages of planning, specification, programming, testing, commissioning, documentation, operation, monitoring, and modifying [13].

A retrospective validation is applicable to computer systems in use when the VMP is elaborated. In this case, the real use of the system must be pointed out in order to allow a correct test plan.

To produce an adequate validation close cooperation between key personnel and those involved with the computer systems is essential. People in responsible positions should have the appropriate training for the management and use of systems within their field of responsibility which utilizes computers. This should include ensuring that appropriate expertise is available to provide advice on aspects of design, validation, installation, and operation of computerized systems [12].

After the validation procedure and personal characterization, it is necessary to describe how to handle the computer systems to validate them. As previously referred, a computer system is used to execute a specific procedure to give a defined result. Therefore, attention should be paid to siting equipment in suitable conditions where inappropriate factors cannot interfere with the system [13]. Therefore, the physical place of the computer system must be described in the validation protocol, and every time its place is changed a revalidation plan is required.

A written detailed description of the system should be provided (including diagrams) and kept up to date. It should describe the principles, objectives, security measures and scope of the system, the main features of the way in which the computer is used, and how it interacts with other systems and procedures [13]. A computer system is a particular type of equipment, and, as for any equipment, it is required to have a SOP available and, if possible, near the equipment.

The software—a program enabling computer to perform a specific task—is a critical component of a computerized system. Even a simple calculus sheet could be considered software, as it allows a computer to perform a specific task. The user of such software should take all reasonable steps to ensure that it has been produced in accordance with a system of quality assurance [13].

The system should include built-in checks of the correct entry and processing of data. In order to verify the validation data, some computer systems may periodically be submitted to a defined group of inputs for which the result is known and the result must be kept and filed. If the results are acceptable, the computer system is operating well, but if the results do not match the expected ones, the computer system is not working properly and maintenance is required. In this situation, all the results obtained from the referred computer system since the last validation verification are consided questionable and must be reevaluated.

Before a system using a computer is brought into use, it should be thoroughly tested and confirmed as being capable of achieving the desired results. If a manual system is being replaced, the two should be run in parallel for a time as part of this testing and validation [13]. This means that every time maintenance has made an intervention in the equipment and any piece has been adjusted or replaced, a revalidation is required. This maintenance intervention could be in both hardware and software.

Data should only be entered or amended by authorized people. Suitable methods of deterring unauthorized entry of data include the use of keys, pass cards, personal codes, and restricted access to computer terminals. There should be a defined

procedure for the issue, cancellation, and alteration of authorization to enter and amend data, including changing personal passwords. Consideration should be given to systems allowing for recording of attempts to access by unauthorized people [13]. A computer system must have different user levels and the access to each application should be protected by a user name and password. Critical operations, such as method modifications, calibration procedures, and adjustments, must only be possible by specific personnel. Operators must only have access to procedures defined and without any possibility of changes. All the operations must be automatically recorded in the equipment, including operator user name, time, and date. These records may be available for a quality assurance audit.

When critical data are being entered manually (e.g., the weight and batch number of an ingredient during dispensing), there should be an additional check on the accuracy of the record which is made. This check may be done by a second operator or by validated electronic means [13]. Frequently, computer systems require external data to realize the specified task. The introduction of an external data is a critical step in the computer system procedure, because an error in data introduction may produce an erroneous result. To avoid errors one can proceed by double verification followed by the automatic registry prove or, alternatively, double verification consisting of the introduction of data by one operator while another verifies the data introduced and both sign the register. An automatic registry proves that it is available when the values introduced in a computer system came from another computer system registry. For example, in the dissolution test for tablets, tablet weights introduced in the computer system could be available from a balance associated with an automatic registry; that registry is the proof that the data introduced are correct.

The system should record the identity of operators entering or confirming critical data. Authority to amend entered data should be restricted to nominated people. Any change to an entry of critical data should be authorized and recorded with the reason for that change. Consideration should be given regarding the creation of a complete record of all entries and amendments (an "audit trail") [13].

As previously mentioned, changes to a system or to a computer program should only be made in accordance with a defined procedure and should include provision for validating, checking, approving, and implementing the change. Such a change should only be implemented and recorded with the agreement of the person responsible for the part of the system concerned. Every significant modification should be validated.

For quality auditing purposes, it should be possible to obtain clear printed copies of electronically stored data. Data should be secured by physical or electronic means against wilful or accidental damage. Stored data should be checked for accessibility, durability, and accuracy. If changes are proposed to the computer equipment or its programs, the above-mentioned checks should be performed at a frequency appropriate to the storage medium being used. Data should be protected by backing up at regular intervals. Back-up data should be stored as long as necessary at a separate and secure location [13].

