GREEN TEA EXTRACT LOADED LIPOSOMES: FORMATION, CHARACTERIZATION AND STABILITY

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

DAMLA DAĞ

IN PARTIAL FULLFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
FOOD ENGINEERING

JUNE 2017

Approval of the thesis:

GREEN TEA EXTRACT LOADED LIPOSOMES: FORMATION, CHARACTERIZATION AND STABILITY

Submitted by **DAMLA DAĞ** in partial fulfillment of the requirements for the degree of **Master of Science in Food Engineering Department, Middle East Technical University** by,

Prof. Dr. Gülbin Dural Ünver
Dean, Graduate School of **Natural and Applied Sciences** _____

Prof. Dr. Serpil Şahin
Head of Department, **Food Engineering** _____

Asst. Prof. Dr. Mecit Halil Öztop
Supervisor, **Food Engineering Dept., METU** _____

Examining Committee Members:

Prof. Dr. Esra Yener
Food Engineering Dept., METU _____

Asst. Prof. Dr. Mecit Halil Öztop
Food Engineering Dept., METU _____

Prof. Dr. Serpil Şahin
Food Engineering Dept., METU _____

Prof. Dr. Servet Gülüm Şumnu
Food Engineering Dept., METU _____

Asst. Prof. Dr. Elif Yolaçaner
Food Engineering Dept., Hacettepe University _____

Date: 13.06.2017

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name, Last name : Damla Dağ

Signature :

ABSTRACT

GREEN TEA EXTRACT LOADED LIPOSOMES:
FORMATION, CHARACTERIZATION AND STABILITY

Dağ, Damla
M.S., Department of Food Engineering
Supervisor: Asst. Prof. Dr. Mecit Halil Öztop

June 2017, 268 pages

Polyphenol-rich green tea extract was encapsulated into liposomes using microfluidization and ultrasonication at two different mediums (acetate buffer and distilled water) to overcome the instability towards oxygen, light, temperature and alkaline conditions. The liposomes loaded with green tea extract by microfluidization were further coated with anionic biopolymers (gum arabic, whey protein) and cationic biopolymer (lysozyme, chitosan) to provide a protective layer over the liposomal surface. The stability of both uncoated and coated liposomes was explored by particle size, zeta potential, transmission electron microscopy, total phenolic content, antioxidant activity and NMR Relaxometry experiments during 28-days storage at 4°C. Moreover, in vitro digestion in the simulated gastric and intestinal juice was performed for uncoated liposomes. The results indicated the biopolymer coated liposomes showed better stability compared to uncoated liposomes during storage. Addition of lysozyme, gum arabic and whey protein to uncoated liposomes increased the particle size from 35 to 43 nm while the increase was recorded as 38 nm to 356 nm after chitosan addition. The zeta potential measurements of uncoated liposomes prepared in distilled water decreased from -30.2 to -23.2 mV at the end of 28^{th} day. This decrease in zeta potential was eliminated by coating of liposomes with biopolymers. The biopolymer layer around the liposomes was also investigated through transmission electron microscope images. Results indicated that lysozyme, gum arabic, whey protein could provide increased stability to liposome possessing fragile structure.

Keywords: Green tea extract, liposome, encapsulation, biopolymer coating, stability

ÖZ

YEŞİL ÇAY ÖZÜTÜ İLE DOLDURULMUŞ LİPOZOM SİSTEMLERİNİN OLUŞTURULMASI, KARAKTERİZASYONU VE DAYANIKLILIĞI

Dağ, Damla
Yüksek Lisans, Gıda Mühendisliği Bölümü
Tez Yöneticisi:Yrd. Doç. Dr. Mecit Halil Öztop

Haziran 2017, 268 sayfa

Polifenol bakımından zengin yeşil çayın oksijen, ışık, sıcaklık ve ortamın asiditesine karşı dayanıksızlığının üstesinden gelmek için, yeşil çay özütü iki farklı ortamda (asetat tampon çözeltisi ve saf su) mikroakışkanlaştırma ve ultrasonikasyon yöntemleri kullanılarak lipozomlara enkapsüle edilmiştir. Ek olarak, mikroakışkanlaştırma yöntemi ile hazırlanan yeşil çay özütü ile yüklü lipozomların lipozomal yüzeyleri lipozomlara koruyucu bir katman sağlamak için anyonik biyopolimerler (arap zamkı, peynir altı suyu proteini) ve katyonik biyopolimer (lizozom, kitozan) ile kaplanmıştır. Kaplanmamış ve kaplanmış lipozomların dayanıklılığı, parçacık boyutu, zeta potansiyeli, transmisyon elektron mikroskobu, toplam fenolik madde miktarı, antioksidan aktivite ve NMR Relaksometre deneyleri ile 4° C'de 28 günlük depolama süresi boyunca incelenmiştir. Ayrıca, kaplanmamış lipozomlar için simüle edilmiş gastrik ve bağırsak suyundaki in vitro sindirim deneyleri yapılmıştır. Elde edilen sonuçlar doğrultusunda, biyopolimer kaplı lipozomların kaplanmamış lipozomlara göre depolama süresi boyunca daha dayanıklı olduğu gösterilmiştir. Lipozomların lizozom, arap zamkı ve peynir altı suyu proteini ile kaplanması, lipozomların parçacık boyutunu 35 nm'den 43 nm'ye arttırırken, kitozan ile kaplanması, lipozomların parçacık boyutunu 38 nm'den 356 nm'ye arttığı gözlemlenmiştir. Saf suda hazırlanan kaplanmamış lipozomların zeta potansiyeli ölçümleri 14. günün sonunda -30.2 mV'den -23.2 mV'ye düşmüştür. Zeta potansiyelindeki bu azalma, lipozomların biyopolimerlerle kaplanmasıyla ortadan kaldırılmıştır. Lipozomların etrafındaki biyopolimer katmanı transmisyon elektron mikroskop görüntüleri ile de incelenmiştir. Sonuçlar, lizozom, arap sakızı, peynir altı suyu protein polimerlerinin kırılgan yapıya sahip lipozomların dayanıklılığını arttığını göstermektedir.

Anahtar Kelimeler: Yeşil çay özütü, lipozom, enkapsülasyon, biyopolimer kaplama, dayanıklılık

To my beloved family,

ACKNOWLEDGEMENTS

I would first like to express my sincere gratitude to my advisor Asst. Prof. Mecit Halil Öztop for the continuous support of my thesis study and related research, his patience, motivation, and immense knowledge. The door to Asst. Prof. Öztop office was always open whenever I ran into a trouble spot or had a question about my research or writing. He has been helpful in providing critical advice during my master which enables to pursue a career in research. He was and remains my best role model for a scientist, mentor and teacher. It has been an honor and a privilege being involved in his project under the guidance of him.

Every result described in this thesis was accomplished with the help and support of fellow labmates. I would like to offer my special thank to Şirvan Sultan Uğuz, Pelin Poçan, Elif Akbaş, Mete Kilercioğlu, Simge Tutku Yıldırım, Selen Güner, İrem Alaçık, Çağrı Helin Karaçam and the rest of Öztop Lab members for their endless supports. I would like to thank Gözde Nezihe Tuncer, Cansu Değirmenci, Ayşenur Yaylacı and Büşra Akdeniz who as good friends, were always willing to help and give their best suggestions. I would like to thank Emrah Kırtıl for his contribution to the discussion of the part of my thesis.

This research has been financially supported by The Scientific and Technological Research Council of Turkey (TUBITAK) with proposal number 113O442. I am deeply grateful to them for giving me this opportunity for my research. Additionally, I would like to thank METU Central Laboratory for the experiments performed there.

Finally, it is a pleasure to express my deepest thanks and gratitude to Tayfun Efe Ertop for his continuous support and encouragement throughout my years of study. I owe a deep sense of gratitude to my mother, Huriye Dağ, father, Şaban Dağ, and brother, Uğur Dağ, for providing me with unfailing support and continuous encouragement throughout my life with their endless love.

TABLE OF CONTENTS

LIST OF TABLES

TABLES

LIST OF FIGURES

FIGURES

CHAPTER 1

INTRODUCTION

1.1. Lipid Oxidation

Lipid oxidation is one of the great concern to food industry since it leads to negative impacts on texture, appearance and nutritional value of food by promoting formation of the compounds that negatively impact food quality such as undesirable off-flavors (rancidity) and potentially toxic reaction products (Coupland & McClements, 1996; Gibis, Vogt, & Weiss, 2012; Lemoine, Civello, Martínez, & Chaves, 2007).

The design and development of the systems that prevent and/or retard lipid oxidation in foods depends on better understanding the mechanism of lipid oxidation. The rate of lipid oxidation is affected by several internal and external factors such as fatty acid composition, the content and activity of antioxidant and pro-oxidant, pH and ionic composition of aqueous phase, irradiation, temperature and oxygen concentration, surface area in contact with oxygen and water activity (Waraho, McClements, & Decker, 2011). Lipid oxidation in foods is occurred along a free-radical (autoxidation), photooxidation and/or lipoxygenase. The mechanism of lipid oxidation could be divided into three distinct stages: initiation, propagation, and termination. Photooxidation and lipoxygenase triggered oxidation differ from free-radical reactions at the initiation stages only.

1.1.1. Free Radical Oxidation

Free radical oxidation is a spontaneous reaction of molecular oxygen with lipids, resulting in oxidative deterioration. It proceeds by a free radical chain mechanism involving three steps: initiation, propagation and termination.

Initiation

Free radical oxidation is initiated with the formation of free radicals in the presence of oxygen. An unsaturated lipid form a free radical when in contact with oxygen either by the abstraction of a hydrogen radical from an allylic methylene group of an unsaturated fatty acid or by addition of a radical to a double bond (Madhavi, Deshpande, & Salunkhe, 1996).

$$RH \longrightarrow R^{\bullet} + H^{\bullet} \qquad \text{(Equation 1.1)}$$

$$ROOH \longrightarrow RO^{\bullet} + HO^{\bullet} \qquad \text{(Equation 1.2)}$$

$$2ROOH \longrightarrow RO^{\bullet} + ROO^{\bullet} + H_2O \qquad \text{(Equation 1.3)}$$

The lipid radical $R^{\bullet}$ is usually formed by heat, light, irradiation or presence of trace metals. Furthermore, lipid hydroperoxide (ROOH) breaks down to yield alkoxy ($RO^{\bullet}$) radicals by undergoing homolytic cleavage or biomolecular decomposition as illustrated in eqns (1.2) and (1.3).

Propagation

Free radicals are converted to different forms of radicals in propagation step. The propagation stage is composed of chain reactions where peroxides (ROOH) as in eqns (1.4) and (1.5) are formed and/or yield new free-radicals such as peroxy radicals, $ROO^{\bullet}$ by consuming oxygen (Madhavi et al., 1996).

$$R^{\bullet} + {}^{3}O_2 \longrightarrow ROO^{\bullet} \qquad \text{(Equation 1.4)}$$

$$ROO^{\bullet} + RH \longrightarrow ROOH + R^{\bullet} \qquad \text{(Equation 1.5)}$$

Termination

A free radical is defined as a molecular entity having one or more unpaired electron(s). The fact that free radicals are unstable they tend to fill their electron vacancies to become stable. Once the amount of unsaturated lipids or fatty acids in the environment decreases, free radicals start to bond to one another resulting in a stable non-radical compounds. Therefore, the termination reactions disrupt the repeating sequence of propagating steps explained above (Lemoine et al., 2007).

$R^{\bullet} + R^{\bullet} \longrightarrow R\text{-}R$ (Equation 1.6)

$R^{\bullet} + ROO^{\bullet} \longrightarrow ROOR$ (Equation 1.7)

$ROO^{\bullet} + ROO^{\bullet} \longrightarrow ROOR + O_2$ (Equation 1.8)

1.1.2. Photooxidation

Photooxidation occurs by the formation of hydroperoxides in a direct reaction of singlet oxygen with unsaturated lipids. The singlet oxygen $^1O_2$ that is 1450 times more reactive than molecular oxygen is formed by a reaction of sensitizers such as chlorophyll, hemoglobin, myoglobin and riboflavin with atmospheric oxygen. In photooxidation, the singlet oxygen is attached to carbon of double bond shifted to an allylic position in the trans configuration. Thus, the hydroperoxides formed in photooxidation have an allylic trans double bond differently from the hydroperoxides formed by autooxidation (Sikorski & Kolakowska, 2003).

Formation of Singlet Oxygen by Photosensitization

Photosensitization is the transfer of energy from photosensitizer that is excited by light to atmospheric oxygen resulting in excited singlet state of oxygen. Photosensitizers that are in ground state are excited to singlet state by absorbing light. The fact that excited singlet state has excess energy they rapidly undergo a process known as intersystem crossing that is transition to slightly lower energy level called as triplet energy level. The energy emerged from de-exciting of triplet state of excited of

photosensitizer to its ground state is transferred to ground state molecular oxygen leads to formation of singlet oxygen (Sikorski & Kolakowska, 2003).

1.1.3. Lipoxygenase

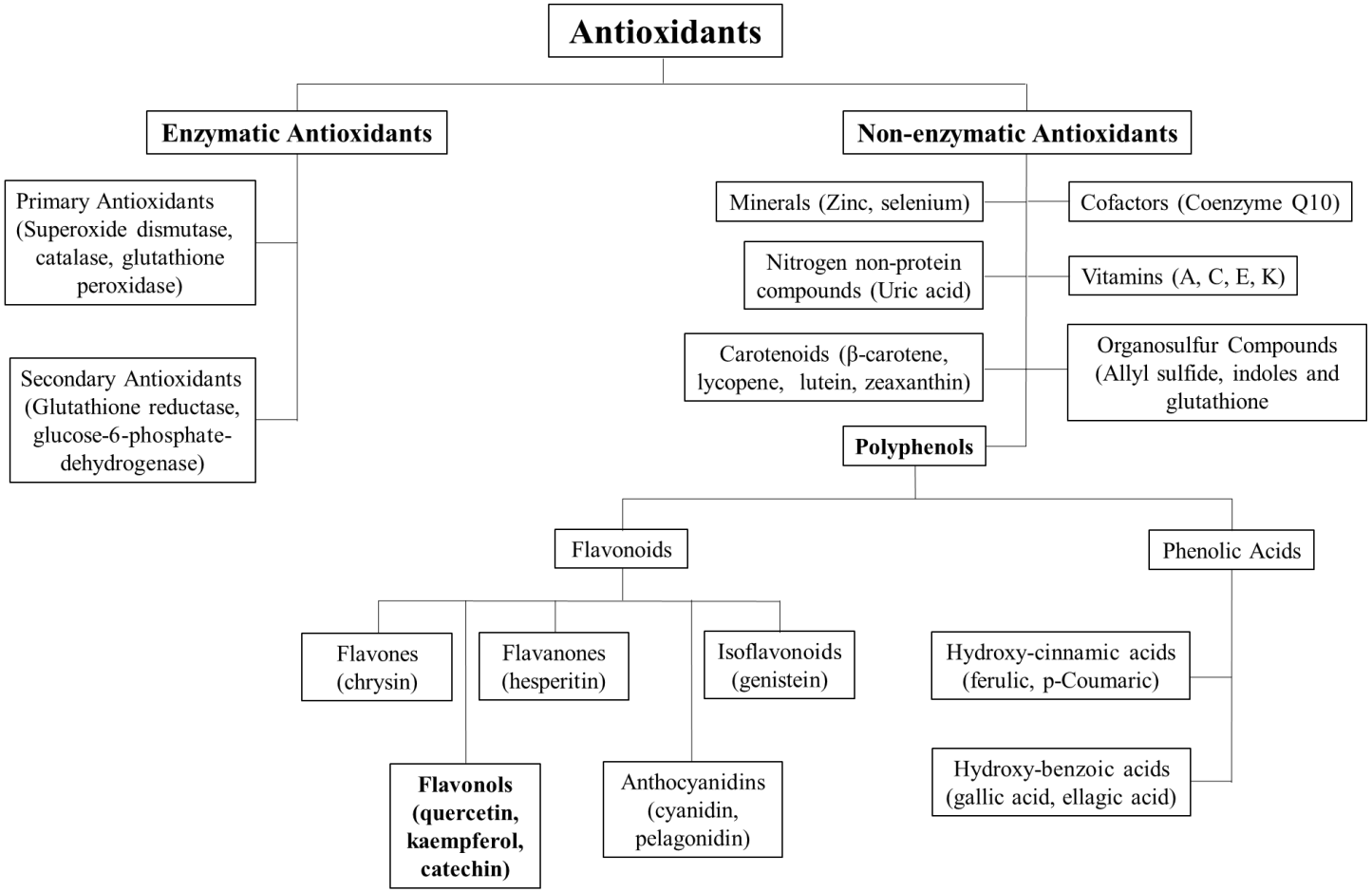
Hydroperoxide can also be formed by the reaction of polyunsaturated lipid with oxygen which is catalyzed by the enzyme called lipoxygenase. Lipoxygenase oxidizes polyethenoid acids containing methylene-interrupted double bonds present in cis geometrical configuration such as in linoleic, linolenic and arachidonic acids. Free radical intermediates are formed during lipoxygenase catalysis which led to cooxidation of easily oxidized compounds such as polyphenols and carotenoids. The enzyme lipoxygenase are present mainly in spices, wheat flour and vegetables and it catalyze the oxidation of unsaturated fats during drying procedure (Madhavi et al., 1996).

1.2. Antioxidants

Although the body possesses its own defense mechanisms against oxidation, adding antioxidants to food or integrating antioxidants to the human diet promotes these defense mechanisms (Mozafari et al., 2006). Antioxidants are defined as any substance that delays or inhibits oxidation of a substrate even at low concentrations (Sindhi et al., 2013).

Antioxidants can be classified as enzymatic and non-enzymatic antioxidants as illustrated in Table 1.1. The main enzymatic antioxidants are catalase, superoxide dismutase and glutathione peroxidase as primary enzymes and glutathione reductase, glucose-6-phosphate-dehydrogenase as secondary enzymes. The non-enzymatic antioxidants have several sub-groups as cofactors (coenzyme Q10), vitamins (A, C, E, K) and their derivatives, minerals (zinc and selenium), carotenoids (β-carotene, lycopene, lutein and zeaxanthin), organosulfur compounds (allyl sulfide, indoles and glutathione), phenolic acids (ferulic acid, p-coumaric, gallic acid and ellagic acid), nitrogen non-protein compounds (uric acid) and flavonoids (genistein, catechin, cyanidin etc.) (Carocho & Ferreira, 2013).

Table 1.1 Classification of Antioxidants (Bunaciu, Danet, Fleschin, & Aboul-Enein, 2016).



The main role of the antioxidants is to inhibit and/or delay the oxidation by coping with free radicals that have an unpaired or unmatched electron. This lack of electron balance of free radicals leads to them to be very reactive compounds. It should be noted the right amount of free radicals is essential for many important immune system functions and for other vital activities within cells. Moreover, free radicals formation is a part of the process of aerobic combustion of glucose. Free radicals also destroy the pathogen invaders such as virus and bacteria to defense the living cells. But unfortunately, overabundance of free radicals can cause damage either by joining with other chemicals resulting in change in their chemistry and/or producing a chain reaction by creating new free radicals.

The antioxidants stabilize free radicals by giving electrons to affected molecules (as illustrated in Fig. 1.1). Thus, they interrupt the oxidizing chain reaction to minimize the damages caused by free radicals. While primary and non-enzymatic antioxidants scavenge free radicals directly, secondary antioxidants manage by series of mechanism including binding metal ions, scavenging oxygen, converting hydroperoxides to non-radical species, absorbing UV radiation or deactivating singlet oxygen (Mozafari et al., 2006).

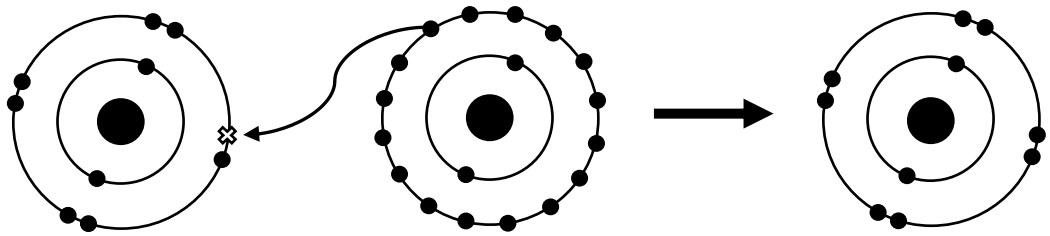


Figure 1.1 Scavenge of antioxidants with free radicals.

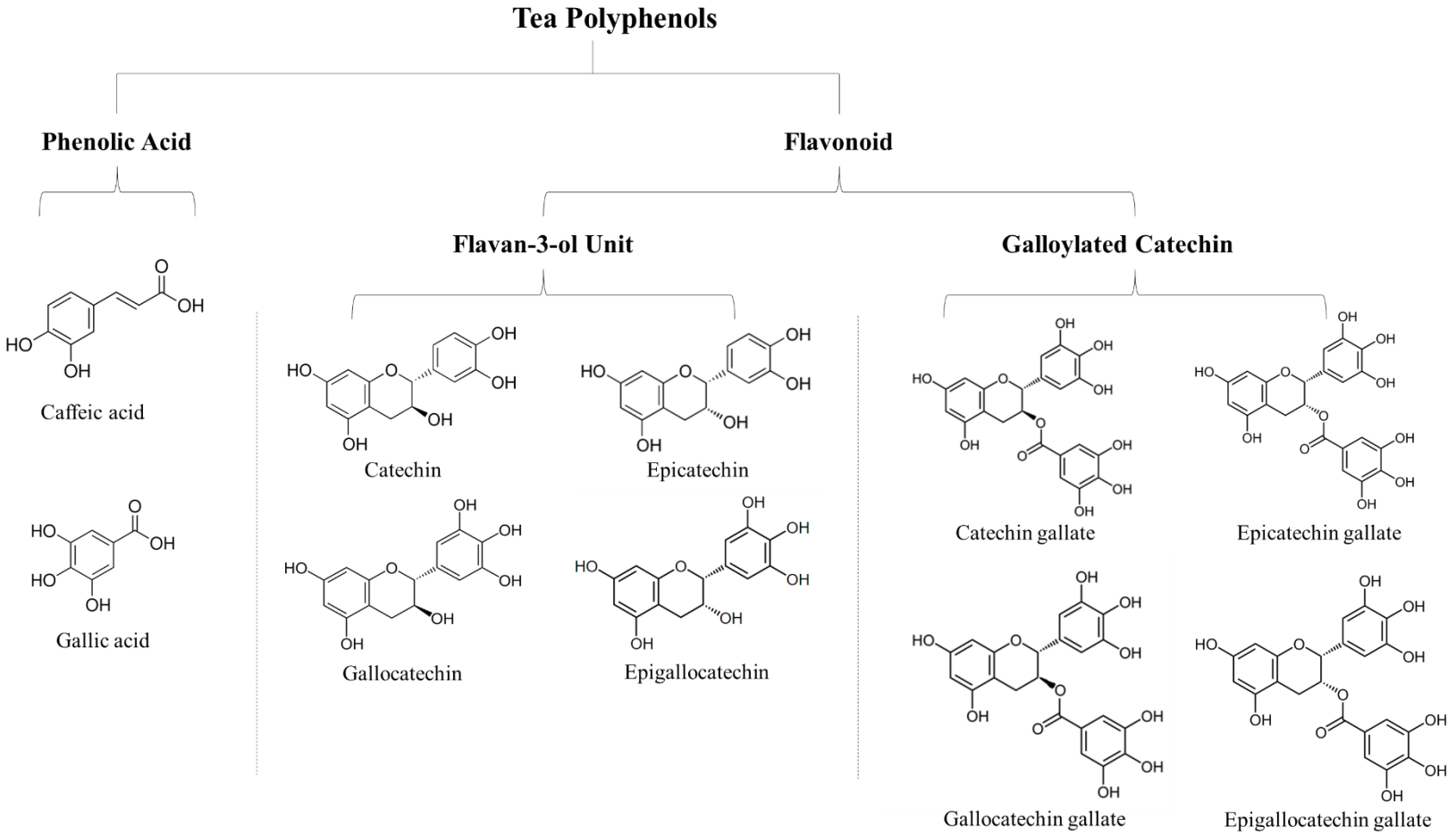
1.3. Green Tea as an Antioxidant

Green tea (*Camellia sinensis*) has become as one of the most important and commonly consumed herb due to health benefits associated with its high catechin content (Hosseini, Gorjian, Rasouli, & Shirali, 2015; Labbé, Têtu, Trudel, & Bazinet, 2008). Recent scientific studies have investigated health benefits of green tea including protection against cancer and cardiovascular diseases, the anti-inflammatory, antiarthritic, antibacterial, antiangiogenic, antioxidative, antiviral, neuroprotective and cholesterol-lowering effects (Chacko, Thambi, Kuttan, & Nishigaki, 2010). The major chemical components of the green tea leaf that provides all of these health benefit are polyphenols. Main polyphenols in green tea include gallic acid, quercetin, kaempferol, myricetin and their glycosides but the major part of polyphenols is composed of different forms of catechin (shown in Table. 1.2). These include (−)-epicatechin (EC), (−)-epicatechin-3-gallate (ECG), (−)-epigallocatechin (EGC) and (−)-epigallocatechin-3-gallate (EGCG) (Babu & Liu, 2008). EGCG is the most abundant catechin in green tea that is responsible for its high antioxidant activity (Du et al., 2013).

In food applications, green tea have been suggested as a food additive to enhance the antioxidant properties and to extend the shelf life of foods by acting as a free radical scavenger to terminate the radical chain reactions that occur during the oxidation of triglycerides (Siripatrawan & Noipha, 2012). However, the sensitivity of green tea polyphenols towards environmental conditions such as oxygen, light, temperature, pH and moisture and variety in the stability of different forms of catechin has restricted its application in food products although it was shown to exhibit even higher antioxidant properties than α-tocopherol, hydroxyanisolebutylated or hydroxytoluenebutylated (Kailaku, Mulyawanti, & Alamsyah, 2014; Rashidinejad, Birch, Sun-Waterhouse, & Everett, 2014). Furthermore, the interaction of polyphenols with food components such as proteins could lead to significant aggregation and precipitation resulting in quantity and/or functional loss of the polyphenols (Bartolomé, Estrella, & Hernández, 2000; Rashidinejad, Birch, Sun-Waterhouse, & Everett, 2014). Thus, entrapping green

tea into liposomes could bear out to overcome limitations of green tea polyphenols due to the fact that liposomes are considered as a promising delivery system for phenolic compounds (Gibis et al., 2012).

Table 1.2 Tea Polyphenols.



1.4. Encapsulation

Encapsulation is an entrapment of one substance, called as active agent into another substance which is named as wall material creating particles in the nanometer (nano-encapsulation), micrometer (micro-encapsulation) or millimeter scale (Ray, Raychaudhuri, & Chakraborty, 2015). Encapsulation technology has been utilized in the food applications to provide effective barrier to sensitive food ingredients such as colorants, flavors, vitamins, antioxidants etc. against environmental parameters (oxygen, light, heat, free radicals etc.). Different encapsulation techniques have to be considered to meet the physicochemical and molecular characteristics of each bioactive compound.

The main purposes of the encapsulation technology in food industry can be summarized as following (Fang & Bhandari, 2010);

1. Protection of encapsulated component from degradation by reducing its reactivity with environment.
2. Prevention/retardation the vaporization of volatiles by reducing the transfer rate.
3. Modification of physical characteristic of encapsulated component.
4. Achievement the controlled release of encapsulated component.
5. Masking the undesirable flavor of encapsulated component.
6. Separation of components within a mixture which react with each other.
7. Obtainment uniform dispersion of encapsulated component.

In the literature, encapsulation of polyphenols with spray drying (Chiou & Langrish, 2007; Kosaraju, Labbett, Emin, Konczak, & Lundin, 2008), coacervation (Deladino, Anbinder, Navarro, & Martino, 2008; Shutava, Balkundi, & Lvov, 2009), liposome entrapment (Fang, Hwang, Huang, & Fang, 2006; Gibis, Vogt, & Weiss, 2012; Priprem, Watanatorn, Sutthiparinyanont, Phachonpai, & Muchimapura, 2008; Takahashi, Uechi, Takara, Asikin, & Wada, 2009), inclusion complexation (Anselmi et al., 2008; Lucas-Abellán, Fortea, Gabaldón, & Núñez-Delicado, 2008), cocrystallization (Deladino, Anbinder, Navarro, & Martino, 2007), nanoencapsulation (Hu et al., 2008; Shutava, Balkundi, Vangala, et al., 2009), freeze drying (Gradinaru,

Biliaderis, Kallithraka, Kefalas, & Garcia-Viguera, 2003; Laine, Kylli, Heinonen, & Jouppila, 2008), yeast encapsulation (Shi et al., 2007) and emulsion (Almajano, Carbó, Jiménez, & Gordon, 2008; Di Mattia, Sacchetti, Mastrocola, & Pittia, 2009) provide an approach to overcome the limitations along with the enhancement of bioactivity and bioavailability. The superiority of liposome entrapment over the other encapsulation techniques is enabling controlled delivery of both water and oil-soluble functional compounds such as antimicrobials, flavors, antioxidants, and bioactive ingredients owing to the presence of both lipid and aqueous phases in the structure of liposomes (Fang & Bhandari, 2010; Laye, McClements, & Weiss, 2008; Rashidinejad et al., 2014). Furthermore, high loading capacities for water-soluble components and being biocompatible, biodegradable, and nontoxic make liposomes attractive encapsulation systems (Gibis, Vogt, & Weiss, 2012).

1.5. Liposome

Liposomes, spherical-shaped microscopic lipid vesicles, are formed from aqueous dispersions of amphiphilic molecules such as polar lipids that tend to produce bilayer-structures. Liposomes can be composed of natural phospholipids such as cholesterol and lecithin or man-made non-toxic phospholipids. The amphiphilic nature of phospholipids and their capability of forming closed vesicle allow both hydrophobic and hydrophilic components to be entrapped to the liposomes. Thus, the fact that liposomes contain both hydrophilic and hydrophobic parts, they are widely used in pharmaceutical, personal care, chemical and food industrial fields to encapsulate both hydrophobic and hydrophilic compounds such as antimicrobials, flavors, antioxidants, and bioactive ingredients by protecting them against to degradation and release of these components at designated targets (Immordino, Dosio, & Cattel, 2006; Laouini et al., 2012).

Liposomes do not have a thermodynamically stable structure, so that external energy, such as sonication, extrusion, homogenization, is required for their formation. The size of the liposomes varies from the nanometer to micrometer depending on the amount of energy used in the liposome formation. (Taylor, Weiss, Davidson, & Bruce, 2005).

Depending on the preparation method, several types of liposomes can be distinguished. In this way, liposomes can be classified into 4 main categories namely small unilamellar vesicles (SUVs), large unilamellar vesicles (LUVs), multilamellar vesicles (MLVs) and multivesicular vesicles (MVVs), which can be also seen in Figure 1.2. Regardless of type, all liposomes are basically lipid bilayer containers in which several components can be entrapped or at least anchored into their structure (Gómez-Hens & Fernández-Romero, 2005).

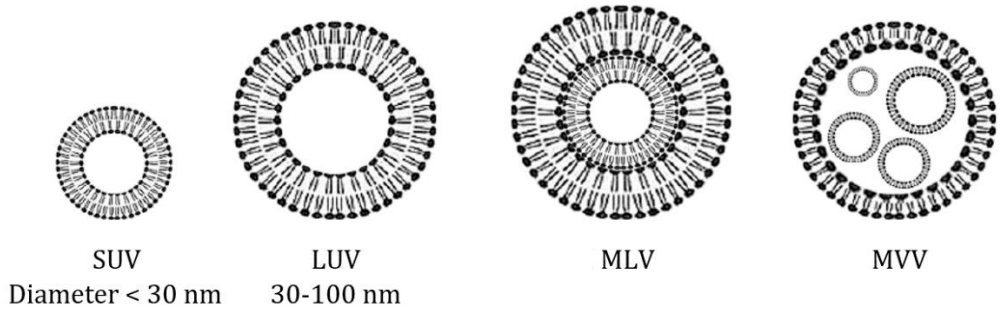


Figure 1.2 Types of liposomes: SUV, small unilamellar vesicle; LUV, large unilamellar vesicle; MLV, multilamellar vesicle; MVV; multivesicular vesicle. (Kırtıl & Öztop, 2014).

1.6. Phospholipids

The liposomes are primarily composed of phospholipids although other lipids such as galactolipids might also be incorporated into the composition. The phospholipids which is the major components of biological membranes can be divided into two categories (glycerophospholipids and sphingomyelins) according to the alcohols contained in phospholipids.

Glycerophospholipids

Glycerophospholipids which are the main phospholipids found in eukaryotic cells contain the glycerol in their backbone. All natural glycerophospholipids are present in α-structure and L-configuration. The head group, the length and the saturation of hydrophobic side chains, the type of bonding between the aliphatic moieties and glycerol backbone, and the number of aliphatic chains determine the chemical structure of glycerophospholipids. According to the head group type, glycerolphospholipids can be grouped as phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidic acid, phosphatidylinositol, phosphatidylglycerol, cardiolipin (Li et al., 2014). The structure of phosphatidylcholine which is the most abundant phospholipids is given in Fig. 1.3. In phosphatidylcholine, two acyl hydrocarbon chains are linked to glycerol at the sn-1 and sn-2 position by an ester linkage. The third glycerol carbon linked to a phosphate at the sn-3 position which is then linked to a choline group. The two hydrocarbon chains provide the hydrophobic tail and the hydrophilic phosphocholine provides the polar head group to phosphatidylcholine.

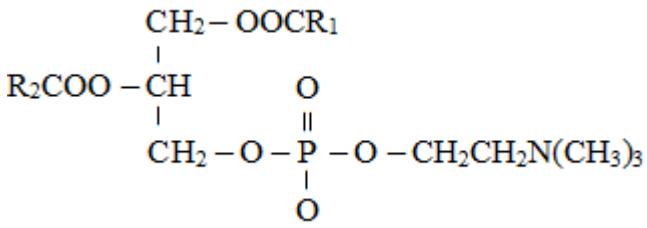


Figure 1.3 Chemical structure of phosphocholine.

Sphingomyelins

Sphingomyelin is one of the major lipids found in the plasma membranes of mammalian cells. Sphingomyelins shows many similarities with phosphatidylcholine in molecular structure. Main differences between sphingomyelins and phosphatidylcholine including the varieties in backbone, the length and the saturation of cis-double bonds, the lengths of the acyl chains, the range of phase transition temperature, the macroscopic properties and the interaction with cholesterol (Li et al., 2014).

According to their sources, phospholipids can be categorized as natural phospholipids and synthetic phospholipids. The main sources of phospholipids are vegetable oils (e.g. soybean, cottonseed, corn, sunflower and rapeseed) and animal tissues (e.g. egg yolk and bovine brain). The natural phospholipid is generally preferred since the cost of phospholipids obtained from natural sources is lower than the synthetic or semi-synthetic phospholipids (Li et al., 2014).

1.7. Food Applications of Liposomes

Based on the results of previous applications of liposome in pharmaceutical and medical research area (drug delivery, cancer treatments, etc.), food scientists have begun to utilize liposomes for controlled delivery of functional components such as proteins, enzymes, antimicrobials, antioxidants, vitamins, and flavors in various food applications (Taylor et al., 2005).

In dairy products, liposome entrapment was utilized in order to decrease processing time of cheese products, fortify the dairy products with vitamins as well as aid in digestion of constituents present in the dairy products. For instance, the entrapment of lipases to improve the production of cheese was one of the recent study in which the application of liposome in the dairy product was investigated. The study showed that the addition of liposome-encapsulated lipases reduced the firmness of Cheddar cheeses while increasing the cohesiveness and elasticity of samples (Kheadr, Vuillemard, & El-Deeb, 2002). Besides the dairy product applications, the liposomes were also used

for the stabilization of food components against degradation. The study carried by Kirby, Whittle, Rigby, Coxon, & Law (1991) indicated that the antioxidant activity of ascorbic acid (Vitamin C) has improved by liposome entrapment. In another study, it was reported that the protection of α-amylase against pepsin attack, storage at low temperature and extreme pH con- ditions was achieced by liposome encapsulation (Hsieh, Chen, Wang, Chang, & Chang, 2002). Lastly, the encapsulation of antimicrobials such as Nisin and lysozyme in order to prevent the spoilage of various food products was another food application of liposome.

1.8. Liposome Formation Mechanism

Previously, it was stated that the phospholipids which are amphiphilic molecules having a hydrophilic head and two apolar hydrophobic chains are the main component of liposomes. Phospholipids have a strong tendency to form membrane in a dispersed aqueous solution due to their amphipathic characteristic. While polar heads of phospholipids prefer to interact with the aqueous medium, the apolar aliphatic chains tend to interact with each other. This behavior of phospholipids is the main explanation for the formation of lipid bilayer in the aqueous solution. In the structure of liposomes, the hydrophobic part of the phospholipids face each other resulting in a lipophilic inner compartment. Thus, it could be concluded that the formation of the lipid bilayers is provided by hydrophobic interaction. Van der Waals interaction, hydrogen bonds and polar interactions between the water molecules of the aqueous medium and the polar heads of lipid strengthen and stabilize this structure.

Although the mathematical descriptions of liposome formation are mostly empirical some thermodynamic concepts provides understanding about the parameters to determine the equilibrium liposome size. The molecules in the lipid bilayer should be in equilibrium with the solvent and lipid in order to possess their thermodynamic equilibrium properties. The probability (ω) of finding liposome of size containing N lipid molecules is described as (Taylor et al., 2005);

$$\omega(N)=\frac{1}{N_0}\exp\left(-N\left(\mu_0-\frac{\mu_1}{kT}\right)\right) \qquad \text{(Equation 1.9)}$$

where μ_0 and μ_1 are the chemical potential of lipid molecule in aqueous phase and in the lipid phase respectively. N_0 is the mean number of lipid molecules in one liposome. The liposome formation is considered as favorable if $\mu_0 - \mu_1 < 0$ and unfavorable if $\mu_0 - \mu_1 > 0$. If the $N=8\pi R^2/a_0$ (where a_0 is the area per lipid molecule) is inserted into equation above the equation will be;

$$\omega(R)=\frac{R}{R_m^2}\exp\left(-\frac{R}{2R_m}\right)^2 \qquad \text{(Equation 1.10)}$$

Where R_m represents the radius at maximum of probability distribution. Unfortunately, this equation does not completely reflect the liposome size due to geometrical constraint of polar lipids (Taylor et al., 2005). The Helfrich has described more accurate relationship by considering the entropic contributions provided by the elastic energy stored in the membrane (Lasic, 1990);

$$\omega(R)=\frac{9R^3}{2R_m^4}\exp\left(-\frac{3R^2}{2R_m^2}\right) \qquad \text{(Equation 1.11)}$$

Eventually, the size distribution of liposomes as a metastable state of aggregation of lipid fragments was described by Tenchov and coworkers as following (Taylor et al., 2005);

$$\omega(d)=\left(\frac{\delta}{\eta}\right)\left(\frac{d-d_0}{\eta}\right)^{\delta-1}\exp\left(\frac{d-d_0}{\eta}\right)^{\delta} \qquad \text{(Equation 1.12)}$$

where d_0 is the minimal size and δ and η are fitting parameters. The limitation of a model due to the lack of any physical description of the liposomal dispersion should be pointed out.

1.9. Liposome Formation Methods

There are several preparation techniques for liposome which can be categorized as mechanical and non-mechanical methods. The method used to form the liposomes is usually chosen by manufacturer depending on the purpose of the use. The main liposome formation methods used in the food industry are discussed in the following section although there are much more preparation methods in the literature.

1.9.1. Mechanical Methods

Microfluidization

Microfluidization is high pressure homogenizer that can rapidly create very small droplet size in high volumes (Thompson & Singh, 2006). It is considered an applicable technique for large scale industrial productions due to its flexibility for control of globule size and the ability to produce fine particles (Jafari, He, & Bhandari, 2007). Moreover, it is suitable for food industry since it does not require alcohol, detergent or solvent (Thompson & Singh, 2006).

As illustrated in Fig. 1.4, microfluidizer consists of a fluid inlet, an air motor that pumps at high pressure and interaction chamber (McClements, 2005). In the case of liposome preparation, phospholipid and material are dispersed in a liquid medium initially. Afterwards, premix solution is pressured into two opposite channels of the microfluidizer and then flow at high velocity. In the interaction chamber, two channels of the inlet fluid collide with each other resulting in kinetic energy that provides the required activation energy to break up the large phospholipid bilayer into smaller size (Taylor et al., 2005).

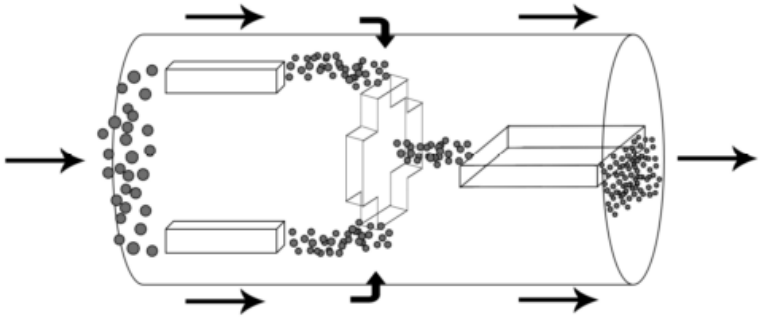


Figure 1.4 Schematic representation of the working principle of microfluidizer (Kırtıl & Öztop, 2014).

