9

Quality control of suppositories

QUALITY CONTROL procedures listed in the US Pharmacopeia (USP30-NF25) for manufactured suppositories include identification, assay, and, in some cases, water content, residual solvent, dissolution, and content uniformity:

• *Identification*: Identification tests are commonly used for the identification and confirmation of official articles.

• *Assay*: Assay and test procedures are used to determine compliance with the pharmacopeial standards of identity, strength, quality, and purity. Chromatographic methods are commonly used for detection and quantitation.

• *Dissolution*: Dissolution testing is used to determine compliance with the dissolution requirements, if present in the individual monographs. The test measures the rate and extent of a drug dissolving in a defined medium under defined conditions.

• *Water*: As many pharmacopeial articles either are hydrates or contain water in adsorbed form, the determination of water content may be important in demonstrating compliance with Pharmacopeial standards. Three methods are commonly used: Method I is a titrimetric method, Method II is an azeotropic method, and Method III is a gravimetric method.

• *Content uniformity*: Content uniformity is required in some monographs to ensure the consistency of dosage units. These dosage units should have a drug substance content within a narrow range around the label claim. Weight variation and content uniformity testing involving groups and individual dosage units are used.

• *Residual solvents*: For pharmacopeial purposes, these are defined as organic volatile chemicals that are used or produced in the manufacture of drug substances or excipients, or in the preparation of drug products. They are not completely removed during processing but should be removed to the extent that is possible and reasonable.

Stability considerations in dispensing practice for suppositories also include observations on excessive softening and oil stains on packaging. Compounded suppositories can be checked for calculations of theoretical and actual weight and weight variation, color, hardness, surface texture, and overall appearance.

Suppository quality control includes physical and chemical aspects of the product (Box 9.1). Physical analysis includes visual examination (physical appearance), uniformity of weight, uniformity of texture, melting point, liquefaction time, melting and solidification time, and mechanical strength. Chemical testing includes analysis of the activity and dissolution testing. The uniformity of texture can be assessed by sectioning a suppository longitudinally and laterally, and ensuring that each section presents a smooth, uniform surface.

A list of official USP suppositories and required quality tests is given in Table 9.1.

Physical analysis

Visual examination

Color and the surface characteristics of the suppository are relatively easy to assess. It is important to check for the absence of fissuring, pitting, fat blooming, exudation, sedimentation, and the migration of the active ingredients. Suppositories can be observed as an intact unit and also by splitting them longitudinally.

Box 9.1 Example standard operating procedure: performing physical quality assessment of compounded suppositories

I. Purpose – The purpose of this procedure is to provide a method of documenting uniformity between batches and physical quality assessment tests of and observations on suppositories.

II. Materials, balance, graduates, beaker, weights.

III. Procedures – Conduct the appropriate tests and record the results/observations on the Physical Quality Assessment Form for Suppositories (Box 9.2).

A. Weight – Accurately weigh the product on a balance.

B. Specific gravity – To calculate the specific gravity, one must know the weight and volume of the product. Record the weight of the individual dosage form or a strip/package of the dosage forms (tared weight). To determine the volume of water displaced, do the following:

Single unit:

1 Place 5.0 mL of water in a 10 mL graduate.

2 Add the dosage form and read the water level.

3 Subtract 5.0 mL from the level in step 2 to determine the volume of water occupied by the dosage form.

4 If the dosage form floats, place a weight attached to a paper clip in the graduate prior to adding the water to the 5.0 mL mark. Then, wrap one end of the paper clip around the dosage form to hold it below the water surface and place it in the graduate. Proceed as in step 3.

Multiple unit packages (suppository strip):

1 Place the empty package in a beaker that will hold it with minimal extra space.

2 Add an exact known quantity of water to cover the product. It may be necessary to add a weight to the package; this same weight should be used for the actual product.

3 With a fine-line marker and with the beaker setting on a level surface, mark the water level on the outside of the beaker.

4 Remove the beaker contents, empty and dry the beaker.

5 Place the dosage form (and the weight if used) into the beaker.

6 Measure the same volume of water as in step 2 into a graduate.

7 Pour the water into the beaker only to the mark from step 3. (Note: Do quickly before the dosage form dissolves.)

8 Measure the volume of water remaining in the beaker.

Calculations:
Now that the weight and volume of the product are obtained, the specific gravity can be calculated by dividing the weight (grams) by the volume (in milliliters).

C. Active drug assay results – As appropriate, have representative samples of the product assayed for active drug content by a contract analytical laboratory. Stability can be assessed by storing the product at room, refrigerated and/or frozen temperatures and having the assay repeated on the stored samples.

D. Color of product – It may be advisable to use a color chart for determining the actual color of the product.

E. Evaluate clarity by visual inspection.

F. Texture of surface – Observe the product to determine smoothness of the surface.

G. Appearance (dry, oily/moist) – Determine whether the product appears dry or oily/moist.

H. Feel (tacky, plastic, elastic) – Touch the product to determine whether it is sticky (tacky) or hard (plastic) or bounces back (elastic).

I. Melting test (for fatty acid, cocoa butter and oil-based products):

Box 9.1 Continued

1 Heat a 200 mL beaker of water to 37°C on a magnetic stirring unit set at about 50 rpm.

2 Add a dosage unit to the water.

3 After 30 minutes, record your observations as yes, no or partially melts on the scale provided.
  NOTE: It may be necessary to add a weight to these dosage units to pull them below the water surface.

J. Dissolution test (for polyethylene glycol, gelatin and water-soluble products):

1 Proceed as in the melting test.

2 After 30 minutes, record your observations as yes, no or partially dissolved on the scale provided.

K. Physical observation – Describe the appearance and organoleptic qualities of the product.

L. Physical stability – Prepare a few additional dosage forms, package and label ("For physical stability observations"). Weekly, observe the product for signs of discoloration, dryness, cracking, mottling, mold growth, etc. Record a descriptive observation on the form at each observation interval. There are sufficient lines for observations for eight weeks (approximately 60 days).

Shape

It is advisable to check the shape of the suppository to see if it is consistent, irrespective of whether the suppository is ogive or torpedo shaped.

Surface condition

The following can be checked: brilliance, dullness, mottling, cracks, dark regions, axial cavities, bursts, air bubbles, holes, etc.

Color

The intensity, nature and homogeneity of the color should be verified.

Odor

Verification of odor can prevent confusion when similar suppositories are being processed. A change in the odor may also be indicative of a degradation process.

Weight

Suppositories can be weighed on an automatic balance, obtaining the weight of 10 suppositories.

If the weight is found to be too small, it is advisable to check whether the mold is being well filled and whether there are axial cavities or air bubbles caused by badly adjusted mechanical stirring or the presence of an undesirable surfactant. It is also important to check that the batch of suppositories is homogeneous. If the weight is found to be too high, check that scraping has been carried out correctly, and also that the mixture is homogeneous. Lastly, the weight may decrease during aging when the suppositories contain volatile substances, especially if the packaging is not airtight.

Melting range (melting point, melting zone)

Melting range or melting zone is the term often preferred by some rather than melting point. Many suppository bases and medicated suppositories are mixtures, and so do not have a precise melting point. Routinely, though, we continue to call the physical phenomenon obtained under rigorous conditions the melting point.