All data generated in a computer system must be available, secure, and safely stored. Unauthorized people cannot have access to data files. It must be impossible to overwrite any data, but all recalculation required must generate a new data and not substitute the previous one. Safety procedures must be available. Data should be backed up periodically following specific SOPs, and backup copies must be iden-

tified and stored in a defined and safe place. All backup data must be evaluated for the backup process, ensuring that the data backup has been well performed. Periodically all data must be checked for integrity in order to ensure that they are well preserved and no data have been lost.

Adequate alternative arrangements should be available for systems which need to be operated in the event of a breakdown. The time required to bring the alternative arrangements into use should be related to the possible urgency of the need. If the system fails or breaks down, the procedures to be followed should be defined and validated. Any failures and corrective actions taken should be recorded [13]. Special cases of breakdowns are power breakdowns. It is required that some computer systems work for 24 h and should not be interrupted after starting data acquisition. Consequently, an extra uninterruptible power supply (UPS) is required to allow the operation of these computerized systems during power breakdowns.

A procedure should be established to record and analyze errors and to enable corrective action to be taken. Every computer system can originate errors that must be documented. Every time an error occurs, it must be analyzed and, if applicable, some adjustments in the computer system may be required. Adjustments have to be well defined and documented; in some cases a revalidation of the system may be required.

When outside agencies are used to provide a computer service, there should be a formal agreement including a clear statement of the responsibilities of that outside agency [13]. When the release of batches for sale or supply is carried out using a computerized system, the system should allow only a qualified person (QP) to release the batches and it should clearly identify and record the person releasing the batches. This is possible by giving the QP an operational level in the computer system that allows it to release batches. No other person should have the same operational level authorization as the QP.

Software Validation Authorities are demanding rules in order to outline the software validation principles used in medical device software or the validation of software used to design, develop, or manufacture medical devices.

Guidances recommend an integration of software life-cycle management and risk management activities. Software validation and verification activities must be conducted throughout the software life cycle [12, 14]. Software verification and validation are terms frequently confused. Software verification is defined as the process that provides objective evidence that the design outputs of a particular phase of the software development life cycle meet all of the specified requirements for that phase [14]. Software verification consists of tasks performed to evaluate if the software is performing desired tasks and providing desirable results. Software verification is part of software validation. Software testing is one of the many verification activities intended to confirm that software development output meets its input requirements. Other verification activities include various static and dynamic analyses, code and document inspections, walkthroughs, and other techniques [14].

Although actual guidelines are only applicable in software used as a component part or accessory of a medical device (e.g., blood establishment software, programmable logic controllers in manufacturing equipment, software that records and maintains the device history record), the validation principles presented in these guidelines could be applicable to any software validation.

Software is not a physical entity and, unlike some hardware failures, software failures occur without advanced warning. One of the most common software failures is branching, that is, the ability to execute alternative series of commands based on differing inputs. The software branching capacity makes the commands extremely complex and difficult to validate once errors occur as an answer of a specific input, and until the introduction of that specific input error has not been detected. Software input can be almost any data and, and since it is impossible to introduce all data into a software, validation of data is extremely difficult. Thus, results are considered to be of high confidence level. The majority of software problems occur as a consequence of errors in the software design and development and are not directly related to the software manufacture. It is simple to manufacture several software copies that work perfectly and as the original one.

Software validation is not separately defined in the quality system regulation. The FDA considers software validation to be the "confirmation by examination and provision of objective evidence that software specifications conform to user needs and intended uses, and that the particular requirements implemented through software can be consistently fulfilled" [14]. Software validations have special concerns on software installation, implementation, and utilization. A software validation consists in several tests, inspections, and verifications performed to assure the adequate installation and use of software and that the tasks performed meet all the specifications defined. Software validations must be performed under the environmental conditions to which software will be submitted. This is particularly important in medical devices that are used under special conditions, such as close to or inside the human body.

A software validation plan must take into account the risk analyses for the software; therefore a critical software must have a high level of confidence and must be submitted to deep validation processes, whereas noncritical software may be submitted to less extensive validation processes.

Seemingly insignificant changes in software code can create unexpected and very significant problems elsewhere in the software program. The software development process should be sufficiently well planned, controlled, and documented to detect and correct unexpected results from software changes [14]. Maintenance of a software must be carefully performed because even a few small changes could develop dramatic software results. Accuracy and thorough documentation are essential in order to assure the software validation.

Software validation is not a simple and easy task; therefore an adequate schedule and task plan for software validation and verification are required to avoid unnecessary time and money expenses. One day of planning with no experiments performed is better than one day of experiments without planning.