Ultrasonication

The fact that ultrasonication is considered a suitable tool for sensitive, non-destructive and non-invasive technique it was became popular in food industry machinery, electronics, oceanography, military, robotics and so on (Mohammadi, Ghasemi-Varnamkhasti, Ebrahimi, & Abbasvali, 2014). The two types of ultrasonication systems used in liposome formation are illustrated in Fig. 1.5. In direct probe system, ultrasonic generator connected to a stainless steel or titanium probe directly contact with the lipid dispersion. Very high energy that leads to very small particles could be supplied to the dispersion by direct probe type ultrasonication. However, it should be noted that energy distribution throughout the system is not homogenous resulting in non-uniformity in particle size of the liposomes. Moreover, overheating of the sample and the eventual degradation of the metal tip of the probe may affect the chemical and physical properties of the food compounds. On the other hand, bath sonicator provides uniform energy to the system which leads to the liposome with uniform particle size distribution. Additionally there is no direct contact between dispersion and probe that can cause the contamination of the system (Taylor et al., 2005).

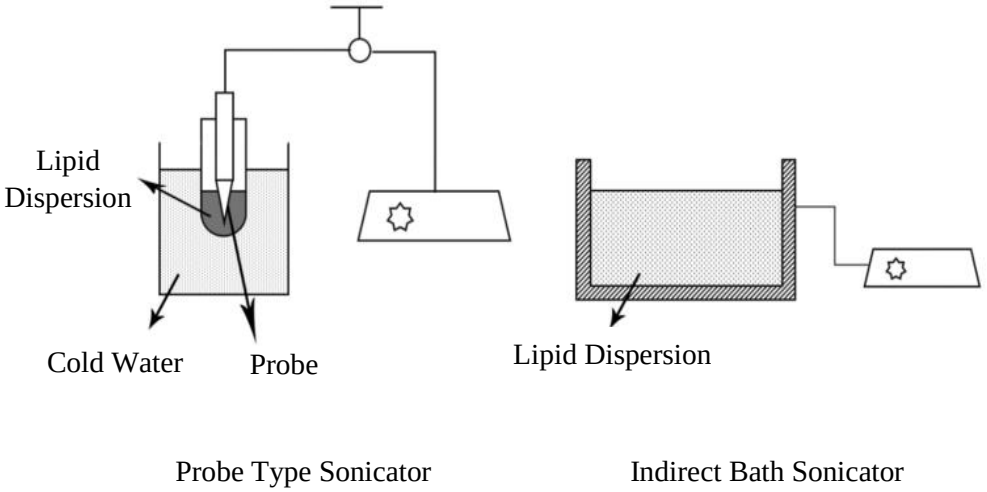


Figure 1.5 The schematic representation of probe type and indirect bath sonicator (Kırtıl & Öztop, 2014; Taylor et al., 2005).

Ultrasonication technique is based on the propagation of ultrasonic waves through aqueous dispersion of polar medium (Taylor et al., 2005). Ultrasonic waves are the waves propagating by particles motion in the medium and travels through the bulk of material at frequencies above 16 kHz (Soria & Villamiel, 2010). In food industry, the applications of ultrasound are classified as low intensity and high intensity ultrasound. While the low intensity ultrasound is considered non-destructive tool with usage of smaller power levels and high frequencies (>100 kHz) high intensity ultrasound disrupt and affect physical, chemical and mechanical properties of foods with high power and low frequencies (between 16 and 100 kHz) (Mohammadi et al., 2014). In high intensity ultrasonication sound waves at frequencies between 16 and 100 kHz are propagating through aqueous medium. The propagation of sound waves through the medium leads to cavitation that is spontaneous generation and collapse of small cavities (Taylor et al., 2005). The cyclic formation and destruction of cavities lead to thermal, mechanical and chemical effects. While the chemical effects include generation of free radicals, mechanical effects include collapse pressure, turbulence

and shear stress (Lateef, Oloke, & Prapulla, 2007; Yusaf & Al-Juboori, 2014). Finally, all of these forces lead to disruption of system and followed by reformation of smaller vesicles.

Extrusion and Membrane Homogenization

The dispersed solution which contains large liposomes formed through a simple mixing process is forced to pass through a membrane/filter with a defined uniform pore size in extrusion or membrane homogenization (Fig. 1.6). The particle size distribution of the liposomes formed by extrusion or membrane homogenization are highly homogenous. In extrusion, the large liposomes are initially ruptured due to the force applied for passage through the capillaries of the membrane and resealed again. Thus, the considerable amount of the encapsulated reagent could be released into the medium during extrusion. It should be also noted that the temperature and extrinsic properties such as the size of the pores, the applied pressure across the membrane of filter and the flow rate should be carefully adjusted to accomplish successful homogenization.

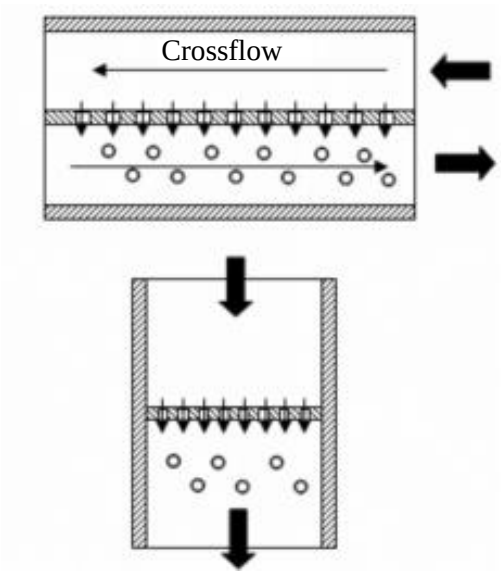


Figure 1.6 The schematic representation of the working principle of extrusion and membrane homogenization (Taylor et al., 2005).

1.9.2. Non-Mechanical Methods

Reverse Phase Evaporation

In the reverse-phase evaporation, the solution of the reagent to be encapsulated is mixed with the lipid dispersion which is prepared at a low boiling point solvent such as diethyl ether, isopropyl ether, chloroform or methanol. Then, the system is homogenized using vortexer or low energy sonicator following by the evaporation of solvent under reduced pressure resulting in the formation of a viscous gel. The formation of the liposomes is achieved by the removal of residual solvent using continued rotary evaporation (Dua, Rana, & Bhandari, 2012). Although the high encapsulation efficiency is obtained by reverse phase evaporation, the removal of organic solvent is not always completely achieved and the non-homogenous particle size distribution could not be attained (Taylor et al., 2005).

Detergent Dialysis Method

The method is based on the solubilization of the lipids by the detergents at their critical micelles concentration. The removal of the detergents by dialysis leads to form the LUVs which are richer in phospholipid. The liposome with uniform particle size distribution are produced by detergent dialysis method. Similar to reverse phase evaporation, the retention of detergent in the liposome is the main disadvantages of the detergent dialysis method. Besides the dialysis, gel chromatography involving a column of Sephadex G- 25, adsorption or binding of Triton X-100 (a detergent) to Bio-Beads SM-210 and binding of octyl glucoside (a detergent) to Amberlite XAD-2 beads could be used to remove the detergents from the liposome (Dua et al., 2012).

Freeze-Drying - Rehydration and Freeze - Thawing

These techniques are preferred to improve the properties of liposomes rather than producing them. Basically, the formation of multivesiculars with high encapsulation efficiency from small preformed liposomes is achieved by these techniques. Dehydration - rehydration cycles are applied to preformed liposome above the gel-

liquid crystalline phase transition temperature resulting in the formation of MLVs from liposomes with smaller particle size. Although the particle size of liposome becomes larger, the amount of the encapsulated component is significantly increased by this technique. Repeated freezing and thawing at a temperature above the phase transition temperature is also used to increase encapsulation efficiency of preformed liposome (Taylor et al., 2005).

1.10. Biopolymer Coating of Liposome

Previously it was emphasized that the liposomes are thermodynamically unstable thus; they have a tendency to burst resulting in the loss of entrapped materials over time to reach their minimum energy state (Guner & Oztop, 2017). The gradual coalescence of liposome might also happen to decrease their curvature leading to a breakdown of liposomal dispersion eventually (Gibis et al., 2012; Laye et al., 2008).

The formation of a polymeric layer around the liposomes could be considered as a solution of this limitation by preventing the liposomes from disruptive forces (Madrigal-Carballo et al., 2010; Wanaga et al., 1999). The biopolymer coating of liposome can be defined as the addition of charged polymers to a charged liposomal structure resulting in the formation of polymeric layer around liposomal surfaces. The adsorption of the biopolymer to the liposomes primarily occurs by electrostatic interactions in the case the biopolymer and liposome are oppositely charged. On the other hand, the polymeric layer around the liposome could be formed by Van der Waals and steric interactions rather than electrostatic interactions if biopolymer and liposomes possess the same electrical charge (Chun, Choi, Min, & Weiss, 2013).

Previously it was pointed out that the biopolymer coating have great potential for use in the food industry for the encapsulation, protection, and release of bioactive lipids (McClements, 2010). Many studies have demonstrated that coating of liposomes with biopolymers improved their ability to deliver and release long-acting drugs due to the longer circulation times associated with the increased thickness of membrane and/or the change on the charge of liposomal surfaces (Chun, Choi, Min, & Weiss, 2013;

Gómez-Hens & Fernández-Romero, 2005; Guzey & McClements, 2007; Lertsutthiwong, Rojsitthisak, & Nimmannit, 2009). Additionally, previous studies revealed that biopolymer coating of liposomes was effective at decreasing internal lipid oxidation by reducing oxygen exposure and improving the stability of liposomes (Gibis et al., 2012).

In the light of the information mentioned above, the biopolymer coating around green tea loaded liposomes was achieved in order to improve the chemical and physical stability of liposomes over time. The hypothetical representation of the formation of biopolymer coating around the liposomes containing green tea extract was shown in Fig. 1.7 in order to visualize the coating process.

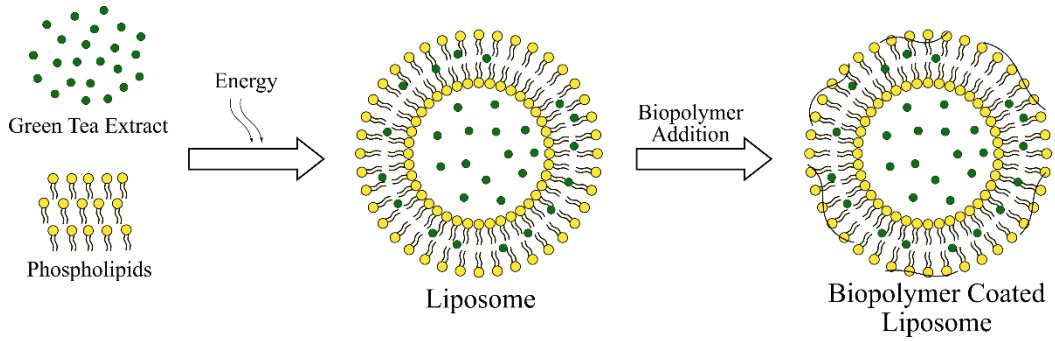


Figure 1.7 The schematic representation of the formation of biopolymer coated liposomes.

1.11. Stability of Liposomes

The liposome stability is a major concern needed to be taken into consideration for the liposome formation, storage and delivery. Thus, the physical and chemical stability of the liposomes should be monitored by characterization of the liposome over a certain time period. Generally, the physical stability refers to the preservation of liposome structure characteristics while the chemical stability is related to the change in molecular structure of liposomes (Chrai, Murari, & Ahmad, 2002). The zeta potential, average particle size, polydispersity index, encapsulation efficiency, lamellarity determination, phase and quantification of the residual solvent are the most common

parameters used in the characterization of liposomes (Laouini et al., 2012). A detailed description of the methods used in the present study are given in the following sections.

1.11.1. Zeta Potential

When a solid surface comes in contact with an aqueous solution, the rearrangement of free ions in the solution occurs resulting in the formation a thin layer with nonzero net charge near the interface. The arrangement of the charges at the solid-liquid interface and the counterions in the liquid is referred to as the electrical double layer (Sze, Erickson, Ren, & Li, 2003).

Zeta potential measurement is based on the movement of charged particles which are suspended in the medium towards to the electrode of the opposite charge when electrical field is applied. Firstly, electrophoretic mobility (U_e) of the particles are calculated by the following equation;

$$U_e = \frac{\text{Velocity of the particles (V)}}{\text{Electric Field (E)}} \qquad \text{(Equation 1.13)}$$

Once the electrophoretic mobility of the particle is measured by the laser Doppler velocimetry, the zeta potential is calculated according to Henry's equation (Robert, 1986);

$$U_e = \frac{2\varepsilon Z f(\kappa a)}{3\eta} \qquad \text{(Equation 1.14)}$$

where Z; zeta potential, ε; the dielectric constant, η; the absolute zero shear viscosity of the medium, f($\boldsymbol{\kappa}$a) is the Henry function, and $\boldsymbol{\kappa}$a is the ratio of the particle radius to electrical double layer thickness.

1.11.2. Particle Size by Dynamic Light Scattering

Dynamic light scattering which is also known as photon correlation spectroscopy is a simple and rapid method used in the characterization the size of colloidal dispersions. The technique is based on the Brownian motion of the particles in the suspension due to collisions between suspended particles and solvent molecules. As a result of Brownian motion of the particles, the time-dependent fluctuations in the intensity of scattered light is observed and these fluctuations are analyzed by an autocorrelator. The scattering vector (q) is calculated by the equation below (Kaszuba, McKnight, Connah, McNeil-Watson, & Nobbmann, 2008);

$$q= \frac{4\pi\tilde{\eta}}{\lambda_0}\sin\left(\frac{\theta}{2}\right) \qquad \text{(Equation 1.15)}$$

where $\tilde{\eta}$; the refractive index of solvent, λ_0; the vacuum wavelength of the laser; θ; the scattering angle.

The speed of particles under Brownian motion is also measured and provides the translational diffusion coefficient D which will be converted into a hydrodynamic diameter (D_H) using the Stokes- Einstein equation (Leal, Rögnvaldsson, Fossheim, Nilssen, & Topgaard, 2008).

$$D_H= \frac{kT}{3\pi\eta D} \qquad \text{(Equation 1.16)}$$

where k; the Boltzmann constant, T; the temperature and η; the dispersant viscosity.

1.11.3. Microscopic Observations

Due to the fact that most particle size determination methods have difficulties to differentiate individual and aggregated liposome, microscopic observation of the liposome is required in order to confirm particle size measurement and to gain valuable information about the structure of the liposomes. Although light microscope provides

several advantages of obtaining vesicle images in a short time using the standard laboratory equipment, information about the vesicle morphology, the sample heterogeneity regarding the shape and size, and differentiation of single and aggregated liposome it is inadequate to gain comprehensive information about the lipid bilayer of liposomes which is offered by other microscope techniques (Bibi et al., 2011).

Electron microscope offers greater magnification which allows to obtain information about lipid bilayer characteristics and the visualization of structure of much smaller unilamellar vesicles which cannot be visualized by light microscope. More specifically, while light microscopes have a resolution of 200 nm, electron microscopes offer a resolution about 0.2 nm. The common electron microscopes used in the characterization of liposome are transmission electron microscope which gives the information about internal structure of particles and scanning electron microscope indicating surface morphology of the particles. In the most basic terms, electron microscope working principle is based on the exposure of the sample to an electron beam which is focused by various lenses. Some electron then collide and displace electrons around the nuclei of atoms in sample while the rest of electron change their path only. Eventually, a projected image is created by focusing and magnifying electrons using a system of magnetic lenses (Bibi et al., 2011).

1.11.4. NMR (Nuclear Magnetic Resonance)

Nuclear Magnetic Resonance is a non-invasive analysis method increasingly employed in colloidal science to gain both structural and dynamic information on a molecular level (Leal et al., 2008). The method is based on the measurement of recovery (or decay) of magnetic signal coming from a sample that is disturbed by a momentary sinusoidal magnetic pulse while the sample was under the effect of another external magnetic field (Hashemi, Bradley, & Lisanti, 2010). High field NMR Spectroscopy despite providing a much more detailed compositional analysis, is limited by its high cost and instrument sizes (Bernewitz, Dalitz, Köhler, Schuchmann, & Guthausen, 2013). Low resolution time domain NMR Relaxometry method, on the

other hand, despite the low field strength, has been proven effective in determination of oil and water contents, dispersed phase ratios, particle size distributions, enclosed water volume in W/O/W emulsions as well as polymer gelation and aggregation in other colloidal systems (Mariette, 2009; Vermeir, Balcaen, Sabatino, Dewettinck, & Van der Meeren, 2014). These bench-top affordable NMR devices that typically operate at frequencies less than 25 MHz monitor the decay and recovery of magnetization in samples via certain sequences such as Free Induction Decay (FID); the Carr-Purcell-Meiboom-Gill (CPMG) and Inversion (or saturation) Recovery. This makes it possible to measure the longitudinal (recovery curve time constant, T_1) and transverse (decay curve time constant, T_2) relaxation times. However, for complex food systems, the different food compartments display different relaxation rates depending on the ^{1}H proton environment (Kirtil et al., 2014; Mariette, 2009; Marigheto et al., 2007). In numerous applications, the decay (or recovery) of magnetization data are inverse Laplace transformed thereby providing the distribution of transverse or longitudinal relaxation times ("relaxation time spectrum") coming from different proton pools that constitute the sample. Especially, T_2 relaxation times and relaxation spectra were numerously used in colloidal system analysis for their power to give detailed information on the state and mobility of water and oil. Owing to the non-invasive nature of the method, reproducible results on the same sample can be gathered which makes the method particularly suitable for time-dependent monitoring of emulsion system dynamics (Bernewitz et al., 2013; Hashemi et al., 2010).

1.11.5. Total Phenolic Content Determination

The quantitative determination of the phenolic compounds using Folin-Ciocalteau reagent has been proposed as a standardized method for the quality control of food products (Ainsworth & Gillespie, 2007). The method was developed by Folin and colleagues at Harvard Medical School and initially used to study the metabolism of proteins in humans. Folin and Denis reported a method in order to detect the tyrosine in protein hydrolysates. Folin and Denis prepared a reagent called as Folin-Denis reagent which is formed by mixing sodium tungstate and (phospho)molybdic acid in

phosphoric acid, boiling it for 2 hours, followed by cooling, diluting and filtering it. The method was then performed for the phenolic determination in urine. Afterwards, Folin and Ciocalteu modified the method and reagent. The new reagent called as Folin-Ciocalteu reagent was prepared by the addition of lithium sulfate and bromine to the phosphotungstic- phosphomolybdic reagent at the end of the boiling period, followed by cooling and dilution. The addition of the lithium leads to the prevention of precipitate formation which may affect the color intensity in the measurement. The modified method and reagent was then used in the determination of tyrosine and tryptophan content in protein hydrolysates as well as in the determination of the phenolic content (Vermerris & Nicholson, 2009).

The Folin-Ciocalteau method is based on the oxidation of phenols in alkaline solution (generally achieved adding sodium carbonate) by the Folin-Ciocalteau reagent and colorimetric measurement of the resultant blue color in the 700–760 nm range (Cicco, Lanorte, Paraggio, Viggiano, & Lattanzio, 2009; Vermerris & Nicholson, 2009). Due to the fact that it is a spectrophotometric assay, a calibration curve preparing with the absorbance values to related concentrations should be drawn. The common compounds used in the calibration curve preparation are chlorogenic acid and gallic acid. The concentration of phenolic compounds in the sample is then expressed as chlorogenic acid or gallic acid equivalents, respectively (Vermerris & Nicholson, 2009).

1.11.6. Antioxidant Activity Determination

Several assays have been widely used to estimate antioxidant activity of fruits and vegetables and their products including 2,2- diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), the oxygen radical absorption capacity (ORAC) and Trolox equivalent antioxidant capacity (TEAC). The methods which will be discussed in the following section are based on the different mechanism of the antioxidant defense system.

2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Method

Due to its easiness and stability of its assay, DPPH radical scavenging method is extensively used to evaluate the free radical scavenging potential of antioxidants present in herbs, wheat grain and bran, flours, vegetables, edible seed oils and conjugated linoleic acids (Mishra, Ojha, & Chaudhury, 2012). DPPH is a stable radical which reacts with hydrogen/electron donor compounds and has a maximum UV–Vis absorption within the range of 515-520 nm (Chen, Bertin, & Froldi, 2013). DPPH radical scavenging method is based on the reducing ability of antioxidants towards DPPH radical resulting into reduction of DPPH• to DPPH2. Upon reduction, a color change of DPPH radical solution from purple to yellow monitored by spectrometer within the range of 515-521 nm (Chen, Bertin, & Froldi, 2013).

Ferric Reducing-Antioxidant Power (FRAP) Method

FRAP method which is another simple and reliable colorimetric method commonly used for measuring the antioxidant activity was also used in the present study. Benzie and Strain developed a method for determination of the reducing ability of plasma in order to measure of its antioxidant power (Pulido, Bravo, & Saura-Calixto, 2000). Later, the method was used to determine the antioxidant activity of other compounds such as tea and wine besides the plasma (Benzie & Strain, 1999). The method measures the ability of antioxidants to reduce ferric iron, Fe^{3+}. It is based on the reduction of the complex of ferric iron (Fe^{3+}) and 2,3,5-triphenyl-1,3,4-triaza-2-azoniacyclopenta-1,4-diene chloride (TPTZ) to the ferrous form (Fe^{2+}) at low pH. This reduction is monitored by measuring the absorption change at 593 nm using spectrophotometer.

The oxygen radical absorption capacity (ORAC)

The ORAC method which is more relevant method among the other antioxidant activity determination methods due to the use of a biologically relevant free radicals was initially developed by Cao, Alessio, & Cutler (1993). This assay is based on generation of free radical using AAPH (2,2-azobis 2-amidopropane dihydrochloride)

and measurement of decrease in fluorescence in the presence of free radical scavengers. In the original method, β-phycoerythrin (β-PE) was used as target protein which will be subject to free radical damage. Later, (β-PE) was replaced with fluorescein (30,60-dihydroxyspiro[isobenzofuran-1[3H], 90[9H]- xanthen]-3-one) by Ou, Hampsch-woodill, & Prior (2001) since β-PE led to some problems such as inconsistency between replicates, photosensitivity and interaction with phenolic compounds owing to nonspecific protein binding. The decrease in the fluorescence is recorder after AAPH is added into the solution. Trolox which is a water-soluble analog of Vitamin E is used as a standard in order to express the results as Trolox Equivalent.

Trolox equivalent antioxidant capacity (TEAC)

The TEAC assay which is also known as 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) assay is based on scavenging of the $ABTS^{\bullet+}$ radicals by the antioxidants in a sample. The $ABTS^{\bullet+}$ is a stable radical with bluish-green color not found in the human body. The reduction of $ABTS^{\bullet+}$ to ABTS in the presence of antioxidants leads to the loss of color which could be monitored spectrometer at 750 nm (Alam, Bristi, & Rafiquzzaman, 2013). The calibration curve is prepared by Trolox in different concentrations. Finally, TEAC values could be expresses as Trolox equivalents (in mM) by using calibration curve.

Objective of the Study

The aim of the present study is to design and characterize the liposomes containing green tea extract during a certain storage period. In the first part of the study, uncoated green tea extract loaded liposomes were formed by microfluidization and ultrasonication in acetate buffer and distilled water. In the second part, to enhance the stability of the liposomes during storage, the effect of additional protective layers (biopolymer coatings) around the liposomes was investigated. Characterization of uncoated and coated liposomes incorporating green tea extract was investigated by conducting zeta potential, mean particle size, transmission electron microscopy, T_2 NMR Relaxometry, total phenolic content, antioxidant activity, in vitro digestion in simulated gastric and intestinal mediums and experiments during 28 day-storage.

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials

Green tea extract was purchased by Spring Valley (Bentonville, Arkansas, U.S.A). Soy lecithin, Lipoid S75 with 70% phosphatidylcholine was purchased from Lipoid GmbH (Ludwigshafen, Germany). Glacial acetic acid was supplied from Merck KGaA (Darmstadt, Germany). Ultra-pure grade lysozyme was obtained from Biomatik Corporation (Wilmington, DE, USA). Whey protein isolate was purchased from Bipro (Hard Line Nutrition, Kavi Food Co., Istanbul, Turkey). The standards of (-)-epigallocatechin-3-gallate (EGCG), (-)-epicatechin (EC), (-)-epicatechin-3-gallate(ECG), (+)-catechin (C), (-)-gallocatechin (GC), (-)-gallocatechin-3-gallate (GCG), (-)-catechin-3-gallate (CG), gum arabic, chitosan (medium molecular weight, viscosity=200-800 cP in 1% acetic acid solution, 75-85% degree of deacetylation), Folin-Ciocalteu reagent, gallic acid, 2,2-diphenyl-1-picrylhydrazyl, 2,4,6-Tris(2-pyridyl)-s-triazine, Iron (III) Chloride Hexahydrate ($FeCl_3.6H_2O$), iron (III) sulfate heptahydrate ($FeS0_4.7H_2O$), sephadex G50, phosphotungstic acid, analytical grade sodium acetate trihydrate, sodium carbonate, methanol, ethanol, and acetic acid were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

2.2. Methods

2.2.1. Preparation of the extract solution

The extract solution was prepared by dissolving 0.1% green tea extract (w/v) in acetate buffer (pH: 3.8) and distilled water around neutral pH (pH:6.5), stirring for 30 minutes at 300 rpm and filtering using a folded cellulose filter paper. A pH of 3.8 was chosen since the stability of tea catechins are very stable when pH < 4 while a pH of 6.5 was determined to observe the stability of green tea catechins in neutral pH that is neither alkaline nor acidic (Ananingsih and others 2013). An acetate buffer was prepared with 1.421 g/L glacial acetic acid and 0.181 g/L analytical grade sodium acetate trihydrate, adjusting final pH to 3.8. Distilled water was obtained using 0.2 µS/cm purity mpMinipure Dest system (mpMinipure Ultrapure Water Systems, Ankara, Turkey).

2.2.2. Preparation of Uncoated Liposomes

To obtain homogenous unilamellar vesicles with small particle diameter (40-50 nm) two-step homogenization process was carried out. 1 % (w/v) soy lecithin was dissolved in extract solution and the obtained coarse dispersion was first blended using an UltraTurrax (WiseTis Homogenizer, Witeg Labortechnik GmbH, Germany) at 20,000 rpm for 2 minutes and then the pre-homogenized solution was subjected to two different homogenization techniques; high pressure microfluidization (Nano Disperser – NLM 100, South Korea) at $13x10^7$ Pascal for five passes and probe type ultrasonicator (Bandelin Sonoplus HD 3100, Bandelin electronic GmbH & Co. KG, Berlin Germany) using sonotrode: TT13 probe at 75% amplitude for 5 minutes separately. The homogenization chamber was cooled during microfluidization technique with ice to prevent the degradation of green tea extract polyphenols. Similarly, the sample was placed into ice bath during ultrasonication to avoid the heating up the sample. As the control, liposomes without green tea extract were prepared in acetate buffer and distilled water were prepared by following same procedure explained above. Each system was prepared in triplicate and stored at 4˚C wrapped in aluminum foil for one-month storage period.

2.2.3. Preparation of Biopolymer Coated Liposomes

Layer-by-Layer (LbL) Biopolymer Coated Liposomes

1 % (w/v) biopolymer stock solutions were prepared by dissolving chitosan in acetate buffer and fish gelatin, whey protein and gum arabic in distilled water. Biopolymer solutions were stirred for 24 h at 400 rpm to ensure complete dissolution. First layer was formed by adding the chitosan and fish gelatin solutions at different amounts (100-2000 µl) to 10 ml uncoated liposomes prepared in acetate buffer and distilled water under continuous stirring (700 rpm, 2 min) respectively. In order to form second layer, gum arabic and whey protein solutions were separately added to the chitosan coated liposomes at different amounts (100-2000 µl) by following same procedure. Whey protein was used to form the second layer of fish gelatin coated liposomes. The schematic representation of LbL deposition technique was illustrated in Fig. 2.1. The dilution, gel separation and ultrafiltration were separately applied to liposome before second layer formation in order to prevent the liposome aggregation.

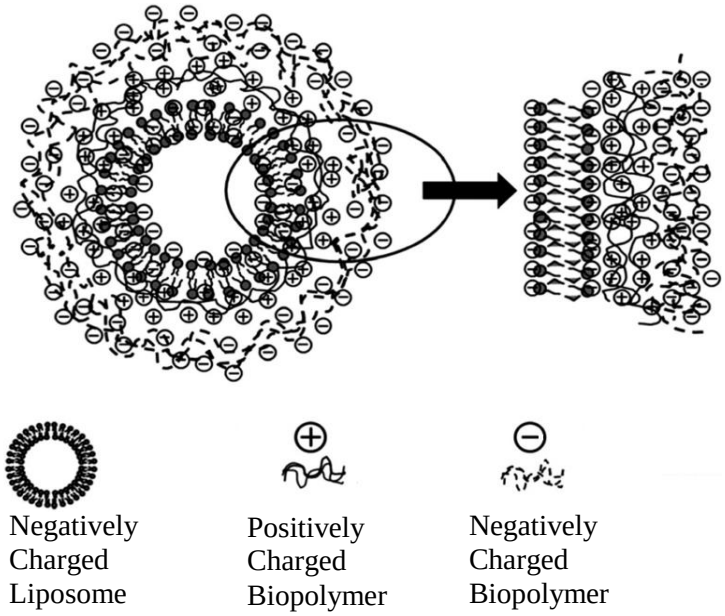


Figure 2.1 The schematic representation of LbL deposition of liposome (Chun et al., 2013).

Dilution

To prevent the aggregation, the liposomes were diluted at 1:5 ratio after the first layer formation.

Gel Filtration of Layer-by-Layer (LbL) Biopolymer Coated Liposomes

Sephadex gel filtration was used to remove the biopoylmers which had not bound to liposomal surfaces and green tea extract which had not been encapsulated in liposomes after first layer formation. Firstly, 10 % w/v Sephadex G50 was dissolved in distilled water and it was allowed to swell for 24 h. The filter paper was placed at the bottom of empty 5 ml syringes. Then, the syringes were filled with 5 ml of Sephadex gel. The syringes were placed in capped 15 ml plastic test tubes before being centrifuged (MF-80, Hanil Science Industrial Co. Ltd., South Korea) at 2000 rpm for 3 min in order to expel the excess water from gel. Then, the syringes containing Sephadex gels were placed into new 15 ml plastic test tubes. 1.5 mL uncoated liposomes were initially added on top of Sephadex G50 column and centrifuge under the same conditions in order to saturate the column with lipid. Finally, 1.5 mL of liposome was added on top of the Sephadex G50 column and the centrifugation step was repeated. Gel-filtered liposomes were collected at the bottom of the test tubes and stored at 4 °C for further analysis (Rashidinejad et al., 2014).

Ultrafiltration

The centrisart centrifugal unit with 100 kDa nominal molecular weight cut off was used for ultrafiltration. 2.5 ml of coated liposome was added into the tube gently. Then, the inner compartment of the tube containing membrane was placed on the top of the sample present in the tube. The tube containing coated liposomes was centrifuged (MF-80, Hanil Science Industrial Co. Ltd., South Korea) at 2,000 rpm for 10 min.

The coating of liposomes by LbL including dilution, gel separation and ultrafiltration was shown in the Fig. 2.1.

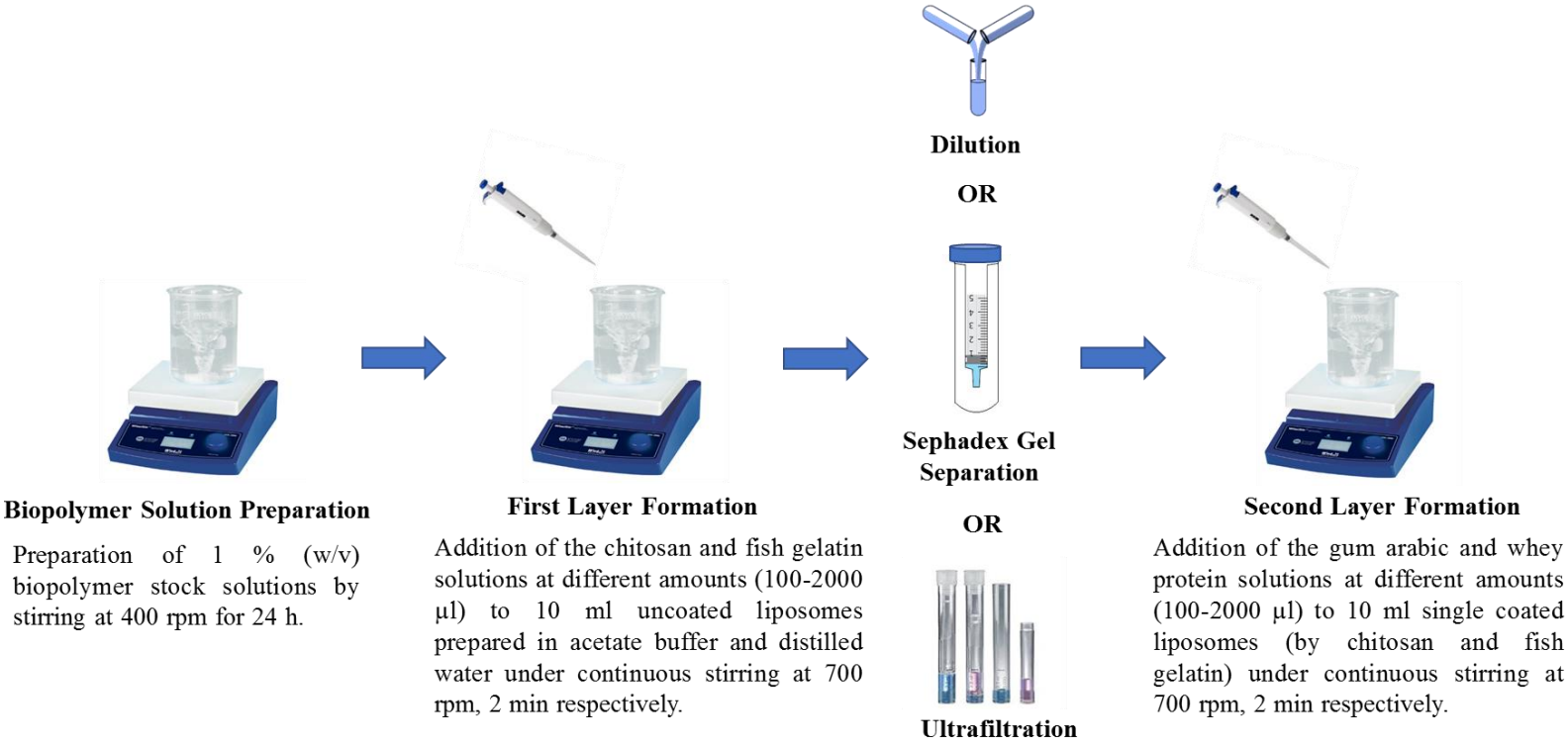


Figure 2.2 Preparation of biopolymer coated liposomes by layer by layer deposition technique.

Single Layer Biopolymer Coated Liposomes

The procedure used in the formation of LbL biopolymer coated liposomes was followed to form single layer biopolymer coated liposomes. 1 % (w/v) biopolymer stock solutions were prepared by dissolving chitosan in acetate buffer and lysozyme, whey protein and gum arabic in distilled water. Biopolymer solutions were stirred for 24 h at 400 rpm to ensure complete dissolution. Biopolymer solutions at different amounts (100-2000 µl) were added dropwise to 10 ml uncoated liposomes under continuous stirring (700 rpm, 2 min) to avoid flocculation depletion and potential cross linking caused by the lacking of the coating materials (Madrigal-Carballo et al., 2010). Uncoated liposomes with green tea extract prepared in acetate buffer and distilled were used as controls. Each system was prepared in triplicate and stored at 4˚C, wrapped in aluminum foil for one-month storage period.

2.2.4. Chemical Characterization of Green Tea Extract by LC-MS/MS

The phenolic acids in green tea were extracted by dissolving 0.1 gram of green tea extract in 5 ml methanol using a vortex for 1 min. Macerate was centrifuged (MF-80, Hanil Science Industrial Co. Ltd., South Korea) at 4,000 x rpm for 10 min and the supernatant was collected. The procedure was repeated for two times and the collected supernatant was filtered through 0.45 µm cellulose-acetate filter before LC-MS/MS analysis.

EGCG, EC, ECG, C, GC, GCG, and CG were identified and quantified by LC/MS/MS system at METU Central Laboratory, Molecular Biology-Biotechnology Research and Development Center, Mass Spectroscopy Laboratory with AGILENT 6460 Triple Quadrupole System (ESI+Agilent Jet Stream) coupled with AGILENT 1200 Series HPLC and equipped with a binary pump delivery system (model G1312B9), a microdegaser (model G1379B), an autosampler (model G1367D), a column compartment (model G1316B). Separation was achieved on a Zorbax SB-C18 (2.1 mm x 50 mm x 1.8 µm) column at 35 °C with an injection volume of 5 µl. Mobile phase A consisted of 0.05 % formic acid and 5 mM ammonium formate and phase B was methanol at 0.5 mL/min flow rate. The operation conditions for the analysis in the

negative mode were as followings: Nebulizing gas 45 psi, sheath gas temperature 350 °C, and sheath gas flow 9 ml/min, capillary voltage 4000 V and nozzle voltage 500 V. The integration and data elaboration were performed by MassHunter Optimizer Software (Agilent G3793AA).

2.2.5. Mean Particle Size and Zeta Potential Measurements

The particle z-average mean diameter and the electrical charge of liposomes were measured periodically during storage using a dynamic light scattering instrument (Zetasizer Nano ZS90, Malvern Instrument, Worcestershire, UK). For the particle size measurement, liposomes were diluted at 1:7 ratio with the solvent to prevent multiple scattering effect. The z-average diameter of liposomes was calculated from their Brownian motion via the Stokes-Einstein equation. For zeta potential measurements, liposomes were directly transferred into a cuvette of the instrument without dilution. The zeta potential was calculated by measuring direction and velocity of the liposomes moved in the applied electric field and applying the Smoluchowski approximation. Measurements were carried out in triplicates at 1^{st}, 7^{th}, 14^{th} and 28^{th} days of storage period.

2.2.6. Transmission Electron Microscopy

Coated and uncoated liposomes were visualized at METU Central Laboratory by transmission electron microscope (FEI Tecnai G2 Spirit BioTwin CTEM, Oregon USA) with Lantanhexaborid (LaB6 electron gun at 120 kV) to investigate the morphology to confirm the particle size measurements. The liposomes were diluted approximately 1:10 with distilled water and one drop of the diluted sample was transferred to a freshly glow discharged TEM copper grid (300 mesh copper Formvar/Carbon). Negative staining with a drop of 1% phosphotungstic acid was applied to reveal the structure of the biopolymer coated liposomes. The excess was drawn off with filter paper and allowed to air dry for 2 min. After washing with distilled water, the excess liquid was again drawn off by filter paper. After waiting 3 min at room temperature, the mesh was examined under transmission electron

microscope at the initial and final day of storage. TEM images were recorded for liposomes prepared with distilled water only to eliminate possible interference of the buffer salts.

2.2.7. Determination of Total Phenolic Content

Phenolic content of both uncoated and biopolymer coated liposomes containing green tea extract was determined by Folin-Ciocalteu method with some slight modifications (Krawitzky et al., 2014). A calibration curve was established with the standard solution of gallic acid at concentrations of 10, 15, 25, 30, 45, 50, 60 ppm. For the measurement, liposomes were diluted with ethanol: acetic acid: water mixture (50:8:42 ml: ml: ml) at a ratio of 1:4 and filtered with a micro filter (0.45 µm Chromafil CA-45/25 S, Düren). 2.5 ml of the diluted Folin-Ciocalteu reagent at a volume ratio of 1:10 with distilled water was added to 0.5 ml diluted samples. After stirring with a vortex, it was left to stand for 5 min in dark. Afterwards, 2 mL of 75 g/L sodium carbonate solution was added to the mixture and vortexed again. After another 60 min of incubation in the dark, its extinction was measured at 760 nm by using UV/VIS Spectrophotometer Optizen Pop Nano Bio (Mecasys Co. LTD, Korea) against a blank prepared by the addition of 2 mL sodium carbonate solution to 2.5 mL diluted Folin-Ciocalteu reagent. The measurement was performed as triplicates at 1^{st}, 7^{th}, 14^{th} and 28^{th} days of storage. The phenolic content was calculated as gallic acid equivalents (GAE) in milligrams per liter sample.

2.2.8. Determination of Antioxidant Activity by DPPH Radical Scavenging Method

AA of uncoated and biopolymer coated liposomes containing green tea extract was measured by DPPH radical scavenging method (Hua Wang, Gao, Zhou, Cai, & Yao, 2008) with some modifications. The standard curve was plotted within 5, 10, 15, 20 and 25 ppm of DPPH. For the experiment, liposomes were diluted with ethanol: acetic acid: water mixture (50:8:42 ml: ml: ml) at a ratio of 1:4 and filtered with 0.45 µm micro filter. DPPH solution was prepared by dissolving 0.0025 grams DPPH in 100

mL methanol and 3.9 ml of DPPH solution was added to 100 µL of diluted liposome and vortexed. After 1 h incubation in the dark, the absorbance was measured at 517 nm against a blank of methanol. As a positive control, 3.9 mL DPPH solution was added to 100 µL methanol and its absorbance was performed immediately without incubation. AA was measured periodically during one-month storage in triplicate and expressed as mg DPPH per L sample.