  The release rate of the suppository is related to its melting point; it is therefore critical that this test be evaluated using a non-destructive method. A number of different techniques are used to study melting behavior, including the open capillary tube, the U-tube, and the drop point methods (Figures 9.1, 9.2, and 9.3). One

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Box 9.2   Physical quality assessment form for compounded suppositories

Product: ————————————————————   Date:
————————————————

Lot/Rx number: ————————————————   Form: Suppository

Characteristic	Theoretical			Actual		Normal Range	
Weight/volume	————————————			————————————			
Specific gravity	————————————			————————————			
Active drug assay results	————————————			————————————			
Initial assay	————————————			————————————			
After storage No. 1	————————————			————————————			
After storage No. 2	————————————			————————————			
Color of product	————————————			————————————			
Clarity	Clear	1	2	3	4	5	Opaque
Texture-surface	Smooth	1	2	3	4	5	Rough
Appears dry	Yes	1	2	3	4	5	No
Appearance	Dry	1	2	3	4	5	Oily/moist
Feels tacky	Yes	1	2	3	4	5	No
Feels plastic	Yes	1	2	3	4	5	No
Feels elastic	Yes	1	2	3	4	5	No
Melting test	Yes	1	2	3	4	5	No
Dissolution test	Yes	1	2	3	4	5	No

Sample set aside for physical observation: Yes   No
If yes, results: Date   Observation

———————   ————————————————————————
———————   ————————————————————————
———————   ————————————————————————
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shortcoming is the use of limited data to describe a continuous, complex melting process occurring in successive steps involving multiple components, including various molecular weight triglycerides, polymers, or other ingredients.

The methods used are similar in principle but include different steps and techniques. In general they include the set-up of the equipment, placement of the suppository dosage unit in the apparatus, followed by the application of heat and observation for a change in the system, such as melting or movement. The results obtained using different methods do not always agree, so it is important to use a consistent method.

In general, the melting point should be equal to or less than 37°C. A non-destructive method must be used because if the suppository is melted before a measurement is made, the suppository constituents may be transformed into a metastable state.

The melting test consists of placing a suppository on the surface of water thermostatically controlled at 37°C and verifying the complete melting of the suppository in a few minutes. This is not so much a measurement as an evaluation.

Melting point determination

The use of a U-shaped capillary tube to determine melting point provides precise information for excipient control and consistency in production

Table 9.1 Official USP suppositories and required quality testing

Monograph	Identification	Assay	Dissolution	Water	Content uniformity	Residual solvents
Acetaminophen	x	x	–	–	–	x
Aminophylline[a]	x	x	–	–	–	
Aspirin[b]	x	x	–	–	–	
Bisacodyl	x	x	–	–	–	
Chlorpromazine[c]	x	x	–	–	–	
Ergotamine tartrate and caffeine	x	x	–	–	–	x
Glycerin	x	x	–	x	–	x
Indometacin	x	x	x	–	x	x
Miconazole nitrate	x	x	–	–	–	x
Morphine sulfate	–	x	–	–	x	x
Nystatin	–	x	–	x	–	
Oxymorphone hydrochloride	x	x	–	–	–	x
Prochlorperazine	x	x	–	–	–	
Progesterone	–	x	–	–	x	x
Promethazine hydrochloride	x	x	–	–	–	
Thiethylperazine maleate	x	x	–	–	x	

[a]Ethylenediamine content required.
[b]Limit of free salicylic acid required.
[c]Other alkylated phenothiazines required.

for those suppositories containing soluble active principles. This method is not suitable when the suppositories have a high powder content, which prevents the fat from sliding inside the capillary tube to give the end-point determination.

When there are more numerous controls, and where studies with a greater precision can be undertaken, an apparatus can be used consisting of a microscope, a heated deck, and a recorder. This provides for a more detailed observation and recording of the melting process.

The melting point can also be determined by placing a small-diameter wire into the mold containing the suppository melt before the form solidifies. The form is then immersed in water, held by the wire, and the temperature of the liquid is raised slowly (about 1°C every 2–3 minutes) until the suppository slips off the wire; this is the melting point of the suppository.

In a study by Coben and Lordi, the changes in the melting range of several semi-synthetic suppository bases over time were investigated. Using X-ray diffraction they characterized the changes

as amorphous to crystalline transitions. They also demonstrated a hardening of the suppository materials over periods of time as short as six weeks, using a modified Krowczynski softening time test and differential scanning calorimetry on freshly solidified, incrementally aged, and equilibrated samples. They also demonstrated that plasticizers inhibited this hardening phenomenon.[1]
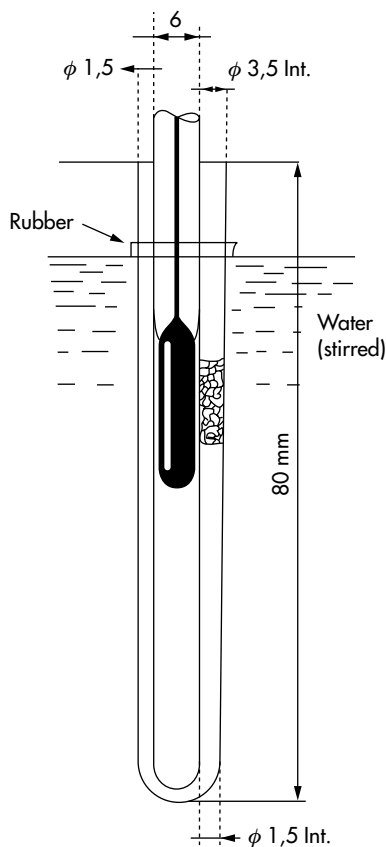
Liquefaction time

Liquefaction testing provides information on the behavior of a suppository when subjected to a maximum temperature of 37°C. The test commonly used is Krowczynski's method (see below), which measures the time required for a suppository to liquefy under pressures similar to those found in the rectum (approximately 30 g) in the presence of water at 37°C. In general, liquefaction should take no longer than about 30 minutes.
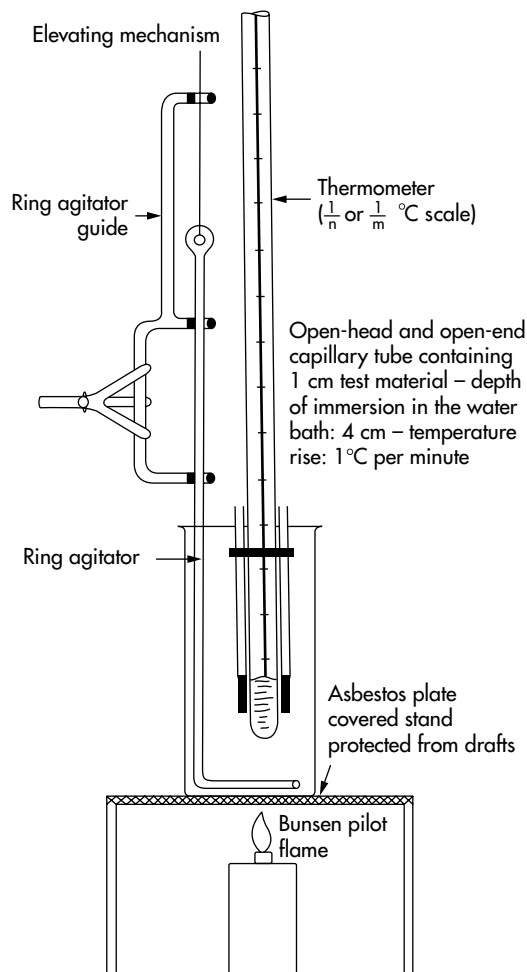
Figure 9.1 Open capillary apparatus for melting point determination.