Electronic Documents Several special cases of electronic documents have been targeted by specific official regulations. These are electronic records and electronic signatures. For instance, the FDA issued regulations that provide criteria for acceptance, under certain circumstances, of electronic records, electronic signatures, and handwritten signatures executed to electronic records as equivalent to paper records and handwritten signatures executed on paper. These regulations, which apply to all FDA program areas, were intended to allow the widest possible use of electronic technology compatible with the FDA's responsibility to protect public health [12].

Some interpretations for this guideline included almost every record generated in a computer as the final target, but another was very simple, considering only a few documents under the scope of this guideline. The records required to be maintained under predicate rules or submitted to the FDA would apply whenever records in electronic format replace paper hardcopies. On the other hand, when the computer is used to generate paper printouts of electronic records and those paper records meet all the requirements of the applicable predicate rules, the FDA would generally not consider people to be "using electronic records in lieu of paper records." The same is considered when people rely on paper records to perform their regulated activities. In these cases, the use of computer systems in the generation of paper records would not trigger the official requirements [12]. Computer systems that generate a document that is printed out and signed by hand are excluded in this guideline. The guideline only regulates documents generated in a computer system that are filed, signed, and modified electronically. Since the documents are not printed and signed, special considerations must be taken in order to assure that an adequate file is performed, undesirable modifications are avoided, and the signature is safe and personalized.

This guideline is only applicable to documents for which it is required to be maintained. All documents created and signed electronically that are not required to be maintained are not under the scope of this guideline. The decision of which documents are under the scope of this guideline is taken by the pharmaceutical industry and must be well documented and justified.

Electronic signatures are intended to be the equivalent of handwritten signatures, initials, and other general signings required by predicate rules. They include electronic signatures that are used, for example, to document the fact that certain events or actions occurred in accordance with the predicate rule (e.g., approved, reviewed, and verified).

Validation Team A well-defined validation team with a well-written description of responsibilities is required and assures the adequate realization of the validation tasks. A validation team should be composed by different responsibilities: responsible-of-validation team, team leader, archive manager, test coordinator, quality assurance member, tester, and witness. The responsible-for-validation team elaborates and approves the VMP, protocols, and reports. The team leader should be responsible for the computer system validation and utilization. An archive manager is responsible for the management of all computer system validation documents. The test coordinator is responsible for the computer system test and coordinates the elaboration and operation of tests for evaluating the performance of the computer system. A quality assurance member is required to periodically inspect and train the personnel and review all the validation documents. The tester is responsible for the execution of the tests required to perform the validation protocol. The witness is responsible for observing and reviewing the operations of the tester.

8.4.9 CLEANING VALIDATION

Cleaning validation is defined as the "the process of providing documented evidence that the cleaning methods employed within a facility consistently controls potential

carryover of product (including intermediates and impurities), cleaning agents and extraneous material into subsequent product to a level, which is below predetermined levels" [15].

The reasons behind the validation of cleaning procedures are the assurance of the safety and purity of the product (customer requirement), it is a regulatory requirement in active pharmaceutical ingredient product manufacture, and it assures the quality of the process from an internal control and compliance point of view [15].

Cleaning should be carried out with relative ease and with the use of standard cleaning materials [7]. Vacuum facilities should be available for cleaning and contact parts fouls be wiped down and sanitized utilizing a sanitizing agent. The equipment should be washed, dried, covered, and stored in an equipment storage area.

As in manufacturing, the packaging operation starts with the generation of the packaging order. This consists of the approved packaging components, the batch number of the product to be packaged, and quantities of each. A supervisor verifies the accuracy and completeness of the packaging order, including expiration date, line being used, and any other special equipment being used for that operation. These steps are accomplished prior to bringing components to the line. The complete line area, including all equipment, is verified as being properly disassembled and cleaned of all product and components from the previous packaging operation. After the QA department has verified that the area has been cleared and cleaned, the components must be brought to the line for mechanical setup. Once the setup mechanic has completed all the adjustments required, a supervisor oversees the prestart procedure. This consists of cleaning all products used during the setup; counting labels used in the label setup; rechecking all components and lot numbers; verifying the bottle count, all stamps, and lot numbers; and signing the packaging order that all is in readiness to start packaging operations.

Nonappropriate cleaning procedures will develop batches of poor quality due to the risk of presence of a number of contaminants, such as precursors of the drug, degradation products, solvents and other materials employed during the manufacturing process, microorganisms, cleaning agents, and lubricants [15].

8.4.10 CONCLUSIONS

Since the middle of the twentieth century the pharmaceutical industry has been a leader in terms of quality and security of manufacturing, associating higher production efficiency with higher profits. Therefore, validation became essential, that is, the confirmation and evidence that all facilities, equipment and processes work as desired, generating quality products.