2.2.9. Antioxidant Activity Determination by Ferric Reducing-Antioxidant Power Method

AA of uncoated liposomes was also measured by FRAP method. First of all, FRAP reagents were prepared as described below: (Benzie & Strain, 1999)

Reagent A: Acetate Buffer (300 mM, pH:3.6): 16 mL glacial acetic acid was added to 3.1 g of sodium acetate trihydrate then the solution was made up to 1 liter using distilled water. pH was adjusted to 3.6.

Reagent B: TPTZ solution: 0.031 g of TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine) was added to 10 mL of 40 mM HCI and dissolved at 50˚C.

Reagent C: 0.054 g of Iron (III) Chloride Hexahydrate ($FeCl_3.6H_2O$) was dissolved in 10 mL of distilled water.

Reagent B and C were freshly prepared for each experiment. FRAP solution was prepared by mixing 2.5 mL of reagent B, 2.5 mL of reagent C and 25 mL of reagent A. This solution was placed in 37˚C water bath for a minimum 10 minutes. The standard curve was prepared with iron (III) sulfate heptahydrate ($FeS0_4.7H_2O$) in 0.1, 0.2, 0.4, 0.6, 0.8 and 1 mM. A standard solution was prepared with ethanol, acetic acid and distilled water with 50:8:42 dilution rate respectively. The samples were diluted at a volume ratio of 1:10 and filtered with micro filter. 2.5 mL of FRAP solution was added to 500 µL of diluted sample and placed in a water bath at 37˚C for 4 minutes. 2.5 mL of FRAP solution was used as a blank. The change in absorbance was measured at 593 nm by using UV/VIS Spectrophotometer. AA was expressed as mM Ferrous

Sulphate per liter sample. AA of the samples by FRAP method was conducted on triplicate at 1^{st}, 7^{th}, 14^{th} and 28^{th} day of storage period.

2.2.10. *In Vitro* Release Studies in the Simulated Gastric (SGF) and Intestinal Fluids (SIF)

In vitro digestion and release of the apparent phenolic compounds by liposomes was investigated by simulation of gastric and intestinal fluid. The simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were prepared as given in U.S. Pharmacopeia (2012).

For SGF preparation, 2.0 g of sodium chloride was mixed with 3.2 g of pepsin from porcine stomach mucosa. Afterward, 7 mL of hydrochloric acid was added to the mixture and volume made up to 1000 mL with water maintaining pH at 1.2. To simulate the digestion in gastric fluid, 1.4 mL of SGF was added to 100 µL liposomes in a 10 mL test tube and incubated in water bath (WB-6, Wisd Co. Ltd., Texas, USA) for 120 min at 37 °C with continuous shaking at 80 rpm. After allowed the tubes to cool down to room temperature, it was filtered through cellulose-acetate 0.45 µm filter and neutralized by adding 0.2 mol/L sodium hydroxide solution.

Similarly, SIF was prepared using monobasic potassium phosphate (6.8 g) dissolved in 250 mL water. Then, 77 mL of 0.2 mol equi/L sodium hydroxide and 500 mL distilled water were added and again mixed. Finally, 10 g of pancreatin was added and the volume of the mixture was made up to 1000 mL and pH was adjusted to 6.8 using 0.2 mol equi/L sodium hydroxide and 0.2 mol equi/L hydrochloric acid. In order to simulate the digestion in the intestinal fluid, 2.4 mL of SIF was taken in a 10 mL test tube and incubated with 100 µL liposomes at 36.6 °C for 120 min without shaking. After cooling down to room temperature, the solution was filtered and enzyme activity was inhibited by decreasing pH to 1.2 using 100 µL of 3 mol/L hydrochloric acid to 2 mL filtrate. After 15 min, the solution was neutralized (pH 7.0) by adding 900 µL of 0.2 mol equi/L sodium hydroxide. Lastly, both the samples of SGF and SIF were analyzed for apparent phenolic content by Folin–Ciocalteu method.

2.2.11. Nuclear Magnetic Resonance (NMR) Relaxometry

NMR experiments were performed by using a 0.5 T (22.40 MHz) low-field bench top ^{1}H nuclear magnetic resonance (LF-NMR) relaxometry instrument (SpinCore Technologies, Inc., Gainesville, USA) with 10 mm r.f. coil. The spin-spin relaxation times (T_2) of liposomes were obtained by using Carr-Purcell-Meiboom-Gill (CPMG) sequence with an echo time (TE) of 1000 µs, spectral width of 300 kHz, repetition delay of 3 s, 24 scans, 512 points, and 3000-6000 number of echoes. The NMR samples were prepared filled into NMR tubes with 10 mm sample size. All T_2 measurements were performed after samples equilibrate to room temperature. Obtained T_2 signals were analyzed with MATLAB to obtain T_2 relaxation curves. Mono and bi-exponential fitting was conducted on relaxation curves. The T_2 relaxation times were measured for every three days during one-month storage period. Non-Negative Least Square (NNLS) was applied to the T_2 decay curves to obtain relaxation spectra. PROSPA software (Magritek Inc., Wellington, New Zealand) was used for 1D-NNLS analysis. All measurements were carried out in triplicate.

2.2.12. Statistical Analysis

All measurements were carried out with three replications. The data for mean particle size, zeta potential, TPC, and AA were tested for normal distribution and equality of variances using Anderson-Darling and Bartlett's test respectively to check the suitability for ANOVA. When the data satisfied the assumptions, the results were analyzed using general linear model tool of Minitab (ver.16.2.0.0, Minitab Inc., United Kingdom) at 5% significance level. Tukey's comparison test was used at 95% confidence interval to determine the statistical significance between results. The results in particle size, zeta potential, TPC and AA experiments represented mean of three replicates ± standard error. One-way ANOVA was performed for each liposome type separately. The letters indicate significant difference of the results at different days within same liposome type ($p < 0.05$).

2.3. Experimental Design Table

Table 2.1. Experimental Design Parameters

UNCOATED AND BIOPOLYMER COATED LIPOSOMES CHARACTERIZATION					
#	**FACTORS**	**LEVELS**		**RESPONSES**	
		Uncoated Liposomes	***Biopolymer Coated Liposomes***	***Uncoated Liposomes***	***Biopolymer Coated Liposomes***
1	Solvent Type	Acetate Buffer (pH:3.8) Distilled Water(pH≈6.5)	Acetate Buffer (pH:3.8) Distilled Water(pH≈6.5)	1. Zeta Potential 2. Mean Particle Size 3. Transmission Electron Microscopy 4. Total Phenolic Content by Folin-Ciocalteu Method 5. Antioxidant Activity by DPPH Radical Scavenging Method 6. Antioxidant Activity by FRAP Method 7. In Vitro Release Studies 8. NMR (Nuclear Magnetic Resonance) Relaxometry Experiments	1. Zeta Potential 2. Mean Particle Size 3. Transmission Electron Microscopy Analysis 4. Total Phenolic Content by Folin- Ciocalteu Method 5. Antioxidant Activity by DPPH Radical Scavenging Method
2	Homogenization Technique	Microfluidization ($13x10^7$ Pascal, 5 passes) Ultrasonication (75% amplitude, 5 min)	Microfluidization ($13x10^7$ Pascal, 5 passes)		
3	Biopolymer Type	-	Lysozyme, Gum arabic, Whey protein, Chitosan		
4	Storage Time* (days)	1^{st}, 4^{th}, 7^{th}, 10^{th}, 14^{th}, 21^{th}, 28^{th}	1^{st}, 4^{th}, 7^{th}, 10^{th}, 14^{th}, 21^{th}, 28^{th}		

*Storage time is different for different experiments.

CHAPTER 3

RESULTS AND DISCUSSION

3.1. Chemical Characterization of Green Tea Extract by LC-MS/MS

LC-MS/MS was preferred for quantitative determination of catechins in green tea extract due to its sensitivity and selectivity through MS/MS experiments. The previous studies showed that the quantitation of catechins in tea extracts by chromatographic analysis was highly depended on sample extraction. The extraction method should allow the complete extraction of the compounds analyzed as well as avoid chemical modification of compounds. The studies revealed that the extraction of catechins with acetonitrile and methanol gives the highest yields among three extraction methods (1) a normal tea brew with boiling water, (2) extraction with acetonitrile or methanol, (3) the official German method (1h boiling under reflux) (Goto, Yoshida, Kiso, & Nagashima, 1996; Horie & Kohata, 2000; Yoshida, Kiso, & Goto, 1999). Furthermore, in the study carried by Zuo, Chen, & Deng (2002) it was demonstrated the multiple step extraction is necessary to increase the extraction efficiency of catechins in teas. In the light of above information, the extraction of catechins in the green tea extract was achieved by two times methanol extraction.

The other critical parameter in LC-MS/MS analysis was the selection of mobile phase for the efficient chromatographic separation of catechins in green tea extract. It was previously reported that 7 catechin types, caffeine, and gallic acid could be successfully separated by using methanol/water mixture as a mobile phase. It is also pointed out that the presence of acid (acetic acid, formic acid, trifluoroacetic acid, orthophosphoric acid etc.) in the mobile phase is crucial for both the complete resolution of catechin present in tea and efficient chromatography of these components without peak tailing (Dalluge, Nelson, Brown Thomas, & Sander, 1998). The effect of the presence of acid in mobile phase was investigated in the study of Wang & Helliwell (2000). Full separation of catechins, caffeine, and gallic acid in green tea was achieved in methanol/water system with the addition of orthophosphoric acid. In that regard for our analysis, the mobile phase was decided as 0.05 formic acid + 5 mM ammonium formate and methanol in order to avoid erroneous results.

In Table 3.1, the retention time and concentration of seven types of catechin in green tea extract are given. The varieties in the selection of solvent type and concentration in both mobile phase and sample extraction, column type and method parameters in chromatographic analysis makes it hard to make a comparison between the results in of our study and the literature. Nevertheless, EGCG, EC, and ECG were identified as the major components while CG was detected in the trace amount in agreement with previous studies (Wang et al., 2000; Zuo et al., 2002).

Table 3.1 Retention time and concentration of seven catechins in green tea extract.

Component	Retention Time (min)	Concentration (mg/g GTE)
(-)-Gallocatechin (GC)	0.797	8.25 ± 0.01
(+)-Catechin (C)	1.718	11.69 ± 0.02
(-)-Epigallocatechin-3-Gallate (EGCG)	2.266	89.84 ± 0.42
(-)-Epicatechin (EC)	2.424	16.06 ± 0.08
(-)-Gallocatechin-3-Gallate (GCG)	2.595	4.80 ± 0.01
(-)-Epicatechine-3-Gallate (ECG)	2.926	14.37 ± 0.05
(-)-Catechine-3-Gallate (CG)	3.135	1.21 ± 0.00

*The results were expressed as mean of three replicates ± standard error.
GTE represented green tea extract.

3.2. Characterization of Uncoated Liposomes

3.2.1. Zeta Potential

The magnitude of zeta potential gives an indication of the surface charge of the particles and often used in the determination of the potential stability of a colloidal system (Gibis, Rahn, & Weiss, 2013; Mady & Darwish, 2010). High negative or positive zeta potentials increase the repulsive interactions to overcome the natural tendency of the liposomes to aggregate. Thus the liposomes with high electrical charges could be expected to show better stability than the liposomes with low electrical charges (Malheiros, Daroit, & Brandelli, 2010).

The phosphatidylcholine was predominantly used in the formation of liposomes due to its great stability against variation in pH or alkaline conditions of medium. The high content of negatively charged phospholipids in lecithin (used in the present study) led to the formation of liposomes with negative electrical charge. The zeta potentials of uncoated liposomes in distilled water and acetate buffer were -30.2 and -10.6 mV at the first day and decreased to -23.2 and -9.48 mV at the end of 14 days respectively (Fig. 3.1). The initial difference in zeta potential of the liposomes in distilled water

and acetate buffer indicated that the electrical charge of the liposomal surfaces was affected by the pH of the medium, the magnitude of electrostatic interactions between different liposomes and liposomes and the phenolic compounds in the extract (Gibis et al., 2012). It should also be noted that the zeta potential of uncoated liposomes decreased at the end of 14 days indicating the degradation and instability over time. Moreover, in agreement with previous studies (Gibis et al., 2012; Rashidinejad et al., 2014) it was observed that the addition of green tea extract did not cause significant change in the zeta potential of liposomes at the initial day of storage.

The fact that the absolute value of the zeta potential could be used as a measure of the stability of the liposome it was suggested that liposomes prepared with distilled water were comparatively more stable than liposomes prepared with acetate buffer. Furthermore, the results showed that zeta potential values decreased at the end of 14^{th} day for distilled water samples that were loaded with the extract ($p<0.05$) while the change was insignificant for other samples. It was suggested that the observed changes in surface charge at the end of 14^{th} day could be an indication of that some modification was taking place in the liposomes. This result was supported by change in particle size, total phenolic content, antioxidant activity and the surface morphology of liposomes at 14^{th} day of storage period that will be discussed in the following sections.

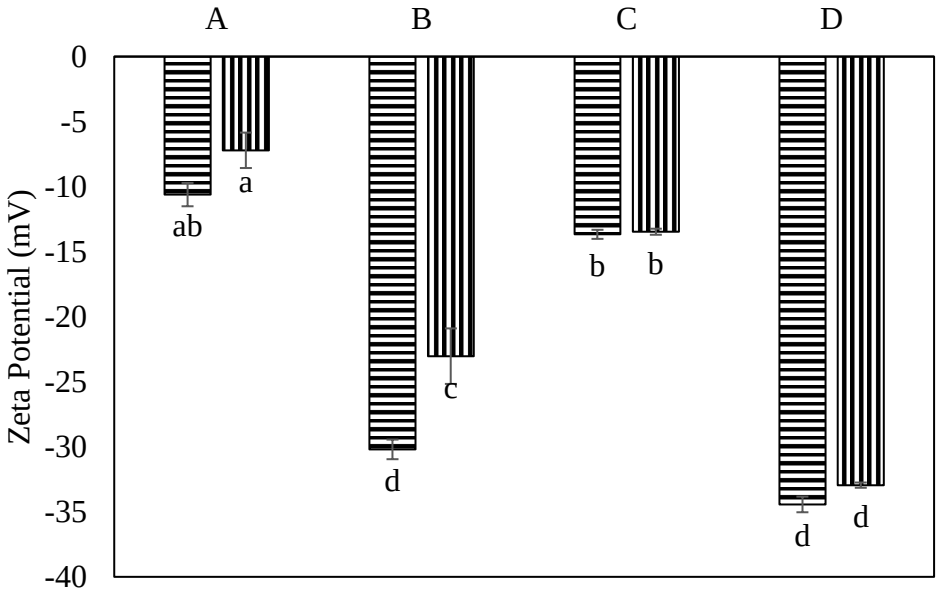


Figure 3.1 Zeta potential of the liposomes prepared by microfluidization during storage period; ▤ Day 1 ▥ Day 14. A, green tea extract loaded liposomes prepared in acetate buffer; B, green tea extract loaded liposomes prepared in distilled water; C, unloaded liposomes prepared in acetate buffer; D, unloaded liposomes prepared in distilled water.

3.2.2. Particle Size Distribution

The mean particle size of green tea extract loaded liposomes prepared by microfluidization and ultrasonication was measured to investigate the stability of the systems. Previous studies indicated the mean particle size was highly dependent on the composition of liposomes. In the study carried by Gibis et al. (2012) it was concluded that the size of the liposomes depended on the material that was encapsulated into liposomes. Similarly, in our study, it was observed the mean particle size of the green tea extract loaded liposomes were higher than the unloaded liposomes' mean particle size. This result might be explained by the fact that phenolic compounds might be incorporated into a lipid bilayer and/or may be absorbed onto the surface of liposomes as well as incorporated into the interior region of the liposomes which could be due to

hydrogen bonding between polar head groups and the phenolic compounds in the extract, hydrophobic interactions between the fatty acid tails of the polar lipid and the more hydrophobic moieties of the phenolic compounds, or thermodynamic driving forces such as the liposomes attaining a more optimal configuration (Gibis et al., 2012).

Particle size results followed mostly a monomodal distribution. The mean particle size of green tea loaded liposomes prepared with distilled water by ultrasonication was around 70 nm (Fig. 3.2) while it was around 130 nm (Fig. 3.3) for liposomes prepared with acetate buffer by ultrasonication. Moreover, the mean particle size of liposomes prepared with distilled water by microfluidization was around 40 nm (Fig. 3.4) whereas it was around 46 nm (Fig. 3.5) for liposomes prepared with acetate buffer by microfluidization on the 1st day of one-month storage time. The mean particle size results for liposomes prepared by microfluidization are supported by the study of Gibis and others (2012) demonstrating very small mean liposomes diameter (50-120 nm) could be achieved by microfluidization technique. By contrast, liposomes with a relatively large mean particle size (70-130 nm) were obtained by ultrasonication. This result is consistent with the previous study of Jafari et al. (2007) showing emulsion with smaller emulsion droplet size was produced by microfluidization compared to ultrasonication.

Moreover, the increase in mean particle size of liposomes prepared by ultrasonication in acetate buffer was observed at 7th day (Fig. 3.3). This result might be explained by that small vesicles formed by sonication are mostly metastable that means vesicles can grow over a time period to decrease the high curvature energy associated with the large bending of the lipid bilayer. The difference in the mean particle size of the liposomes prepared by microfluidization and ultrasonication was due to the fact that energy distribution throughout the solution being non-homogenous during sonication resulting in less uniform or often multi-modal particle size distributions (Taylor et al., 2005).

ANOVA results showed that there was no significant difference ($p>0.05$) on mean particle size at the end of the one-month storage for green tea extract loaded liposomes prepared with distilled water by microfluidization and ultrasonication. In contrast, the significant difference ($p\leq0.05$) was observed in mean particle size for loaded liposomes prepared with acetate buffer by microfluidization and ultrasonication. This could be explained by the pH dependence behavior of liposomes. It was stated that in acidic conditions, rapid release of encapsulated material was observed which did not occur in basic conditions (Janeiro & Oliveira Brett, 2004). It means that liposomes lost their stability in acidic conditions in a short time that results in aggregation of liposomes due to van der Waals interactions and the increased surface area (Cagdas et al., 2014).

The effect of solvent type and homogenization technique on particle size of the liposomes at the initial day of storage was examined by separate statistical analysis. It was investigated that the solvent type had a significant effect ($p\leq0.05$) on particle size of liposomes prepared by microfluidization. The statistical analysis could not be performed in order to investigate the effect of solvent type on particle size of sonicated liposomes since the particle size data did not show normal distribution and transformation even did not solve the problem therefore ANOVA was conducted on whole factors. The same case was observed for the sonicated and microfluidized sample on particle size too. The difference was obvious on the results. A table showing all the parameters together was added to the the Appendix D.

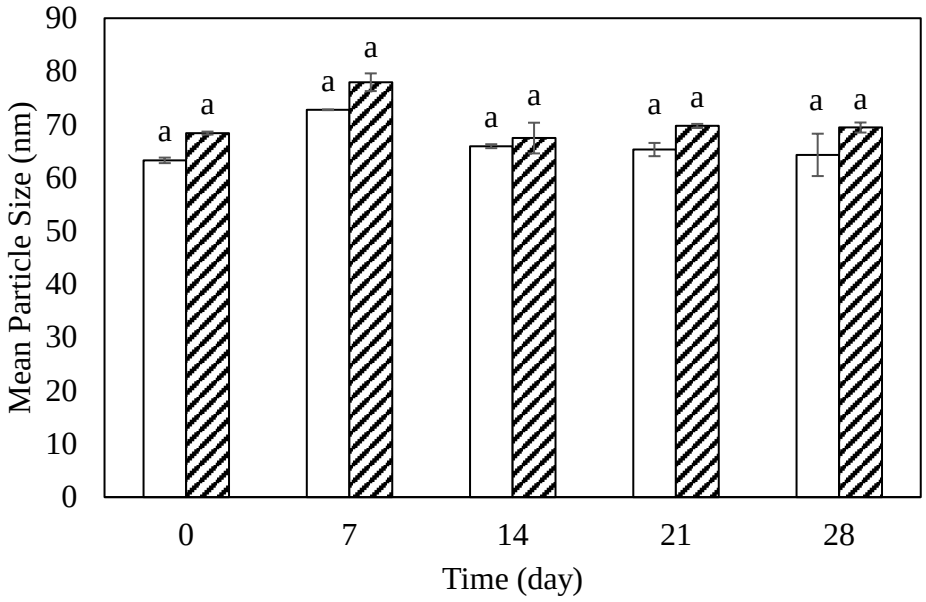


Figure 3.2 Mean particle size of liposomes prepared with distilled water by ultrasonication; ☐ without extract, ▨ with extract.

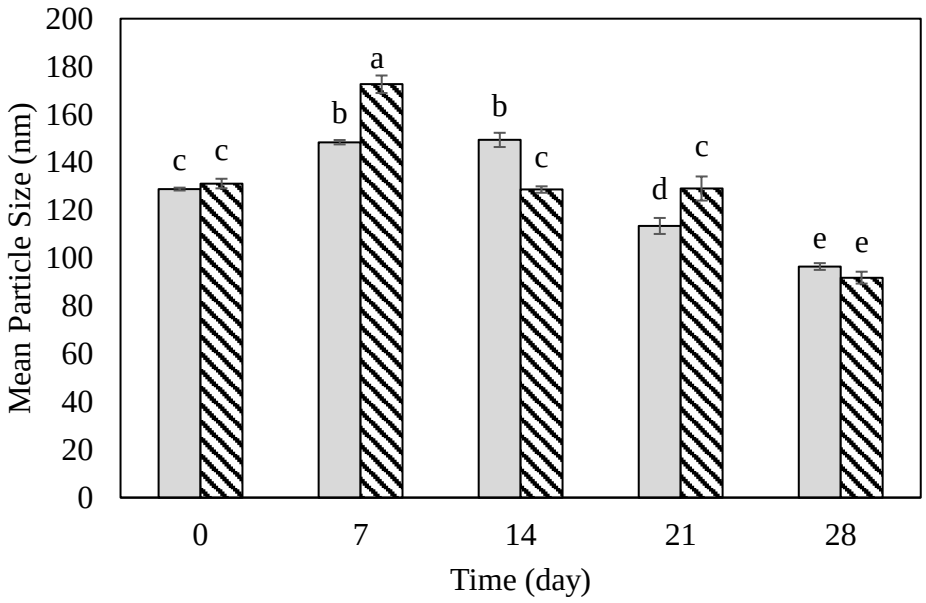


Figure 3.3 Mean particle size of liposomes prepared with acetate buffer by ultrasonication; ☐ without extract, ▧ with extract.

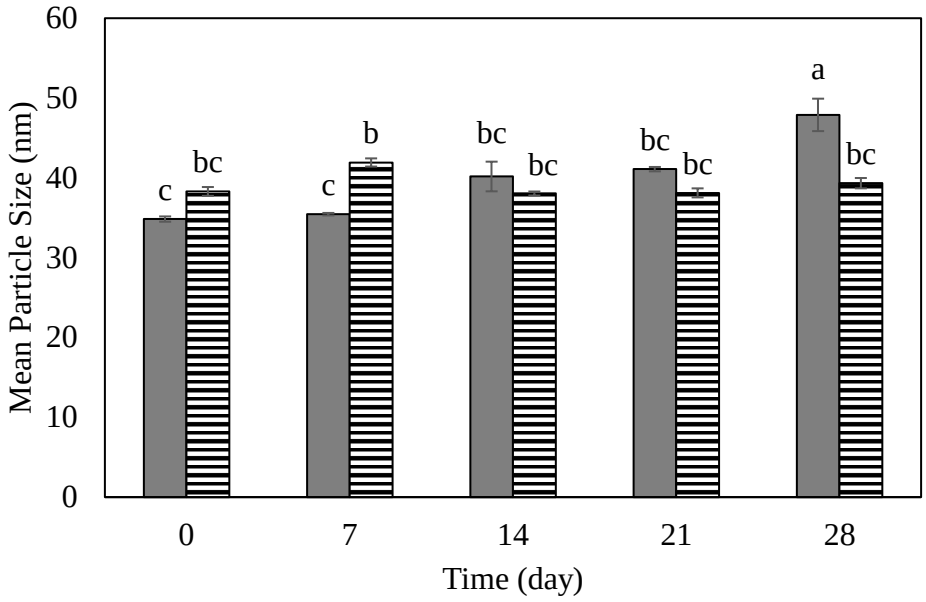


Figure 3.4 Mean particle size of liposomes prepared with distilled water by microfluidization; ▩ without extract, ▤ with extract.

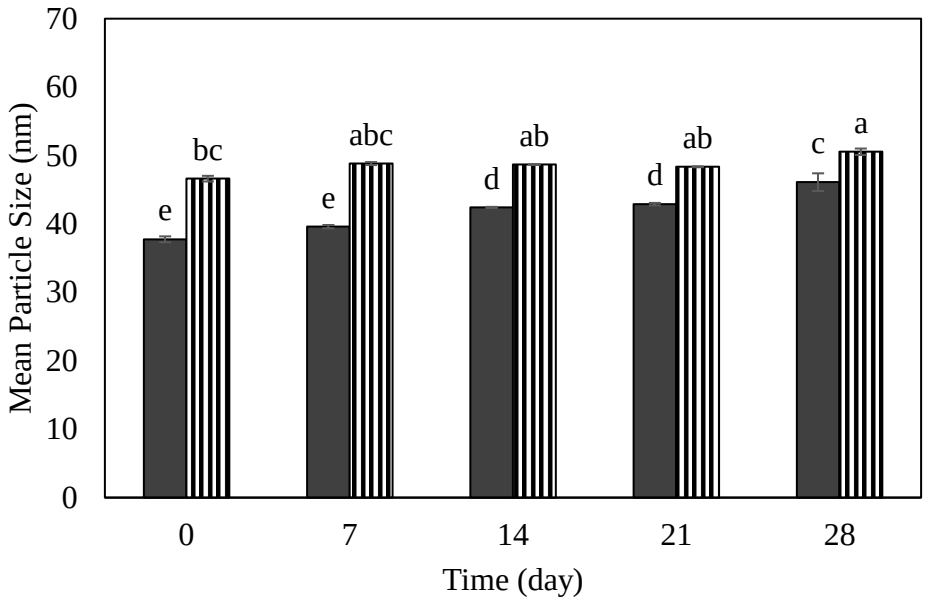


Figure 3.5 Mean particle size of liposomes prepared with acetate buffer by microfluidization; ▩ without extract, ▥ with extract.

3.2.3. Transmission Electron Microscopy (TEM) Analysis

TEM is a common visualization technique performed in the characterization of liposomes as it provides valuable information about the laminar structure of vesicles, as well as the interior and bilayer coatings of liposomes (Chen, Zhu, Huang, Wang, & Yan, 2013; Rashidinejad et al., 2014). TEM analysis was performed for uncoated liposomes to observe the morphology of green tea extract loaded liposomes and confirm the particle size distribution results. The liposomes generally exhibit spherical shape which agreed with the study of Thompson and Singh (2006). Furthermore, the larger particle size and diameter were investigated for liposomes prepared by ultrasonication (as illustrated in Fig. 3.6a) compared to green tea extract loaded liposomes prepared by microfluidization (as seen in Fig. 3.7a) which confirmed the mean particle size results obtained by dynamic light scattering. The morphology of liposomes containing green tea extract was more uniform and showed the regularity in the shape for liposomes prepared by microfluidizer whereas the shape of liposome prepared by ultrasonication was not uniform in size and was partially bound to large or small drops. On 14^{th} day of the one-month storage period, it was observed that the structure of the liposomes began to lose their shape resulting in leakage of the green tea extract from the interior region of the liposome to surrounding (as shown in Fig. 3.6/7b). The tendency of the liposomes to leak due to their fragility could be one reason for the leakage of the liposome on 14^{th} day of the storage period. Alternatively, small liposomes tend to merge in order to decrease their curvature resulting in a breakdown of the liposomal dispersion over time since the liposomes are at their lowest energy state when they are not curved (Gibis et al., 2012).

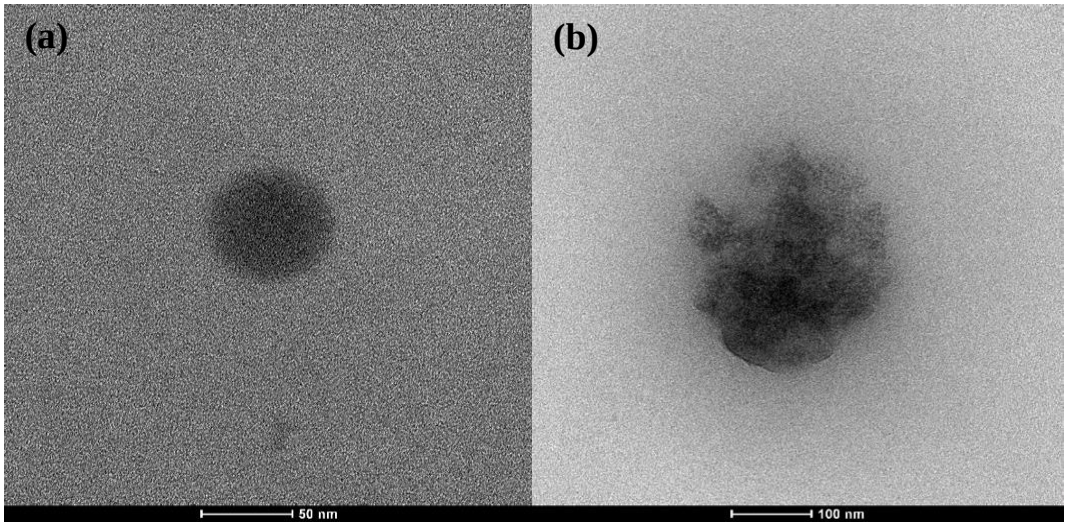


Figure 3.6 Transmission electron microscopy images of green tea extract loaded liposomes prepared by ultrasonication. (a) At the 1st day of storage period. (b) At the 14th day of storage period.

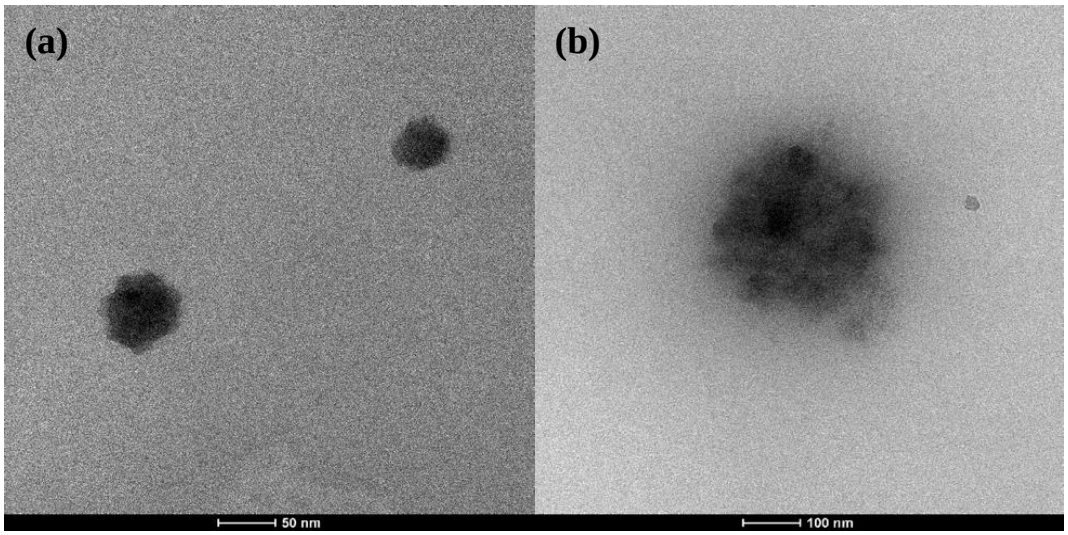


Figure 3.7 Transmission electron microscopy images of green tea extract loaded liposomes prepared by microfluidization. (a) At the 1st day of storage period. (b) At the 14th day of storage period.

3.2.4. Total Phenolic Content by Folin-Ciocalteu Method

TPC of 0.1% green tea extract solution in distilled water and acetate buffer were calculated as 86.195 and 80.481 mg GAE/L sample respectively. TPC of the control samples prepared in acetate buffer by ultrasonication and microfluidization were calculated as 78.795 and 62.340 mg GAE/L sample while TPC of the control samples prepared in distilled water by both homogenization techniques were found as 87.163 and 56.687 mg GAE/L sample. Furthermore, TPC of green tea extract loaded liposome in acetate buffer by ultrasonication and microfluidization were determined as 139.612 and 117.306 mg GAE/L sample (as illustrated in Fig. 3.8) while TPC of green tea extract loaded liposome in distilled water by both homogenization techniques were found as 165.837 and 111.864 mg GAE/L sample (as shown in Fig. 3.9) respectively. These results indicated that both homogenization techniques enhanced the TPC of green tea loaded liposomes compared to 0.1% green tea extract solution. Regarding the effect of sonication and microfluidization on the phenolic compounds, an enhancement of the phenolic content of kasturi lime juice with sonication by Bhat et al. (2011) and positive effect on the extraction of phenolic compounds at high pressure conditions by Karacam et al. (2015) were investigated previously. Furthermore, it can be observed from Fig. 3.8 and 3.9 TPC of green tea extract loaded liposomes prepared by ultrasonication in both acetate buffer and distilled water were higher than liposomes prepared by microfluidization at the beginning of storage.

According to ANOVA results, it was investigated the effect of solvent type on TPC was significant ($p \leq 0.05$) for liposomes prepared by ultrasonication whilst the effect of solvent type was not significant ($p > 0.05$) the TPC of liposomes prepared by microfluidization. The significant effect ($p \leq 0.05$) of homogenization technique on TPC was observed for both liposomes prepared in acetate buffer and distilled water at the first day of the storage period. A table showing all the parameters together was added to the Appendix D. Moreover, the significant difference ($p \leq 0.05$) on TPC between first and final day was observed for the liposomes prepared by ultrasonication. On the other hand, there was no significant difference ($p > 0.05$) on TPC between the

first and final day of one-month storage for liposomes prepared by microfluidization. It can be concluded microfluidization was less effective in the enhancement of TPC although it formed relatively stable liposomes compared to ultrasonication. This result is also supported by the study of Thompson and Singh (2006) concluding liposomes produced by microfluidization were relatively stable without rapid aggregation or fusion.

An increase in TPC on 14th day during one-month storage time was investigated for all liposomes except the liposomes prepared in distilled water by ultrasonication. This may be attributed to the loss of structural integrity of the liposomes on the 14th day of storage. The tendency of liposomes to burst resulting in loss of the encapsulated material over time was also reported in the study of Gibis et al. (2012). The loss in integrity was also supported by the TEM images of the liposomes prepared in distilled water by microfluidization and ultrasonication discussed in the previous section. Spherical shape with dimensions in the nanometer range was observed for liposomes at the first day of storage period whereas it was investigated they began to burst and release green tea extract into medium resulting in an increase in phenolic content on 14th day due to their fragility. It was hypothesized that green tea extract TPC was enhanced by homogenization but also might have decreased due to encapsulation in the liposomes through binding in the phospholipid bilayer. On the other hand, a gradual decrease in TPC was observed for liposomes prepared with distilled water by ultrasonication. This was attributed to sonication conditions being not sufficient to form stable liposomes in distilled water. TEM images supported this explanation by showing non-uniformity in shape and size for the same system even at the first day. It was concluded that certain amount of green tea extract could not be encapsulated into liposomes by ultrasonication. Since liposomes could not preserve non-encapsulated green tea extract against pH, light and temperature decrease in TPC was observed over time.

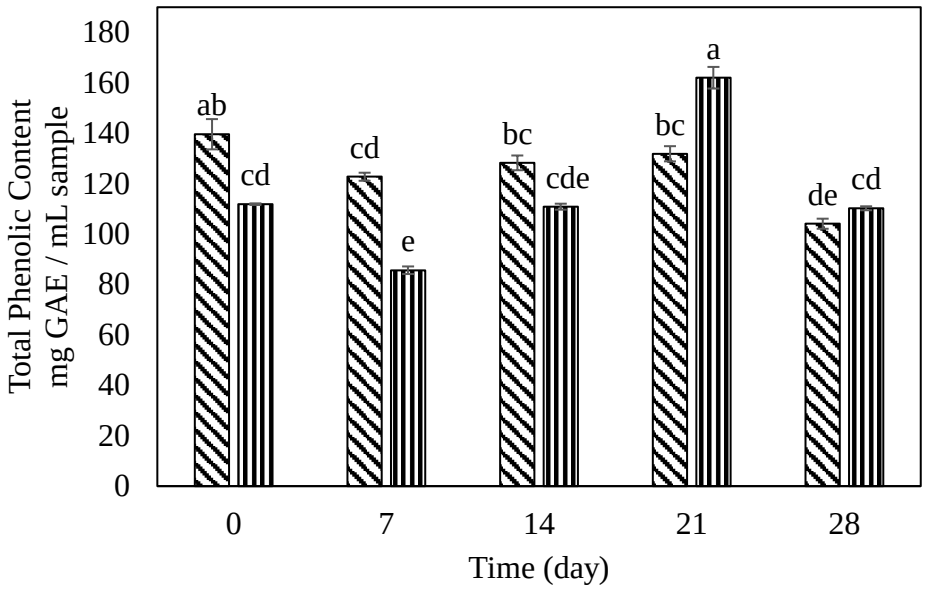


Figure 3.8 Total phenolic content of green tea extract loaded liposomes prepared in acetate buffer ▧ by ultrasonication ▥ by microfluidization.

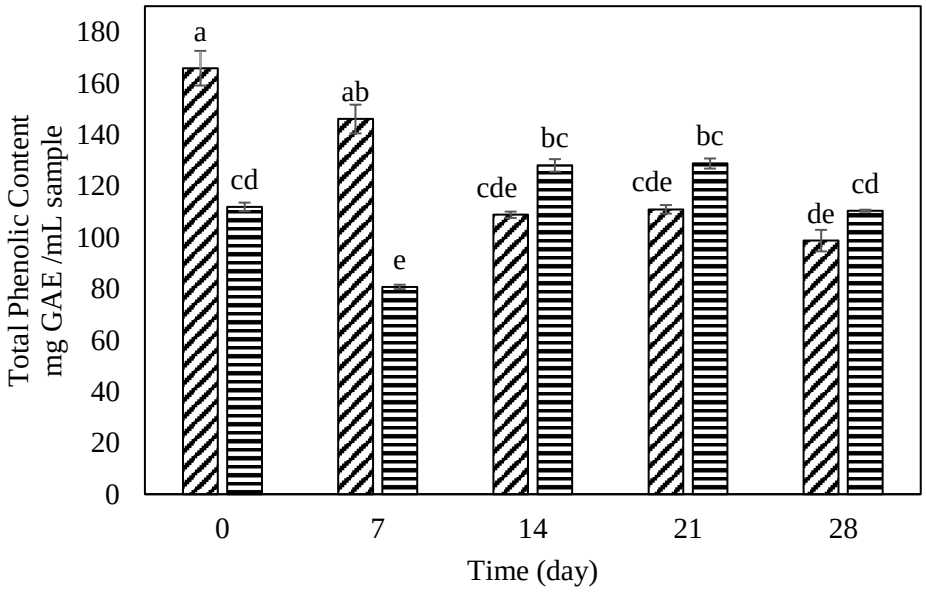


Figure 3.9 Total phenolic content of green tea extract loaded liposomes prepared in distilled water ▨ by ultrasonication ▤ by microfluidization.

3.2.5. Antioxidant Activity Determination by DPPH Radical Scavenging and Ferric Reducing-Antioxidant Power (FRAP) Method

AA of green tea loaded liposomes was determined by both DPPH radical scavenging and ferric reducing-antioxidant power (FRAP) methods. Furthermore, AA of green tea extract used in the present study was determined by DPPH radical scavenging method. AA of green tea extract loaded liposomes was calculated as 25.969 mg DPPH/g extract and 0.1% green tea extract solution in distilled water and acetate buffer was calculated as 24.664 and 24.447 mg DPPH/L sample respectively. AA by DPPH method of the control samples prepared in acetate buffer by ultrasonication and microfluidization were determined as 1.149 and 0.167 mg DPPH/L sample while TPC of the control samples prepared in distilled water by both homogenization techniques were found as 1.521 and 0.105 mg DPPH/L sample respectively. AA by DPPH method of green tea extract loaded liposome in acetate buffer by ultrasonication and microfluidization were found as 21.753 and 14.989 mg DPPH/L sample (Fig. 3.10) while it was determined as 20.451 and 15.051 mg DPPH/L sample (Fig. 3.11) for green tea extract loaded liposome in distilled water by ultrasonication and microfluidization respectively. In addition, AA by FRAP method of the control samples prepared in acetate buffer by ultrasonication and microfluidization were determined as 4.689 and 1.037 mM ferrous sulphate/L sample while AA of the control samples prepared in distilled water by both homogenization techniques were found as 4.827 and 1.547 mM ferrous sulphate/L sample respectively. AA by FRAP method of green tea extract loaded liposome in acetate buffer by ultrasonication and microfluidization were determined as 18.923 and 13.491 mM ferrous sulphate/L sample (Fig. 3.12) while it was measured as 16.688 and 13.909 mM ferrous sulphate/L sample (Fig. 3.13) for green tea extract loaded liposome in distilled water by both homogenization techniques. High, positive and significant correlation (R^2=0.863, $p<0.05$) was observed between AA results calculated by DPPH and FRAP methods for liposomes prepared in distilled water by microfluidization.