Figure 9.2 U-Tube apparatus for melting point determination.

Two example set-ups are shown in Figures 9.4 and 9.5. Numerous techniques have been developed and used over the years. Setnikar and Fantelli's method, for example, consists of measuring the liquefaction of the suppositories in a cellophane gut immersed in water at 37°C, at the same pressure as in the rectum. This technique has been described in detail.[2]
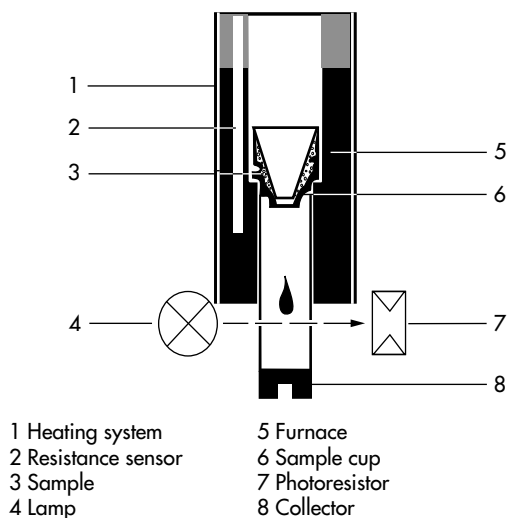
For Krowczynski's method, the apparatus consists of a 16 mm diameter glass tube, 235 mm long with an approximately 6 mm diameter reduction at the base. One end is blocked with a small rubber stopper to facilitate cleaning after use. A thermostat graduated in tenths of a centigrade is used. The tube and thermometer are held in place by means of a large rubber stopper with two holes in a 225 mm long tube with a 50 mm diameter, fitted with lateral tubes to allow the water at 37°C from a constant-temperature water bath to circulate.

Another apparatus is equipped with a 30 g glass stem 180 mm long and 9 mm wide. The base has a ring form with a 14 mm diameter. The ring of the stem has a cuneiform shape opening to allow the melted excipient to escape upwards during the test. At a distance of approximately 100 mm from the ring, three glass projections support the stem in a vertical position in the tube. The stem is also marked with a dash corresponding to its position with respect to the upper level of the tube.

1 Heating system   5 Furnace
2 Resistance sensor   6 Sample cup
3 Sample   7 Photoresistor
4 Lamp   8 Collector

Figure 9.3 Drop point apparatus for melting point determination.

The directions for use are as follows: (1) obtain a constant temperature in the circulating water bath of 37°C, (2) pour approximately 5 mL of water down the tube so that all the tube is filled below the narrowed part (and so that the suppository to be tested is in relatively humid conditions similar to those in the rectum, (3) after 5 minutes (the time necessary to bring the 5 mL of water to 37°C), insert a suppository with the end pointed downward into the glass tube, insert the glass stem so that it is resting on top of the suppository and start the timer, (4) note the time required for the mark on the glass stem to drop and come in line with the upper edge of the tube, (5) repeat this for two more suppositories, (6) if the difference between the three time measurements is greater than 105 seconds, start again on two more suppositories (making a total of five suppositories), and (7) determine the average liquefaction time.

Apparatus using a cellophane bag (Figure 9.5) consists of a glass cylinder with an external diameter of 50 mm, narrowing down to 22 mm at either end for a length of 30 mm. The cylinder is fitted with two connections through which water that is maintained at 37°C can circulate. A 34–35 cm length of cellulose dialyzer tubing, size-inflated diameter of 1.12 inch (2.8 cm), is moistened, opened and placed in the cylinder.

The tube is drawn out of either end of the cylinder and secured with two elastic bands. Tubing is attached to allow the warm water to circulate, maintaining the temperature. When the appropriate temperature is reached, the suppository is placed in the dialysis tubing and the time to liquefaction measured. The apparatus can also be used to determine the melting point of suppositories made with both water-soluble and water-insoluble bases. This can be accomplished by increasing the temperature of the water at a set rate, for example one degree every 10 minutes until the suppository melts.[3]

Suppository penetration test

A suppository penetration test can be used to determine the temperature at which the suppository becomes sufficiently soft for a penetrating rod to drop through its length. The apparatus used is shown in Figure 9.6. The temperature is adjusted to that required for the test, generally about 37°C. The suppository is placed in the device and the penetration rod gently moved into place. The device holding the suppository and penetration rod is lowered into the constant temperature bath and a stopwatch is started. When the penetration rod drops through the softened suppository the time is recorded.

Melting and solidification time

There is a relationship between melting and solidification that is important to characterize. The release of the active ingredient from the vehicle is related to the melting point of the vehicle and the solubility of the drug in the vehicle. Suppositories undergo three changes in phase during their "life." First, they are melted and then solidified; upon administration, they are again melted. An understanding of these factors and their relationships is critical for evaluating the bioavailability of the final suppository formulation. The higher the melting point, the later the drug effects appear. If too high, the drug effect does not appear.

Melting and solidification is a complex process and difficulties in measurement can arise,
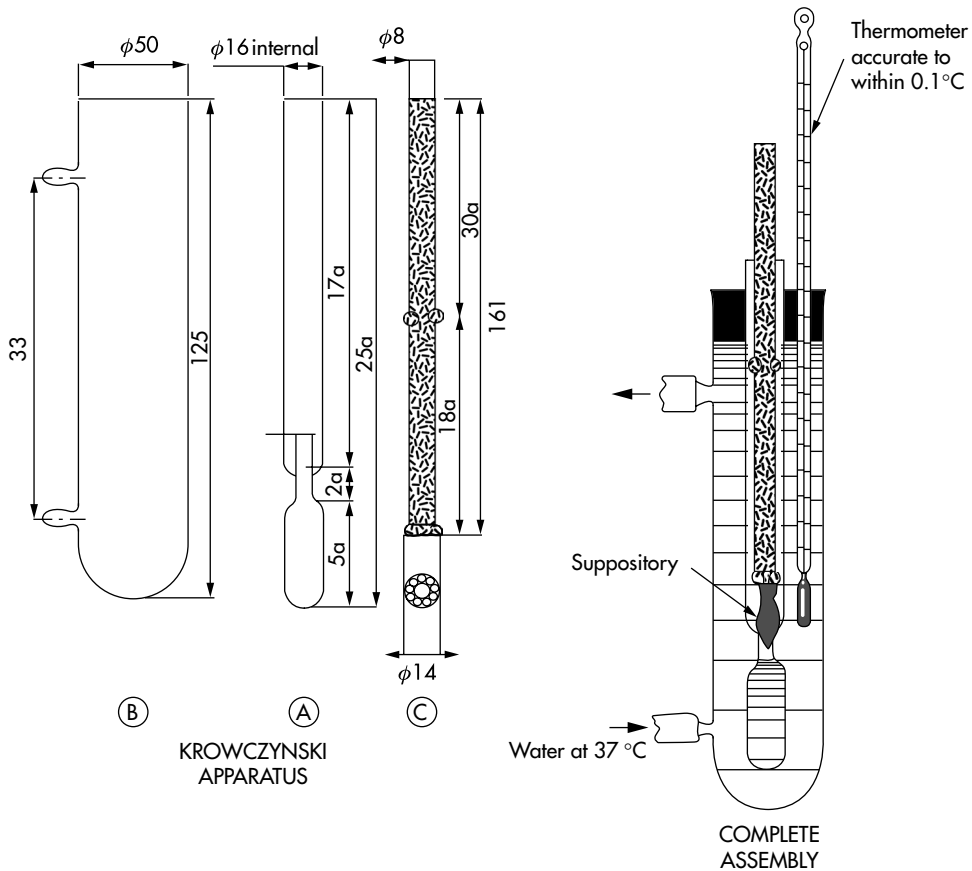
Figure 9.4 Liquefaction time apparatus.

leading to different results obtained using different methods. The solidification temperature is defined as the highest temperature occurring during the solidification of a supercooled liquid. Various methods are available to measure it, including Shukoff's method,[4] in which the liquid is shaken in an evacuated flask until turbid and the temperature noted at which a transitory rise in temperature occurs during cooling.