In the present chapter, the pharmaceutical industry validation system has been reviewed. To have an appropriate validation system it is first required to define which equipment, facilities, and processes will be validated, when they will be validated, and by whom this must be performed. This definition is based on a risk assessment priority and is written in a specific document, the so-called MVP. In order to generate an adequate validation report, all the validation activities should be described in the validation protocols, SOPs, and specific procedures.

Facilities validation is a critical process in a pharmaceutical industry and the types of pharmaceutical forms produced must be considered. Facilities that produce different pharmaceutical forms have different specific requirements and different critical parameters based on risk assessment. All facilities must have an adequate flow of people, raw materials, bulk products, and finished products. These flows must be created in order to avoid cross-contamination. Additionally, pressurized rooms and adequate SOPs should be supplied to minimize the risk of cross-contamination. Controlled air temperature and humidity are also required and should be validated to ensure adequate stability of the product.

MVP is a crucial procedure to confirm that the product manufacture is adequate and is generated in a consistent manner with the same quality. Definitions of equipment, rooms, quality of raw materials, and process parameters are necessary to validate the manufacturing process. Maintaining these definitions ensures that the product has the same quality after the process validation. Changes in equipment, raw materials, or process parameters require a manufacturing process revalidation.

Analytical methods validation is one of the most regulated validation processes in the pharmaceutical industry. Analytical validations are required to demonstrate that the methods employed are the most indicated for each product and that the results obtained are reliably correct. All methods employed in raw and finished product materials analysis are required to be validated.

Equipment validation is comprised of four critical operations: design, installation, operational, and performance qualifications. These operations will confirm that the equipment has adequate specifications, installation, and functions, manufacturing a product with adequate properties. After these procedures, whenever equipment is installed, it must be periodically verified and calibrated in order to ensure adequate performance.

Computer systems are a specific type of equipment that must be validated as well. These systems have specific requirements if they are used to collect and process data. Associated with computer hardware is the software, carefully validated in order to prove that the data generated by them are correct.

In order to avoid cross-contamination, another concern with respect to equipment is the cleaning process, which must comprise cleaning SOPs to ensure adequate cleanliness. Cleaning validation must be performed based on risk assessment and worst-case scenarios.

REFERENCES

1. World Health Organization (WHO) (2006), WHO expert committee on specifications for pharmaceutical preparations (fortieth report)—Supplementary guidelines on good manufacturing practices (GMP): Validation, WHO, Geneva.
2. Thomas, E., Grochulski, A., Patel, R., George, S. M., and Zhang L. (2006, Sept.), The laboratory control system: Fulfilling cGMP requirements, *BioPharm Int.*, 26–32.
3. Slater, S. (1999), *Biopharmaceutical Validation: An Overview in Biopharmaceuticals, an Industrial Perspective*, Springer-Verlag.
4. Chaloner-Larsson, G., Anderson, R., and Egan, A. *A WHO Guide to Good Manufacturing Practice (GMP) Requirements*, 1997, World Health Organization, Geneva.

5. International Conference on Harmonization (ICH) (2000), Good manufacturing practice guide for active pharmaceutical ingredients, ICH, Geneva.
6. European Medicines Evaluation Agency (EMEA) (2003), *Good Manufacturing Practices*, Chapter 4: Building and facilities, EMEA.
7. Nash, R. A. (1990), The essential of process validation, in *Pharmaceutical Dosage Forms, Tablets*, Marcel Dekker Inc, New York.
8. Hanna, S. A. (1990), Quality assurance, in *Pharmaceutical Dosage Forms, Tablets*, Marcel Dekker Inc, New York.
9. Connolly, R. J., Berstler, F. A., and Coffin-Beach, D. (1990), Tablet production, in *Pharmaceutical Dosage Forms, Tablets*, Marcel Dekker Inc, New York.
10. European Medicines Evaluation Agency (EMEA) (2003), *Good Manufacturing Practices*, Chapter 3: Premise and equipment, EMEA.
11. U.S. Food and Drug Administration (FDA) (1987), Guideline on general principles of process validation, FDA, Rockville, MD.
12. U.S. Food and Drug Administration (FDA) (2003), Guidance for industry, CFR Part 11, Electronic records; electronic signatures, good manufacturing practices, FDA, Rockville, MD.
13. European Medicines Evaluation Agency (EMEA) (2003), *Good Manufacturing Practices*, Annex 11: Computerised systems, EMEA.
14. U.S. Food and Drug Administration (FDA) (2002), general principles of software validation, good manufacturing practices, Rockville, MD.
15. Active Pharmaceutical Ingredients Committee (APIC) (1999), Cleaning validation in active pharmaceutical ingredient manufacturing plants, APIC.

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