From the results, a decrease was observed in the AA of liposomes formed by ultrasonication and microfluidization compared to 0.1% green tea extract solution. Similar results were observed by a previous study of Sun et al. (2015) demonstrating a decrease in AA of fresh apple due to the synergic effect of the ultrasound and temperature increase. Moreover, a decrease in AA due to microfluidization could be explained by overheating of the sample during microfluidization. High temperature, i.e. above 40˚ C, leads to epimerization defined as the conversion of epicatechins in cis structure to epimers that are non-epicatechins. This process caused a change in the epi-structured catechin to non-epi-structured catechin resulting in a decrease in the concentration of catechins in green tea (Ananingsih et al., 2013). This hypothesis was also supported by the study carried by Réblová (2012) that showed a decrease in AA with an increase in temperature.

The fact that AA of the green tea loaded liposomes decreased whereas TPC of the systems increased could be explained by that not all phenolic compounds exhibited antioxidant activity (Giada, 2013). Similar to TPC results, the significant difference ($p \leq 0.05$) on AA for the loaded liposomes prepared by ultrasonication was observed while no significant difference ($p > 0.05$) was found for the systems prepared by microfluidization between the first and final day of one-month storage. Furthermore, an increase in AA on 14^{th} day due to the leakage of green tea extract from liposomes was observed for all systems in both methods. The effect of solvent type on AA was significant ($p \leq 0.05$) for liposomes prepared by ultrasonication while the solvent type was not significantly ($p > 0.05$) alter the AA of liposomes prepared by microfluidization at the initial day of storage. Additionally, homogenization technique has a significant change ($p \leq 0.05$) in AA results for both liposomes prepared in acetate buffer and distilled water at the first day of the storage. A table showing all the parameters together was added to the Appendix D. It should be noted that the statistical analysis of AA data calculated by FRAP method could not be performed since AA results did not show normal distribution. Similarly, AA results of liposomes prepared by ultrasonication and microfluidization in distilled water at 7^{th} day calculated by DPPH

method could not analyzed statistically since the data did not indicate normal distribution.

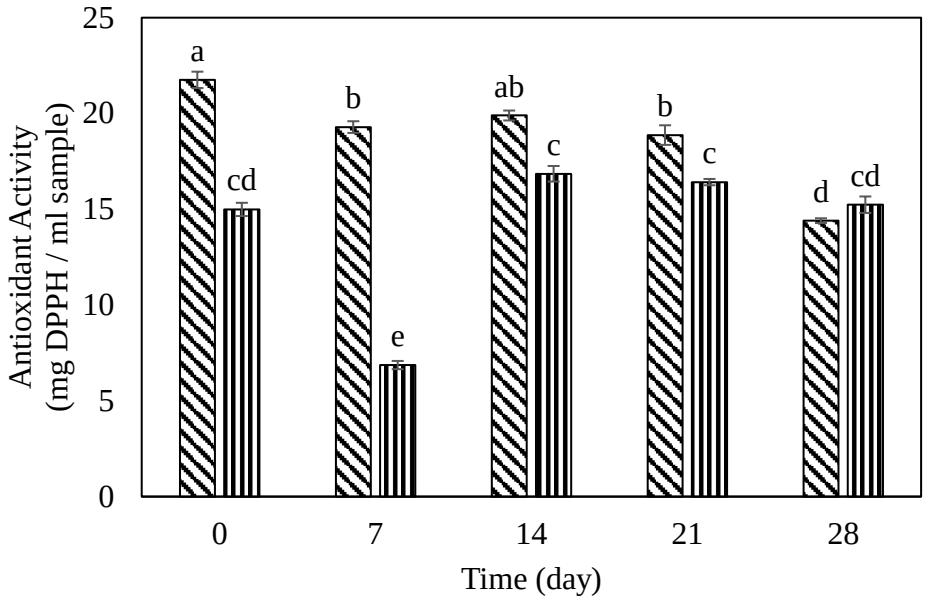


Figure 3.10 Antioxidant activity of green tea extract loaded liposomes prepared in acetate buffer ▧ by ultrasonication ▥ by microfluidization by DPPH Radical Scavenging Method.

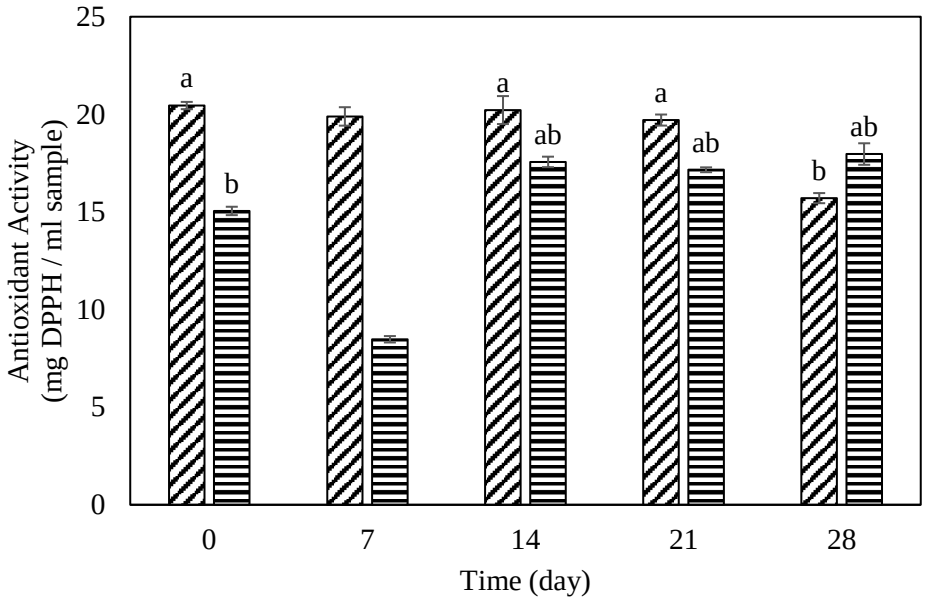


Figure 3.11 Antioxidant activity of green tea extract loaded liposomes prepared in distilled water ▨ by ultrasonication ▤ by microfluidization by DPPH Radical Scavenging Method.

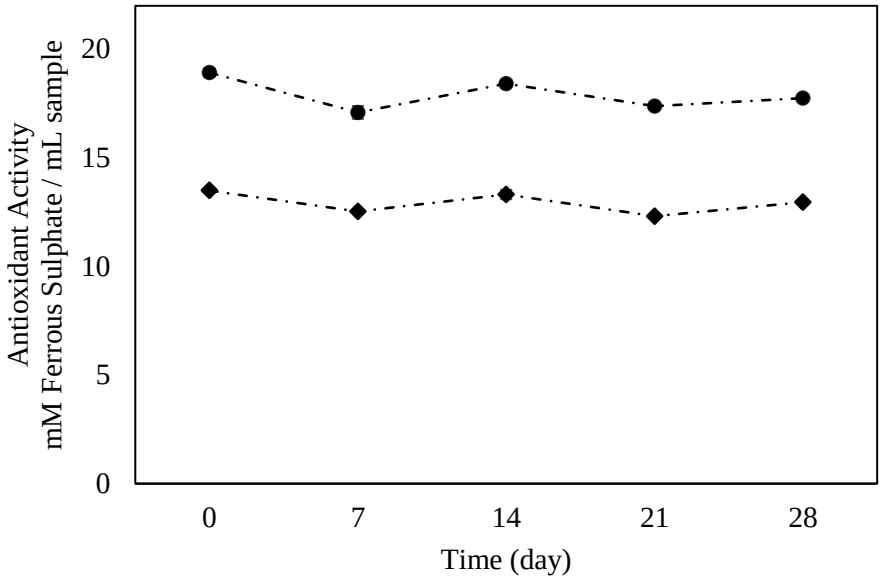


Figure 3.12 Antioxidant activity of green tea extract loaded liposomes prepared in acetate buffer; (●) by ultrasonication (◆) by microfluidization by Ferric Reducing-Antioxidant Power (FRAP) Method.

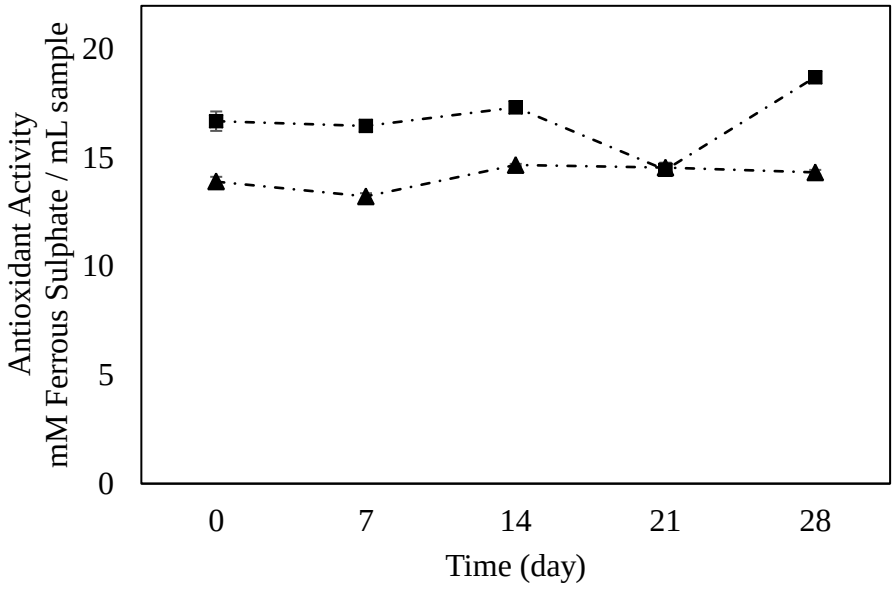


Figure 3.13 Antioxidant activity of green tea extract loaded liposomes prepared in distilled water; (■) by ultrasonication (▲) by microfluidization by Ferric Reducing-Antioxidant Power (FRAP) Method.

3.2.6. *In Vitro* Release Studies

The bioaccessibility of polyphenols depends on their structure as well as on the food matrix. A good carrier system should provide the protection for the encapsulated material from environmental conditions as well as give controlled release and enhancement of bioavailability. Among the approaches that have been applied to improve the intestinal absorption (i.e. the use of enzyme inhibitors, permeation enhancers), nano- and submicron-sized particles are believed to enhance the bioavailability of encapsulated materials by virtue of their small size and high surface area (Li, Paulson, & Gill, 2015). In the present study, the relationship between the stability of liposomes and in vitro digestion was monitored to gain some information for further developing more stable liposomes in the gastro-intestinal tract. Digestion experiments were performed on the liposomes that were freshly prepared.

The in vitro release profiles obtained with different uncoated liposome formulations in gastric and intestinal medium were shown in Fig. 3.14. The decrease in total phenolic content of uncoated liposomes after in vitro digestion could be interpreted as the poor stability of uncoated liposomes in the simulated gastric and intestinal fluids. Generally, uncoated liposomes were relatively stable in mildly acidic environments. However, it should be also noted that the acidic conditions of gastric medium could cause hydrolysis of the saturated phospholipids bilayer leading to destabilization of liposomes (Li et al., 2015).

The poor stability of uncoated liposomes in the simulated intestinal fluid was probably due to the pancreatin which is a proteolytic mixture containing the enzymes pancreatic lipase, phospholipase A_2 and cholesterol esterase (Liu, Ye, Liu, Liu, & Singh, 2012). In the light of this information, the uncoated liposomes could be disrupted by phospholipase hydrolysis resulting in the leakage of encapsulated green tea extract through pores formed on the lipid bilayer (Li et al., 2015).

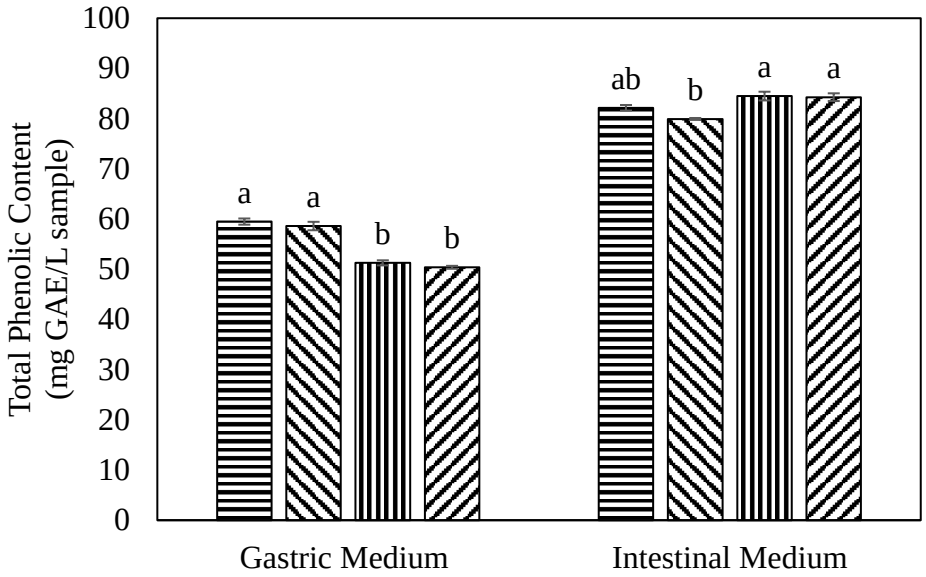


Figure 3.14 In vitro release profile of uncoated liposomes prepared by microfluidization in the simulated gastric and intestinal fluids; ▤ green tea extract loaded liposomes prepared in acetate buffer, ▧ green tea extract loaded liposomes prepared in distilled water, ▥ unloaded liposomes prepared in acetate buffer, ▨ unloaded liposomes prepared in distilled water.

3.2.7. NMR (Nuclear Magnetic Resonance) Relaxometry Experiments

Mono-exponential NMR T_2 Relaxation Times

NMR T_2 relaxation times were acquired by fitting NMR transversal decay signal data to a mono- or bi-exponential model. The model that gave a higher coefficient of determination (R^2) coupled with meaningful T_2 and M_0 (associated with proton density) values were chosen. Data that display very low M_0 or T_2 values (even lower than TE set during measurement which was 1000 µs) were considered to be spectral formations, hence were ignored.

Figure 3.15 and 3.16 shows T_2 relaxation times of unloaded and loaded liposomes prepared in distilled water and acetate buffer by different homogenization techniques, microfluidization and ultrasonication respectively. Moreover, the T_2 relaxation times values are illustrated in Table 3.2. As evident from the Figure 3.15 and 3.16 mean T_2 relaxation times of all liposomal dispersions displayed a mono-exponential behavior at day 1 right after preparation. This was indicative of the sufficiency of homogenization process. At first, liposomes were evenly dispersed inside the buffer solutions.

This finding is related to the diffusion of water between different compartment effecting T_2 relaxation signal. For two different proton environments A and B, where the relaxation behavior of water is defined with T_{21} and T_{22}, if the diffusion coefficient between these two compartments (D_{AB} (m^2/s)) are faster compared to the difference in relaxation rate ΔR_2 (with $R_2=1/T_2$), water relaxation will be averaged and a single mono-exponential signal will be achieved. As the difference between relaxation rates increases between different water states in sample, multiple peaks appear (as discussed in T_2 relaxation spectra) (Mariette, 2009). This mechanism is exploited to gain information on emulsion and colloidal system stability using NMR Relaxometry.

In NMR, magnetic relaxation signal coming from of ^1H protons of the whole sample is measured. However, the magnetic relaxation of non-exchangeable protons (such as the ones coming from non-exchangeable CH bonds in solids) do not contribute to the signal; owing to the measurement delay limitation of low-field NMR systems. The liquid signal and the exchangeable solid signal (such as the ones coming from ~OH groups) was dominated by lipids and water for our case. Thus, for this study almost all explanations will be related to state of lipids and water in solution (Kirtil & Oztop, 2015; Mariette, 2009; Marigheto et al., 2007).

The effect of green tea extract loading on T_2 relaxation was also investigated in the study. Unloaded liposomes had a mean T_2 time of 1711 ms whereas loaded ones had a mean T_2 time of 976 ms (Figure 3.16, mean of acetate buffer and distilled water T_2

values at day 1). Meanwhile unloaded and loaded liposomes had a surface mean particle size of 38 and 47 nm, respectively (illustrated in previous section). The dominant influence of particle size on T_2 relaxation times were previously underlined previously, which makes us believe that something else was in effect that decreased T_2 times and was sufficient enough to compensate for the effect of increasing particle sizes. Green tea extract contains relatively high amounts of catechin and many other polyphenols whose ~OH side chains interact favorably with polar water molecules (Ananingsih, Sharma, & Zhou, 2013; Chacko et al., 2010; Hosseini et al., 2015). This result shows that encapsulation was not %100 efficient. Some of the extract could not be entrapped and was dissolved within to solution which restricted the availability of free water outside the capsules. This is a promising result in that it shows that NMR could be used to estimate (or even quantify) encapsulation efficiency. In a number of studies, NMR was used to calculate dispersed phase ratio (Di Bari, Macnaughtan, Norton, Sullo, & Norton, 2016; Fridjonsson, Graham, Akhfash, May, & Johns, 2014; Ling, Haber, Fridjonsson, May, & Johns, 2016) and to find the enclosed water volume in W/O/W emulsions using a paramagnetic ion to extinguish the signal coming from the outer water (Benichou, Aserin, & Garti, 2007; Bernewitz et al., 2013; Bernewitz, Schmidt, Schuchmann, & Guthausen, 2014; Vermeir, Balcaen, et al., 2014). However, to best our knowledge, there currently isn't any study that uses time domain NMR relaxometry for encapsulation efficiency estimation in emulsions. Upon manipulation of parameters such as echo and delay time, it might be possible to isolate the T_2 relaxation signal to only be affected by the amount of active agent released to the environment, which could prove to be a potential future study.

Time dependent behavior of unloaded and loaded liposomes can be seen in Table 3.2. The aim here was to see whether it was possible to monitor a possible instability occurrence in liposomes via NMR T_2 relaxometry. For unloaded samples, acetate buffer solvent ones did not display a statistically significant change in T_2 times with respect to time ($p>0.05$) despite a few exceptions. There definitely wasn't a trend for acetate buffer samples with respect to time. The physical and chemical stability of the almost all of these samples were confirmed in our previous study with TEM images

and particle size. In the previous section, it was found that the particle sizes of acetate buffer samples did not differ significantly between the first and final days of measurement. So this trend could not be related with a physical instability.

Overall the increasing trend could be observed for all samples which could be related with an increase in particle sizes. The sudden leaps in T_2 times especially within the 2^{nd} week period (between the 7^{th} and 14^{th} days) could be related with leakage of liposomes internal encapsulated water into the environment. The liberation of water from the constraints of the liposomal core could have caused the sudden increases in T_2 times. This behavior will be discussed in more detail with loaded liposomes.

For loaded liposomes, the T_2 times in line with the particle sizes did not display an apparent trend within the course of 30 days both for distilled water and acetate buffer samples. There were no sudden leaps in T_2 times during the 2^{nd} week period. Yet this is to be expected. Even if there was some leakage of green tea extract due to liposomal instability, the green tea extract when dissolved in the continuous water phase could have increased the T_2 relaxation rate hence diminishing the possible differentiation in T_2 times. Actually around the 14^{th} day mark, the phenolic content measurements from a previous study we have conducted, revealed a sudden increase in free phenolic content released into the liposomal dispersion. This finding confirmed liposome leakage explanation previously suggested for unloaded liposomes as well.

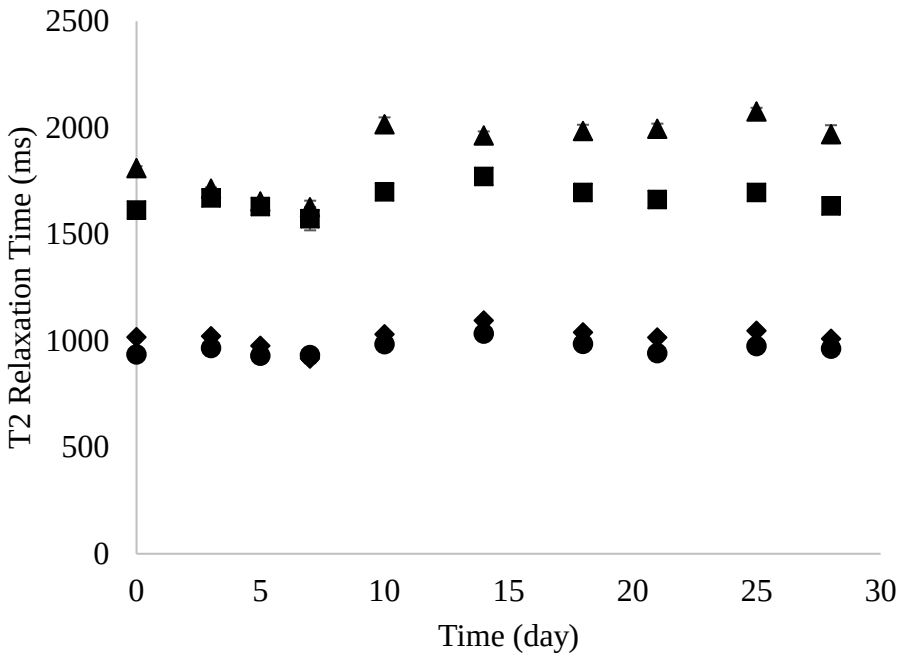


Figure 3.15 T_2 relaxation time of liposomes. (●) green tea extract loaded liposomes in acetate buffer; (◆) green tea extract loaded liposomes in distilled water; (■) unloaded liposomes in acetate buffer; (▲) unloaded liposomes in distilled water by microfluidization.

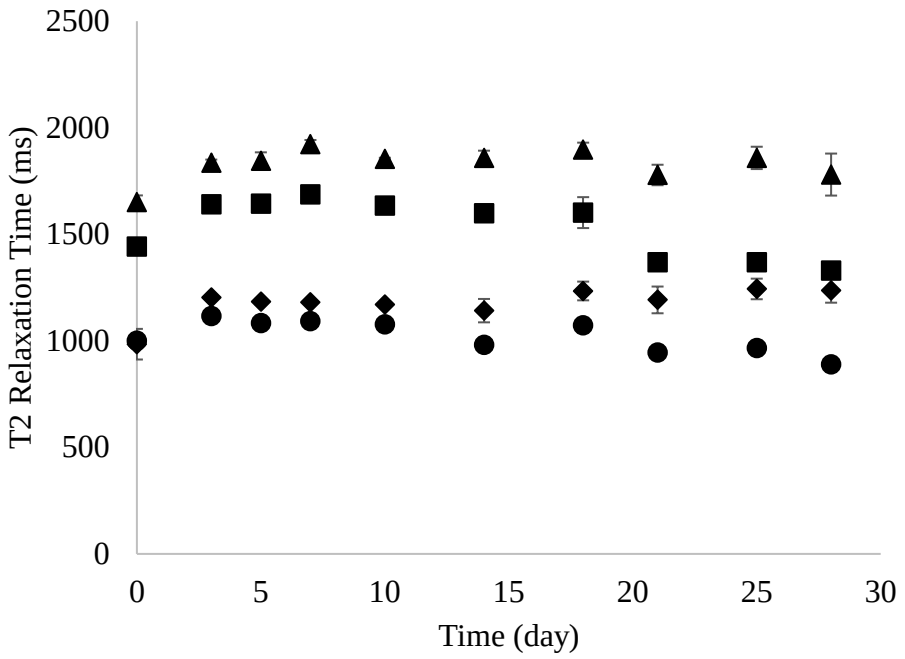


Figure 3.16 T_2 relaxation time of liposomes. (●) green tea extract loaded liposomes in acetate buffer; (◆) green tea extract loaded liposomes in distilled water; (■) unloaded liposomes in acetate buffer; (▲) unloaded liposomes in distilled water by ultrasonication.

Table 3.2 T_2 changes of microfluidized and sonicated liposomes with time, analyzed by 1-Way ANOVA where A: green tea extract loaded liposomes in acetate buffer; B: green tea extract loaded liposomes in distilled water, C; unloaded liposomes in acetate buffer, D; unloaded liposomes in distilled water.

Time (days)		T_2 (ms)			
		A	**B**	**C**	**D**
Microfluidization	**1**	$935.82^{bc} \pm 4.39$	$1017.09^{bc} \pm 9.37$	$1612.86^{bc} \pm 20.50$	$1810.06^{b} \pm 9.24$
	3	$965.78^{bc} \pm 3.37$	$1021.53^{abc} \pm 6.22$	$1670.11^{bc} \pm 5.44$	$1714.34^{bc} \pm 5.27$
	5	$929.27^{c} \pm 6.38$	$975.84^{bc} \pm 5.58$	$1629.56^{c} \pm 13.62$	$1653.59^{c} \pm 8.99$
	7	$931.70^{c} \pm 4.59$	$916.35^{c} \pm 21.79$	$1573.04^{d} \pm 54.77$	$1627.16^{c} \pm 30.41$
	10	$983.49^{ab} \pm 0.32$	$1030.11^{ab} \pm 3.52$	$1699.43^{b} \pm 8.09$	$2015.29^{a} \pm 33.16$
	14	$1033.01^{a} \pm 9.69$	$1095.27^{a} \pm 0.97$	$1770.80^{a} \pm 11.09$	$1963.09^{a} \pm 19.63$
	18	$984.47^{ab} \pm 0.52$	$1038.97^{ab} \pm 13.23$	$1695.14^{b} \pm 7.19$	$1983.80^{a} \pm 30.10$
	21	$940.96^{bc} \pm 3.17$	$1015.31^{abc} \pm 12.42$	$1663.32^{bc} \pm 18.78$	$1994.64^{a} \pm 24.48$
	25	$974.18^{bc} \pm 12.13$	$1046.75^{ab} \pm 7.76$	$1695.67^{ab} \pm 21.97$	$2076.39^{a} \pm 17.04$
	28	$962.06^{bc} \pm 10.41$	$1008.66^{bc} \pm 3.15$	$1633.36^{bc} \pm 17.04$	$1969.67^{a} \pm 41.98$
Ultrasonication	**1**	$1000.29^{bc} \pm 0.54$	$984.14^{b} \pm 71.87$	$1442.78^{b} \pm 25.96$	$1651.38^{b} \pm 31.06$
	3	$1116.59^{a} \pm 8.44$	$1202.94^{a} \pm 9.74$	$1640.51^{ab} \pm 8.18$	$1835.62^{ab} \pm 14.90$
	5	$1082.85^{ab} \pm 24.10$	$1183.48^{a} \pm 14.92$	$1643.28^{ab} \pm 21.70$	$1844.03^{ab} \pm 40.55$
	7	$1092.25^{a} \pm 23.86$	$1180.04^{a} \pm 4.97$	$1686.79^{ab} \pm 29.98$	$1922.59^{a} \pm 19.92$
	10	$1077.07^{ab} \pm 5.05$	$1169.64^{a} \pm 10.08$	$1634.89^{ab} \pm 11.65$	$1853.61^{ab} \pm 5.49$
	14	$980.68^{c} \pm 14.29$	$1141.62^{a} \pm 54.85$	$1598.75^{ab} \pm 12.26$	$1858.25^{ab} \pm 34.13$
	18	$1072.77^{ab} \pm 30.13$	$1233.92^{a} \pm 43.92$	$1601.68^{a} \pm 72.54$	$1896.95^{a} \pm 33.03$
	21	$945.42^{cd} \pm 10.53$	$1192.05^{b} \pm 62.72$	$1368.49^{ab} \pm 11.42$	$1778.31^{ab} \pm 48.10$
	25	$966.59^{cd} \pm 14.71$	$1243.41^{b} \pm 48.32$	$1367.81^{a} \pm 24.76$	$1858.44^{ab} \pm 53.38$
	28	$889.31^{d} \pm 19.79$	$1236.49^{b} \pm 57.51$	$1329.25^{a} \pm 0.88$	$1780.21^{ab} \pm 98.60$

T_2 Relaxation Spectra

For a further, more detailed analysis, it is a common application for magnetization decay data to be inverse Laplace transformed into a continuous 1-dimensional distribution of transverse magnetization thereby acquiring a relaxation spectrum. This method is ideal for quantitative analysis of NMR signals from complex products with multi-componential structure such as ice cream, cake, cheese, etc (Mariette, 2009). The method has been applied for analysis of emulsions numerous times and were proven effective in determination of water and fat content, measurement of the solid fat index, droplet size measurement, quantification of the amount of the solid and liquid phase, identification of polymorphic state of the lipid and measurement of dispersed phase ratios (Bernewitz et al., 2013, 2014; Mariette, 2009). For our analysis, a Non-Negative Least Squares based, Matlab (Mathworks, 2009) function was utilized.

T_2 relaxation spectrum data for microfluidized samples can be seen in Table 3.3 and Table 3.4. The samples yielded 3 peaks, each referring to components with varying ^1H relaxation rates. The number of peaks did not seem to change over the course of 30 days. Peak formations with smaller T_2 values than CPMG pulse spacing of the instrument were identified as artefacts and not included in analysis. These spectral peaks were known to commonly occur due to problems with the mathematical fitting of data (Kirtil et al., 2014; Marigheto et al., 2007).

The most crucial step in T_2 relaxation spectrum analysis is the assignment of peaks to particular components in the sample. The mathematical transformation involves the fitting of T_2 relaxation data into the signal equation (given in Eqn 1) as a sum of Gaussian functions for the solid part and the sum of exponential functions for the liquid part.

$$Y(t)=\sum S_i \exp - \left(\frac{t}{T_2}\right)^2 + \sum L_i \exp - \left(\frac{t}{T_2}\right)^2 \qquad (1)$$

According to the composition of the sample, the solid signal (Gaussian relaxation component of signal) can be attributed to non-exchangeable protons from solid fat, ice, protein and polysaccharides. The exponential liquid signal is attributed to liquid water, fat and exchangeable protons from solids. The exchangeable solid protons have very fast proton relaxations compared to the liquids (Mariette, 2009). With the set echo delay of 1000 μs, the signal was assured to only come from liquid components in the sample. So we know that the green tea extract molecules despite having a direct effect on liquid signal, will not contribute to the NMR signal themselves.

The liquid components in green tea extract loaded liposomes were liquid fat and water. However, the surrounding matrix greatly effects water and fat peak location. The water could display peaks anywhere between 10 – 3,000 ms, while the oil peaks are in the range of 0.2 – 20 ms at the specific temperature of 30° C (Allsopp, Wright, Lastockin, Mirotchnik & Kantzas, 2005). Water and fat relaxation can be influenced by numerous factors. This high information content presents many opportunities for sample characterization but also makes it difficult to assign the peaks to particular proton pools especially when no previous knowledge of the sample is available, as is the case with liposomes. To best our knowledge, liposomal characterization with NMR T_2 relaxation spectrum was not previously carried out. However, the fact that liposomal dispersions resemble emulsions, eased interpretation by collation with emulsion studies.

For peak 1 the T_2s ranged from 9-20 ms for microfluidized samples. This peak with the lowest T_2 and a low relative peak area (which provides an estimate of the amount of water in a particular environment (Liu et al., 2016)), was associated with the ^1H protons in the lipid bilayer. In a number of studies involving emulsions, a peak with similar T_2s were attributed to liquid fat (Jones & Taylor, 2015; Miklos et al., 2014; Vermeir, Sabatino, Balcaen, Van Ranst, & Van der Meeren, 2014; Zhang et al., 2016). Peak 3, which displays the highest T_2s (ranging between 1000-1800 ms) and relative peak areas (~87-95%) was attributed to external water hosting the liposomes. The external water in emulsions were previously assigned to components with T_2 of similar ranges. In W/O/W emulsion studies the external water peak was confirmed to reside

around 1000 ms by addition of $MnCl_2$. Paramagnetic ion addition almost completely distinguished this peak while not affecting others (Vermeir, Balcaen, et al., 2014; Vermeir, Sabatino, et al., 2014). Peak 2 with intermediate T_2s (~75-365 ms) and low peak relative areas; was most likely related with protons of water residing inside the liposomes. The internal water cannot be identified with the term "bound" water which generally display T_2 values of ms to a few tens of ms (Y. Liu et al., 2016). The ill-defined term "bound" water commonly is used for water that is H-bonded to a polymer's polar groups. The other extreme "free" or "bulk" water is the water that is not bound to any polymers thus is not restricted in mobility in any regard. Water may exist in countless states that reside between these two extremes. In these states, water though not directly bonded to a polymer could be restricted by H-bonding with the first hydration layer (Y. Liu et al., 2016). The bulk of water inside the liposomes are both physically and chemically restricted in that regard by the liposomal layers, which explains the T_2 values of around a few hundred ms range. Multiple studies in emulsions have also previously associated entrapped water with peak residing around 100 ms (Vermeir, Balcaen, et al., 2014; Vermeir, Sabatino, et al., 2014) and have claimed the water internal water molecules to be only marginally less mobile than bulk water, hence not being "bound". (Mariette, 2009).

The T_2 times of internal and external water peak seem to be significantly different for loaded and unloaded samples ($p<0.05$), but the effect of buffer could not be observed with T_2 times. Upon green tea extract loading, both water peaks shift to lower T_2 times. Green tea extract loading was expected to decrease T_2 time of internal water, yet the fact that T_2 times of external water decreases significantly as well confirms that some of the green tea was not encapsulated and was most likely either suspended in the solution or adsorbed on the liposome surface. The higher concentration of green tea extract results in higher relaxation rates induced by slower reorientation of water and cross exchange of hydration water with the extremely fast relaxing exchangeable ~OH groups in the green tea extract.

There doesn't seem to be any significant change with respect to time for loaded microfluidized samples in peak 1 & peak 2 (Table 3.3 and 3.4). This result was consistent with the samples' stability with time. Physical stability measurements (such as particle size measurements, zeta potential measurements and TEM images) confirmed that both loaded and unloaded microfluidized samples were mostly stable for the 1-month period they were examined (Guner & Oztop, 2017; Dag & Oztop, 2016). However, in terms of chemical stability (pursued via hydroperoxide formation), loaded and unloaded samples yielded very different results with green tea extract loaded ones showing superior chemical stability (Dag & Oztop, 2016). This result was demonstrated in T_2 relaxation spectra, as fluctuations in T_2 values of internal water. With the accumulation of hydroperoxide and oxidation end products inside the liposomes, T_2 of internal water seemed to decrease in the first weeks, yet the increasing molecular concentration inside the liposomes most likely created an osmotic pressure induced driving force for water transfer into the liposomes from the external dilute water. This could have resulted the increase in T_2 times in the following days. The ongoing oxidation further decreased the T_2 times, and the process most likely repeated which resulted in fluctuations in T_2 times. In other studies on the subject, researchers have associated the stability of internal water T_2 times and peak areas with overall emulsion stability (Onuki, Kida, Funatani, Hayashi, & Takayama, 2016; Zhang et al., 2016).

Another finding worth noting, is that peak 3, which is the external water peak, seemed to increase for both loaded and unloaded samples around the 7^{th}-14^{th} day mark. This result supports the previous explanation on leakage of internal water from the liposome to the environment which was confirmed by phenolic content measurements discussed peviously. The increase in T_2 times, is the result of liberation of water from constraints of lipid bilayers, hence increasing the amount of total bulk water in the system. This increases the T_2 of external water to around 2000 ms which is lower than 3000 ms of pure water. This is because of the high interfacial contact area between the phospholipids' polar heads and the water molecules resulting in the molecular mobility

of water adjacent to oil droplets being more restricted than that of bulk water (Onuki et al., 2016).

Table 3.3 T2 relaxation spectrum data (Time 1, Time 2, Time 3) for liposomes prepared by microfluidization. A; green tea extract loaded liposomes in acetate buffer, B; green tea extract loaded liposomes in distilled water, C; unloaded liposomes in acetate buffer, D; unloaded liposomes in distilled water.

	Time 1 (ms)									
	0	**3**	**5**	**7**	**10**	**14**	**18**	**21**	**24**	**28**
A	9.3 a,A	12.4 a,A	9.6 a,A	8.5 a,A	18.0 a,A	15.0 a,AB	8.3 a,AB	11.2 a,A	10.2 a,A	10.3 a,A
B	19.3 a,A	15.1 a,A	17.5 a,A	14.2 a,A	24.6 a,A	11.0 a,B	15.26 a,B	15.4 a,A	23.0 a,A	11.4 a,A
C	10.6 b,A	13.3 ab,A	16.7 ab,A	13.7 ab,A	17.6 ab,A	22.2 ab,A	24.0 a,A	13.7 ab,A	16.4 ab,A	18.3 ab,A
D	11.7 a,A	13.3 a,A	13.2 a,A	16.3 a,A	24.3 a,A	14.7 a,AB	22.3 a,AB	20.7 a,A	20.7 a,A	17.3 a,A
	Time 2 (ms)									
	0	**3**	**5**	**7**	**10**	**14**	**18**	**21**	**24**	**28**
A	77.0 a,C	99.0 a,B	107.3 a,AB	86.3 a,A	114.5 a,C	101.0 a,B	60.0 a,C	71.0 a,B	107.0 a,B	62.7 a,C
B	127.3 a,BC	172.7 a,AB	70.0 a,B	174.3 a,A	158.3 a,BC	95.3 a,B	50.3 a,C	134.0 a,B	116.0 a,B	56.3 a,C
C	363.3 a,A	206.7 b,AB	233.3 ab,A	286.7 ab,A	246.7 ab,AB	300.0 ab,A	233.3 ab,B	246.7 ab,AB	216.7 b,B	273.3 ab,B
D	293.3 abc,AB	293.3 abc,A	226.7 c,A	253.3 bc,A	340.0 ab,A	333.3 abc,A	383.3 a,A	350.0 ab,A	340.0 ab,A	350.0 ab,A
	Time 3 (ms)									
	0	**3**	**5**	**7**	**10**	**14**	**18**	**21**	**24**	**28**
A	920.0 c,D	920.0 c,C	920.0 c,B	920.0 c,B	1000.0 ab,C	1050.0 a,C	1000.0 ab,C	920.0 c,D	973.3 bc,C	920.0 c,C
B	1000.0 b,C	1000.0 b,B	973.3 b,B	920.0 c,B	1000.0 b,C	1100.0 a,C	1000.0 b,C	1000.0 b,C	1000.0 b,C	1000.0 b,C
C	1700.0 a,B	1700.0 a,A	1633.3 a,A	1633.3 a,A	1700.0 a,B	1800.0 a,B	1700.0 a,B	1700.0 a,B	1700.0 a,B	1633.3 a,B
D	1800.0 bc,A	1700.0 c,A	1700.0 c,A	1633.3 c,A	2066.7 a,A	2000.0 ab,A	2000.0 ab,A	2000.0 ab,A	2066.7 a,A	1933.3 ab,A

*Different small letters show the significant difference ($p<0.05$) between the storage days while different capital letters indicate the significant difference ($p<0.05$) between liposome types.

Table 3.4 T2 relaxation spectrum data (Area 1, Area 2, Area 3) for liposomes prepared by microfluidization. A; green tea extract loaded liposomes in acetate buffer, B; green tea extract loaded liposomes in distilled water, C; unloaded liposomes in acetate buffer, D; unloaded liposomes in distilled water.

	Area 1 (%)									
	0	**3**	**5**	**7**	**10**	**14**	**18**	**21**	**24**	**28**
A	4.04 ab,AB	3.52 ab,A	4.34 ab,A	2.61 ab,A	1.17 b,A	2.71 ab,A	2.08 ab,A	2.57 ab,B	4.72 a,A	3.57 ab,A
B	1.76 a,B	3.06 a,A	1.60 a,C	3.28 a,A	3.16 a,A	2.46 a,A	3.04 a,A	2.70 a,AB	2.97 a,A	4.17 a,A
C	3.81 a,A	3.96 a,A	2.58 a,BC	4.92 a,A	3.22 a,A	2.49 a,A	5.45 a,A	5.56 a,A	4.49 a,A	3.38 a,A
D	5.21 a,AB	5.02 a,A	3.19 a,AB	3.62 a,A	3.30 a,A	4.29 a,A	5.11 a,A	3.60 a,AB	3.95 a,A	4.58 a,A
	Area 2 (%)									
	0	**3**	**5**	**7**	**10**	**14**	**18**	**21**	**24**	**28**
A	1.99 a,B	1.03 a,B	1.18 a,AB	2.03 a,A	1.45 a,BC	1.03 a,B	2.54 a,A	1.71 a,A	1.23 a,B	1.13 a,B
B	2.04 b,B	1.75 b,AB	0.57 b,B	5.72 a,A	1.12 b,C	1.67 b,B	1.69 b,A	1.81 b,A	1.41 b,B	1.16 b,B
C	6.70 a,A	3.08 a,AB	2.47 a,AB	4.28 a,A	3.01 a,AB	3.21 a,A	2.52 a,A	2.51 a,A	3.18 a,A	3.17 a,A
D	3.86 a,A	3.61 a,A	2.97 a,A	3.49 a,A	3.88 a,A	3.11 a,A	3.54 a,A	2.74 a,A	2.57 a,AB	3.90 a,A
	Area 3 (%)									
	0	**3**	**5**	**7**	**10**	**14**	**18**	**21**	**24**	**28**
A	93.97 a,A	95.45 a,A	93.06 a,B	96.23 a,A	97.38 a,A	96.26 a,A	92.51 a,A	95.72 a,A	95.62 a,A	90.79 a,A
B	96.21 ab,A	95.19 ab,A	97.83 a,A	91.00 b,A	95.72 ab,AB	95.87 ab,A	95.27 ab,A	95.49 ab,A	96.09 ab,A	94.66 ab,A
C	89.49 a,A	92.96 a,AB	94.95 a,AB	90.81 a,A	93.77 a,AB	94.30 a,A	92.03 a,A	91.93 a,B	92.33 a,A	93.45 a,A
D	90.93 a,A	91.37 a,B	93.84 a,B	92.89 a,A	92.82 a,B	92.61 a,A	91.36 a,A	93.66 a,B	93.49 a,A	91.51 a,A

*Different small letters show the significant difference ($p<0.05$) between the storage days while different capital letters indicate the significant difference ($p<0.05$) between liposome types.