The European Pharmacopoeia also describes a procedure that involves heating the material, then allowing it to cool slowly while stirring. The temperature is recorded at 1 minute intervals. The cooling curve normally passes through a minimum, which indicates a supercooled melt. Heat is liberated during crystallization and the temperature–time curve rises. The maximum temperature in this phase is the solidification temperature.

Mechanical strength/crushing test

Suppositories can be classified as brittle or elastic by evaluating the mechanical force required to break them. Tests are used that measure the mass (in kilograms) that a suppository can bear without breaking. A good result is at least 1.8–2 kg pressure. In the example laboratory set-up shown in Figure 9.7, the suppository is positioned in an upright position and increasing weights are placed on it until it loses its structure and collapses. The purpose of the test is to verify that the suppository can be transported under normal conditions, and administered to the patient.[5]
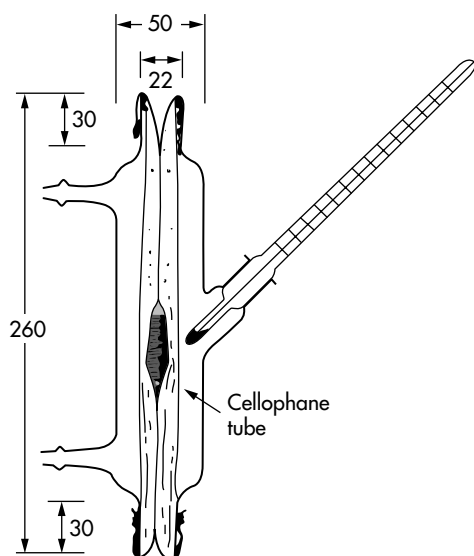
Figure 9.5 Liquefaction time apparatus using cellophane bag.

Chemical testing

Dissolution testing

One of the most important quality control tools available for *in vitro* assessment is dissolution testing. Dissolution testing is often required for suppositories to test for hardening and polymorphic transitions of active ingredients and suppository bases. However, unlike for tablets and capsule dosage forms, there are not enough dissolution testing methods or validations for suppositories. This can be partly attributable to the immiscibility of some of the suppository vehicles in water.

If the drug is immiscible in an aqueous dissolution fluid then it may require a partitioning step; unfortunately this involves extra time, which alters the dissolution rate calculation.

If a filtration step is involved in dissolution testing, the filtration membrane may introduce an erroneous result between actual and obtained results as it may clog. Variations in density between the suppository and the receiving fluid must also be considered.

Dissolution testing methods include the paddle method, basket method, membrane diffusion
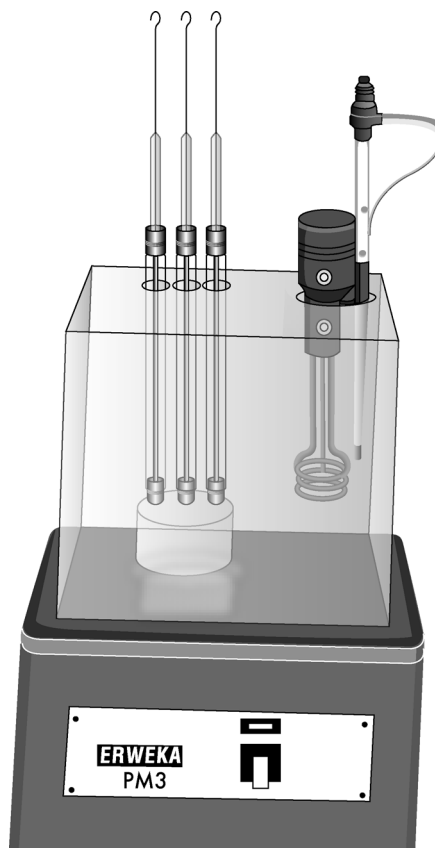
Figure 9.6 Suppository penetration apparatus.

method/dialysis method, and the continuous flow/bead method. The equipments for these various methods are shown in Figures 9.8–9.11.

The application of the paddle, basket, and flow-through dissolution methods was studied by Gjellan and Graffner for seven different rectal compositions of hydrophilic and lipophilic-type suppositories. The formulations were (1) lipophilic, melting–Witepsol, (2) lipophilic, melting–Witepsol with 2% Tween 85, (3) lipophilic melting–Novata, (4) hydrophilic dissolving–polyethylene glycol (PEG) 3350 + 1500, (5) hydrophilic dissolving–PEG 3350 + 1500 with 2% Myrj 51, (6) hydrophilic dissolving–gelatin capsule, and (7) lipophilic melting–gelatin capsule with a surfactant.[6]
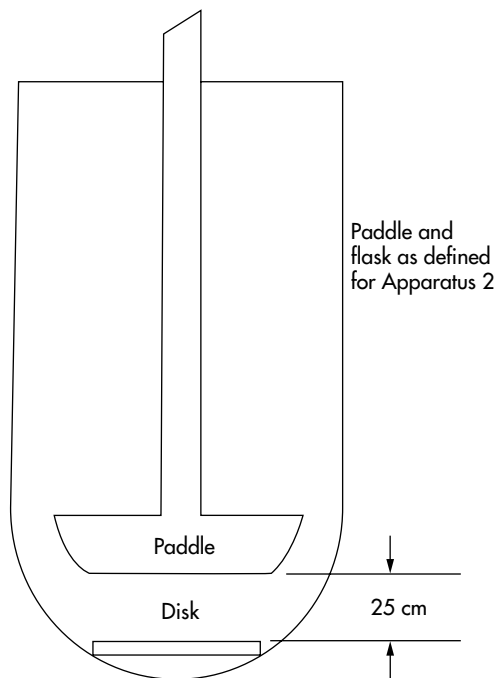
The melting suppositories with the paddle method showed fat floating rapidly to the surface
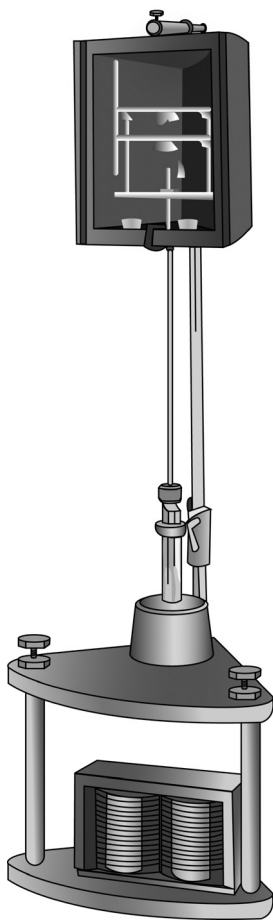
Figure 9.7 Mechanical strength/breaking apparatus.