3.3. Characterization of Biopolymer Coated Liposomes

In the previous section, it was observed that liposomes prepared by microfluidization showed better stability during storage period. Therefore, the addition of biopolymeric layer around liposomal surface was performed for liposomes prepared by microfluidization only. The biopolymer coated liposomes were characterized by conducting the zeta potential, mean particle size distribution, transmission electron microscopy, total phenolic content and antioxidant activity experiments during 28 days of the storage.

3.3.1. Formation of Multiple-Layered Liposomes

The liposomes were initially coated using the layer-by-layer (LbL) deposition technique to increase their stability over time. The LbL deposition is a technique that involves the coating of the particles, liposomes in our case, with two or more interfacial membrane layers (Gibis et al., 2013). The coating of liposomes by the LbL deposition technique is based on the electrostatic attraction between oppositely charged polyelectrolytes. Positively and negatively charged biopolymers were alternatingly added to liposomes to build up to several sequentially-stacked interfacial layers on top of the phospholipid membranes (Chun, Choi, Min, & Weiss, 2013).

In the present study, the positively charged biopolymers (chitosan, fish gelatin) and negatively charged biopolymers (gum arabic and whey protein) were used to form the multiple-layered liposomes. Due to phospolipids and measured zeta potential values of -30.2 and -10.6 mV in distilled and acetate buffer respectively, the surface of the liposomes is known to be negatively charged. The overall goal of biopolymer coating was to obtain a liposome with 4 layers using one anionic and one cationic polymers. And for that purpose chitosan coated liposomes (cationic) were further coated with gum arabic and whey protein and the fish gelatin coated liposomes were further coated with whey protein.

Due to the fact that chitosan was soluble in acidic condition, it was used to coat liposomes prepared in acetate buffer. On the other hand, since fish gelatin was positively charged in neutral pH it was utilized in the preparation of multiple-layered liposomes in distilled water.

In the LbL formulations, the first layer was formed by the addition of 1% chitosan and fish gelatin as the cationic polymers at different amounts to 10 mL liposomes prepared in acetate buffer and distilled water respectively. Afterwards, gum arabic and whey protein were added to each system at different amounts to decide the proper formulation. For each layer, the amount of biopolymer giving the highest zeta potential was determined. To confirm the anionic/cationic nature and the concentrations of the polymers to be used in multiple layer formation, zeta potential experiments were conducted for the polymers initially. The zeta potential of the liposomes after the addition of chitosan as first layer and gum arabic or whey protein as the second layer was shown in Table 3.5 while the zeta potential of the liposomes after the addition of fish gelatin as first layer and whey protein as the second layer was given in Table 3.6. It should be noted that the addition of whey protein to positively charged chitosan coated liposomes could not alter the charge of the liposome from positive to negative. Due to the fact that the isoelectric point of whey protein is near pH 4.5, the whey protein prepared in acetate buffer (near the isoelectric point of whey protein, pH:3.8) could not shift the liposomes from positive charge to negative charge at the concentration used in the study (Pelegrine & Gasparetto, 2005).

Table 3.6 The effect of chitosan, gum arabic and whey protein concentration on the zeta potential of liposomes.

First Layer		Second Layer		
Chitosan (1%)			Gum Arabic (1%)	Whey Protein (1%)
Amount of Biopolymer (µl)	**Zeta Potential (mV)**	**Amount of Biopolymer (µl)**	**Zeta Potential (mV)**	**Zeta Potential (mV)**
150	8.83	100	-11.9	10.2
200	7.99	200	-15.9	9.14
250	7.8	300	-15.1	6.3
300	9.32	400	-16.6	8.51
400	9.41	500	-19.7	9.61
500	10.2	600	-18.4	10.7
600	8.88	700	-17.9	7.91
700	7.4	1000	-16.1	8.91
800	9.85	2000	-14.8	10

Table 3.8 The effect of fish gelatin and whey protein concentration on the zeta potential of liposomes.

First Layer		Second Layer	
Fish Gelatin		Whey Protein	
Amount of Biopolymer (µl)	Zeta Potential (mV)	Amount of Biopolymer (µl)	Zeta Potential (mV)
25	-4.53	100	-9.12
50	0.0489	200	-13.6
1000	5.07	300	-21.9
1500	0.966	400	-20.1
2000	3.91	500	-16.3
-	-	600	-20.8
-	-	700	-19.5
-	-	1000	-29
-	-	1500	-26.1
-	-	2000	-29

After the coating of one anionic and one cationic polymer, problems were observed on the formulated systems. The main problem in the LbL deposition technique was the aggregation (as can be seen in Fig. 3.17). The aggregation was associated with several factors such as the presence of uncoated, coated liposomes, the excess amount of biopolymer that was not coated to the liposomes and unencapsulated green tea extract in the environment.

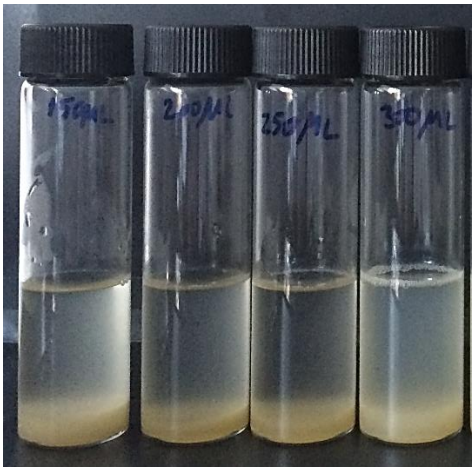


Figure 3.17 The aggregation of the liposome after second layer formation.

To prevent the aggregation, the liposomes were firstly diluted before the new layer formation. Unfortunately, this dilution step decreased the amount of total phenolic content of the system significantly so that the absorbance values in TPC experiments could not be read by spectrometer. Then, it was decided to perform the gel filtration before the new layer formation to separate the biopolymer coated liposome from uncoated liposomes, excess amount of the biopolymers and unencapsulated green tea extract. Basically, in the gel separation, the molecules in the solution are separated according to differences in their sizes by passing through a column packed with a chromatographic medium which is called as a gel. The pores in the gel matrix are comparable in size to the molecules which is desired to separate (Laouini et al., 2012). Relatively small molecules move slowly through gel matrix since they diffuse into the gel. On the other hand, relatively large molecules move fast through the gel since they are not able to pass through the pores of gel. This difference allows to separate the molecules possessing different size by collecting the relatively big molecules firstly. In this regard, it was hypothesized the biopolymer coated liposomes could be separated since they had a large particle size compared to uncoated liposomes. Meanwhile, it was aimed to the removal of the excess biopolymers and unencapsulated green tea extract from the coated liposomes. Unfortunately, the total phenolic content experiment results after gel filtration indicated that the biopolymer coated liposomes containing green tea extract could not be obtained using this method. No phenolic compounds were detected after gel filtration. Lastly, ultrafiltration was conducted for the separation step. Ultrafiltration is used for the physical separation of particles by a semi-permeable membrane with an appropriately selected molecular weight cut-off. In the principle, the particles having lower molecular weight than molecular weight cut-off of membrane across the membrane, the particles having higher molecular weight are unable to penetrate the membrane (Wallace, Li, Nation, & Boyd, 2012). The supernatant was again characterized by total phenolic content to ensure liposome containing green tea extract was present in the solution. Similar to previous observations, the TPC of the supernatant was low so that its absorbance values can not be read by spectrometer. Since a stable LbL coated liposome system can not obtained

by using these 4 polymers, eventually, it was decided to form a single layer around the liposome rather than multiple layers.

3.3.2. Zeta Potential of Single Layer Biopolymer Coated Liposomes

According to the results of uncoated liposomes showing poor stability during 28 day-storage at 4 ^{o}C (discussed in section 3.2) and LbL coating did not work efficiently, it was hypothesized that the single layer biopolymer coating would improve the storage stability of the liposomes by creating a protective layer over liposomal surface. Thus, to increase the thermodynamic stability of liposomes, the effect of chitosan, lysozyme, gum arabic and whey protein coating was explored. This time lysozyme rather than the fish gelatin as the 2nd cationic polymer was selected since fish gelatin was forming gel particles when stored in refrigerator. The following thawing could have disrupt the liposomes that is why fish gelatin was not used among the single biopolymer coating formulations.

The change in the electrical surface charge of liposomes as a function of final biopolymer concentration was monitored. Using the information provided by zeta potential measurements (Table 3.7), the biopolymer concentration giving the highest electrical surface charge was determined. As can be seen in Table 3.7, the coating of the liposomes with chitosan shifted the zeta potential of negatively charged liposomes to positive indicating chitosan was adsorbed to the liposomal surface. On the other hand, lysozyme, gum arabic and whey protein did not alter the zeta potential of liposomes to positive values. It should be pointed out that Van der Waals and steric interactions besides electrostatic interactions should be also considered in the liposomal layer formation (Chun et al., 2013). In that regard, it was hypothesized that lysozyme, gum arabic and whey protein might be adsorbed to the liposomal surface by Van der Waals and steric interactions rather than electrostatic interactions. This hypothesis will be confirmed by the particle size measurement results and TEM micrographs as will be discussed in the following sections.

Decrease in zeta potential values of biopolymer coated liposomes at the end of the storage period (Fig. 3.18/19) was not observed which was an indication that the improvement in the physical stability of uncoated liposomes was achieved via biopolymer coating. Although lysozyme, gum arabic and whey protein coated liposomes were not studied previously, there are several studies that reported the stability of liposomes was enhanced by other biopolymers such as chitosan, pectin and polygalacturonic acid coating which provided physical and chemical protection of the encapsulated compounds (Gibis et al., 2012; Laye et al., 2008; Lopes, Pinilla, & Brandelli, 2017; Madrigal-Carballo et al., 2010).

In general, the systems having a zeta potential of more than 61 mV was expected to show excellent stability; 41-60 mV good stability; 31-40 mV moderate stability and 10-30 mV instability (Du Plessis, Ramachandran, Weiner, & Müller, 1996). It should also definitely be noted that the stability of the liposomes over time does not just depend on its zeta potential. In the study of Makino et al. (1991) it was shown that the liposomal suspension might remain stable even at low zeta potential. The fact that zeta potential was not a direct indicator of the stability of liposomes particle size measurement and TEM imaging were also conducted to assess the physical stability of the coated liposomes.

Table 3.9 The effect of lysozyme, gum arabic, whey protein and chitosan concentration on the zeta potential of liposomes.

	Lysozyme	**Gum Arabic**	**Whey Protein**		**Chitosan**
Amount of Biopolymer (µl)	**Zeta Potential (mV)**			**Amount of Biopolymer (µl)**	**Zeta Potential (mV)**
100	**-35.0**	-25.3	-21.5	150	8.83
200	-30.3	-24.7	**-33.5**	200	7.99
300	-30.9	**-25.4**	-13.3	250	7.8
400	-27.3	-25.4	-27.5	300	9.32
500	-19.8	-24.4	-31.9	400	9.41
600	-16.3	-25.4	-29.9	500	**10.2**
700	-14.8	-21.4	-14.9	600	8.88
1000	-5.83	-23.1	-20.8	700	7.4
1500	-4.24	-22.6	-29.2	800	9.85

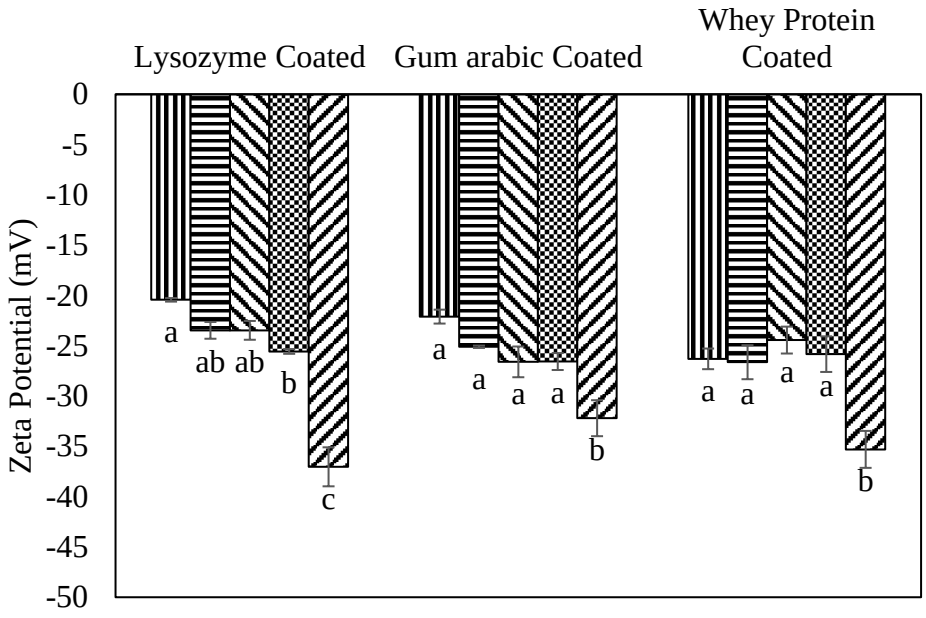


Figure 3.18 Zeta Potential of lysozyme, gum arabic and whey protein coated liposomes; ▥ day 0; ▤ day 7; ▧ day 14; ▩ day 21; ▨ day 28.

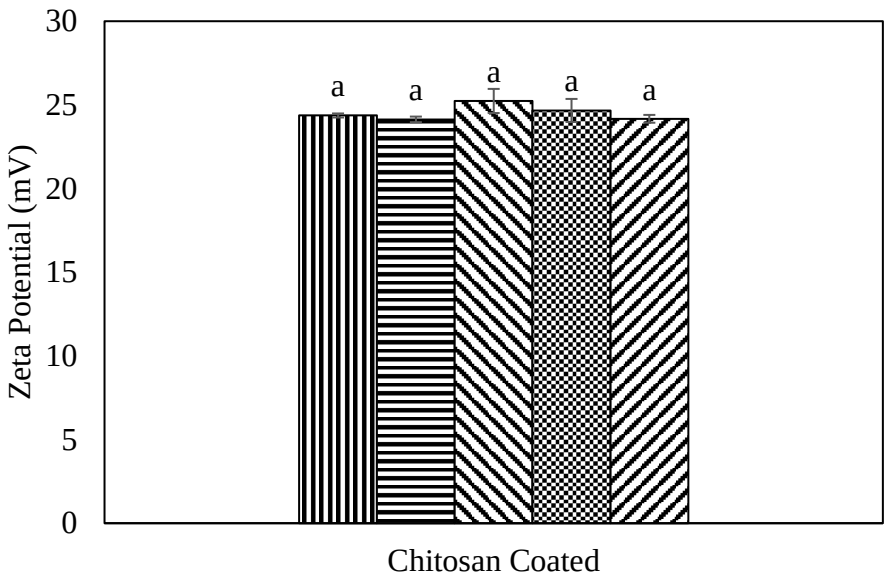


Figure 3.19 Zeta Potential of chitosan coated liposomes; ▥ day 0; ▤ day 7; ▧ day 14; ▩ day 21; ▨ day 28.

3.3.3. Particle Size Distribution

Similar to uncoated liposomes, the physical stability of coated liposomes was investigated by measuring their particle size during 28 days. Fig. 3.20 and 3.21 shows uncoated liposomes containing green tea extract both in distilled water (Uncoated I) and acetate buffer (Uncoated II) having smaller particle size (35 and 38 nm respectively) compared to biopolymer coated liposomes (ranging 43 to 356 nm depending on polymer type). The observed change in particle size suggested that biopolymers were successfully adsorbed to the surface of liposomes and formed a protective layer. As illustrated in Fig. 3.22, the coating of liposomes by chitosan led to marginal increase in the particle size (from 38 to 356 nm) in comparison with lysozyme, gum arabic and whey protein (from 34 to 58 nm). The possible explanation for this result might be the strong electrostatic interaction between positively charged chitosan and negatively charged liposomes (Gibis et al., 2012; Laye et al., 2008; Madrigal-Carballo et al., 2010). Notwithstanding, chitosan coated liposomes showed

fluctuations in particle size during 28 days which was an indicator for poor physical stability. The increase in the particle size of chitosan coated liposomes at 14th day could be explained by depletion flocculation resulting from exceeding free polymer concentration a particular value (McClements, 2005). Eventually, chitosan coated liposomes having large particle size began to breakdown at 28th day of the storage period. On the other hand, the particle size of the lysozyme, gum arabic and whey protein coated liposomes remained constant compared to uncoated liposomes during 28 days. From particle size and zeta potential results, it could be concluded that the stability of the liposomes was improved by coating liposomes with lysozyme, gum arabic and whey protein through increased electrostatic repulsion between the particles (Lopes et al., 2017).

Previously, many studies reported that the physical and chemical stability of liposomes could be increased by coating liposomes with chitosan, a positively charged biopolymer, via electrostatic deposition (Gibis et al., 2013, 2012; Laye et al., 2008; Madrigal-Carballo et al., 2010; Mady & Darwish, 2010). Nowadays, the studies that explored the coating of liposomes with negatively charged biopolymers have also begun to be investigated. In the study of Lopes et al. (2017) negatively charged polysaccharides was utilized as a coating material in the layer formation of nisin loaded liposomes. The results demonstrated that the stability of liposomes incorporating nisin during 28 day-storage at room temperature increased with pectin and polygalacturonic acid coating. In that regard, this study also elucidated that lysozyme, gum arabic and whey protein might be an alternative to chitosan in the formation of liposome layer to obtain liposome better stability over time.

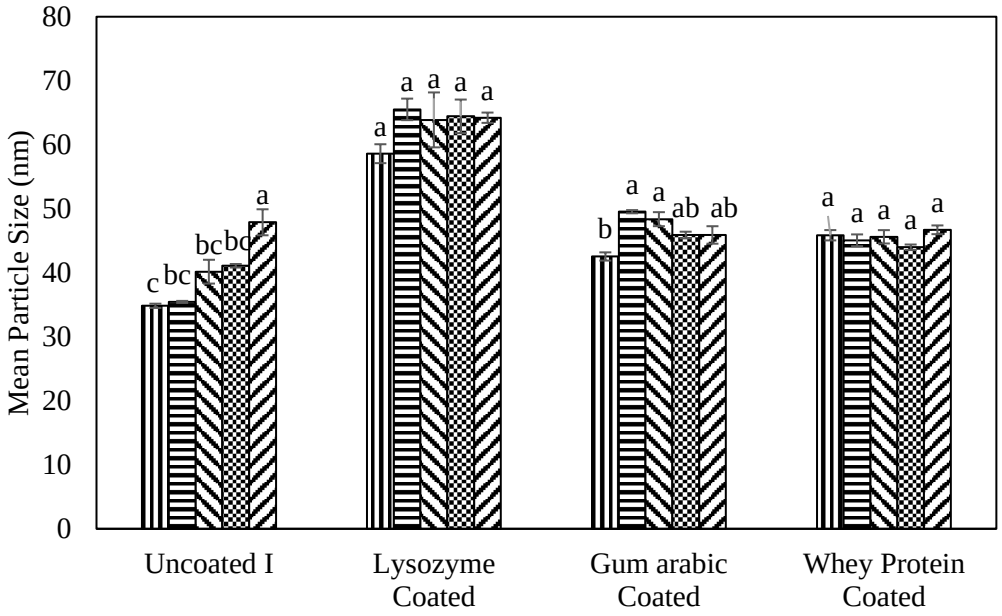


Figure 3.20 Mean particle size of uncoated I, lysozyme, gum arabic and whey protein coated liposomes; ▥ day 0; ▤ day 7; ▧ day 14; ▩ day 21; ▨ day 28.

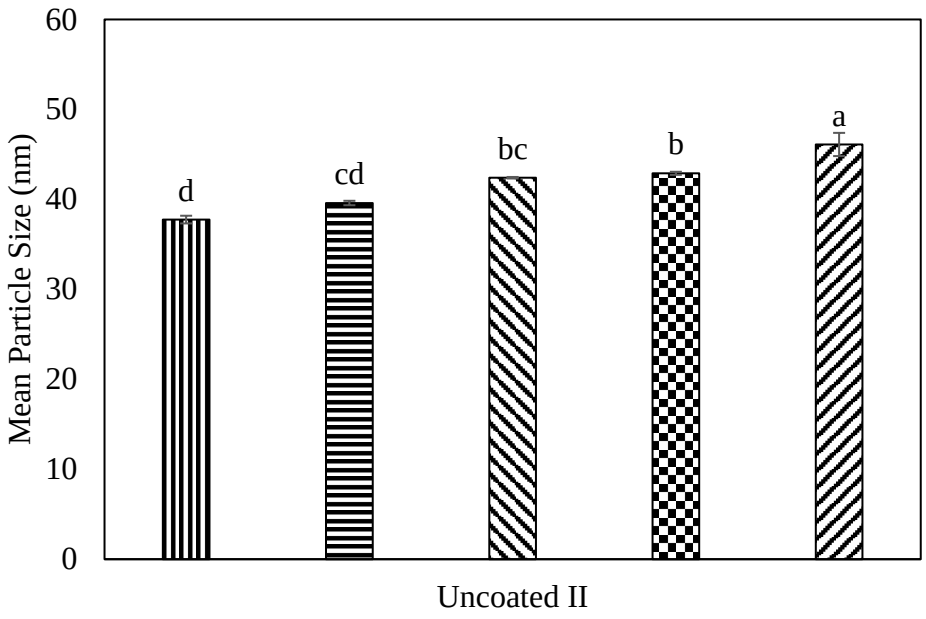


Figure 3.21 Mean particle size of uncoated II; ▥ day 0; ▤ day 7; ▧ day 14; ▩ day 21; ▨ day 28.

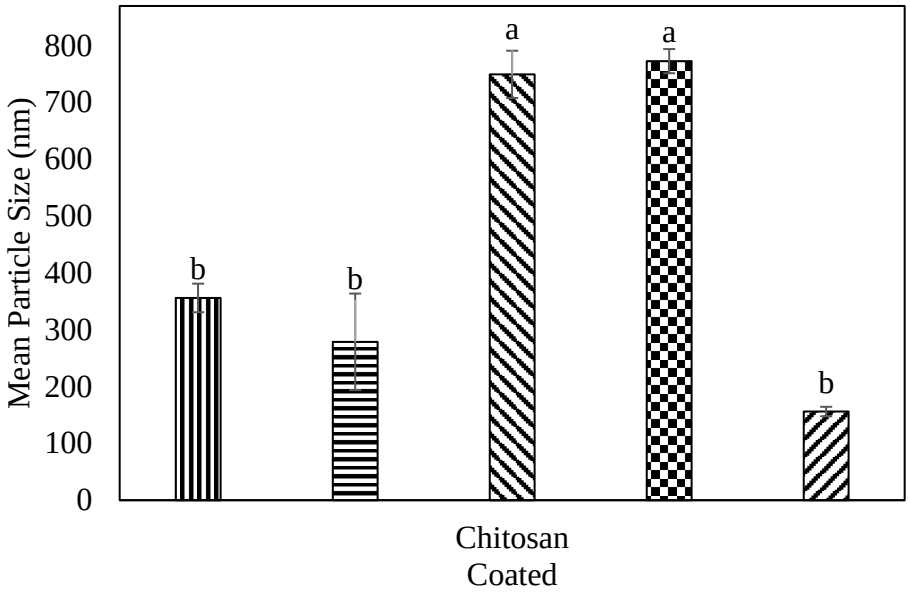


Figure 3.22 Mean particle size of chitosan coated liposomes; ▥ day 0; ▤ day 7; ▧ day 14; ▩ day 21; ▨ day 28.

3.3.4. Transmission Electron Microscopy

TEM analysis was also performed to observe the morphology of uncoated and coated liposomes containing green tea extract. It was necessary to use negative staining with phosphotungstic acid to increase the contrast in TEM images for biopolymer coated liposomes. By using a negative stain, the background is stained rather than the liposomes; thus the outer region of vesicles seems black while the inner region of the liposomes seems white. However, it should be considered the use of a negative stain might cause changes in the vesicular structure of liposome and the stain agent could bring out the formation of dark and light fringes hence the lamellar structure of the liposome could be misinterpreted. Besides, subsequent vacuuming in the electron microscope chamber could lead to dehydration of the sample causing a change in the morphology and poorly represented images (Bibi et al., 2011; Chen et al., 2013).

The uncoated and biopolymer coated liposomes incorporating green tea extract were visualized by TEM and their morphological characteristic are seen in Fig. 3.23a-h. The microscopic observations of liposomes indicated that the uncoated and coated liposomes were unilamellar in nature with dimensions in the nanometer range. The liposomes at the first day could easily be identified as discrete particles that were predominantly spherical in shape (Fig. 3.23a-d). At the 28^{th} day of storage uncoated liposomes begun to be physically destabilized resulting in loss of encapsulated green tea extract and increase in particle size (Fig. 3.23a/e). On the other hand, the biopolymer coated liposomes retained their spherical shapes with a few aggregated or semifused vesicles (Fig. 3.23/f-h).

Among the biopolymer coated liposomes, the contrast could not be achieved with negative staining for lysozyme coated liposomes. The possible reason might be the washing step of excess stain which could have cause complete washing off the stain from the background. In Fig. 3.23c/d, the existence of dense gum arabic and whey protein layer that was well visualized on the surface of the liposomes supported the hypothesis suggesting biopolymers might have adsorbed to the liposomal surface through Van der Waals and steric interactions.

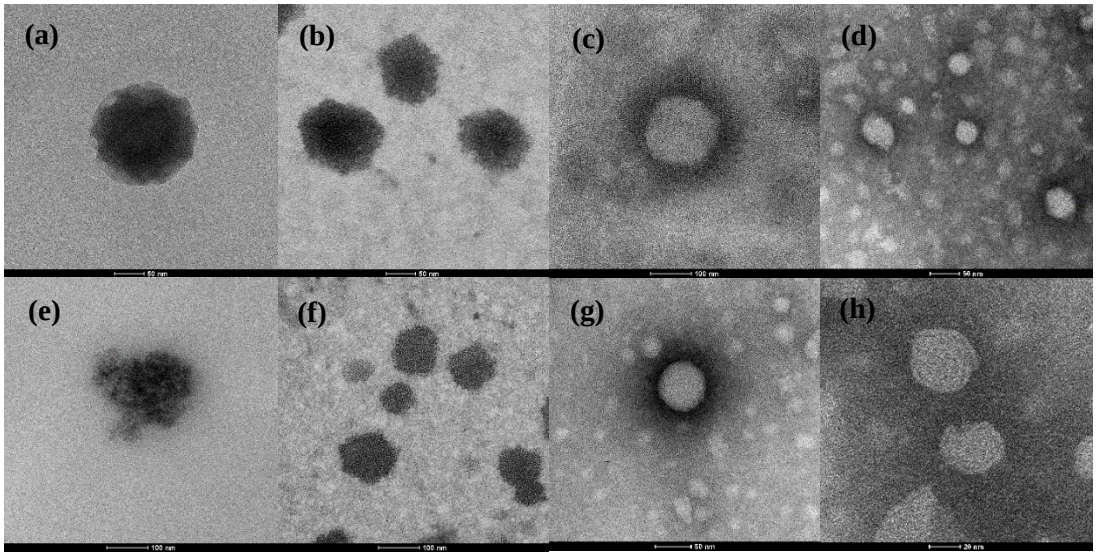


Figure 3.23 Transmission electron micrographs of: (a) uncoated liposome at 1st day (b) lysozyme coated liposome at 1st day (c) gum arabic coated liposome at 1st day (d) whey protein coated liposome at 1st day (e) uncoated liposome at 28th day (f) lysozyme coated liposome at 28th day (g) gum arabic coated liposome at 28th day (h) whey protein coated liposome at 28th day.

3.3.5. Determination of Total Phenolic Content

The content of phenolic compounds in coated liposomes incorporating green tea extract was quantified using Folin-Ciocalteau method. As can be seen in Fig. 3.24, the fluctuations in TPC for both uncoated and biopolymer coated liposomes during 28 days storage at 4 °C were detected. For uncoated liposomes, the significant decrease in TPC results was observed at 7th day followed by the significant increase at 14th day of storage. Similar observation was made in the study carried by Klimczak, Małecka, Szlachta, & Gliszczyńska-Świgło (2007) reporting a decrease in TPC after 4 months of storage of orange juice followed by a significant increase at the end of 6 months.

Similar to uncoated liposomes, the increase in TPC until 14th day of storage with the decrease in the following days was noticed for the biopolymer coated liposomes except gum arabic coated ones. Piljac-Žegarac, Valek, Martinez, & Belščak (2009) also revealed an increase in TPC in the first days of storage with decrease in the following days. During storage, some compounds that have ability to react with Folin–Ciocalteu reagent might be formed leading to the increase in TPC (Piljac-Žegarac et al., 2009). On the other hand, the degradation of some phenolic compounds could induce the decrease in TPC. Therefore, the fluctuation behavior of phenolic compounds during storage might be attributed to a possible formation of compounds reacting with Folin–Ciocalteu reagent as well as the degradation of some phenolic compounds simultaneously. At the end of the 28-day storage, the final TPC values of both uncoated and coated liposomes reached initial TPC values except with an enhancement in TPC of lysozyme coated liposomes.

Results suggested that the storage during 28 days at 4°C did not lead to reduction in TPC of green tea phenols which was in agreement with the observations of Kevers et al. (2007) and Piljac-Žegarac et al. (2009) who demonstrated that the phenolic compounds of many fruits/vegetables and six types of fruit juice remained stable during storage.

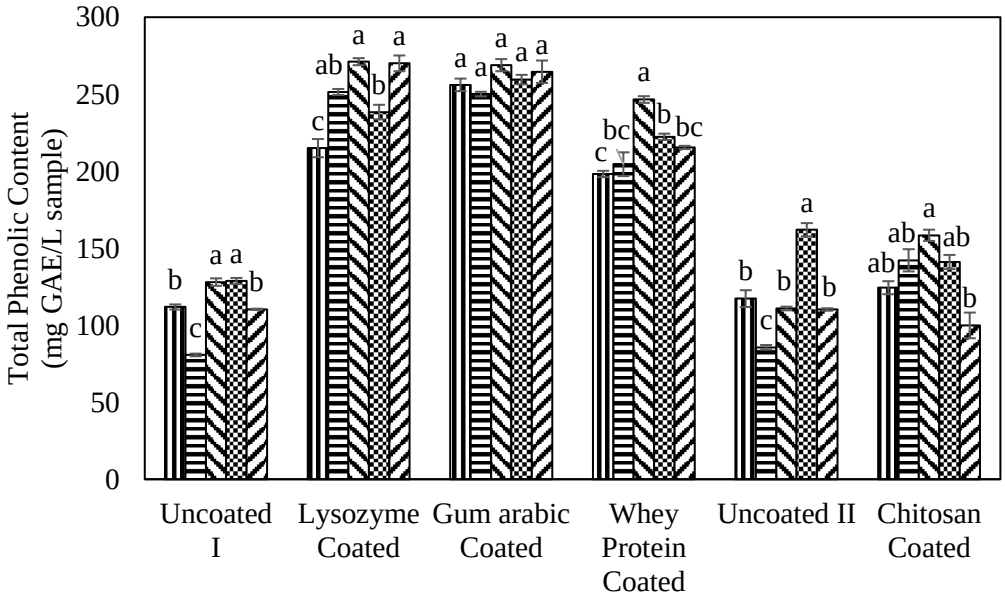


Figure 3.24 Total phenolic content of green tea loaded liposomes during 28-day storage; ▥ day 0; ▤ day 7; ▧ day 14; ▩ day 21; ▨ day 28.

3.3.6. Determination of Antioxidant Activity

The AA of the biopolymer coated liposomes containing green tea extract was evaluated by DPPH radical scavenging method. FRAP method was not performed in AA determination of biopolymer coated liposomes due to the high correlation between DPPH radical scavenging and FRAP methods (see section 3.2.5).

Similar to TPC results, the fluctuations in AA were also investigated for both uncoated and biopolymer coated liposomes during 28 days of storage at 4 °C. As illustrated in Fig. 3.25, a decrease in the AA at 7th day was followed by an increase at 14th day of storage for both uncoated and coated liposomes. After 14th day, AA of coated liposomes began to decrease continuously until the end of the storage period whilst AA of the uncoated liposomes remained unchanged.

The fluctuations in AA was previously reported for catechin, resveratrol and grape extract (Pinelo, Rubilar, Sineiro, & Nuñez, 2005), apple juice (Pinelo, Manzocco, Nuñez, & Nicoli, 2004), refrigerated celery (Vina & Chaves, 2006) and black carrot, cranberry, blueberry, pomegranate, strawberry and cherry juice (Piljac-Žegarac et al., 2009). The observed increase in AA was explained by the possible polymerization reactions of polyphenols resulting in oligomers possessing larger area for charge delocalization. Once the degree of polymerization exceeds a critical value, the increase in molecular complexity and steric hindrance reduces the availability of hydroxyl groups in phenolic compounds to react with the DPPH radicals resulting in lower AA (Hagerman et al., 1998; Piljac-Žegarac et al., 2009). This could be a possible reason for the decrease in AA of coated liposomes followed after the increase at 14^{th} day.

Furthermore, Pinelo, Rubilar, Sineiro, & Nuñez (2005) indicated that the temperature, incubation time and chemical characteristic of medium containing phenols influence the reactivity of polyphenols against free radicals. In their study, the effect of storage temperature 22, 37, 60 °C, the chemical characteristics of the medium (ethanol, methanol and water) and the reaction time on the AA of catechin, resveratrol and grape extract were investigated. The results revealed that longer incubation time was required to obtain maximum AA value at low temperatures. This might explain the reason why the liposomes containing green tea extract showed an increase in AA at longer incubation times compared to the previous studies demonstrating an increase in AA at the first few days of incubation.

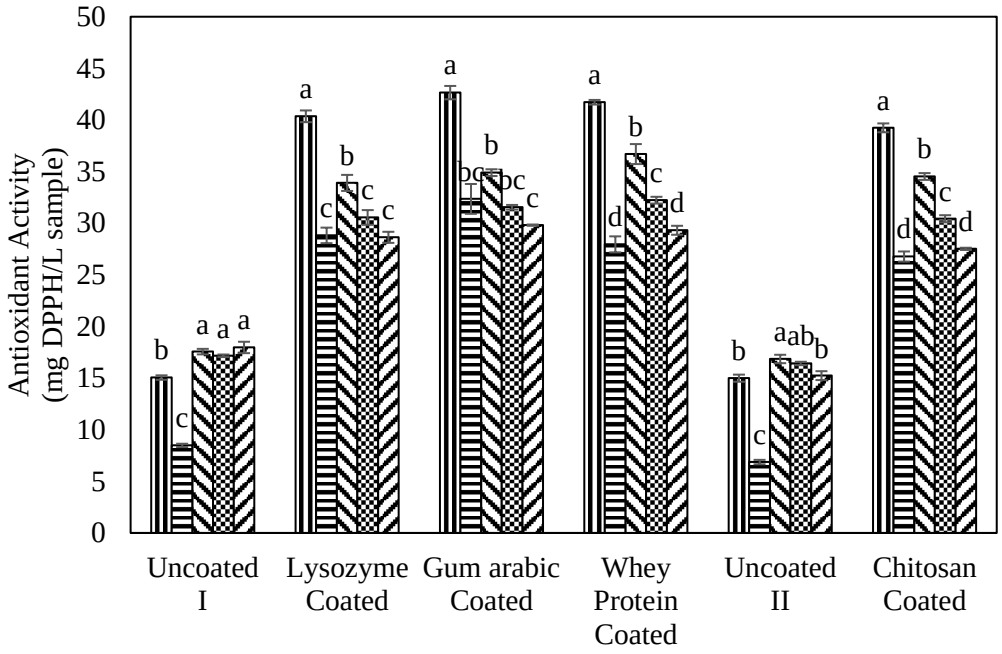


Figure 3.25 Antioxidant activity of green tea loaded liposomes during 28-day storage; ▥ day 0; ▤ day 7; ▧ day 14; ▩ day 21; ▨ day 28.

CHAPTER 4

CONCLUSION AND RECOMMENDATIONS

In the scope of this study, green tea extract (0.1% w/v) was encapsulated into liposomes by dispersing 1% (w/v) soy lecithin in distilled water and acetate buffer through microfluidization and ultrasonication techniques in order to enhance the stability of AA of green tea polyphenols and protect their functional properties. In the first part of the current study, the effects of homogenization type and pH of the medium on the stability of uncoated green tea extract during one-month storage period at 4°C were investigated. Uncoated liposomes with and without green tea extract were characterized by conducting zeta potential, mean particle size, transmission electron microscope, total phenolic content, antioxidant activity, in vitro release in the simulated gastric and intestinal fluids and nuclear magnetic resonance relaxometry experiments.

From the first part, it was concluded that among uncoated liposomes formulations, liposomes containing green tea extract prepared in distilled water (pH: 6.5) by microfluidization technique have shown better stability during one-month storage period which provided no significant difference ($p>0.05$) on mean particle size, total phenolic content, antioxidant activity at the end of the one-month storage. Additionally, uniform size in regular shape on transmission electron microscopy result

was investigated for liposome with the same composition. According to the results obtained in the first part of the study, the liposome prepared with microfluidization technique was used for the following part of the study.

In the second part of the study, the uncoated liposomes were coated by anionic biopolymers (gum arabic and whey protein) and cationic biopolymer (lysozyme, chitosan) in order to enhance the stability of uncoated liposomes during storage period. Whether the lysozyme, gum arabic and whey protein could be an alternative to cationic biopolymer (chitosan) for the coating of anionic liposomes were investigated. Similar to first part, zeta potential, mean particle size, transmission electron microscope, total phenolic content, antioxidant activity and nuclear magnetic resonance relaxometry experiments were conducted in order to characterize the biopolymer coated liposomes.

The increase in particle size, the stability in zeta potentials during refrigerated storage and the investigation of biopolymer layers around liposomes by transmission electron microscopy confirmed that the coating with biopolymers was successfully achieved. The coated liposomes exhibited better stability than uncoated liposomes regarding particle size, zeta potential, total phenolic content and antioxidant activity during 28-day storage at 4°C. The fluctuations in total phenolic content and antioxidant activity were observed for all liposome types during storage. Time domain low resolution NMR relaxometry measurements were found to be efficient for liposome characterization. Signals acquired from samples showed a similar trend with particle size changes due to deformation of liposome structure wshich increases the bulk water in the environment.

The results of the study indicate that green tea extract is a potential source of antioxidants, with possible applications in food industry as a functional food ingredient. Furthermore, it was demonstrated encapsulation of green tea extract in liposomes is a promising technique to protect its antioxidant. Furthermore, although more research is still needed to elucidate the exact mechanism of biopolymers for coating of negatively charged liposomes, our findings indicated that coating negatively

charged liposomes with lysozyme, gum arabic and whey protein improved the stability of green tea loaded liposome during 28-day storage at 4°C.

REFERENCES

Ainsworth, E. A., & Gillespie, K. M. (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. *Nature Protocols*, *2*(4), 875–877. https://doi.org/10.1038/nprot.2007.102

Alam, M. N., Bristi, N. J., & Rafiquzzaman, M. (2013). Review on in vivo and in vitro methods evaluation of antioxidant activity. *Saudi Pharmaceutical Journal*, *21*(2), 143–152. https://doi.org/10.1016/j.jsps.2012.05.002

Allsopp, K., Wright, I., Lastockin, D., Mirotchnik, K., & Kantzas, A. (2005). Determination of oil and water compositions of oil / water emulsions using low field NMR relaxometry. *Journal of Canadian Petroleum Technology*, *40*(7), 58–61. https://doi.org/10.2118/01-07-05

Almajano, M. P., Carbó, R., Jiménez, J. A. L., & Gordon, M. H. (2008). Antioxidant and antimicrobial activities of tea infusions. *Food Chemistry*, *108*(1), 55–63. https://doi.org/10.1016/j.foodchem.2007.10.040

Ananingsih, V. K., Sharma, A., & Zhou, W. (2013a). Green tea catechins during food processing and storage: A review on stability and detection. *Food Research International*, *50*(2), 469–479. https://doi.org/10.1016/j.foodres.2011.03.004

Ananingsih, V. K., Sharma, A., & Zhou, W. (2013b). Green tea catechins during food processing and storage: A review on stability and detection. *Food Research International*, *50*(2), 469–479. https://doi.org/10.1016/j.foodres.2011.03.004

Anselmi, C., Centini, M., Maggiore, M., Gaggelli, N., Andreassi, M., Buonocore, A., … Facino, R. M. (2008). Non-covalent inclusion of ferulic acid with ??-

cyclodextrin improves photo-stability and delivery: NMR and modeling studies. *Journal of Pharmaceutical and Biomedical Analysis*, *46*(4), 645–652. https://doi.org/10.1016/j.jpba.2007.11.037

Babu, P. V. A., & Liu, D. (2008). Green tea catechins and cardiovascular health: an update. *Current Medicinal Chemistry*, *15*(18), 1840–50. https://doi.org/10.1016/j.ejheart.2008.04.008.The

Bartolomé, B., Estrella, I., & Hernández, M. T. (2000). Interaction of low molecular weight phenolics with proteins (BSA). *Journal of Food Science*, *65*(4), 617–621. https://doi.org/10.1111/j.1365-2621.2000.tb16060.x

Benichou, A., Aserin, A., & Garti, N. (2007). W/O/W double emulsions stabilized with WPI-polysaccharide complexes. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *294*(1–3), 20–32. https://doi.org/10.1016/j.colsurfa.2006.07.056

Benzie, I. F. F., & Strain, J. J. (1999). Ferric reducing (antioxidant) power as a measure of antioxidant capacity: the FRAP assay. *Methods Enzymol*, *299*, 15–36.