Figure 9.8 Dissolution apparatus using the paddle method.

of the fluid instead of staying below the water surface. With the basket method, the surfactant produced small droplets of the fat that were dispersed into the medium almost immediately. Some of the fat particles also blocked the basket mesh. When a surfactant was not used, the basket served as the container for all the melted fat. In the flow-through cell, the two suppository compositions behaved differently. The surfactant makes the fat more sensitive to agitation. A deformation of the suppository is seen as a fast release of small droplets of fat when the surfactant is incorporated. For both compositions, the melting fat accumulates on the top of the chamber during the dissolution process.[6]

The melting suppositories without the surfactant in the basket method simply stayed in the basket. In the paddle method, small pieces of melted fat accumulated about the helix and small pieces were continuously floating to the surface of the dissolution medium. In the flow-through system, there was a spreading of the base at the cell walls in the first chamber where it was then forced into the second chamber and collected at the top of that chamber.

The dissolving suppositories with and without surfactant behaved similarly in all the different techniques. The suppositories gradually decreased in size, just like dissolving tablets, and the content of the surfactant made no difference.

With the melting suppositories, the dissolution rates for those containing surfactant were faster than those without surfactant. With surfactant, the dissolution profiles using the different techniques were approximately the same.

With the dissolving suppositories, the paddle method at 50 rpm and the flow-through method at 16 mL/min produced the same dissolution profiles with and without surfactant.

The investigators also concluded that suppositories containing a surfactant behave differently
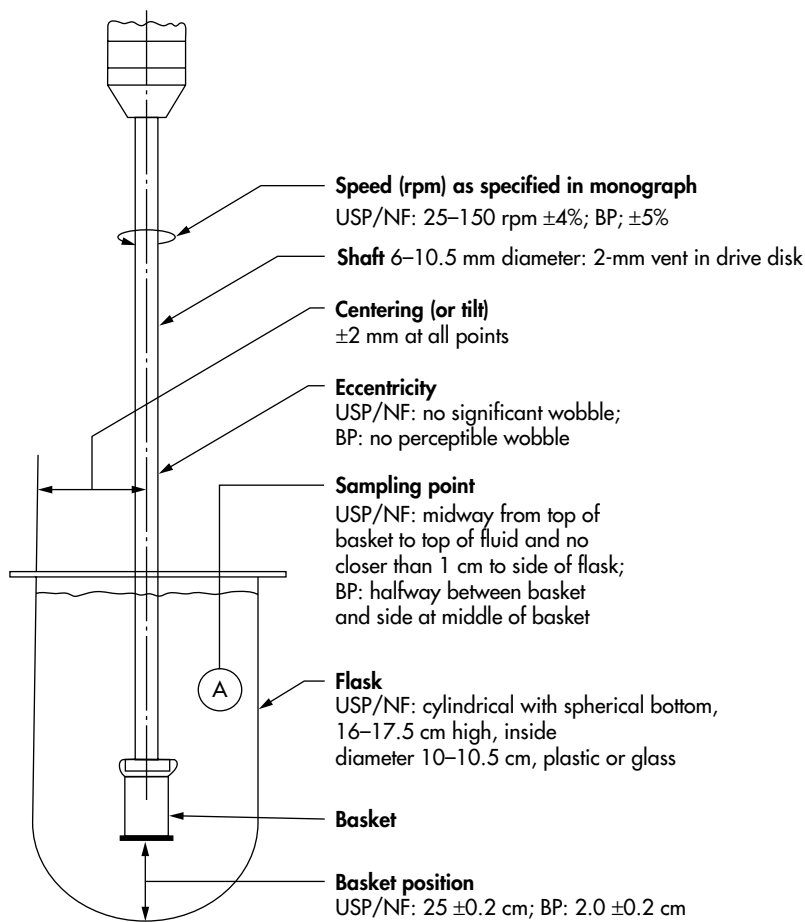
Speed (rpm) as specified in monograph
USP/NF: 25–150 rpm ±4%; BP; ±5%

Shaft 6–10.5 mm diameter: 2-mm vent in drive disk

Centering (or tilt)
±2 mm at all points

Eccentricity
USP/NF: no significant wobble;
BP: no perceptible wobble

Sampling point
USP/NF: midway from top of
basket to top of fluid and no
closer than 1 cm to side of flask;
BP: halfway between basket
and side at middle of basket

Flask
USP/NF: cylindrical with spherical bottom,
16–17.5 cm high, inside
diameter 10–10.5 cm, plastic or glass

Basket

Basket position
USP/NF: 25 ±0.2 cm; BP: 2.0 ±0.2 cm

Figure 9.9   Dissolution apparatus using the basket method.

37°C from
water bath

C

A

DIALYSIS
APPARATUS

B

D   to
Apparatus

Pump

attached to A   attached to B

pH meter

Na⁺ electrode
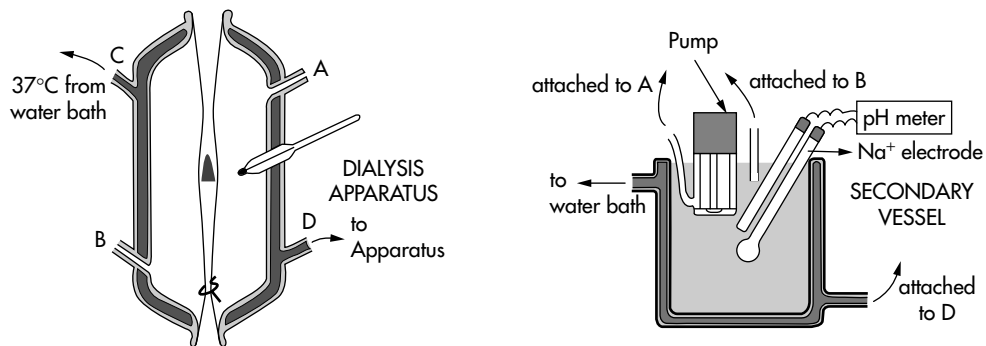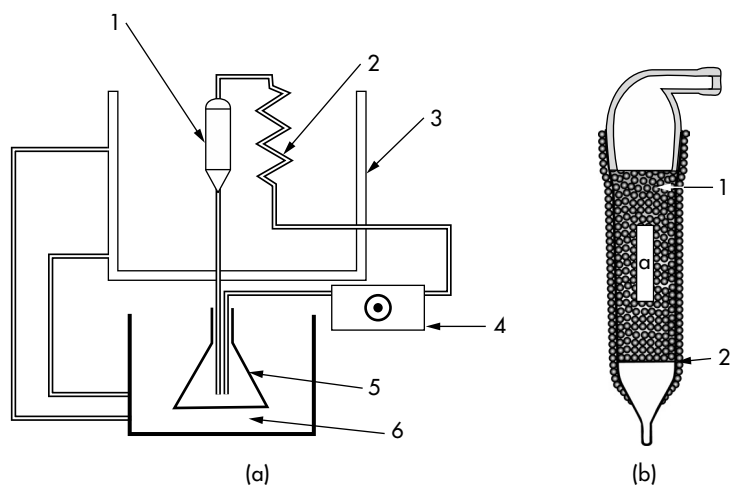
to
water bath

SECONDARY
VESSEL

attached
to D

Figure 9.10   Dissolution apparatus using the membrane/dialysis diffusion method.

Figure 9.11 Dissolution apparatus using the continuous flow method: (a) 1 – schematic drawing with the cell in the upper middle of the diagram; 2 – tubing for moving the dissolution fluid; 3 – constant temperature bath; 4 – pump; 5 – receiver flask for dissolution fluid; 6 – constant temperature bath; (b) continuous flow cell; 1 – beads; 2 – suppository being tested; 3 – filter (glass filter) to retain the beads.

from those without and produce the fastest dissolution rates of paracetamol. The presence of the surfactant makes the suppository more sensitive to the differences in the dissolution techniques.[6]

The flow-through method was slower, possibly as a result of the delayed spreading of the melted mass in the dissolution cell. The subsequent contact area with the dissolution fluid is smaller than that achieved with either the paddle method or the basket method.[6]

In a series of workshops held under the auspices of the Fédération International Pharmaceutique (FIP) and co-sponsored by the Royal Pharmaceutical Society, the Bundesverband der Pharmazeutischen Industrie, Colloquium Pharmaceuticum, the American Association of Pharmaceutical Scientists, and the US Food and Drug Administration, it was concluded that hydrophilic suppositories that release the drug by dissolving in the rectal fluids can be evaluated by the basket, paddle, or flow-through cell methods. Lipophilic suppositories, on the other hand, release the drug after melting in the rectal cavity and are significantly affected by rectal temperature. After determining the proper temperature to use, consideration must be given to the drug partitioning between the melted lipophilic base and the receptor fluid. This may result in a distribution equilibrium between the phases as opposed to complete dissolution. In these cases, sink conditions are essential in order to simulate the *in vivo* situation. In this situation membranes provide a more elegant way to obtain a filtrate for immediate assay; however, because this poses an artificial condition, it is not generally recommended. Recommended equipment for lipophilic suppositories, therefore, includes a modified basket method, a paddle method with a wired screen and a sinker, and a modified flow-through cell with a specific dual-chamber suppository cell. The flow cell tends to generate more variability in the data due to the behavior of the suppository within the cell, especially in formulations that contain spreading agents. In these difficult situations, the membrane method may be preferable.[7]

It is evident that no single method of dissolution testing is suitable for all the various suppository formulations and types of suppositories. However, from the methods available, the authors concluded that a method can generally be selected that will be adequate. Even though it is difficult to make a recommendation,

one might consider starting with the basket or paddle method for hydrophilic suppositories and the modified flow-through cell for lipophilic suppositories.[7]

A bead-bed suppository dissolution apparatus was evaluated by measuring the release of benzocaine from various suppository vehicles. This proposed method using beads as the bed and placing the suppository within the beads was designed to provide greater constancy of the exposed suppository area for dissolution. The liquid passed through the bed at a constant rate. Direct contact of the suppository was maintained with the dissolution medium, confining the suppository within the beads.[8]

A study to determine the feasibility of using the European Pharmacopeia flow-through cell for dissolution testing of compounded rectal suppositories containing indometacin or sodium diclofenac investigated various suppository bases. The fastest and most repeatable release rates were from the hydrophilic bases; the macrogols had more than 80% of the drug released within 60 minutes. The lipophilic bases were somewhat slower, showing that after 350 minutes, only 18.5–50% of the total amount was released, with somewhat non-reproducible results. This demonstrates the slow and non-reproducible release when the lipophilic suppository base does not melt. The authors concluded that the use of the test for formulations that do not melt is not recommended.[9]

One study using the paddle dissolution method on water- and fat-soluble indometacin suppositories for rectal administration in rats determined that the choice of the membrane is critical. When a cellulose or artificial sausage membrane of cow protein was used, the amount of indometacin released from fatty base suppositories was higher than that from water-soluble bases. But, the results were reversed when a natural sausage membrane of pig colon was used. Selection of the proper model system is important.[10]

A comparison of different dissolution and permeation methods was undertaken using diaphyllin, piroxicam, naproxen, and tenoxicam commercial standard suppositories. Dissolution tests were conducted with the Desaga-type flow cell. Permeation studies were conducted using the Mühlemann tester and a laboratory-designed membrane-diffusion cell. The values for the dissolution rates were faster than the permeation rates, as would be anticipated. There was good correlation between the Mühlemann tester and the laboratory-designed membrane-diffusion cell. Based on this study, the authors concluded that it is possible to obtain standardized and automated results that show good reproducibility.[11]

An investigation was conducted to determine the *in vitro* dissolution behavior of piroxicam suppositories using the flow-through method, conventional stirred vessel–USP paddle and basket methods. The results indicate that the flow-through method seems useful for studying the dissolution process of suppositories containing piroxicam, resulting in reproducible dissolution data. The flow-through cell method gives more rapid *in vitro* dissolution rates for piroxicam than the USP paddle method, possibly due to the steeper concentration gradient in the vicinity of the suppositories as well as a better ability to thoroughly wet the suppositories. The USP basket method gave substantially slower dissolution rates than either of the other two methods.[12,13]

Using a dissolution chamber apparatus for suppositories introduced in the European Pharmacopoeia and the British Pharmacopoeia, one study of commercial paracetamol suppositories found that the results obtained are clearly dependent upon the temperature maintained in the dissolution chamber. Complete melting of a suppository in the dissolution chamber was required for an appropriate dissolution of paracetamol *in vitro*. Associated tests were compared for relevance, including disintegration time, softening time, drop point, and particle size.[14]

The release rates of fluconazole in different types of suppository bases was studied using the USP dissolution apparatus I and the permeation was studied using Franz diffusion cells with rat rectal membrane with normal saline as the receptor medium. The different bases used included hydrophilic (PEG), lipophilic (cocoa butter or Witepsol W45), and amphiphilic (Suppocire AP, a polyglycolized glyceride). The results of the dissolution test concluded that dissolution of fluconazole from the bases, in decreasing order, was PEG > Suppocire AP = Witepsol W45 > cocoa butter. The permeation studies showed Suppocire

AP > PEG = Witepsol W45 > cocoa butter. It was mentioned that the increased permeation characteristics seen with the Suppocire AP base are probably due to an alteration of the membrane characteristics because of the surface active properties of the base.[15]

In another study a case was presented for two rotating flask methods (simple and compartmental) for evaluating the liberation of drugs from commercially available acetaminophen suppositories. The authors compared numerous suppository test methods but decried their complicated construction and difficulty in handling. They concluded that the two rotating flask methods correlated well with each other and had relevant characteristics and bioavailability.[16]

Content uniformity testing

In order to ensure content uniformity, individual suppositories must be analyzed to provide information on dose-to-dose uniformity. Testing is based on the assay of the individual content of drug substance(s) in a number of individual dosage units to determine whether the individual content is within the limits set. "Acceptance value calculations are not required for suppositories. Assay 10 units individually as directed in the *Assay* in the individual monograph, unless otherwise specified in the *Procedure for content uniformity*" (ref. 17, p. 383).