Bernewitz, R., Dalitz, F., Köhler, K., Schuchmann, H. P., & Guthausen, G. (2013). Characterisation of multiple emulsions by NMR spectroscopy and diffusometry. *Microporous and Mesoporous Materials*, *178*, 69–73. https://doi.org/10.1016/j.micromeso.2013.02.049

Bernewitz, R., Schmidt, U. S., Schuchmann, H. P., & Guthausen, G. (2014). Structure of and diffusion in O/W/O double emulsions by CLSM and NMR-comparison with W/O/W. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *458*(1), 10–18. https://doi.org/10.1016/j.colsurfa.2014.01.002

Bhat, R., Kamaruddin, N. S. B. C., Min-Tze, L., & Karim, A. A. (2011). Sonication

improves kasturi lime (Citrus microcarpa) juice quality. *Ultrasonics Sonochemistry*, *18*(6), 1295–1300. https://doi.org/10.1016/j.ultsonch.2011.04.002

Bibi, S., Kaur, R., Henriksen-Lacey, M., McNeil, S. E., Wilkhu, J., Lattmann, E., … Perrie, Y. (2011). Microscopy imaging of liposomes: From coverslips to environmental SEM. *International Journal of Pharmaceutics*, *417*(1–2), 138–150. https://doi.org/10.1016/j.ijpharm.2010.12.021

Bunaciu, A. A., Danet, A. F., Fleschin, Ş., & Aboul-Enein, H. Y. (2016). Recent Applications for in Vitro Antioxidant Activity Assay. *Critical Reviews in Analytical Chemistry*, *46*(5), 389–399. https://doi.org/10.1080/10408347.2015.1101369

Cagdas, M., Sezer, A. D., & Bucak, S. (2014). Liposomes as Potential Drug Carrier Systems for Drug Delivery. *Nanotechnology and Nanomaterials » "Application of Nanotechnology in Drug Delivery,"* 1–50. https://doi.org/10.5772/57028

Cao, G., Alessio, H. M., & Cutler, R. G. (1993). Oxygen-radical absorbance capacity assay for antioxidants. *Free Radical Biology and Medicine*, *14*(3), 303–311. https://doi.org/https://doi.org/10.1016/0891-5849(93)90027-R

Carocho, M., & Ferreira, I. C. F. R. (2013). A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. *Food and Chemical Toxicology*, *51*(1), 15–25. https://doi.org/10.1016/j.fct.2012.09.021

Chacko, S. M., Thambi, P. T., Kuttan, R., & Nishigaki, I. (2010). Beneficial effects of green tea: a literature review. *Chinese Medicine*, *5*(13), 1–9. https://doi.org/10.1186/1749-8546-5-13

Chen, C., Zhu, S., Huang, T., Wang, S., & Yan, X. (2013). Analytical Methods Analytical techniques for single-liposome characterization. *Analytical Methods*,

5(2150), 2150–2157. https://doi.org/10.1039/c3ay40219c

Chen, Z., Bertin, R., & Froldi, G. (2013). EC50 estimation of antioxidant activity in DPPH* assay using several statistical programs. *Food Chemistry*, *138*(1), 414–420. https://doi.org/10.1016/j.foodchem.2012.11.001

Chiou, D., & Langrish, T. A. G. (2007). Development and characterisation of novel nutraceuticals with spray drying technology. *Journal of Food Engineering*, *82*(1), 84–91. https://doi.org/10.1016/j.jfoodeng.2007.01.021

Chrai, S. S., Murari, R., & Ahmad, I. (2002). Liposomes : A Review Part I : Manufacturing Issues. *Biotech Trends*, (April), 30–34.

Chun, J. Y., Choi, M. J., Min, S. G., & Weiss, J. (2013a). Formation and stability of multiple-layered liposomes by layer-by-layer electrostatic deposition of biopolymers. *Food Hydrocolloids*, *30*(1), 249–257. https://doi.org/10.1016/j.foodhyd.2012.05.024

Chun, J. Y., Choi, M. J., Min, S. G., & Weiss, J. (2013b). Formation and stability of multiple-layered liposomes by layer-by-layer electrostatic deposition of biopolymers. *Food Hydrocolloids*, *30*(1), 249–257. https://doi.org/10.1016/j.foodhyd.2012.05.024

Cicco, N., Lanorte, M. T., Paraggio, M., Viggiano, M., & Lattanzio, V. (2009). A reproducible, rapid and inexpensive Folin-Ciocalteu micro-method in determining phenolics of plant methanol extracts. *Microchemical Journal*, *91*(1), 107–110. https://doi.org/10.1016/j.microc.2008.08.011

Coupland, J. N., & McClements, D. J. (1996). Lipid oxidation in food emulsions. *Trends in Food Science & Technology*, *7*(3), 83–91. https://doi.org/10.1016/0924-2244(96)81302-1

Dalluge, J. J., Nelson, B. C., Brown Thomas, J., & Sander, L. C. (1998). Selection of column and gradient elution system for the separation of catechins in green tea

using high-performance liquid chromatography. *Journal of Chromatography A*, *793*(2), 265–274. https://doi.org/10.1016/S0021-9673(97)00906-0

Deladino, L., Anbinder, P. S., Navarro, A. S., & Martino, M. N. (2007). Co-crystallization of yerba mate extract (Ilex paraguariensis) and mineral salts within a sucrose matrix. *Journal of Food Engineering*, *80*(2), 573–580. https://doi.org/10.1016/j.jfoodeng.2006.06.016

Deladino, L., Anbinder, P. S., Navarro, A. S., & Martino, M. N. (2008). Encapsulation of natural antioxidants extracted from Ilex paraguariensis. *Carbohydrate Polymers*, *71*(1), 126–134. https://doi.org/10.1016/j.carbpol.2007.05.030

Di Bari, V., Macnaughtan, W., Norton, J., Sullo, A., & Norton, I. (2016). Crystallisation in water-in-cocoa butter emulsions: Role of the dispersed phase on fat crystallisation and polymorphic transition. *Food Structure*. https://doi.org/10.1016/j.foostr.2016.10.001

Di Mattia, C. D., Sacchetti, G., Mastrocola, D., & Pittia, P. (2009). Effect of phenolic antioxidants on the dispersion state and chemical stability of olive oil O/W emulsions. *Food Research International*, *42*(8), 1163–1170. https://doi.org/10.1016/j.foodres.2009.05.017

Du, G.-J., Wang, C.-Z., Qi, L.-W., Zhang, Z.-Y., Calway, T., He, T.-C.,Yuan, C.-S. (2013). The synergistic apoptotic interaction of panaxadiol and epigallocatechin gallate in human colorectal cancer cells. *Phytotherapy Research : PTR*, *27*(2), 272–7. https://doi.org/10.1002/ptr.4707

Dua, J. S., Rana, A. C., & Bhandari, A. K. (2012). Review Article Liposome : Methods of Preparation and Applications. *International Journal of Pharmaceutical Studies and Research*, *III*, 7. https://doi.org/E-ISSN 2229-4619

Du Plessis, J., Ramachandran, C., Weiner, N., & Müller, D. G. (1996). The influence

of lipid composition and lamellarity of liposomes on the physical stability of liposomes upon storage. *International Journal of Pharmaceutics*, *127*(2), 273–278. https://doi.org/10.1016/0378-5173(95)04281-4

Fang, J. Y., Hwang, T. L., Huang, Y. L., & Fang, C. L. (2006). Enhancement of the transdermal delivery of catechins by liposomes incorporating anionic surfactants and ethanol. *International Journal of Pharmaceutics*, *310*(1–2), 131–138. https://doi.org/10.1016/j.ijpharm.2005.12.004

Fang, Z., & Bhandari, B. (2010). Encapsulation of polyphenols - A review. *Trends in Food Science and Technology*, *21*(10), 510–523. https://doi.org/10.1016/j.tifs.2010.08.003

Fridjonsson, E. O., Graham, B. F., Akhfash, M., May, E. F., & Johns, M. L. (2014). Optimized droplet sizing of water-in-crude oil emulsions using nuclear magnetic resonance. *Energy and Fuels*, *28*(3), 1756–1764. https://doi.org/10.1021/ef402117k

Giada, M. (2013). Food Phenolic Compounds: Main Classes, Sources and Their Antioxidant Power. *Oxidative Stress and Chronic Degenerative Diseases - A Role for Antioxidants*, 87–112. https://doi.org/10.5772/51687

Gibis, M., Rahn, N., & Weiss, J. (2013). Physical and oxidative stability of uncoated and chitosan-coated liposomes containing grape seed extract. *Pharmaceutics*, *5*(3), 421–433. https://doi.org/10.3390/pharmaceutics5030421

Gibis, M., Vogt, E., & Weiss, J. (2012a). Encapsulation of polyphenolic grape seed extract in polymer-coated liposomes. *Food & Function*, *3*, 246. https://doi.org/10.1039/c1fo10181a

Gibis, M., Vogt, E., & Weiss, J. (2012b). Encapsulation of polyphenolic grape seed extract in polymer-coated liposomes. *Food Funct.*, *3*(3), 246–254. https://doi.org/10.1039/C1FO10181A

Gómez-Hens, A., & Fernández-Romero, J. M. (2005). The role of liposomes in analytical processes, *24*(1), 9–19. https://doi.org/10.1016/j.trac.2004.07.017

Goto, T., Yoshida, Y., Kiso, M., & Nagashima, H. (1996). Simultaneous analysis of individual catechins and caffeine in green tea. *Journal of Chromatography A*, *749*(1–2), 295–299. https://doi.org/10.1016/0021-9673(96)00456-6

Gradinaru, G., Biliaderis, C. G., Kallithraka, S., Kefalas, P., & Garcia-Viguera, C. (2003). Thermal stability of Hibiscus sabdariffa L. anthocyanins in solution and in solid state: Effects of copigmentation and glass transition. *Food Chemistry*, *83*(3), 423–436. https://doi.org/10.1016/S0308-8146(03)00125-0

Guner, S., & Oztop, M. H. (2017). Food grade liposome systems: Effect of solvent, homogenization types and storage conditions on oxidative and physical stability. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *513*, 468–478. https://doi.org/10.1016/j.colsurfa.2016.11.022

Guzey, D., & Mcclements, D. J. (2007). Formation , stability and properties of multilayer emulsions for application in the food industry, *130*(2006), 227–248. https://doi.org/10.1016/j.cis.2006.11.021

Hagerman, A. E., Riedl, K. M., Jones, G. A., Sovik, K. N., Ritchard, N. T., Hartzfeld, P. W., & Riechel, T. L. (1998). High Molecular Weight Plant Polyphenolics (Tannins) as Biological Antioxidants. *Journal of Agricultural and Food Chemistry*, *46*(5), 1887–1892. https://doi.org/10.1021/jf970975b

Hashemi, R. H., Bradley, W. G., & Lisanti, C. J. (2010). *MRI: The Basics*. Lippincott Williams & Wilkins.

Horie, H., & Kohata, K. (2000). Analysis of tea components by high-performance liquid chromatography and high-performance capillary electrophoresis. *Journal of Chromatography A*, *881*(1–2), 425–438. https://doi.org/10.1016/S0021-9673(99)01345-X

Hosseini, S. A., Gorjian, M., Rasouli, L., & Shirali, S. (2015). A comparison between the effect of green tea and Kombucha prepared from green tea on the weight of diabetic rats. *Biomedical and Pharmacology Journal*, *12*(January), 141–146. https://doi.org/10.13005/bbra/1616

Hsieh, Y. F., Chen, T. L., Wang, Y. T., Chang, J. H., & Chang, H. M. (2002). Properties of liposomes prepared with various lipids. *Journal of Food …*, *67*(8), 1–6. https://doi.org/10.1111/j.1365-2621.2002.tb08820.x

Hu, B., Pan, C., Sun, Y., Hou, Z., Ye, H., Hu, B., & Zeng, X. (2008). Optimization of fabrication parameters to produce chitosan-tripolyphosphate nanoparticles for delivery of tea catechins. *Journal of Agricultural and Food Chemistry*, *56*(16), 7451–7458. https://doi.org/10.1021/jf801111c

Immordino, M. L., Dosio, F., & Cattel, L. (2006). Stealth liposomes: Review of the basic science, rationale, and clinical applications, existing and potential. *International Journal of Nanomedicine*, *1*(3), 297–315. https://doi.org/10.1023/A:1020134521778

Jafari, S. M., He, Y., & Bhandari, B. (2007). Production of sub-micron emulsions by ultrasound and microfluidization techniques. *Journal of Food Engineering*, *82*(4), 478–488. https://doi.org/10.1016/j.jfoodeng.2007.03.007

Janeiro, P., & Oliveira Brett, A. M. (2004). Catechin electrochemical oxidation mechanisms. *Analytica Chimica Acta*, *518*(1–2), 109–115. https://doi.org/10.1016/j.aca.2004.05.038

Jones, M., & Taylor, S. E. (2015). NMR relaxometry and diffusometry in characterizing structural, interfacial and colloidal properties of heavy oils and oil sands. *Advances in Colloid and Interface Science*, *224*, 33–45. https://doi.org/10.1016/j.cis.2015.07.007

Kailaku, S. I., Mulyawanti, I., & Alamsyah, A. N. (2014). Formulation of

Nanoencapsulated Catechin with Chitosan as Encapsulation Material. *Procedia Chemistry*, *9*, 235–241. https://doi.org/10.1016/j.proche.2014.05.028

Karacam, C. H., Sahin, S., & Oztop, M. H. (2015). Effect of high pressure homogenization (microfluidization) on the quality of Ottoman Strawberry (F. Ananassa) juice. *LWT - Food Science and Technology*, *64*(2), 932–937. https://doi.org/10.1016/j.lwt.2015.06.064

Kaszuba, M., McKnight, D., Connah, M. T., McNeil-Watson, F. K., & Nobbmann, U. (2008). Measuring sub nanometre sizes using dynamic light scattering. *Journal of Nanoparticle Research*, *10*(5), 823–829. https://doi.org/10.1007/s11051-007-9317-4

Kevers, C., Falkowski, M., Tabart, J., Defraigne, J. O., Dommes, J., & Pincemail, J. (2007). Evolution of antioxidant capacity during storage of selected fruits and vegetables. *Journal of Agricultural and Food Chemistry*, *55*(21), 8596–8603. https://doi.org/10.1021/jf071736j

Kheadr, E. E., Vuillemard, J. C., & El-Deeb, S. A. (2002). Acceleration of Cheddar Cheese Lipolysis by Using Liposome-entrapped Lipases. *Journal of Food Science*, *67*(2), 485–492.

Kirby, C. J., Whittle, C. J., Rigby, N., Coxon, D. T., & Law, B. A. (1991). Stabilization of ascorbic acid by microencapsulation in liposomes. *International Journal of Food Science & Technology*, *26*(5), 437–449. https://doi.org/10.1111/j.1365-2621.1991.tb01988.x

Kirtil, E., & Oztop, M. H. (2015). 1H Nuclear Magnetic Resonance Relaxometry and Magnetic Resonance Imaging and Applications in Food Science and Processing. *Food Engineering Reviews*, (January), 1–22. https://doi.org/10.1007/s12393-015-9118-y

Kirtil, E., Oztop, M. H., Sirijariyawat, A., Ngamchuachit, P., Barrett, D. M., &

McCarthy, M. J. (2014). Effect of pectin methyl esterase (PME) and CaCl2 infusion on the cell integrity of fresh-cut and frozen-thawed mangoes: An NMR relaxometry study. *Food Research International*, *66*(October), 409–416. https://doi.org/10.1016/j.foodres.2014.10.006

Klimczak, I., Małecka, M., Szlachta, M., & Gliszczyńska-Świgło, A. (2007). Effect of storage on the content of polyphenols, vitamin C and the antioxidant activity of orange juices. *Journal of Food Composition and Analysis*, *20*(3–4), 313–322. https://doi.org/10.1016/j.jfca.2006.02.012

Kosaraju, S. L., Labbett, D., Emin, M., Konczak, I., & Lundin, L. (2008). Delivering polyphenols for healthy ageing. *Nutrition and Dietetics*, *65*(SUPPL. 3). https://doi.org/10.1111/j.1747-0080.2008.00261.x

Krawitzky, M., Arias, E., Peiro, J. M., Negueruela, A. I., Val, J., & Oria, R. (2014). Determination of color, antioxidant activity, and phenolic profile of different fruit tissue of spanish verde doncella apple cultivar. *International Journal of Food Properties*, *17*(10), 2298–2311. https://doi.org/10.1080/10942912.2013.792829

Labbé, D., Têtu, B., Trudel, D., & Bazinet, L. (2008). Catechin stability of EGC- and EGCG-enriched tea drinks produced by a two-step extraction procedure. *Food Chemistry*, *111*(1), 139–143. https://doi.org/10.1016/j.foodchem.2008.03.048

Laine, P., Kylli, P., Heinonen, M., & Jouppila, K. (2008). Storage stability of microencapsulated cloudberry (Rubus chamaemorus) phenolics. *Journal of Agricultural and Food Chemistry*, *56*(23), 11251–11261. https://doi.org/10.1021/jf801868h

Laouini, A., Jaafar-Maalej, C., Limayem-Blouza, I., Sfar, S., Charcosset, C., & Fessi, H. (2012). Preparation, Characterization and Applications of Liposomes: State of the Art. *Journal of Colloid Science and Biotechnology*, *1*(2), 147–168. https://doi.org/10.1166/jcsb.2012.1020

Lasic, D. D. (1990). On the thermodynamic stability of liposomes. *Journal of Colloid and Interface Science*, *140*(1), 302–304. https://doi.org/DOI: 10.1016/0021-9797(90)90348-R

Lateef, A., Oloke, J. K., & Prapulla, S. G. (2007). The effect of ultrasonication on the release of fructosyltransferase from Aureobasidium pullulans CFR 77. *Enzyme and Microbial Technology*, *40*, 1067–1070. Retrieved from http://dx.doi.org/10.1016/j.enzmictec.2006.08.008

Laye, C., McClements, D. J., & Weiss, J. (2008). Formation of biopolymer-coated liposomes by electrostatic deposition of chitosan. *Journal of Food Science*, *73*(5), N7-15. https://doi.org/10.1111/j.1750-3841.2008.00747.x

Leal, C., Rögnvaldsson, S., Fossheim, S., Nilssen, E. A., & Topgaard, D. (2008). Dynamic and structural aspects of PEGylated liposomes monitored by NMR. *Journal of Colloid and Interface Science*, *325*(2), 485–493. https://doi.org/10.1016/j.jcis.2008.05.051

Lemoine, M. L., Civello, P. M., Martínez, G. A., & Chaves, A. R. (2007). Influence of postharvest UV-C treatment on refrigerated storage of minimally processed broccoli (Brassica oleracea var. Italica). *Journal of the Science of Food and Agriculture*, *87*(6), 1132–1139. https://doi.org/10.1002/jsfa

Lertsutthiwong, P., Rojsitthisak, P., & Nimmannit, U. (2009). Preparation of turmeric oil-loaded chitosan-alginate biopolymeric nanocapsules. *Materials Science & Engineering C*, *29*(3), 856–860. https://doi.org/10.1016/j.msec.2008.08.004

Li, J., Wang, X., Zhang, T., Wang, C., Huang, Z., Luo, X., & Deng, Y. (2014). A review on phospholipids and their main applications in drug delivery systems. *Asian Journal of Pharmaceutical Sciences*, *10*(2), 81–98. https://doi.org/10.1016/j.ajps.2014.09.004

Li, Z., Paulson, A. T., & Gill, T. A. (2015). Encapsulation of bioactive salmon protein hydrolysates with chitosan-coated liposomes. *Journal of Functional Foods*, *19*, 733–743. https://doi.org/10.1016/j.jff.2015.09.058

Ling, N. N. A., Haber, A., Fridjonsson, E. O., May, E. F., & Johns, M. L. (2016). Shear-induced emulsion droplet diffusion studies using NMR. *Journal of Colloid and Interface Science*, *464*, 229–237. https://doi.org/10.1016/j.jcis.2015.11.013

Liu, W., Ye, A., Liu, C., Liu, W., & Singh, H. (2012). Structure and integrity of liposomes prepared from milk- or soybean-derived phospholipids during in vitro digestion. *Food Research International*, *48*(2), 499–506. https://doi.org/10.1016/j.foodres.2012.04.017

Liu, Y., Gajewicz, A. M., Rodin, V., Soer, W. J., Scheerder, J., Satgurunathan, G., … Keddie, J. L. (2016). Explanations for water whitening in secondary dispersion and emulsion polymer films. *Journal of Polymer Science, Part B: Polymer Physics*, 1658–1674. https://doi.org/10.1002/polb.24070

Lopes, N. A., Pinilla, C. M. B., & Brandelli, A. (2017). Pectin and polygalacturonic acid-coated liposomes as novel delivery system for nisin: Preparation, characterization and release behavior. *Food Hydrocolloids*, *70*, 1–7. https://doi.org/10.1016/j.foodhyd.2017.03.016

Lucas-Abellán, C., Fortea, I., Gabaldón, J. A., & Núñez-Delicado, E. (2008). Encapsulation of quercetin and myricetin in cyclodextrins at acidic pH. *Journal of Agricultural and Food Chemistry*, *56*(1), 255–259. https://doi.org/10.1021/jf0720640

Madhavi, D. L., Deshpande, S. S., & Salunkhe, D. K. (1996). *Food Antioxidants: Technological: Toxicological and Health Perspectives*. New York: Marcel Dekker Inc.

Madrigal-Carballo, S., Lim, S., Rodriguez, G., Vila, A. O., Krueger, C. G., Gunasekaran, S., & Reed, J. D. (2010). Biopolymer coating of soybean lecithin liposomes via layer-by-layer self-assembly as novel delivery system for ellagic acid. *Journal of Functional Foods*, *2*(2), 99–106. https://doi.org/10.1016/j.jff.2010.01.002

Mady, M. M., & Darwish, M. M. (2010). Effect of chitosan coating on the characteristics of DPPC liposomes. *Journal of Advanced Research*, *1*(3), 187–191. https://doi.org/10.1016/j.jare.2010.05.008

Makino, K., Yamada, T., Kimura, M., Oka, T., Ohshima, H., & Kondo, T. (1991). Temperature- and ionic strength-induced conformational changes in the lipid head group region of liposomes as suggested by zeta potential data. *Biophysical Chemistry*, *41*(2), 175–183. https://doi.org/https://doi.org/10.1016/0301-4622(91)80017-L

Malheiros, P. S., Daroit, D. J., & Brandelli, A. (2010). Food applications of liposome-encapsulated antimicrobial peptides. *Trends in Food Science and Technology*, *21*(6), 284–292. https://doi.org/10.1016/j.tifs.2010.03.003

Mariette, F. (2009). Investigations of food colloids by NMR and MRI. *Current Opinion in Colloid and Interface Science*, *14*(3), 203–211. https://doi.org/10.1016/j.cocis.2008.10.006

Marigheto, N., Venturi, L., Hibberd, D., Wright, K. M., Ferrante, G., & Hills, B. P. (2007). Methods for peak assignment in low-resolution multidimensional NMR cross-correlation relaxometry. *Journal of Magnetic Resonance*, *187*(2), 327–342. https://doi.org/10.1016/j.jmr.2007.04.016

McClements, D. J. (2005). Theoretical analysis of factors affecting the formation and stability of multilayered colloidal dispersions. *Langmuir : The ACS Journal of Surfaces and Colloids*, *21*(21), 9777–9785. https://doi.org/10.1021/la0512603

McClements, D. J. (2010). Design of nano-laminated coatings to control bioavailability of lipophilic food components. *Journal of Food Science*, *75*(1), 30–42. https://doi.org/10.1111/j.1750-3841.2009.01452.x

Miklos, R., Mora-Gallego, H., Larsen, F. H., Serra, X., Cheong, L. Z., Xu, X., … Lametsch, R. (2014). Influence of lipid type on water and fat mobility in fermented sausages studied by low-field NMR. *Meat Science*, *96*(1), 617–622. https://doi.org/10.1016/j.meatsci.2013.08.025

Mishra, K., Ojha, H., & Chaudhury, N. K. (2012). Estimation of antiradical properties of antioxidants using DPPH - assay: A critical review and results. *Food Chemistry*, *130*(4), 1036–1043. https://doi.org/10.1016/j.foodchem.2011.07.127

Mohammadi, V., Ghasemi-Varnamkhasti, M., Ebrahimi, R., & Abbasvali, M. (2014). Ultrasonic techniques for the milk production industry. *Measurement*, *58*(February 2016), 93–102. https://doi.org/10.1016/j.measurement.2014.08.022

Mozafari, M., Flanagan, J., Matia-Merino, L., Awati, A., Omri, A., Suntres, Z. E., & Singh, H. (2006). Recent trends in the lipid- based nanoencapsulation of antioxidants and their role in foods. *Journal of the Science of Food and Agricultire*, *86*(December 2005), 2038–2045. https://doi.org/10.1002/jsfa

Onuki, Y., Kida, C., Funatani, C., Hayashi, Y., & Takayama, K. (2016). MRI as a promising tool for evaluation of the stability of cosmetic emulsions. *International Journal of Cosmetic Science*, *38*(3), 272–278. https://doi.org/10.1111/ics.12290

Ou, B., Hampsch-woodill, M., & Prior, R. L. (2001). Development and Validation of an Improved Oxygen Radical Absorbance Capacity Assay Using Fluorescein as the Fluorescent Probe Development and Validation of an Improved Oxygen Radical Absorbance Capacity Assay Using Fluorescein as the Fluorescent, *49*, 4619–4626. https://doi.org/10.1021/jf010586o

Pelegrine, D. H. G., & Gasparetto, C. A. (2005). Whey proteins solubility as function of temperature and pH. *LWT - Food Science and Technology*, *38*(1), 77–80. https://doi.org/10.1016/j.lwt.2004.03.013

Piljac-Žegarac, J., Valek, L., Martinez, S., & Belščak, A. (2009). Fluctuations in the phenolic content and antioxidant capacity of dark fruit juices in refrigerated storage. *Food Chemistry*, *113*(2), 394–400. https://doi.org/10.1016/j.foodchem.2008.07.048

Pinelo, M., Manzocco, L., Nuñez, M. J., & Nicoli, M. C. (2004). Interaction among phenols in food fortification: negative synergism on antioxidant capacity. *Journal of Agricultural and Food Chemistry*, *52*(5), 1177–1180. https://doi.org/10.1021/jf0350515

Pinelo, M., Rubilar, M., Sineiro, J., & Nuñez, M. J. (2005). A thermal treatment to increase the antioxidant capacity of natural phenols: Catechin, resveratrol and grape extract cases. *European Food Research and Technology*, *221*(3–4), 284–290. https://doi.org/10.1007/s00217-005-1159-7

Priprem, A., Watanatorn, J., Sutthiparinyanont, S., Phachonpai, W., & Muchimapura, S. (2008). Anxiety and cognitive effects of quercetin liposomes in rats. *Nanomedicine: Nanotechnology, Biology, and Medicine*, *4*(1), 70–78. https://doi.org/10.1016/j.nano.2007.12.001

Pulido, R., Bravo, L., & Saura-Calixto, F. (2000). Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *Journal of Agricultural and Food Chemistry*, *48*(8), 3396–3402. https://doi.org/10.1021/jf9913458

Rashidinejad, A., Birch, E. J., Sun-Waterhouse, D., & Everett, D. W. (2014a). Delivery of green tea catechin and epigallocatechin gallate in liposomes incorporated into low-fat hard cheese. *Food Chemistry*, *156*(February), 176–183. https://doi.org/10.1016/j.foodchem.2014.01.115

Rashidinejad, A., Birch, E. J., Sun-Waterhouse, D., & Everett, D. W. (2014b). Delivery of green tea catechin and epigallocatechin gallate in liposomes incorporated into low-fat hard cheese. *Food Chemistry*, *156*, 176–183. https://doi.org/10.1016/j.foodchem.2014.01.115

Ray, S., Raychaudhuri, U., & Chakraborty, R. (2015). An overview of encapsulation of active compounds used in food products by drying technology. *Food Bioscience*, *13*, 76–83. https://doi.org/10.1016/j.fbio.2015.12.009

Réblová, Z. (2012). Effect of Temperature on the Antioxidant Activity of Phenolic Acids. *Czech Journal of Food Science*, *30*(2), 171–177.

Robert, J. H. (1986). *Foundations of colloid science* (1st ed.). Oxford: Oxford University Press.

Shi, G., Rao, L., Yu, H., Xiang, H., Pen, G., Long, S., & Yang, C. (2007). Yeast-cell-based microencapsulation of chlorogenic acid as a water-soluble antioxidant. *Journal of Food Engineering*, *80*(4), 1060–1067. https://doi.org/10.1016/j.jfoodeng.2006.06.038

Shutava, T. G., Balkundi, S. S., & Lvov, Y. M. (2009). (-)-Epigallocatechin gallate/gelatin layer-by-layer assembled films and microcapsules. *Journal of Colloid and Interface Science*, *330*(2), 276–283. https://doi.org/10.1016/j.jcis.2008.10.082

Shutava, T. G., Balkundi, S. S., Vangala, P., Steffan, J. J., Bigelow, R. L., Cardelli, J. A., … Lvov, Y. M. (2009). Layer-by-layer-coated gelatin nanoparticles as a vehicle for delivery of natural polyphenols. *ACS Nano*, *3*(7), 1877–1885. https://doi.org/10.1021/nn900451a

Sikorski, Z. E., & Kolakowska, A. (2003). *Chemical and Functional Properties of Food Lipids*. Florida: CRC Press LCL.

Sindhi, V., Gupta, V., Sharma, K., Bhatnagar, S., Kumari, R., & Dhaka, N. (2013).

Potential applications of antioxidants - A review. *Journal of Pharmacy Research*, *7*(9), 828–835. https://doi.org/10.1016/j.jopr.2013.10.001

Siripatrawan, U., & Noipha, S. (2012). Active film from chitosan incorporating green tea extract for shelf life extension of pork sausages. *Food Hydrocolloids*, *27*(1), 102–108. https://doi.org/10.1016/j.foodhyd.2011.08.011

Soria, A. C., & Villamiel, M. (2010). Effect of ultrasound on the technological properties and bioactivity of food: A review. *Trends in Food Science and Technology*, *21*(7), 323–331. https://doi.org/10.1016/j.tifs.2010.04.003

Sun, Y., Zhong, L., Cao, L., Lin, W., & Ye, X. (2015). Sonication inhibited browning but decreased polyphenols contents and antioxidant activity of fresh apple (malus pumila mill, cv. Red Fuji) juice. *Journal of Food Science and Technology*, *52*(12), 8336–8342. https://doi.org/10.1007/s13197-015-1896-y

Sze, A., Erickson, D., Ren, L., & Li, D. (2003). Zeta-potential measurement using the Smoluchowski equation and the slope of the current-time relationship in electroosmotic flow. *Journal of Colloid and Interface Science*, *261*(2), 402–410. https://doi.org/10.1016/S0021-9797(03)00142-5

Takahashi, M., Uechi, S., K., T., Asikin, Y., & Wada, K. (2009). Evaluation of an Oral Carrier System in Rats: Bioavailability and Antioxidant Properties of Liposome-Encapsulated Curcumin. *Journal of Agricultural and Food Chemistry*, *57*(19), 9141–9146.

Taylor, T. M., Weiss, J., Davidson, P. M., & Bruce, B. D. (2005). Liposomal Nanocapsules in Food Science and Agriculture. *Critical Reviews in Food Science and Nutrition*, *45*(7–8), 587–605. https://doi.org/10.1080/10408390591001135

Thompson, A. K., & Singh, H. (2006). Preparation of liposomes from milk fat globule membrane phospholipids using a microfluidizer. *Journal of Dairy*

Science, *89*(2), 410–419. https://doi.org/10.3168/jds.S0022-0302(06)72105-1

Vermeir, L., Balcaen, M., Sabatino, P., Dewettinck, K., & Van der Meeren, P. (2014). Influence of molecular exchange on the enclosed water volume fraction of W/O/W double emulsions as determined by low-resolution NMR diffusometry and T2-relaxometry. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *456*(1), 129–138. https://doi.org/10.1016/j.colsurfa.2014.05.022

Vermeir, L., Sabatino, P., Balcaen, M., Van Ranst, G., & Van der Meeren, P. (2014). Evaluation of the effect of homogenization energy input on the enclosed water volume of concentrated W/O/W emulsions by low-resolution T2-relaxometry. *Food Hydrocolloids*, *34*, 34–38. https://doi.org/10.1016/j.foodhyd.2013.01.024

Vermerris, W., & Nicholson, R. (2009). *Phenolic Compound Biochemistry*. *Springer* (Vol. 53). https://doi.org/10.1017/CBO9781107415324.004

Vina, S. Z., & Chaves, A. R. (2006). Antioxidant responses in minimally processed celery during refrigerated storage. *Food Chemistry*, *94*(1), 68–74. https://doi.org/10.1016/j.foodchem.2004.10.051

Wallace, S. J., Li, J., Nation, R. L., & Boyd, B. J. (2012). Drug release from nanomedicines: Selection of appropriate encapsulation and release methodology. *Drug Delivery and Translational Research*, *2*(4), 284–292. https://doi.org/10.1007/s13346-012-0064-4

Wanaga, K. A. I., No, S. A. O., Arioka, K. O. N., Akemi, M. A. K., Orimoto, K. A. M., Amashita, S. H. Y., … Ku, N. A. O. (1999). Application of Surface-Coated Liposomes for Oral Delivery of Peptide : Effects of Coating the Liposome ’ s Surface on the GI Transit of Insulin, *88*(2), 0–4.

Wang, H., Gao, X. D., Zhou, G. C., Cai, L., & Yao, W. B. (2008). In vitro and in vivo antioxidant activity of aqueous extract from Choerospondias axillaris fruit.

Food Chemistry, *106*(3), 888–895. https://doi.org/10.1016/j.foodchem.2007.05.068

Wang, H., & Helliwell, K. (2000). Epimerisation of catechins in green tea infusions. *Food Chemistry*, *70*(3), 337–344. https://doi.org/10.1016/S0308-8146(00)00099-6

Wang, H., Helliwell, K., & You, X. (2000). Isocratic elution system for the determination of catechins, caffeine and gallic acid in green tea using HPLC. *Food Chemistry*, *68*(1), 115–121. https://doi.org/10.1016/S0308-8146(99)00179-X

Waraho, T., Mcclements, D. J., & Decker, E. A. (2011). Mechanisms of lipid oxidation in food dispersions. *Trends in Food Science and Technology*, *22*(1), 3–13. https://doi.org/10.1016/j.tifs.2010.11.003

Yoshida, Y., Kiso, M., & Goto, T. (1999). Efficiency of the extraction of catechins from green tea. *Food Chemistry*, *67*, 429–433.

Yusaf, T., & Al-Juboori, R. A. (2014). Alternative methods of microorganism disruption for agricultural applications. *Applied Energy*, *114*, 909–923. Retrieved from http://dx.doi.org/10.1016/j.apenergy.2013.08.085

Zhang, R., Huo, J., Peng, Z., Feng, Q., Wang, J., & Zhang, J. (2016). Research on Oil-Based Drilling Fluids Emulsion Droplet by Low-Field NMR. *Applied Magnetic Resonance*, *47*(12), 1339–1352. https://doi.org/10.1007/s00723-016-0845-x

Zuo, Y., Chen, H., & Deng, Y. (2002). Simultaneous determination of catechins, caffeine and gallic acids in green, oolong, black and pu-erh teas using HPLC with a photodiode array detector. *Talanta*, *57*(2), 307–316. https://doi.org/10.1016/S0039-9140(02)00030-9

APPENDIX A

LC-MS/MS CHOROMATOGRAM

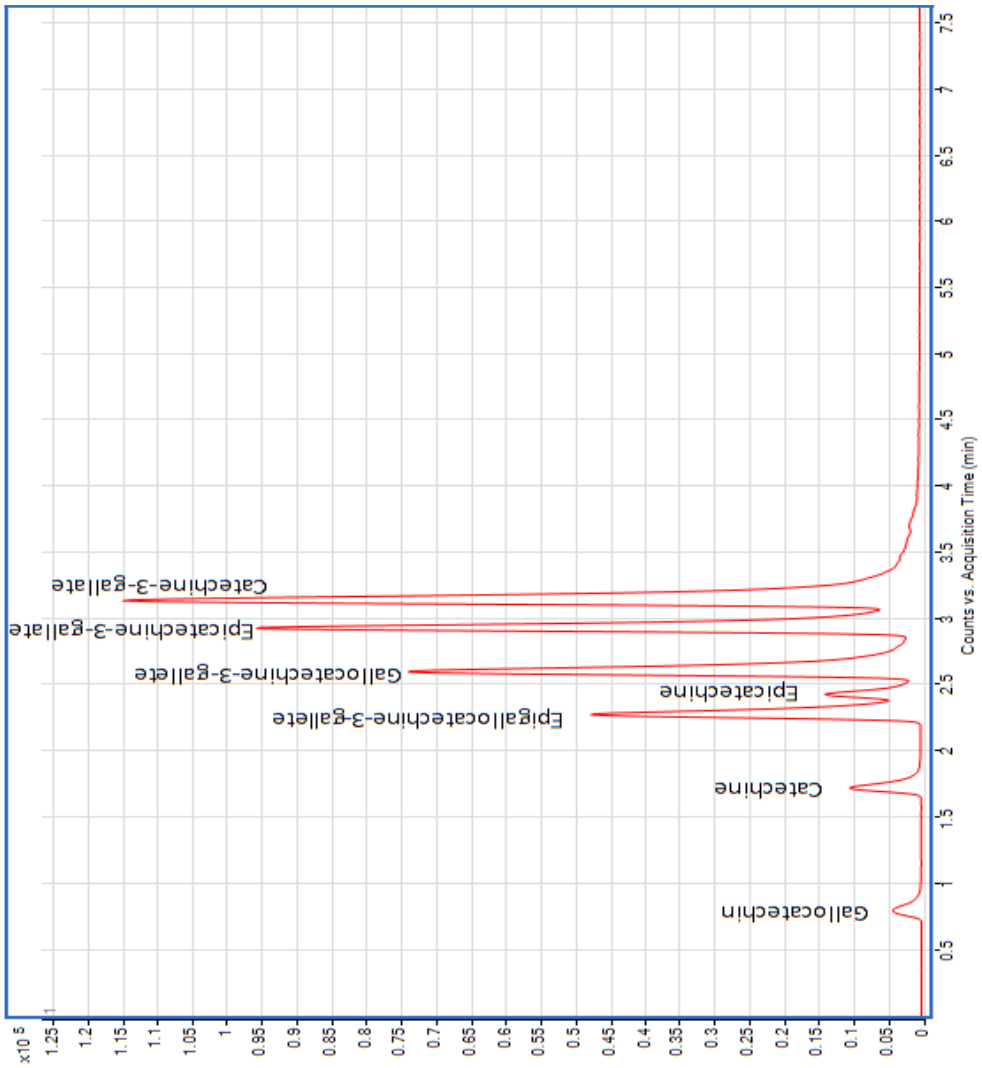


Figure A.1 LC-MS/MS chromatogram of green tea extract.

APPENDIX B

PARTICLE SIZE DISTRIBUTION

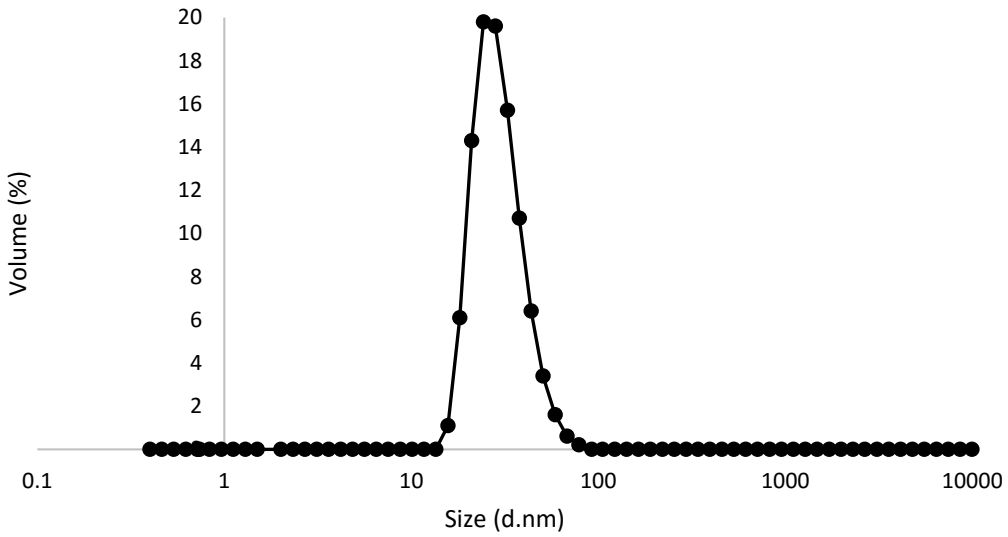


Figure B.1 The percentage volume particle size distribution of the liposomes prepared in acetate buffer by microfluidization at the first day of storage period.

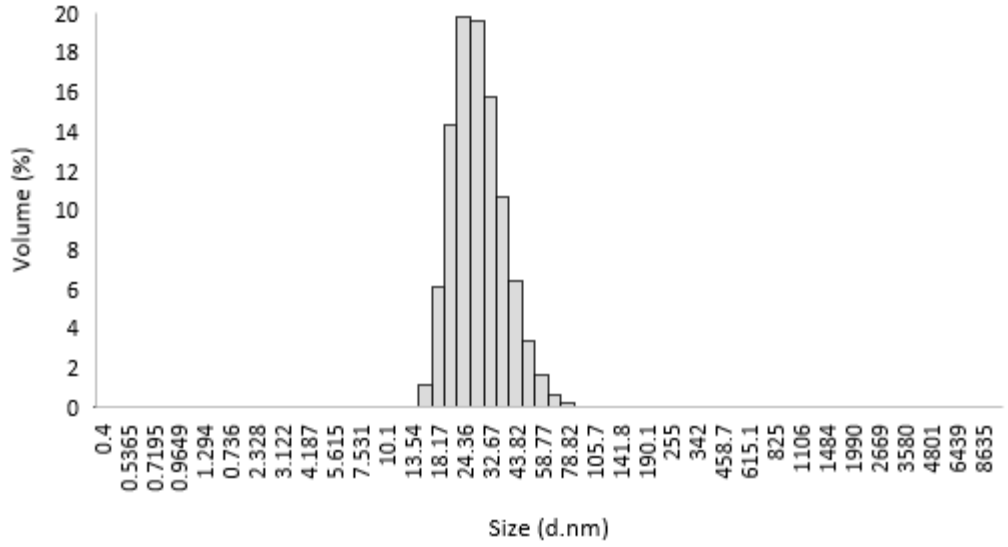


Figure B.2 The percentage volume particle size distribution of the liposomes prepared in acetate buffer by microfluidization at the first day of storage period.

APPENDIX C

CALIBRATION CURVES

Absorbance (at 760 nm) = 0.012 (mg gallic acid/L) - 0.0798

(R^2=0.9943)

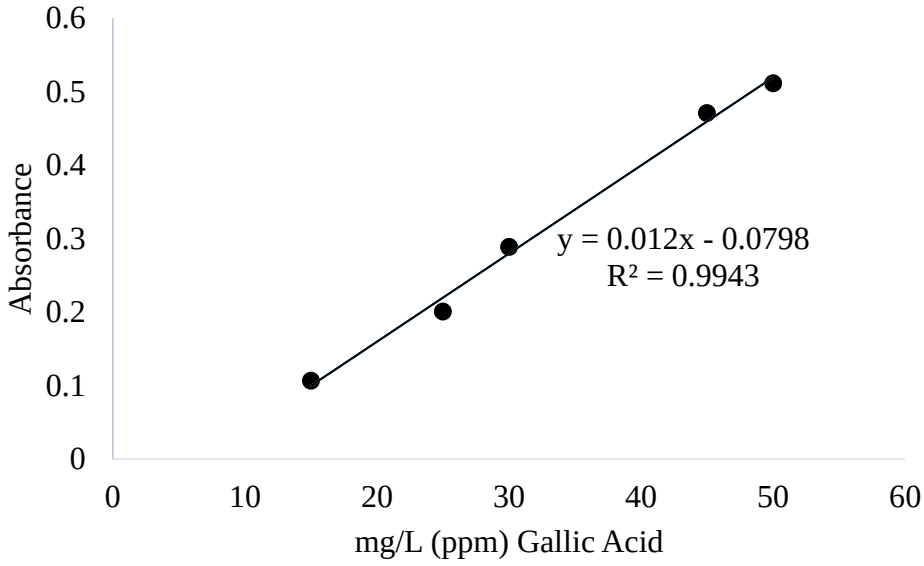


Figure C.1 Calibration Curve for Folin-Ciocalteau Method.

Absorbance (at 517 nm) = 0.0289 (mg DPPH/L) + 0.0093

(R^2=0.9999)

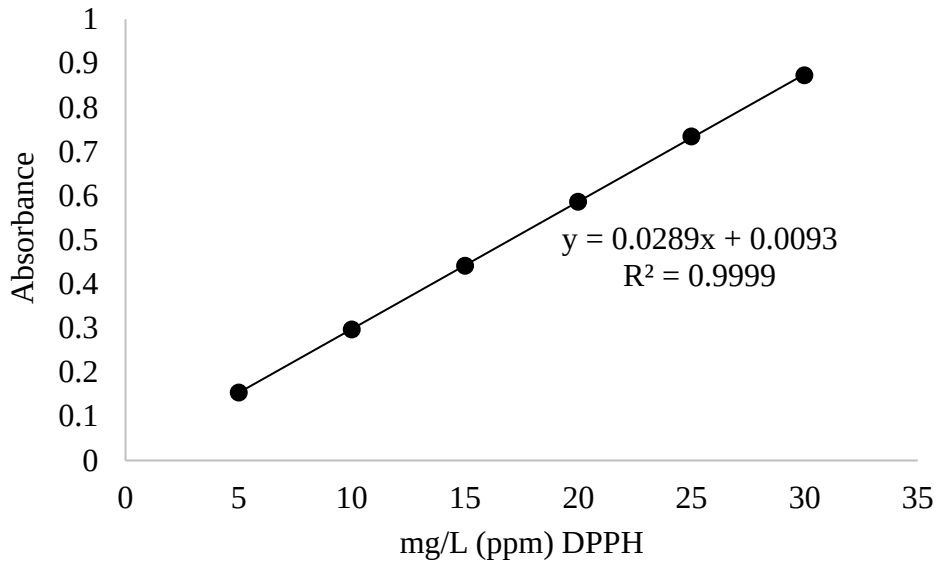


Figure C.2 Calibration Curve for DPPH Scavenging Method.

Absorbance (at 593 nm) = 0.4384 (mM Ferrous Sulphate) + 0.0022

(R^2=0.9972)

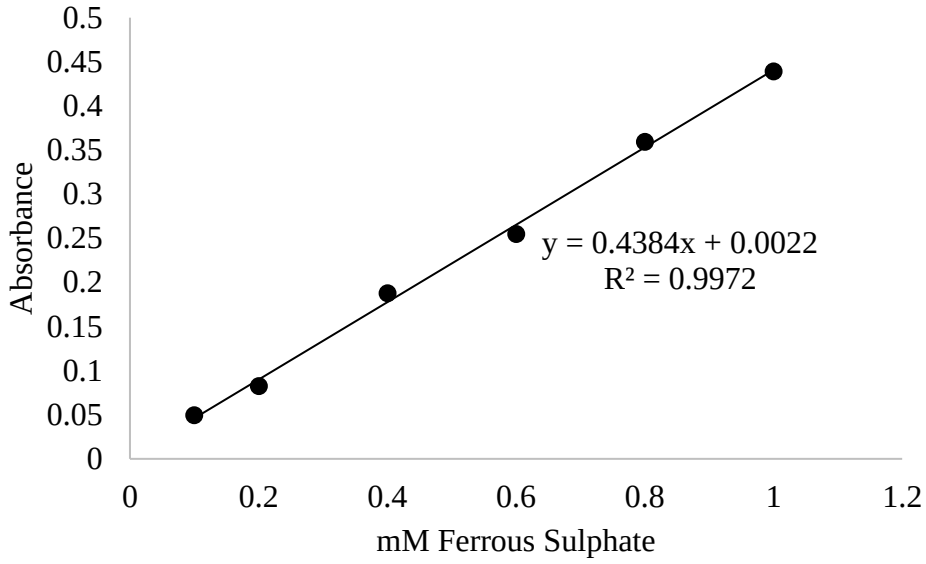


Figure C.3 Calibration Curve for FRAP Method.

APPENDIX D

COMPARATIVE

MEAN PARTICLE SIZE, TPC and AA TABLES

Table D.1 The mean particle size (nm) of green tea extract loaded liposomes.

	Microfluidization		**Ultrasonication**	
	Acetate Buffer	**Distilled Water**	**Acetate Buffer**	**Distilled Water**
Day 0	$46.61^{bc} \pm 0.41$	$38.29^{bc} \pm 0.55$	$131.13^{c} \pm 2.00$	$68.41^{a} \pm 0.28$
Day 7	$48.82^{abc} \pm 0.22$	$41.93^{b} \pm 0.51$	$172.6^{a} \pm 3.66$	$77.97^{a} \pm 1.66$
Day 14	$48.67^{ab} \pm 0.06$	$38.04^{bc} \pm 0.25$	$128.63^{c} \pm 1.34$	$67.48^{a} \pm 2.88$
Day 21	$48.35^{ab} \pm 0.07$	$38.12^{bc} \pm 0.57$	$129.07^{c} \pm 5.03$	$69.75^{a} \pm 0.38$
Day 28	$50.55^{a} \pm 0.47$	$39.33^{bc} \pm 0.65$	$91.85^{e} \pm 2.48$	$69.46^{a} \pm 0.94$

*The statistical analysis was performed separately for each liposomes type prepared in acetate buffer and distilled water.

Table D.2 The total phenolic content (mg GAE/L sample) of green tea extract loaded liposomes.

	Microfluidization		Ultrasonication	
	Acetate Buffer	**Distilled Water**	**Acetate Buffer**	**Distilled Water**
Day 0	111.86^{cd}±0.31	111.86^{cd}±1.66	139.61^{ab}±5.99	165.84^{a}±6.75
Day 7	85.65^{e}±1.51	80.71^{e}±0.86	122.74^{cd}±1.59	146.07^{ab}±5.58
Day 14	110.87^{cde}±1.19	128.01^{bc}±2.44	128.25^{bc}±2.92	108.79^{cde}±1.22
Day 21	162.02^{a}±4.29	128.73^{bc}±1.97	131.86^{bc}±3.00	110.9^{cde}±1.67
Day 28	110.23^{cd}±0.79	110.37^{cd}±0.39	104.1^{de}±1.99	98.72^{de}±4.18

*The statistical analysis was performed separately for each liposomes type prepared in acetate buffer and distilled water.

Table D.3 The antioxidant activity (mg DPPH/L sample) of green tea extract loaded liposomes.

	Microfluidization		Ultrasonication	
	Acetate Buffer	**Distilled Water**	**Acetate Buffer**	**Distilled Water**
Day 0	14.98^{cd} ± 0.35	15.05^{b} ± 0.22	21.75^{a} ± 0.43	20.45^{a} ± 0.18
Day 7	6.87^{e} ± 0.21	8.48 ± 0.16	19.29^{b} ± 0.30	19.89 ± 0.47
Day 14	16.85^{c} ± 0.41	17.56^{ab} ± 0.26	19.89^{ab} ± 0.26	20.22^{a} ± 0.71
Day 21	16.42^{c} ± 016	17.16^{ab} ± 0.12	18.87^{b} ± 0.51	19.71^{a} ± 0.28
Day 28	15.24^{cd} ± 0.43	17.97^{ab} ± 0.55	14.4^{d} ± 0.12	15.71^{ab} ± 0.26

*The statistical analysis was performed separately for each liposomes type prepared in acetate buffer and distilled water.

Table D.4 The antioxidant activity (mM Ferrous Sulphate/L sample) of green tea extract loaded liposomes.

	Microfluidization		**Ultrasonication**	
	Acetate Buffer	**Distilled Water**	**Acetate Buffer**	**Distilled Water**
Day 0	13.49 ± 0.07	13.91 ± 0.22	18.92 ± 0.19	16.69 ± 0.45
Day 7	12.53 ± 0.08	13.23 ± 0.15	17.09 ± 0.29	16.46 ± 0.11
Day 14	13.31 ± 0.21	14.67 ± 0.05	18.42 ± 0.08	17.32 ± 0.07
Day 21	12.31 ± 0.05	14.55 ± 0.23	17.38 ± 0.11	14.45 ± 0.18
Day 28	12.96 ± 0.11	14.34 ± 0.11	17.75 ± 0.13	18.72 ± 0.06

*The statistical analysis was performed separately for each liposomes type prepared in acetate buffer and distilled water.

APPENDIX E

ANOVA TABLES

Table E.1 Analysis of Variance for uncoated liposomes produced by microfluidization and ultrasonication in distilled water and acetate buffer. Effect of the homogenization type, medium and storage time on zeta potential, particle size, antioxidant activity, total phenolic content and T2 relaxation times using adjusted SS for Tests.

General Linear Model: Zeta Potential versus Day; Liposome Type

```
Factor  Type   Levels  Values
Day     fixed       2  0; 14
Type    fixed       4  A; B; C; D


Analysis of Variance for Zeta, using Adjusted SS for Tests

Source    DF   Seq SS   Adj SS  Adj MS       F      P
Day        1    57.23    57.23   57.23   18.30  0.001
Type       3  2354.52  2354.52  784.84  250.99  0.000
Day*Type   3    41.01    41.01   13.67    4.37  0.020
Error     16    50.03    50.03    3.13
Total     23  2502.79


S = 1.76833   R-Sq = 98.00%   R-Sq(adj) = 97.13%


Unusual Observations for Zeta

Obs      Zeta       Fit  SE Fit  Residual  St Resid
 16  -27.3000  -23.0333  1.0209   -4.2667     -2.96 R

R denotes an observation with a large standardized residual.
```

Grouping Information Using Tukey Method and 95.0% Confidence

Day	N	Mean	Grouping
14	12	-19.1	A
0	12	-22.2	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Type	N	Mean	Grouping
A	6	-8.9	A
C	6	-13.6	B
B	6	-26.6	C
D	6	-33.6	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Day	Type	N	Mean	Grouping
14	A	3	-7.2	A
0	A	3	-10.6	A B
14	C	3	-13.5	B
0	C	3	-13.7	B
14	B	3	-23.0	C
0	B	3	-30.2	D
14	D	3	-32.9	D
0	D	3	-34.4	D

Means that do not share a letter are significantly different.

General Linear Model: Particle Size versus Day, Type (unloaded and loaded liposomes prepared in acetate buffer by ultrasonication.)

Factor	Type	Levels	Values
DAY	fixed	5	0, 7, 14, 21, 28
TYPE	fixed	2	Loaded, Unloaded

Analysis of Variance for Particle Size, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
DAY	4	14197.0	14197.0	3549.3	160.32	0.000
TYPE	1	84.2	84.2	84.2	3.80	0.065
DAY*TYPE	4	1846.0	1846.0	461.5	20.85	0.000
Error	20	442.8	442.8	22.1		
Total	29	16570.0				

S = 4.70515 R-Sq = 97.33% R-Sq(adj) = 96.13%

Unusual Observations for Particle Size

Obs	Particle Size	Fit	SE Fit	Residual	St Resid
27	139.100	129.067	2.717	10.033	2.61 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95.0% Confidence

DAY	N	Mean	Grouping
7	6	160.5	A
14	6	139.0	B
0	6	130.0	C
21	6	121.3	D
28	6	94.2	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

TYPE	N	Mean	Grouping
Loaded	15	130.7	A
Unloaded	15	127.3	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

DAY	TYPE	N	Mean	Grouping
7	Loaded	3	172.6	A
14	Unloaded	3	149.4	B
7	Unloaded	3	148.4	B
0	Loaded	3	131.1	C
21	Loaded	3	129.1	C
0	Unloaded	3	128.8	C
14	Loaded	3	128.6	C
21	Unloaded	3	113.4	D
28	Unloaded	3	96.5	E
28	Loaded	3	91.9	E

Means that do not share a letter are significantly different.

General Linear Model: Particle Size versus Day, Type (unloaded and loaded liposomes prepared in distilled water by ultrasonication.)

Factor	Type	Levels	Values
TYPE	fixed	2	Loaded, Unloaded
DAY	fixed	5	0, 7, 14, 21, 28

Analysis of Variance for Particle Size, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
TYPE	1	128.330	130.988	130.988	14.20	0.001
DAY	4	365.387	365.385	91.346	9.90	0.000
TYPE*DAY	4	14.801	14.801	3.700	0.40	0.805
Error	19	175.224	175.224	9.222		
Total	28	683.741				

S = 3.03682 R-Sq = 74.37% R-Sq(adj) = 62.23%

Unusual Observations for Particle Size

Obs	Particle Size	Fit	SE Fit	Residual	St Resid	
13	71.6200	64.3167	1.7533	7.3033	2.95	R
15	57.9300	64.3167	1.7533	-6.3867	-2.58	R
23	73.2400	67.4767	1.7533	5.7633	2.32	R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95.0% Confidence

TYPE	N	Mean	Grouping
Loaded	15	70.6	A
Unloaded	14	66.3	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

DAY	N	Mean	Grouping
7	6	75.4	A
21	5	67.5	B
28	6	66.9	B
14	6	66.7	B
0	6	65.8	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

TYPE	DAY	N	Mean	Grouping
Loaded	7	3	78.0	A
Unloaded	7	3	72.8	A
Loaded	21	3	69.8	A
Loaded	28	3	69.5	A
Loaded	0	3	68.4	A
Loaded	14	3	67.5	A
Unloaded	14	3	65.9	A
Unloaded	21	2	65.3	A
Unloaded	28	3	64.3	A
Unloaded	0	3	63.3	A

Means that do not share a letter are significantly different.

General Linear Model: Particle Size versus Day, Type (unloaded and loaded liposomes prepared in acetate buffer by microfluidization.)

```
Factor  Type   Levels  Values
DAY     fixed       5  0, 7, 14, 21, 28
TYPE    fixed       2  Loaded, Unloaded


Analysis of Variance for Particle Size, using Adjusted SS for Tests

Source    DF   Seq SS   Adj SS   Adj MS       F      P
DAY        4  120.894  120.894   30.224   42.09  0.000
TYPE       1  351.782  351.782  351.782  489.92  0.000
DAY*TYPE   4   26.629   26.629    6.657    9.27  0.000
Error     20   14.361   14.361    0.718
Total     29  513.666


S = 0.847374   R-Sq = 97.20%   R-Sq(adj) = 95.95%

Unusual Observations for Particle Size

     Particle
Obs      Size      Fit  SE Fit  Residual  St Resid
 14   48.5900  46.1033  0.4892    2.4867      3.59 R
 15   44.2600  46.1033  0.4892   -1.8433     -2.66 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95.0% Confidence

DAY  N  Mean  Grouping
28   6  48.3  A
21   6  45.6    B
14   6  45.5    B
 7   6  44.2    B
 0   6  42.2      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

TYPE       N  Mean  Grouping
Loaded    15  48.6  A
Unloaded  15  41.8    B

Means that do not share a letter are significantly different.
```

Grouping Information Using Tukey Method and 95.0% Confidence

DAY	TYPE	N	Mean	Grouping
28	Loaded	3	50.5	A
7	Loaded	3	48.8	A B
14	Loaded	3	48.7	A B
21	Loaded	3	48.3	A B C
0	Loaded	3	46.6	B C
28	Unloaded	3	46.1	C
21	Unloaded	3	42.9	D
14	Unloaded	3	42.4	D
7	Unloaded	3	39.6	E
0	Unloaded	3	37.8	E

Means that do not share a letter are significantly different.

General Linear Model: Particle Size versus Day, Type (unloaded and loaded liposomes prepared in distilled water by microfluidization.)

Factor	Type	Levels	Values
DAY	fixed	5	0, 7, 14, 21, 28
TYPE	fixed	2	Loaded, Unloaded

Analysis of Variance for Particle Size, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
DAY	4	157.942	157.942	39.486	14.36	0.000
TYPE	1	4.144	4.144	4.144	1.51	0.234
DAY*TYPE	4	206.605	206.605	51.651	18.79	0.000
Error	20	54.975	54.975	2.749		
Total	29	423.666				

S = 1.65794 R-Sq = 87.02% R-Sq(adj) = 81.18%

Unusual Observations for Particle Size

Obs	Particle Size	Fit	SE Fit	Residual	St Resid	
9	36.5200	40.1700	0.9572	-3.6500	-2.70	R
13	44.3900	47.8867	0.9572	-3.4967	-2.58	R
15	51.4300	47.8867	0.9572	3.5433	2.62	R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95.0% Confidence

DAY	N	Mean	Grouping
28	6	43.6	A
21	6	39.6	B
14	6	39.1	B C
7	6	38.7	B C
0	6	36.6	C

Means that do not share a letter are significantly different.

```
Grouping Information Using Tukey Method and 95.0% Confidence

TYPE       N  Mean  Grouping
Unloaded  15  39.9  A
Loaded    15  39.1  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95.0% Confidence

DAY  TYPE      N  Mean  Grouping
28   Unloaded  3  47.9  A
 7   Loaded    3  41.9    B
21   Unloaded  3  41.1    B C
14   Unloaded  3  40.2    B C
28   Loaded    3  39.3    B C
 0   Loaded    3  38.3    B C
21   Loaded    3  38.1    B C
14   Loaded    3  38.0    B C
 7   Unloaded  3  35.4      C
 0   Unloaded  3  34.8      C

Means that do not share a letter are significantly different.
```

General Linear Model: Total Phenolic Content versus Homogenization type; Day (for liposomes prepared in acetate buffer by both microfluidization and ultrasonication.)

```
Factor  Type   Levels  Values
HT      fixed       2  M; U
DAY     fixed       5  0; 7; 14; 21; 28


Analysis of Variance for FOLIN, using Adjusted SS for Tests

Source  DF   Seq SS  Adj SS  Adj MS      F      P
HT       1    414.1   414.1   414.1   5.32  0.032
DAY      4   7742.7  7742.7  1935.7  24.87  0.000
HT*DAY   4   5497.2  5497.2  1374.3  17.65  0.000
Error   20   1556.9  1556.9    77.8
Total   29  15211.0


S = 8.82300   R-Sq = 89.76%   R-Sq(adj) = 85.16%
Unusual Observations for FOLIN

Obs    FOLIN      Fit  SE Fit  Residual  St Resid
 16  132.265  148.932   5.094   -16.667     -2.31 R
 18  167.571  148.932   5.094    18.639      2.59 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95.0% Confidence

HT   N   Mean  Grouping
U   15  125.9  A
M   15  118.5    B
```

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

DAY	N	Mean	Grouping
21	6	146.9	A
0	6	133.1	A B
14	6	119.6	B C
28	6	107.1	C D
7	6	104.2	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

HT	DAY	N	Mean	Grouping
M	21	3	162.0	A
U	0	3	148.9	A B
U	21	3	131.9	B C
U	14	3	128.3	B C
U	7	3	122.7	C D
M	0	3	117.3	C D
M	28	3	116.5	C D
M	14	3	110.9	C D E
U	28	3	97.7	D E
M	7	3	85.7	E

Means that do not share a letter are significantly different.

General Linear Model: Total Phenolic Content versus Homogenization type; Day (for liposomes prepared in distilled water by both microfluidization and ultrasonication.)

Factor	Type	Levels	Values
HT	fixed	2	M; U
DAY	fixed	5	0; 7; 14; 21; 28

Analysis of Variance for FOLIN, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
HT	1	704.1	704.1	704.1	8.72	0.008
DAY	4	2068.5	2068.5	517.1	6.40	0.002
HT*DAY	4	9450.6	9450.6	2362.6	29.26	0.000
Error	20	1614.7	1614.7	80.7		
Total	29	13837.9				

S = 8.98536 R-Sq = 88.33% R-Sq(adj) = 83.08%

Unusual Observations for FOLIN

Obs	FOLIN	Fit	SE Fit	Residual	St Resid	
13	134.993	118.576	5.188	16.417	2.24	R
17	174.102	156.143	5.188	17.959	2.45	R
18	136.755	156.143	5.188	-19.388	-2.64	R

R denotes an observation with a large standardized residual.

```
Grouping Information Using Tukey Method and 95.0% Confidence

HT   N   Mean  Grouping
U   15  124.1  A
M   15  114.4    B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

DAY  N   Mean  Grouping
 0   6  134.0  A
21   6  119.8  A B
14   6  118.4    B
 7   6  115.5    B
28   6  108.7    B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

HT  DAY  N   Mean  Grouping
U    0   3  156.1  A
U    7   3  146.1  A B
M   21   3  128.7    B C
M   14   3  128.0    B C
M   28   3  118.6      C D
M    0   3  111.9      C D
U   21   3  110.9      C D E
U   14   3  108.8      C D E
U   28   3   98.7        D E
M    7   3   85.0          E

Means that do not share a letter are significantly different.
```

General Linear Model: Antioxidant Activity by DPPH Scavenging Method versus Homogenization type; Day (for liposomes prepared in acetate buffer by both microfluidization and ultrasonication.)

```
Factor  Type   Levels  Values
HT      fixed       2  M; U
DAY     fixed       5  0; 7; 14; 21; 28


Analysis of Variance for DPPH, using Adjusted SS for Tests

Source  DF   Seq SS   Adj SS   Adj MS       F      P
HT       1  162.791  162.791  162.791  368.51  0.000
DAY      4  126.082  126.082   31.521   71.35  0.000
HT*DAY   4  140.983  140.983   35.246   79.79  0.000
Error   20    8.835    8.835    0.442
Total   29  438.691


S = 0.664644   R-Sq = 97.99%   R-Sq(adj) = 97.08%
```

```
Unusual Observations for DPPH

Obs    DPPH     Fit  SE Fit  Residual  St Resid
  6  8.5395  7.4233  0.3837    1.1163      2.06 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95.0% Confidence

HT   N  Mean  Grouping
U   15  18.8  A
M   15  14.2    B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

DAY  N  Mean  Grouping
14   6  18.4  A
 0   6  18.4  A
21   6  17.6  A
28   6  14.8    B
 7   6  13.4      C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

HT  DAY  N  Mean  Grouping
U    0   3  21.8  A
U   14   3  19.9  A B
U    7   3  19.3    B
U   21   3  18.9    B
M   14   3  16.8      C
M   21   3  16.4      C
M   28   3  15.2      C D
M    0   3  15.0      C D
U   28   3  14.4        D
M    7   3   7.4          E

Means that do not share a letter are significantly different.
```

General Linear Model: Antioxidant Activity by DPPH Scavenging Method versus Homogenization type; Day (for liposomes prepared in distilled water by both microfluidization and ultrasonication.)

```
Factor  Type   Levels  Values
HT      fixed       2  M; U
DAY     fixed       4  0; 14; 21; 28


Analysis of Variance for DPPH, using Adjusted SS for Tests

Source  DF  Seq SS  Adj SS  Adj MS     F      P
HT       1  13.305  13.305  13.305  8.73  0.009
```

```
DAY      3  22.921  22.921   7.640  5.01  0.012
HT*DAY   3  35.607  35.607  11.869  7.79  0.002
Error   16  24.381  24.381   1.524
Total   23  96.215


S = 1.23442   R-Sq = 74.66%   R-Sq(adj) = 63.57%


Unusual Observations for DPPH

Obs     DPPH      Fit  SE Fit  Residual  St Resid
  6  21.7488  18.9581  0.7127    2.7907      2.77 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95.0% Confidence

HT   N  Mean  Grouping
U   12  19.0  A
M   12  17.5    B

Means that do not share a letter are significantly different.



Grouping Information Using Tukey Method and 95.0% Confidence

DAY  N  Mean  Grouping
14   6  19.6  A
21   6  18.4  A B
 0   6  18.2  A B
28   6  16.8    B
Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

HT  DAY  N  Mean  Grouping
U    0   3  20.5  A
U   14   3  20.2  A
U   21   3  19.7  A
M   14   3  19.0  A B
M   28   3  18.0  A B
M   21   3  17.2  A B
M    0   3  16.0    B
U   28   3  15.7    B

Means that do not share a letter are significantly different.
```

General Linear Model: In vitro digestion in the simulated gastric and intestinal medium versus Day; Type (for liposomes prepared in acetate buffer and distilled water by microfluidization.)

```
Factor  Type   Levels  Values
day     fixed       2  0; 30
Type    fixed       4  A; B; C; D


Analysis of Variance for Phenolic, using Adjusted SS for Tests

Source    DF   Seq SS   Adj SS   Adj MS       F      P
day        1  1312.76  1312.76  1312.76  485.75  0.000
Type       3   304.91   304.91   101.64   37.61  0.000
day*Type   3   528.36   528.36   176.12   65.17  0.000
Error     16    43.24    43.24     2.70
Total     23  2189.27

S = 1.64394   R-Sq = 98.02%   R-Sq(adj) = 97.16%

Unusual Observations for Phenolic

Obs  Phenolic      Fit  SE Fit  Residual  St Resid
 16   60.6000  65.0444  0.9491   -4.4444     -3.31 R
 18   68.2667  65.0444  0.9491    3.2222      2.40 R

R denotes an observation with a large standardized residual.
Grouping Information Using Tukey Method and 95.0% Confidence

Type  N  Mean  Grouping
D     6  65.7  A
A     6  65.0  A
B     6  61.8    B
C     6  56.7      C

Means that do not share a letter are significantly different.
Grouping Information Using Tukey Method and 95.0% Confidence

day   N  Mean  Grouping
30   12  69.7  A
 0   12  54.9    B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

day  Type  N  Mean  Grouping
30   D     3  81.1  A
30   A     3  70.6    B
30   B     3  65.0      C
30   C     3  62.2      C D
 0   A     3  59.5        D
 0   B     3  58.6        D
 0   C     3  51.3          E
 0   D     3  50.4          E

Means that do not share a letter are significantly different.
```

Effect of solvent type on the particle size of microfluidized liposome;

General Linear Model: Particle Size versus MEDIUM

```
Factor  Type   Levels  Values
MEDUIM  fixed       2  Acetate Buffer; Distilled Water


Analysis of Variance for Particle Size, using Adjusted SS for Tests

Source  DF  Seq SS  Adj SS  Adj MS       F      P
MEDIUM   1  103.75  103.75  103.75  145.43  0.000
Error    4    2.85    2.85    0.71
Total    5  106.60


S = 0.844620   R-Sq = 97.32%   R-Sq(adj) = 96.65%


Grouping Information Using Tukey Method and 95.0% Confidence

MEDIUM           N  Mean  Grouping
Acetate Buffer   3  46.6  A
Distilled Water  3  38.3    B

Means that do not share a letter are significantly different.
```

General Linear Model: TPC versus MEDIUM

```
Factor  Type   Levels  Values
MEDUIM  fixed       2  Acetate Buffer; Distilled Water


Analysis of Variance for TPC, using Adjusted SS for Tests

Source  DF  Seq SS  Adj SS  Adj MS     F      P
MEDIUM   1   44.43   44.43   44.43  0.91  0.394
Error    4  194.88  194.88   48.72
Total    5  239.31


S = 6.98000   R-Sq = 18.56%   R-Sq(adj) = 0.00%


Grouping Information Using Tukey Method and 95.0% Confidence

MEDIUM           N   Mean  Grouping
Acetate Buffer   3  117.3  A
Distilled Water  3  111.9  A

Means that do not share a letter are significantly different.
```

General Linear Model: DPPH versus MEDIUM

```
Factor  Type   Levels  Values
MEDUIM  fixed       2  Acetate Buffer; Distilled Water

Analysis of Variance for DPPH, using Adjusted SS for Tests

Source  DF  Seq SS  Adj SS  Adj MS     F      P
MEDIUM   1  0.0058  0.0058  0.0058  0.02  0.886
Error    4  0.9922  0.9922  0.2481
Total    5  0.9980
S = 0.498058   R-Sq = 0.58%   R-Sq(adj) = 0.00%

Grouping Information Using Tukey Method and 95.0% Confidence

MEDIUM           N  Mean  Grouping
Distilled Water  3  15.1  A
Acetate Buffer   3  15.0  A

Means that do not share a letter are significantly different.
```

General Linear Model: FRAP versus MEDIUM

```
Factor  Type   Levels  Values
MEDUIM  fixed       2  Acetate Buffer; Distilled Water


Analysis of Variance for FRAP, using Adjusted SS for Tests

Source  DF   Seq SS   Adj SS   Adj MS     F      P
MEDIUM   1  0.26232  0.26232  0.26232  3.39  0.139
Error    4  0.30941  0.30941  0.07735
Total    5  0.57173


S = 0.278123   R-Sq = 45.88%   R-Sq(adj) = 32.35%


Grouping Information Using Tukey Method and 95.0% Confidence

MEDIUM           N  Mean  Grouping
Distilled Water  3  13.9  A
Acetate Buffer   3  13.5  A

Means that do not share a letter are significantly different.
```

Effect of medium on the particle size of sonicated liposome;

General Linear Model: sqrt(tpc) versus MEDIUM

```
Factor  Type   Levels  Values
MEDUIM  fixed       2  Acetate Buffer; Distilled Water
```

Analysis of Variance for sqrt(tpc), using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
MEDUIM	1	1.6920	1.6920	1.6920	16.94	0.015
Error	4	0.3996	0.3996	0.0999		
Total	5	2.0915				

S = 0.316057 R-Sq = 80.90% R-Sq(adj) = 76.12%

Grouping Information Using Tukey Method and 95.0% Confidence

MEDUIM	N	Mean	Grouping
Distilled Water	3	12.9	A
Acetate Buffer	3	11.8	B

Means that do not share a letter are significantly different.

General Linear Model: DPPH versus MEDIUM

Factor	Type	Levels	Values
MEDIUM	fixed	2	Acetate Buffer; Distilled Water

Analysis of Variance for DPPH, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
MEDIUM	1	2.5441	2.5441	2.5441	7.84	0.049
Error	4	1.2980	1.2980	0.3245		
Total	5	3.8421				

S = 0.569649 R-Sq = 66.22% R-Sq(adj) = 57.77%

Grouping Information Using Tukey Method and 95.0% Confidence

MEDIUM	N	Mean	Grouping
Acetate Buffer	3	21.8	A
Distilled Water	3	20.5	B

Means that do not share a letter are significantly different.

General Linear Model: FRAP versus MEDIUM

Factor	Type	Levels	Values
MEDIUM	fixed	2	Acetate Buffer; Distilled Water

Analysis of Variance for FRAP, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
MEDIUM	1	4.3511	4.3511	4.3511	12.17	0.025
Error	4	1.4305	1.4305	0.3576		
Total	5	5.7816				

```
S = 0.598016   R-Sq = 75.26%   R-Sq(adj) = 69.07%


Grouping Information Using Tukey Method and 95.0% Confidence

MEDIUM           N  Mean  Grouping
Acetate Buffer   3  19.2  A
Distilled Water  3  17.5    B

Means that do not share a letter are significantly different.
```

Effect of Homogenization Technique for Liposome Prepared in Distilled Water;

General Linear Model: TPC versus HT

```
Factor  Type   Levels  Values
HT      fixed       2  M; U


Analysis of Variance for TPC, using Adjusted SS for Tests

Source  DF  Seq SS  Adj SS  Adj MS       F      P
HT       1  4369.6  4369.6  4369.6  114.08  0.000
Error    4   153.2   153.2    38.3
Total    5  4522.8
S = 6.18903   R-Sq = 96.61%   R-Sq(adj) = 95.77%


Grouping Information Using Tukey Method and 95.0% Confidence

HT  N   Mean  Grouping
U   3  165.8  A
M   3  111.9    B
Means that do not share a letter are significantly different.
```

General Linear Model: DPPH versus HT

```
Factor  Type   Levels  Values
HT      fixed       2  M; U


Analysis of Variance for DPPH, using Adjusted SS for Tests

Source  DF  Seq SS  Adj SS  Adj MS       F      P
HT       1  43.740  43.740  43.740  361.05  0.000
Error    4   0.485   0.485   0.121
Total    5  44.225


S = 0.348061   R-Sq = 98.90%   R-Sq(adj) = 98.63%


Grouping Information Using Tukey Method and 95.0% Confidence

HT  N  Mean  Grouping
U   3  20.5  A
M   3  15.1    B

Means that do not share a letter are significantly different.
```

General Linear Model: FRAP versus HT

```
Factor  Type   Levels  Values
HT      fixed       2  M; U


Analysis of Variance for FRAP, using Adjusted SS for Tests

Source  DF  Seq SS  Adj SS  Adj MS      F      P
HT       1  19.107  19.107  19.107  51.42  0.002
Error    4   1.486   1.486   0.372
Total    5  20.593


S = 0.609578   R-Sq = 92.78%   R-Sq(adj) = 90.98%


Grouping Information Using Tukey Method and 95.0% Confidence

HT  N  Mean  Grouping
U   3  17.5  A
M   3  13.9    B

Means that do not share a letter are significantly different.
```

Effect of Homogenization Technique for Liposome Prepared in Acetate Buffer;

General Linear Model: TPC versus HT

```
Factor  Type   Levels  Values
HT      fixed       2  M; U


Analysis of Variance for TPC, using Adjusted SS for Tests

Source  DF   Seq SS  Adj SS  Adj MS      F      P
HT       1   746.34  746.34  746.34  10.43  0.032
Error    4   286.25  286.25   71.56
Total    5  1032.59


S = 8.45948   R-Sq = 72.28%   R-Sq(adj) = 65.35%


Grouping Information Using Tukey Method and 95.0% Confidence

HT  N   Mean  Grouping
U   3  139.6  A
M   3  117.3    B

Means that do not share a letter are significantly different.
```

General Linear Model: DPPH versus HT

```
Factor  Type   Levels  Values
HT      fixed       2  M; U


Analysis of Variance for DPPH, using Adjusted SS for Tests

Source  DF  Seq SS  Adj SS  Adj MS       F      P
HT       1  68.634  68.634  68.634  152.04  0.000
Error    4   1.806   1.806   0.451
Total    5  70.440


S = 0.671874   R-Sq = 97.44%   R-Sq(adj) = 96.80%


Grouping Information Using Tukey Method and 95.0% Confidence

HT  N  Mean  Grouping
U   3  21.8  A
M   3  15.0    B

Means that do not share a letter are significantly different.
```

General Linear Model: T_2 Relaxation Time versus Day (for green tea extract loaded liposomes prepared in acetate buffer by microfluidization.)