The USP 30 "Criteria"[17] for suppositories states the following:

> Limit A (if the average of the limits specified in the potency definition in the individual monograph is 100.0% or less) – Unless otherwise specified in the individual monograph, the requirements for dosage uniformity are met if the amount of the drug substance in each of the 10 dosage units as determined from the Content Uniformity method lies within the range of 85.0% to 115.0% of the label claim, and the RSD is less than or equal to 6.0%.
>
> If 1 unit is outside the range of 85.0% to 115.0% of label claim, and no unit is outside the range of 75.0% to 125.0% of label claim, or if the relative standard deviation is greater than 6.0%, or if both conditions prevail, test 20 additional units. The requirements are met if not more than

1 unit of the 30 is outside the range of 85.0% to 115.0% of label claim, and no unit is outside the range of 75.0% to 125.0% of label claim and the RSD of the 30 dosage units does not exceed 7.8%.

> Limit B (if the average of the limits specified in the potency definition in the individual monograph is greater than 100.0 percent).
>
> If the average value of the dosage units tested is 100.0 percent or less, the requirements are as in Limit A.
>
> If the average value of the dosage units tested is greater than or equal to the average of the limits specified in the potency definition in the individual monograph, the requirements are as specified under Limit A, except that the words "label claim" are replaced by the words "label claim multiplied by the average of the limits specified in the potency definition in the monograph divided by 100.
>
> If the average value of the dosage units tested is between 100 percent and the average of the limits specified in the potency definition in the individual monograph, the requirements are as specified under Limit A, except that the words "label claim" are replaced by the words "label claim multiplied by the average value of the dosage units tested (expressed as a percent of label claim) divided by 100 (p. 383).

Content uniformity is important not only between suppositories, but also within suppositories in the event that a suppository is halved for administration. A method of determining the distribution of paracetamol in suppositories using differential scanning calorimetry has been reported. In this study, samples of 2.5–4.0 mg were carefully removed from the tip, middle, and base sections of individual paracetamol suppositories using a stainless steel scalpel. The contents of paracetamol in the suppositories were determined by differential scanning calorimetry and ultraviolet spectrophotometry. The results obtained were $10.1 \pm 0.2\%$, $10.1 \pm 0.2\%$, and $10.3 \pm 0.2\%$ w/w paracetamol, indicating that the paracetamol was uniformly distributed throughout the suppositories.[18]

One study reported difficulty in obtaining content uniformity between dispensatory-prepared rectal suppositories when sampling numbers were only 10% of a batch of 300 (N = 30). This was solved by increasing the number

of suppositories tested to 15% ($N = 45$) of the total batch prepared.[19]

Content uniformity tests can be used to optimize production techniques and sampling methods. In one study a sampling plan was developed, combining practicality for the consumer and a satisfactory discrimination between "good" and "bad" batches. Samples of 50 suppositories obtained from a year's production of 42 batches were studied. There was no difference between the weights of the suppositories obtained during the production process. The intent was to determine the variation in content as there was no uniform standard. At the time of this study (1970), the German and Russian pharmacopeias allowed weight variations of rectal suppositories of within ±5% of the average weight. The Pharmacopoeia Nordica allowed weight deviation up to ±10% of the average weight for 90% of the suppositories, provided that the variations do not exceed ±20%. The International (1967), NF XII, BP (1963), Italian VII, Austrian IX, Gallica VIII and Japanese VII pharmacopeias gave no specification for weight uniformity of suppositories.[20]

Conductivity

Siegmund and Leuenberger studied the conductivity of binary mixtures of different volume to volume ratios of liquid PEG 200 and solid PEG 6000 in an attempt to relate this to dissolution rate processes. The conductivity was measured using a specially designed cell, which could be filled with the sample to be analyzed. The results were analyzed using the percolation theory and its relation to the dissolution rate of binary mixtures. They demonstrated that the conductivity and the dissolution rate process can be successfully modeled by the basic equation of percolation theory and that both processes can be correlated.[21,22]

Chemical testing procedures

Prior to chemical testing, the size of the manufacturing batch must be defined. This may invoke specific requirements on sample size and when

and where samples are obtained. Here we are concerned with the general homogeneous nature of mixed products in a single system or unity, or in a single operation being used for the manufacture of suppositories under constant modalities.

Active drug analysis is important for batch-to-batch uniformity and can also be used for within-batch production control. The analysis of active drugs in suppositories presents some interesting and sometimes difficult problems due to the matrix in which they are contained, such as fatty acids and long-chain polymers. Once assay methods have been developed, they can be used to also determine content uniformity and dissolution testing parameters.

Sampling and sample preparation

In general, the analytical methods involved require sample preparation prior to using an instrumental method technique. Sample preparation involves the preparation of a suitable and uniform sample composite and the extraction of the drug from the excipients.

For manufactured suppositories, preparation of a uniform sample may require a minimum of five to 30 individual doses which are weighed and combined together, such as by melting in a beaker. From this composite, the required quantity of sample is removed for analysis.

The quantities for sampling are usually a minimum of 10–15 suppositories. The samples are taken from the first ten molds, from the beginning, from the middle of the manufacturing and finally from the last ten molds, and are placed in impervious packing, preferably made of glass, and then labeled. The samples can also be taken from directly packaged suppositories. Special care must be taken when labeling the sample receptacles, making sure that the date when the sample was taken, and the batch number are included.

Sample preparation techniques may require extraction, heating, the addition of electrolytes to the aqueous phase (salting out), modification of the surface tension by addition of silicones, ricinoleate, octylic alcohol or others, centrifugation, acidification (particularly anionic type emulsion of the soap type) or the addition of an

organic solvent. Often it is necessary to combine the techniques described above.

Extraction

The method used to extract the active drug from the sample varies depending upon the physicochemical characteristics of the drug and the vehicle. Many extraction procedures require partitioning into an aqueous medium after mixing with either hexane, pentane, chloroform, ether, or another suitable organic solvent. In some methods, multiple extractions and back extractions may be required.

Extraction by water at 37°C method

One suppository per test is placed in a tube containing 10 mL of water at 37°C. After stirring in a constant temperature water bath, the tubes are withdrawn at 10-minute intervals and immediately placed in an ice bath to solidify the melted excipient. The concentration of the active drug in the supernatant liquid is then determined.

Extraction by dialysis across a cellophane membrane

Several methods studying the diffusion of active principles originating from melted suppositories at a temperature near 37°C across a semipermeable membrane have been used.[23–25]

Other methods

There is another method that deserves close attention as it seems to give excellent correlation with *in vivo* absorption results obtained with different suppository formulas, notably with thiazinamium and indometacin. The apparatus consists of a cylindrical dialysis cell placed in a water bath at 37°C. The water bath has a circulation of liquid assured by thermostatic control and a water pump. The dialysis cell has three orifices: the first holds a precision thermometer used to control the temperature of the dialysis liquid; the second holds a cellophane gut (diameter 18 mm, thickness of 25 μm), which is placed in

the water 15 minutes before introducing the suppository to be tested; and the third is used to sample the dialysis water at regular intervals. These dialysis samples are then quantitatively analyzed for the active principle that has diffused from the suppository.

The test liquid (350 mL), from which the samples are obtained, with a pH of 7.38, is used in each test. Samples to be analyzed are taken at 30, 60, 90, 120, 150, 180, 210, and 240 minutes. The composition of the test liquid is as follows: $Na_2HPO_4.2H_2O$ (9.50 g), $NaH_2PO_4.2H_2O$ (2.08 g), NaCl (4.60 g), polyvinylpyrrolidone (35.0 g), purified water to 1000 mL.

Dialysis

A modification of the dialysis membrane method for drug release from suppositories was used to analyze dissolution from 50 mg indometacin suppositories. The system consisted of a dialysis membrane (17 cm long, 28 mm wide), suspended in 1000 mL of 50 mM phosphate buffer maintained at 37°C. This technique also uses a clamp to bind the ends of the tubing rather than thread (which tends to wrinkle the tubing at the ends).

The results using the apparatus allowed correlation of the release rate with *in vivo* data (AUC, C_{max} and T_{max} in rabbits). In this method, any water remaining in the dialysis tubing after hydration resulted in a decrease in the rate of drug release due to lost sample.[26]

Example set-ups are shown in Figure 9.12.

Preparation for analysis

Once the sample has been extracted, the next step is the analytical method. Analytical methods commonly include high-performance liquid chromatography (HPLC), gas chromatography, and spectroscopy. Ideally, all methods should be capable of indicating stability.

In selecting the analytical method, important factors to be evaluated include (1) the solubility characteristics of the active ingredients, (2) particle fineness, (3) the hydrophilic or lipophilic character of the excipients, (4) the ability to spread on the rectal mucous membrane, (5) the
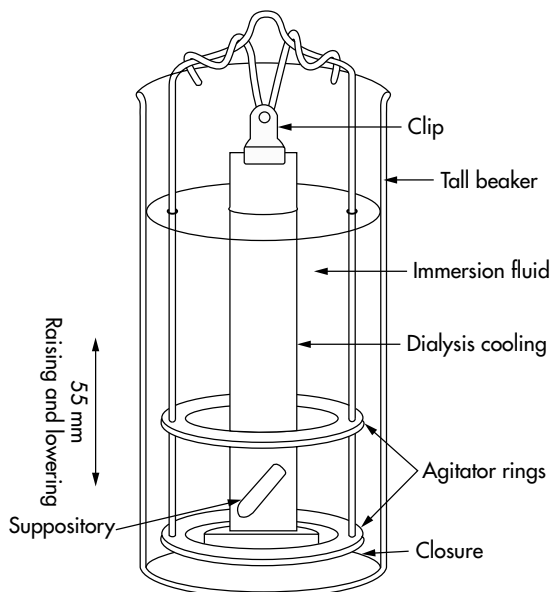
Figure 9.12 Dialysis apparatus.

tendency to give water-in-oil emulsions in the presence of emulsifying agents, and (6) viscosity.

Sample preparation may involve concentration, dilution, solution in various solvents, or other manipulation depending upon the analytical method selected.

Other chemical tests

Any developed and validated analytical method should be as simple as possible to help ensure precision and accuracy. The development of a method can involve many steps. An example of a "methods development" procedure and the data generated follows. A similar approach can be used for different chromatographic and spectroscopic methods.

A methods development study was conducted to determine the feasibility of using reverse phase high-performance liquid chromatography (RP-HPLC) for *in vitro* evaluation of the distribution behavior of nine common drugs (paracetamol, caffeine, diclofenac, propyphenazone, indometacin, codeine base, codeine phosphate, phenobarbital acid, and phenobarbital sodium) between one of the generally used suppository

bases (Witepsol H15) and simulated rectal fluid imitated by a phosphate buffer at pH 7.2 utilizing the routine shaker flask method. Capacity factors (log k') of the drugs were determined on a reverse-phase C18 column using various methanol/5 mM phosphate buffer pH 7.2 mobile phases containing different percentages of methanol. Apparent capacity factors (log $k(w)_{app}$) were derived by extrapolation of the methanol concentration to zero and, using the correction for ionization, the real capacity factors log $k'(w)$ were calculated. The lipophilicity of the compounds was assessed by the partition coefficients ClogP (calculated log octanol/water partition coefficient) and the distribution coefficients ClogD7.2, calculated for the *n*-octanol/water system. Correlations between log $k'(w)$ and ClogP, log $k(w)_{app}$ and ClogD7.2, log $k(w)_{app}$ and log K were found. This last correlation suggested that the parameter log $k(w)_{app}$ was suitable for evaluating the distribution behavior of the studied drugs in the examined Witepsol H15/rectal liquid system. The applicability of this was tested for the nine different drugs used in this study. It was demonstrated that the classical shaker flask method for determination of distribution behavior, or partition coefficients, of the studied drugs between suppository base Witepsol H15 and pH 7.2 phosphate buffer might be replaced by the RP-HPLC technique, which is rapid, stable and reproducible.[27]

A rapid method was developed for the determination of miconazole nitrate in suppositories. It consisted of dissolving the sample in ethanol, dilution in acetonitrile/water 1:1) and injection onto a C18 column. The mobile phase consisted of 55% acetonitrile, a triethylammonium phosphate buffer and an ion-pairing agent and detection at 214 nm. The mean recovery was 98.8% for the suppositories and a total run time of less than 4 minutes.[28]

Other methods of analysis reported include HPLC and pulsed proton NMR.[29,30]

A method was developed for the determination of bisacodyl and its degradation products using RP-HPLC. A C18 column, ultraviolet detector at 254 nm and a mobile phase of methanol/acetonitrile/0.02 M citric acid (1:1:2) was used. The mean recovery for bisacodyl from the commercial suppositories was 99.7%.[31,32]

In another study, methods were developed for investigating the release of carbon dioxide from effervescent suppositories containing sodium bicarbonate and anhydrous sodium dihydrogen phosphate for the purposes of formulation development and quality control during production. Method 1 was conducted without stirring and method 2 was with stirring; the evolution of carbon dioxide was measured using a gas burette. Three lots of commercial suppositories were used. Using method 1, only 60% of the carbon dioxide was released in the medium without polysorbate 80; since no stirring was involved, this was a "native" release profile. In method 2, 100% was released containing 1% polysorbate 80 with comparatively low standard deviations. The authors concluded that method 1 would be most beneficial for comparing the effects of various factors, such as additives and melting points, on the release profiles of carbon dioxide from the suppositories; however, this method would not be practical for quality control as only about 60% of the carbon dioxide was released. Method 2, on the other hand, would be useful because of its complete release and low standard deviations. These methods are applicable, therefore, to early and late formulation studies of effervescent suppositories, respectively.[33]

In summary, many different analytical methods from wet chemistry to modern instrumental methods can be used for analytical testing of suppositories, after sampling and sample preparation. A complete methods development is required to ensure accuracy, precision, and reproducibility.

Aging and aging tests

Changes over time may alter the physical and/or chemical properties of a suppository. Melting point fluctuations, for example, may occur either as a result of polymorphic changes in the excipient, in which case the temperature variation will be between 1 and 1.5°C maximum, or as a result of evaporation of a volatile medicament or because of physical or chemical reactions between medicaments or excipients.

Some problems associated with aging include the following:

- An odor may emanate from suppositories with vegetable extracts due to fungal contamination.
- Suppositories with some dyes may discolor due to oxidation of the dyes.
- The shape of some suppositories may be altered during storage at incorrect temperatures, especially if they contain essential oils.
- Suppositories containing vegetable extracts or caffeine base may exhibit whitening on the surface.
- Suppositories containing camphor, menthol, or other volatile substances that may be lost due to vaporization over time may lose weight during storage.
- Other aging phenomena, such as hardening, softening, bloom, mottling, discoloration and cracking may occur over time depending upon the composition of the suppositories and storage conditions.

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