One-way ANOVA: T_2 versus day

```
Source  DF     SS    MS     F      P
day      9  27740  3082  9.76  0.000
Error   20   6319   316
Total   29  34059

S = 17.77   R-Sq = 81.45%   R-Sq(adj) = 73.10%

                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev  ---+---------+---------+---------+------
 0     3   935.8    7.6    (----*----)
 3     3   965.8    5.8              (----*-----)
 5     3   929.3   11.1  (----*-----)
 7     3   931.7    7.9   (----*----)
10     3   983.5    0.4                (----*----)
14     3  1033.0   45.7                           (----*-----)
18     3   984.5    0.9                (----*----)
21     3   941.0    5.5     (----*-----)
24     3   974.2   21.0               (-----*----)
28     3   962.1   18.0          (-----*----)
                          ---+---------+---------+---------+------
                           920       960      1000      1040

Pooled StDev = 17.8
```

Grouping Information Using Tukey Method

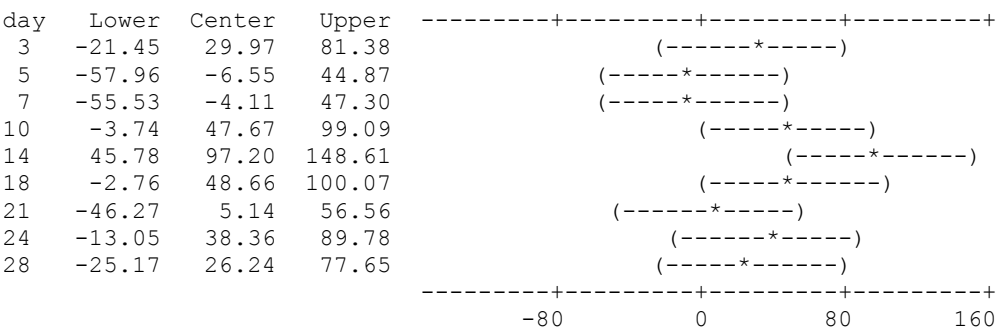
day	N	Mean	Grouping
14	3	1033.01	A
18	3	984.47	A B
10	3	983.49	A B
24	3	974.18	B C
3	3	965.78	B C
28	3	962.06	B C
21	3	940.96	B C
0	3	935.82	B C
7	3	931.70	C
5	3	929.27	C

Means that do not share a letter are significantly different.
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of day

Individual confidence level = 99.80%

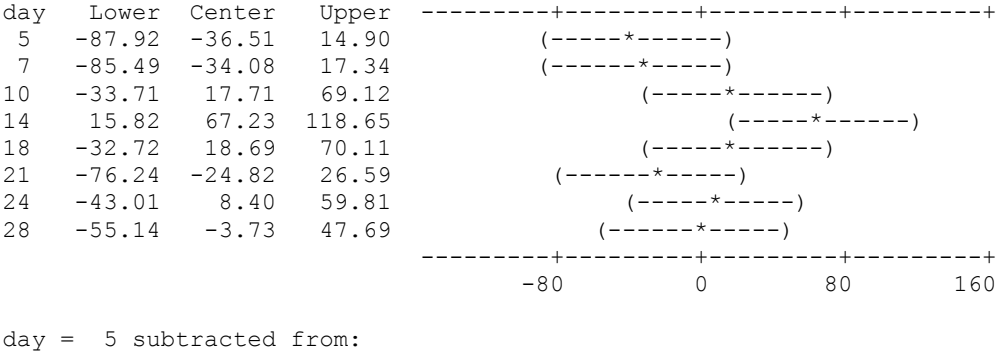
day = 0 subtracted from:

day	Lower	Center	Upper
3	-21.45	29.97	81.38
5	-57.96	-6.55	44.87
7	-55.53	-4.11	47.30
10	-3.74	47.67	99.09
14	45.78	97.20	148.61
18	-2.76	48.66	100.07
21	-46.27	5.14	56.56
24	-13.05	38.36	89.78
28	-25.17	26.24	77.65



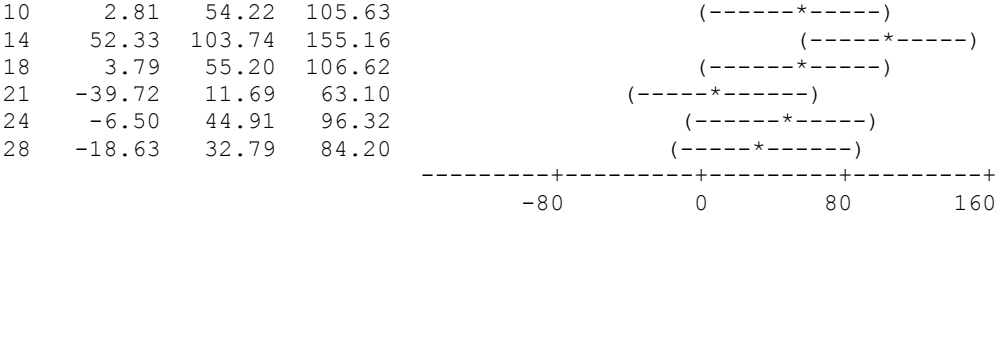
day = 3 subtracted from:

day	Lower	Center	Upper
5	-87.92	-36.51	14.90
7	-85.49	-34.08	17.34
10	-33.71	17.71	69.12
14	15.82	67.23	118.65
18	-32.72	18.69	70.11
21	-76.24	-24.82	26.59
24	-43.01	8.40	59.81
28	-55.14	-3.73	47.69



day = 5 subtracted from:

day	Lower	Center	Upper
7	-48.98	2.43	53.85
10	2.81	54.22	105.63
14	52.33	103.74	155.16
18	3.79	55.20	106.62
21	-39.72	11.69	63.10
24	-6.50	44.91	96.32
28	-18.63	32.79	84.20



```
day =  7 subtracted from:

day   Lower  Center   Upper  ---------+---------+---------+---------+
10     0.37   51.79  103.20                   (-----*------)
14    49.90  101.31  152.72                         (------*-----)
18     1.36   52.77  104.18                   (------*-----)
21   -42.16    9.26   60.67              (-----*------)
24    -8.94   42.48   93.89                  (-----*------)
28   -21.06   30.35   81.77                (------*-----)
                           ---------+---------+---------+---------+
                                  -80         0        80       160


day = 10 subtracted from:

day   Lower  Center   Upper  ---------+---------+---------+---------+
14    -1.89   49.52  100.94                   (-----*------)
18   -50.43    0.98   52.40              (-----*------)
21   -93.94  -42.53    8.88         (------*-----)
24   -60.72   -9.31   42.10             (------*-----)
28   -72.85  -21.43   29.98           (-----*------)
                           ---------+---------+---------+---------+
                                  -80         0        80       160
day = 14 subtracted from:

day    Lower  Center   Upper  ---------+---------+---------+---------+
18    -99.95  -48.54    2.87         (-----*-----)
21   -143.47  -92.05  -40.64   (-----*------)
24   -110.25  -58.83   -7.42       (------*-----)
28   -122.37  -70.96  -19.54     (-----*------)
                            ---------+---------+---------+---------+
                                   -80         0        80       160


day = 18 subtracted from:

day   Lower  Center  Upper  ---------+---------+---------+---------+
21   -94.93  -43.51   7.90         (------*-----)
24   -61.71  -10.29  41.12             (------*-----)
28   -73.83  -22.42  29.00           (-----*------)
                           ---------+---------+---------+---------+
                                  -80         0        80       160


day = 21 subtracted from:

day   Lower  Center  Upper  ---------+---------+---------+---------+
24   -18.19   33.22  84.63                  (-----*------)
28   -30.32   21.10  72.51                (------*-----)
                           ---------+---------+---------+---------+
                                  -80         0        80       160

day = 24 subtracted from:

day   Lower  Center  Upper  ---------+---------+---------+---------+
28   -63.54  -12.12  39.29            (-----*------)
                           ---------+---------+---------+---------+
                                  -80         0        80       160
```

General Linear Model: T_2 Relaxation Time versus Day (for green tea extract loaded liposomes prepared in distilled water by microfluidization.)

One-way ANOVA: T_2 Relaxation Time versus day

```
Source  DF      SS    MS     F      P
day      9   83061  9229  6.27  0.000
Error   20   29459  1473
Total   29  112519

S = 38.38   R-Sq = 73.82%   R-Sq(adj) = 62.04%

                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev  ---------+---------+---------+---------+
 0     3  1612.9   35.5       (-----*----)
 3     3  1670.1    9.4             (-----*-----)
 5     3  1629.6   23.6         (-----*----)
 7     3  1573.0   94.9  (-----*----)
10     3  1699.4   14.0                  (----*-----)
14     3  1770.8   19.2                           (----*-----)
18     3  1695.1   12.5                (-----*-----)
21     3  1663.3   32.5             (-----*-----)
24     3  1695.7   38.1                (-----*-----)
28     3  1633.4   24.4         (-----*-----)
                          ---------+---------+---------+---------+
                                1600      1680      1760      1840
Pooled StDev = 38.4


Grouping Information Using Tukey Method

day  N     Mean  Grouping
14   3  1770.80  A
10   3  1699.43  A B
24   3  1695.67  A B
18   3  1695.14  A B
 3   3  1670.11  A B C
21   3  1663.32  A B C
28   3  1633.36    B C
 5   3  1629.56    B C
 0   3  1612.86    B C
 7   3  1573.04      C

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of day

Individual confidence level = 99.80%
```

```
day =  0 subtracted from:

day    Lower  Center   Upper  ---------+---------+---------+---------+
 3    -53.76   57.25  168.26                (------*------)
 5    -94.31   16.71  127.72             (------*------)
 7   -150.83  -39.82   71.19          (------*-----)
10    -24.44   86.57  197.58                (------*------)
14     46.94  157.95  268.96                     (------*------)
18    -28.73   82.28  193.29                (------*------)
21    -60.55   50.46  161.47             (------*------)
24    -28.20   82.82  193.83                (------*------)
28    -90.51   20.50  131.51             (------*------)
                          ---------+---------+---------+---------+
                                -160         0       160       320


day =  3 subtracted from:

day    Lower  Center   Upper  ---------+---------+---------+---------+
 5   -151.56  -40.54   70.47          (-----*------)
 7   -208.08  -97.07   13.94       (------*------)
10    -81.69   29.32  140.33             (------*------)
14    -10.31  100.70  211.71                 (------*------)
18    -85.98   25.03  136.04             (------*------)
21   -117.80   -6.79  104.22          (------*------)
24    -85.45   25.56  136.58             (------*------)
28   -147.76  -36.75   74.26          (------*------)
                          ---------+---------+---------+---------+
                                -160         0       160       320

day =  5 subtracted from:

day    Lower  Center   Upper  ---------+---------+---------+---------+
 7   -167.54  -56.53   54.48         (-----*------)
10    -41.15   69.86  180.88                (------*------)
14     30.23  141.24  252.25                     (------*------)
18    -45.44   65.58  176.59                (------*------)
21    -77.26   33.76  144.77             (------*------)
24    -44.90   66.11  177.12                (------*------)
28   -107.22    3.80  114.81          (------*------)
                          ---------+---------+---------+---------+
                                -160         0       160       320
day =  7 subtracted from:

day   Lower  Center   Upper  ---------+---------+---------+---------+
10    15.38  126.39  237.40                   (------*------)
14    86.76  197.77  308.78                       (------*------)
18    11.09  122.10  233.11                   (------*------)
21   -20.73   90.28  201.29                (------*------)
24    11.62  122.64  233.65                   (------*------)
28   -50.69   60.32  171.33                (------*------)
                          ---------+---------+---------+---------+
                                -160         0       160       320
```

```
day = 10 subtracted from:

day    Lower  Center   Upper  ---------+---------+---------+---------+
14    -39.63   71.38  182.39                  (-----*------)
18   -115.30   -4.29  106.72              (------*------)
21   -147.12  -36.11   74.90            (------*------)
24   -114.77   -3.76  107.26              (------*------)
28   -177.08  -66.07   44.94          (------*------)
                              ---------+---------+---------+---------+
                                    -160         0       160       320


day = 14 subtracted from:

day    Lower   Center   Upper  ---------+---------+---------+---------+
18   -186.68   -75.67   35.34         (------*------)
21   -218.50  -107.49    3.52       (------*------)
24   -186.15   -75.13   35.88         (------*------)
28   -248.46  -137.45  -26.44     (------*------)
                               ---------+---------+---------+---------+
                                     -160         0       160       320


day = 18 subtracted from:

day    Lower  Center   Upper  ---------+---------+---------+---------+
21   -142.83  -31.82   79.19           (------*------)
24   -110.48    0.53  111.55             (------*------)
28   -172.79  -61.78   49.23          (------*------)
                              ---------+---------+---------+---------+
                                    -160         0       160       320


day = 21 subtracted from:

day    Lower  Center   Upper  ---------+---------+---------+---------+
24    -78.66   32.35  143.36               (------*------)
28   -140.97  -29.96   81.05           (------*------)
                              ---------+---------+---------+---------+
                                    -160         0       160       320


day = 24 subtracted from:

day    Lower  Center  Upper  ---------+---------+---------+---------+
28   -173.32  -62.31  48.70          (------*------)
                              ---------+---------+---------+---------+
                                    -160         0       160       320
```

General Linear Model: T_2 Relaxation Time versus Day (for unloaded liposomes prepared in acetate buffer by microfluidization.)

One-way ANOVA: T_2 Relaxation Time versus day

```
Source  DF     SS    MS      F      P
day      9  58744  6527  20.82  0.000
Error   20   6270   313
Total   29  65013

S = 17.71   R-Sq = 90.36%   R-Sq(adj) = 86.02%


                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean  StDev   -+---------+---------+---------+--------
 0     3  1017.1   16.2                   (---*--)
 3     3  1021.5   10.8                    (--*---)
 5     3   975.8    9.7             (---*--)
 7     3   916.3   37.7   (---*--)
10     3  1030.1    6.1                     (---*--)
14     3  1095.3    1.2                               (---*--)
18     3  1039.0   22.9                       (--*---)
21     3  1015.3   21.5                   (--*---)
24     3  1046.7   13.4                        (--*---)
28     3  1008.7    5.5                  (--*---)
                        -+---------+---------+---------+--------
                        900       960      1020      1080

Pooled StDev = 17.7


Grouping Information Using Tukey Method

day  N     Mean  Grouping
14   3  1095.27  A
24   3  1046.75  A B
18   3  1038.97    B
10   3  1030.11    B
 3   3  1021.53    B C
 0   3  1017.09    B C
21   3  1015.31    B C
28   3  1008.66    B C
 5   3   975.84      C
 7   3   916.35        D

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of day

Individual confidence level = 99.80%
```

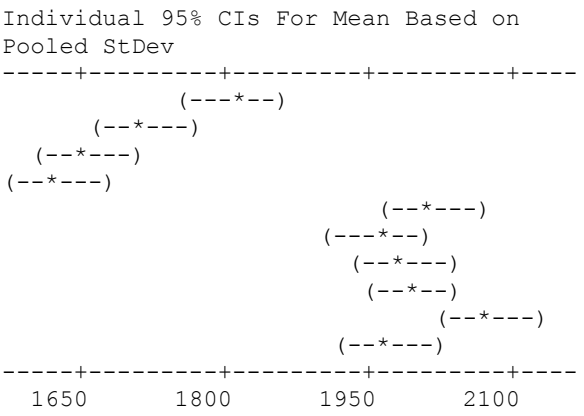
```
day =  0 subtracted from:

day    Lower   Center   Upper  ---------+---------+---------+---------+
 3    -46.78     4.44   55.65               (---*----)
 5    -92.47   -41.26    9.96          (----*---)
 7   -151.96  -100.74  -49.53       (----*---)
10    -38.20    13.02   64.23                (---*---)
14     26.96    78.17  129.39                     (----*---)
18    -29.34    21.87   73.09                 (---*---)
21    -53.00    -1.78   49.43               (---*---)
24    -21.56    29.66   80.87                 (---*----)
28    -59.64    -8.43   42.79              (---*----)
                          ---------+---------+---------+---------+
                                -120         0       120       240

day =  3 subtracted from:

day    Lower   Center   Upper  ---------+---------+---------+---------+
 5    -96.90   -45.69    5.52            (---*---)
 7   -156.39  -105.18  -53.97       (---*----)
10    -42.63     8.58   59.79                (----*---)
14     22.52    73.74  124.95                     (---*---)
18    -33.78    17.44   68.65                (---*----)
21    -57.43    -6.22   45.00              (---*----)
24    -25.99    25.22   76.43                 (---*---)
28    -64.08   -12.86   38.35              (---*---)
                          ---------+---------+---------+---------+
                                -120         0       120       240

day =  5 subtracted from:

day    Lower  Center   Upper  ---------+---------+---------+---------+
 7   -110.70  -59.49   -8.28           (---*---)
10      3.06   54.27  105.49                    (----*---)
14     68.22  119.43  170.64                          (---*---)
18     11.92   63.13  114.34                    (---*----)
21    -11.74   39.47   90.69                  (---*----)
24     19.70   70.91  122.13                     (---*---)
28    -18.39   32.83   84.04                  (----*---)
                           ---------+---------+---------+---------+
                                 -120         0       120       240

day =  7 subtracted from:

day   Lower  Center   Upper  ---------+---------+---------+---------+
10    62.55  113.76  164.97                         (---*----)
14   127.70  178.92  230.13                               (---*---)
18    71.40  122.62  173.83                          (---*---)
21    47.75   98.96  150.17                        (---*----)
24    79.19  130.40  181.61                           (---*---)
28    41.10   92.32  143.53                       (----*---)
                           ---------+---------+---------+---------+
                                 -120         0       120       240
```

```
day = 10 subtracted from:

day   Lower  Center   Upper  ---------+---------+---------+---------+
14    13.94   65.16  116.37                    (---*----)
18   -42.36    8.86   60.07                 (----*---)
21   -66.01  -14.80   36.41              (----*---)
24   -34.57   16.64   67.85                 (---*----)
28   -72.66  -21.44   29.77              (---*---)
                            ---------+---------+---------+---------+
                                  -120         0       120       240

day = 14 subtracted from:

day    Lower  Center   Upper  ---------+---------+---------+---------+
18   -107.51  -56.30   -5.09           (---*----)
21   -131.17  -79.96  -28.74         (---*----)
24    -99.73  -48.52    2.70            (---*---)
28   -137.81  -86.60  -35.39         (---*---)
                             ---------+---------+---------+---------+
                                   -120         0       120       240
day = 18 subtracted from:

day   Lower  Center  Upper  ---------+---------+---------+---------+
21   -74.87  -23.66  27.56             (---*---)
24   -43.43    7.78  59.00               (----*---)
28   -81.51  -30.30  20.91            (---*----)
                           ---------+---------+---------+---------+
                                 -120         0       120       240


day = 21 subtracted from:

day   Lower  Center  Upper  ---------+---------+---------+---------+
24   -19.77   31.44  82.65                  (----*---)
28   -57.86   -6.65  44.57               (---*----)
                           ---------+---------+---------+---------+
                                 -120         0       120       240


day = 24 subtracted from:

day   Lower  Center  Upper  ---------+---------+---------+---------+
28   -89.30  -38.08  13.13             (---*---)
                           ---------+---------+---------+---------+
                                 -120         0       120       240
```

General Linear Model: T_2 Relaxation Time versus Day (for unloaded liposomes prepared in distilled water by microfluidization.)

One-way ANOVA: T_2 Relaxation Time versus day

```
Source  DF      SS     MS      F      P
day      9  729758  81084  43.86  0.000
Error   20   36972   1849
Total   29  766730

S = 43.00   R-Sq = 95.18%   R-Sq(adj) = 93.01%
```




```
Pooled StDev = 43.0


Grouping Information Using Tukey Method

day  N     Mean  Grouping
24   3  2076.39  A
10   3  2015.29  A
21   3  1994.64  A
18   3  1983.80  A
28   3  1969.67  A
14   3  1963.09  A
 0   3  1810.06    B
 3   3  1714.34    B C
 5   3  1653.59      C
 7   3  1627.16      C

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of day

Individual confidence level = 99.80%
```

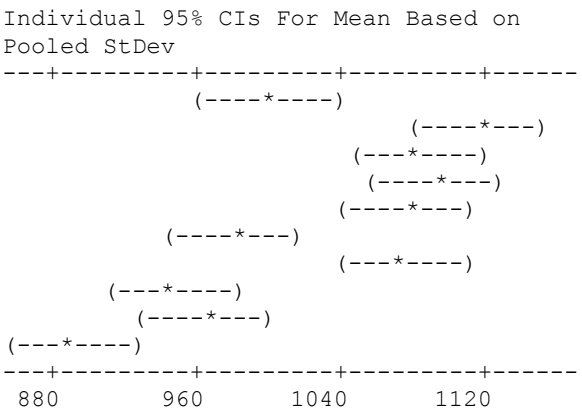
```
day =  0 subtracted from:

day    Lower   Center   Upper  ---------+---------+---------+---------+
 3   -220.09   -95.73   28.64              (---*---)
 5   -280.84  -156.48  -32.11            (---*---)
 7   -307.27  -182.90  -58.54           (---*---)
10     80.87   205.23  329.60                      (---*---)
14     28.66   153.03  277.39                    (---*---)
18     49.37   173.74  298.10                     (---*---)
21     60.21   184.58  308.94                     (---*---)
24    141.96   266.32  390.69                        (---*---)
28     35.24   159.60  283.97                    (---*---)
                              ---------+---------+---------+---------+
                                    -300         0       300       600


day =  3 subtracted from:

day    Lower  Center   Upper  ---------+---------+---------+---------+
 5   -185.12  -60.75   63.62              (---*---)
 7   -211.54  -87.17   37.19             (---*---)
10    176.59  300.96  425.32                         (---*---)
14    124.39  248.75  373.12                       (---*---)
18    145.10  269.46  393.83                        (---*---)
21    155.94  280.30  404.67                        (---*---)
24    237.68  362.05  486.42                           (---*---)
28    130.97  255.33  379.70                       (----*---)
                           ---------+---------+---------+---------+
                                 -300         0       300       600


day =  5 subtracted from:

day    Lower  Center   Upper  ---------+---------+---------+---------+
 7   -150.79  -26.42   97.94               (---*---)
10    237.34  361.71  486.07                           (---*---)
14    185.14  309.50  433.87                         (---*---)
18    205.85  330.21  454.58                          (---*---)
21    216.69  341.05  465.42                          (---*----)
24    298.43  422.80  547.16                             (---*---)
28    191.72  316.08  440.45                         (----*---)
                           ---------+---------+---------+---------+
                                 -300         0       300       600


day =  7 subtracted from:

day   Lower  Center   Upper  ---------+---------+---------+---------+
10   263.77  388.13  512.50                           (---*---)
14   211.56  335.93  460.29                         (---*---)
18   232.27  356.64  481.00                          (---*---)
21   243.11  367.48  491.84                          (---*---)
24   324.86  449.22  573.59                             (---*---)
28   218.14  342.51  466.87                         (---*----)
                          ---------+---------+---------+---------+
                                -300         0       300       600
```

```
day = 10 subtracted from:

day    Lower  Center   Upper  ---------+---------+---------+---------+
14   -176.57  -52.21   72.16              (---*---)
18   -155.86  -31.49   92.87               (---*---)
21   -145.02  -20.66  103.71               (---*---)
24    -63.27   61.09  185.46                  (---*---)
28   -169.99  -45.63   78.74              (---*----)
                               ---------+---------+---------+---------+
                                      -300         0       300       600


day = 14 subtracted from:

day    Lower  Center   Upper  ---------+---------+---------+---------+
18   -103.65   20.71  145.08                (---*---)
21    -92.81   31.55  155.92                (---*---)
24    -11.07  113.30  237.66                   (---*---)
28   -117.79    6.58  130.94               (---*---)
                               ---------+---------+---------+---------+
                                      -300         0       300       600


day = 18 subtracted from:

day    Lower  Center   Upper  ---------+---------+---------+---------+
21   -113.53   10.84  135.20               (---*----)
24    -31.78   92.59  216.95                  (---*---)
28   -138.50  -14.13  110.23              (----*---)
                               ---------+---------+---------+---------+
                                      -300         0       300       600


day = 21 subtracted from:

day    Lower  Center   Upper  ---------+---------+---------+---------+
24    -42.62   81.75  206.11                  (---*---)
28   -149.34  -24.97   99.39              (---*---)
                               ---------+---------+---------+---------+
                                      -300         0       300       600


day = 24 subtracted from:

day    Lower   Center  Upper  ---------+---------+---------+---------+
28   -231.08  -106.72  17.65           (---*----)
                               ---------+---------+---------+---------+
                                      -300         0       300       600
```

General Linear Model: T_2 Relaxation Time versus Day (for green tea extract loaded liposomes prepared in acetate buffer by ultrasonication.)

One-way ANOVA: T_2 Relaxation Time versus day

```
Source  DF      SS     MS      F      P
day      9  155743  17305  18.74  0.000
Error   20   18471    924
Total   29  174215

S = 30.39   R-Sq = 89.40%   R-Sq(adj) = 84.63%
```




```
Pooled StDev = 30.4


Grouping Information Using Tukey Method

day  N     Mean  Grouping
 3   3  1116.60  A
 7   3  1092.25  A
 5   3  1082.85  A B
10   3  1077.07  A B
18   3  1072.77  A B
 0   3  1000.29    B C
14   3   980.68      C
24   3   966.59      C D
21   3   945.42      C D
28   3   889.31        D

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of day

Individual confidence level = 99.80%
```

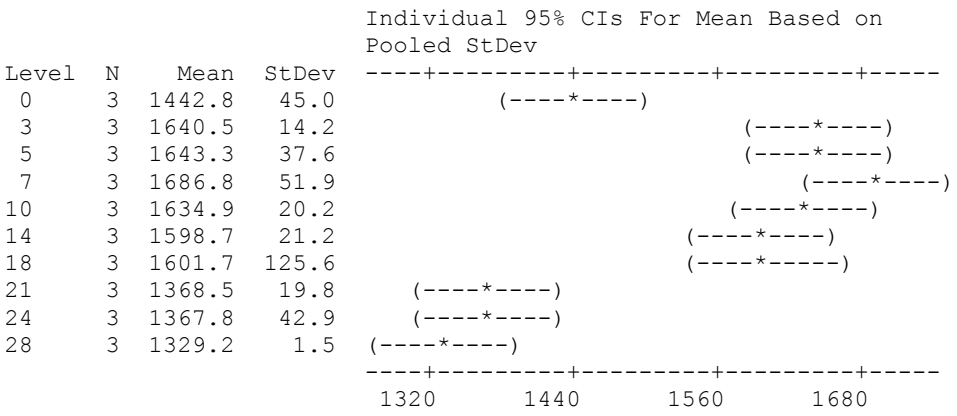
```
day =  0 subtracted from:

day    Lower   Center   Upper     +---------+---------+---------+---------
 3     28.40   116.30  204.21                        (----*-----)
 5     -5.35    82.56  170.46                      (----*-----)
 7      4.05    91.96  179.86                      (-----*----)
10    -11.13    76.78  164.68                     (-----*----)
14   -107.52   -19.62   68.29                (-----*----)
18    -15.43    72.48  160.38                     (-----*----)
21   -142.78   -54.88   33.03             (-----*----)
24   -121.61   -33.71   54.20              (-----*----)
28   -198.88  -110.98  -23.07          (----*-----)
                                  +---------+---------+---------+---------
                               -320      -160         0       160


day =  3 subtracted from:

day    Lower   Center    Upper     +---------+---------+---------+---------
 5   -121.65   -33.75    54.16              (-----*----)
 7   -112.25   -24.35    63.56               (----*-----)
10   -127.43   -39.52    48.38              (-----*----)
14   -223.82  -135.92   -48.01          (-----*----)
18   -131.73   -43.82    44.08              (----*-----)
21   -259.08  -171.18   -83.27         (----*-----)
24   -237.91  -150.01   -62.10          (-----*----)
28   -315.18  -227.28  -139.38     (-----*----)
                                   +---------+---------+---------+---------
                                -320      -160         0       160


day =  5 subtracted from:

day    Lower   Center    Upper     +---------+---------+---------+---------
 7    -78.50     9.40    97.30                  (-----*----)
10    -93.68    -5.78    82.13                (-----*----)
14   -190.08  -102.17   -14.27            (-----*----)
18    -97.98   -10.08    77.83                (----*-----)
21   -225.33  -137.43   -49.53          (----*-----)
24   -204.17  -116.26   -28.36           (-----*----)
28   -281.44  -193.53  -105.63       (-----*----)
                                   +---------+---------+---------+---------
                                -320      -160         0       160


day =  7 subtracted from:

day    Lower   Center    Upper     +---------+---------+---------+---------
10   -103.08   -15.18    72.73                (----*-----)
14   -199.48  -111.57   -23.67            (----*-----)
18   -107.38   -19.48    68.43                 (-----*----)
21   -234.73  -146.83   -58.93          (-----*----)
24   -213.57  -125.66   -37.76            (----*-----)
28   -290.84  -202.93  -115.03       (----*-----)
                                   +---------+---------+---------+---------
                                -320      -160         0       160
```

```
day = 10 subtracted from:

day    Lower   Center   Upper     +---------+---------+---------+---------
14   -184.30   -96.39   -8.49              (-----*----)
18    -92.20    -4.30   83.60                    (-----*----)
21   -219.56  -131.65  -43.75           (-----*----)
24   -198.39  -110.48  -22.58             (----*-----)
28   -275.66  -187.76  -99.85         (----*-----)
                                +---------+---------+---------+---------
                              -320      -160         0       160

day = 14 subtracted from:

day    Lower  Center   Upper     +---------+---------+---------+---------
18      4.19   92.09  180.00                       (-----*----)
21   -123.16  -35.26   52.65                (-----*----)
24   -101.99  -14.09   73.81                  (----*-----)
28   -179.27  -91.36   -3.46              (----*-----)
                              +---------+---------+---------+---------
                            -320      -160         0       160
day = 18 subtracted from:

day    Lower   Center   Upper     +---------+---------+---------+---------
21   -215.26  -127.35  -39.45            (----*-----)
24   -194.09  -106.18  -18.28             (----*-----)
28   -271.36  -183.46  -95.55         (-----*----)
                               +---------+---------+---------+---------
                             -320      -160         0       160


day = 21 subtracted from:

day    Lower  Center   Upper     +---------+---------+---------+---------
24    -66.74   21.17  109.07                   (----*-----)
28   -144.01  -56.10   31.80               (----*-----)
                              +---------+---------+---------+---------
                            -320      -160         0       160


day = 24 subtracted from:

day    Lower  Center  Upper     +---------+---------+---------+---------
28   -165.18  -77.27  10.63              (----*-----)
                             +---------+---------+---------+---------
                           -320      -160         0       160
```

General Linear Model: T_2 Relaxation Time versus Day (for green tea extract loaded liposomes prepared in distilled water by ultrasonication.)

One-way ANOVA: T_2 Relaxation Time versus day

```
Source  DF      SS     MS      F      P
day      9  512323  56925  22.58  0.000
Error   20   50415   2521
Total   29  562738
S = 50.21   R-Sq = 91.04%   R-Sq(adj) = 87.01%
```




```
Pooled StDev = 50.2
```

```
Grouping Information Using Tukey Method

day  N     Mean  Grouping
 7   3  1686.79  A
 5   3  1643.28  A
 3   3  1640.51  A
10   3  1634.90  A
18   3  1601.68  A
14   3  1598.75  A
 0   3  1442.78    B
21   3  1368.49    B
24   3  1367.81    B
28   3  1329.25    B

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of day

Individual confidence level = 99.80%
```

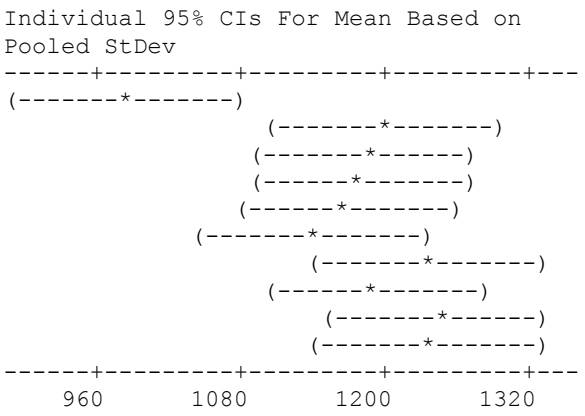
```
day =  0 subtracted from:

day    Lower   Center   Upper  -------+---------+---------+---------+--
 3     52.50   197.73  342.95                    (----*---)
 5     55.27   200.49  345.72                    (----*----)
 7     98.78   244.01  389.23                     (----*----)
10     46.89   192.12  337.34                    (---*----)
14     10.74   155.97  301.19                  (----*----)
18     13.68   158.90  304.13                  (----*----)
21   -219.51   -74.29   70.94           (----*---)
24   -220.20   -74.98   70.25           (----*---)
28   -258.76  -113.53   31.69         (----*----)
                                -------+---------+---------+---------+--
                                    -300         0       300       600


day =  3 subtracted from:

day    Lower   Center    Upper  -------+---------+---------+---------+--
 5   -142.46     2.76   147.99              (----*----)
 7    -98.95    46.28   191.50                (----*---)
10   -150.84    -5.61   139.61              (----*----)
14   -186.99   -41.76   103.46            (----*---)
18   -184.05   -38.83   106.40            (----*----)
21   -417.24  -272.02  -126.79     (----*----)
24   -417.93  -272.71  -127.48     (----*----)
28   -456.49  -311.26  -166.04    (----*---)
                               -------+---------+---------+---------+--
                                   -300         0       300       600


day =  5 subtracted from:

day    Lower   Center    Upper  -------+---------+---------+---------+--
 7   -101.71    43.51   188.74               (---*----)
10   -153.60    -8.38   136.85              (----*----)
14   -189.75   -44.53   100.70            (----*---)
18   -186.82   -41.59   103.63            (----*---)
21   -420.01  -274.78  -129.56     (----*----)
24   -420.69  -275.47  -130.24     (----*----)
28   -459.25  -314.03  -168.80    (----*---)
                               -------+---------+---------+---------+--
                                   -300         0       300       600


day =  7 subtracted from:

day    Lower   Center    Upper  -------+---------+---------+---------+--
10   -197.12   -51.89    93.33           (----*----)
14   -233.26   -88.04    57.19          (----*----)
18   -230.33   -85.11    60.12          (----*----)
21   -463.52  -318.30  -173.07    (---*----)
24   -464.21  -318.98  -173.76    (---*----)
28   -502.76  -357.54  -212.31  (----*----)
                               -------+---------+---------+---------+--
                                   -300         0       300       600
```

```
day = 10 subtracted from:

day    Lower   Center    Upper  -------+---------+---------+---------+--
14   -181.37   -36.15   109.08            (----*----)
18   -178.44   -33.22   112.01            (----*----)
21   -411.63  -266.41  -121.18     (----*----)
24   -412.32  -267.09  -121.87     (----*----)
28   -450.87  -305.65  -160.42    (----*----)
                                -------+---------+---------+---------+--
                                    -300         0       300       600

day = 14 subtracted from:

day    Lower   Center    Upper  -------+---------+---------+---------+--
18   -142.29     2.93   148.16             (----*----)
21   -375.48  -230.26   -85.03      (----*----)
24   -376.17  -230.94   -85.72      (----*----)
28   -414.72  -269.50  -124.27     (----*----)
                                -------+---------+---------+---------+--
                                    -300         0       300       600
day = 18 subtracted from:

day    Lower   Center    Upper  -------+---------+---------+---------+--
21   -378.41  -233.19   -87.96      (----*----)
24   -379.10  -233.88   -88.65      (----*----)
28   -417.66  -272.43  -127.21     (----*----)
                                -------+---------+---------+---------+--
                                    -300         0       300       600


day = 21 subtracted from:

day    Lower  Center   Upper  -------+---------+---------+---------+--
24   -145.91   -0.69  144.54             (----*----)
28   -184.47  -39.24  105.98           (----*----)
                               -------+---------+---------+---------+--
                                   -300         0       300       600


day = 24 subtracted from:

day    Lower  Center   Upper  -------+---------+---------+---------+--
28   -183.78  -38.56  106.67           (----*----)
                               -------+---------+---------+---------+--
                                   -300         0       300       600
```

General Linear Model: T_2 Relaxation Time versus Day (for unloaded liposomes prepared in acetate buffer by ultrasonication.)

One-way ANOVA: T_2 Relaxation Time versus day

```
Source  DF      SS     MS     F      P
day      9  151918  16880  2.80  0.027
Error   20  120743   6037
Total   29  272661

S = 77.70   R-Sq = 55.72%   R-Sq(adj) = 35.79%
```




```
Level  N    Mean  StDev
 0     3   984.1  124.5
 3     3  1202.9   16.9
 5     3  1183.5   25.8
 7     3  1180.0    8.6
10     3  1169.6   17.5
14     3  1141.6   95.0
18     3  1233.9   76.1
21     3  1192.1  108.6
24     3  1243.4   83.7
28     3  1236.5   99.6

Pooled StDev = 77.7
Grouping Information Using Tukey Method

day  N     Mean  Grouping
24   3  1243.41  A
28   3  1236.49  A
18   3  1233.92  A
 3   3  1202.94  A B
21   3  1192.05  A B
 5   3  1183.48  A B
 7   3  1180.05  A B
10   3  1169.64  A B
14   3  1141.62  A B
 0   3   984.14    B

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of day

Individual confidence level = 99.80%
```

```
day =  0 subtracted from:

day   Lower  Center   Upper  ---+---------+---------+---------+------
 3    -5.95  218.80  443.54              (--------*--------)
 5   -25.41  199.34  424.09             (--------*--------)
 7   -28.84  195.91  420.65             (--------*--------)
10   -39.25  185.50  410.24            (--------*--------)
14   -67.26  157.48  382.23           (--------*--------)
18    25.03  249.78  474.52               (--------*--------)
21   -16.83  207.91  432.66             (--------*--------)
24    34.53  259.27  484.02               (--------*--------)
28    27.60  252.35  477.09               (--------*--------)
                           ---+---------+---------+---------+------
                          -250         0       250       500


day =  3 subtracted from:

day    Lower  Center   Upper  ---+---------+---------+---------+------
 5   -244.20  -19.45  205.29     (--------*--------)
 7   -247.63  -22.89  201.86     (--------*--------)
10   -258.05  -33.30  191.45     (--------*--------)
14   -286.06  -61.31  163.43    (--------*--------)
18   -193.77   30.98  255.73       (--------*--------)
21   -235.63  -10.88  213.86      (--------*--------)
24   -184.27   40.48  265.22        (--------*--------)
28   -191.20   33.55  258.30      (--------*--------)
                           ---+---------+---------+---------+------
                          -250         0       250       500


day =  5 subtracted from:

day    Lower  Center   Upper  ---+---------+---------+---------+------
 7   -228.18   -3.43  221.31      (--------*--------)
10   -238.59  -13.84  210.90     (--------*--------)
14   -266.61  -41.86  182.89    (--------*--------)
18   -174.31   50.43  275.18        (--------*--------)
21   -216.18    8.57  233.32      (--------*--------)
24   -164.82   59.93  284.68        (--------*--------)
28   -171.74   53.01  277.75        (--------*--------)
                           ---+---------+---------+---------+------
                          -250         0       250       500


day =  7 subtracted from:

day    Lower  Center   Upper  ---+---------+---------+---------+------
10   -235.16  -10.41  214.33      (--------*--------)
14   -263.17  -38.43  186.32    (--------*--------)
18   -170.88   53.87  278.61        (--------*--------)
21   -212.74   12.00  236.75      (--------*--------)
24   -161.38   63.36  288.11         (--------*--------)
28   -168.31   56.44  281.18        (--------*--------)
                           ---+---------+---------+---------+------
                          -250         0       250       500
```

```
day = 10 subtracted from:

day    Lower  Center   Upper  ---+---------+---------+---------+------
14   -252.76  -28.01  196.73     (--------*--------)
18   -160.47   64.28  289.03         (--------*--------)
21   -202.33   22.42  247.16       (--------*--------)
24   -150.97   73.78  298.52         (--------*--------)
28   -157.90   66.85  291.60         (--------*--------)
                           ---+---------+---------+---------+------
                           -250         0       250       500
day = 14 subtracted from:

day    Lower  Center   Upper  ---+---------+---------+---------+------
18   -132.45   92.29  317.04          (--------*--------)
21   -174.32   50.43  275.18        (--------*--------)
24   -122.96  101.79  326.54          (--------*--------)
28   -129.88   94.86  319.61          (--------*--------)
                           ---+---------+---------+---------+------
                           -250         0       250       500


day = 18 subtracted from:

day    Lower  Center   Upper  ---+---------+---------+---------+------
21   -266.61  -41.86  182.88    (--------*--------)
24   -215.25    9.50  234.24      (--------*--------)
28   -222.18    2.57  227.32      (--------*--------)
                           ---+---------+---------+---------+------
                           -250         0       250       500


day = 21 subtracted from:

day    Lower  Center   Upper  ---+---------+---------+---------+------
24   -173.39   51.36  276.11        (--------*--------)
28   -180.31   44.43  269.18        (--------*--------)
                           ---+---------+---------+---------+------
                           -250         0       250       500

day = 24 subtracted from:

day    Lower  Center   Upper  ---+---------+---------+---------+------
28   -231.67   -6.93  217.82      (--------*--------)
                           ---+---------+---------+---------+------
                           -250         0       250       500
```

General Linear Model: T_2 Relaxation Time versus Day (for unloaded liposomes prepared in distilled water by ultrasonication.)

One-way ANOVA: T_2 Relaxation Time versus day

```
Source  DF      SS     MS     F      P
day      9  157386  17487  2.87  0.024
Error   20  121759   6088
Total   29  279146

S = 78.03   R-Sq = 56.38%   R-Sq(adj) = 36.75%
```

```
                          Individual 95% CIs For Mean Based on Pooled StDev
Level  N    Mean  StDev     +---------+---------+---------+---------
 0     3  1651.4   53.8     (-------*------)
 3     3  1835.6   25.8                (-------*-------)
 5     3  1844.0   70.2                 (-------*------)
 7     3  1922.6   34.5                       (-------*-------)
10     3  1853.6    9.5                  (------*-------)
14     3  1858.3   59.1                  (-------*-------)
18     3  1897.0   57.2                     (-------*-------)
21     3  1778.3   83.3              (-------*-------)
24     3  1858.4   90.7                     (-------*-------)
28     3  1780.2  170.8               (------*-------)
                                     +---------+---------+---------+---------
                                    1560      1680      1800      1920
Pooled StDev = 78.0


Grouping Information Using Tukey Method

day  N     Mean  Grouping
 7   3  1922.60  A
18   3  1896.95  A
24   3  1858.44  A B
14   3  1858.25  A B
10   3  1853.61  A B
 5   3  1844.03  A B
 3   3  1835.62  A B
28   3  1780.21  A B
21   3  1778.31  A B
 0   3  1651.38    B
Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of day

Individual confidence level = 99.80%


day =  0 subtracted from:

day   Lower  Center   Upper  -----+---------+---------+---------+----
 3   -41.46  184.23  409.92              (--------*--------)
 5   -33.04  192.65  418.34               (--------*--------)
 7    45.53  271.22  496.91                  (--------*--------)
10   -23.46  202.23  427.92               (--------*--------)
14   -18.82  206.87  432.56               (--------*--------)
18    19.88  245.57  471.26                 (--------*--------)
21   -98.76  126.93  352.62            (--------*--------)
24   -18.63  207.06  432.75               (--------*--------)
28   -96.86  128.83  354.52            (--------*--------)
                              -----+---------+---------+---------+----
                                -250         0       250       500
```

```
day =  3 subtracted from:

day    Lower  Center   Upper  -----+---------+---------+---------+----
 5   -217.28    8.41  234.10        (--------*--------)
 7   -138.71   86.98  312.67           (--------*---------)
10   -207.70   17.99  243.68         (--------*--------)
14   -203.06   22.64  248.33         (--------*--------)
18   -164.35   61.34  287.03          (--------*--------)
21   -282.99  -57.30  168.39      (--------*--------)
24   -202.86   22.83  248.52         (--------*--------)
28   -281.09  -55.40  170.29      (--------*--------)
                             -----+---------+---------+---------+----
                               -250         0       250       500

day =  5 subtracted from:

day    Lower  Center   Upper  -----+---------+---------+---------+----
 7   -147.12   78.57  304.26           (--------*--------)
10   -216.11    9.58  235.27        (--------*--------)
14   -211.47   14.22  239.91         (--------*--------)
18   -172.77   52.92  278.61          (--------*--------)
21   -291.41  -65.72  159.97     (--------*--------)
24   -211.28   14.41  240.10         (--------*--------)
28   -289.51  -63.82  161.88     (--------*--------)
                             -----+---------+---------+---------+----
                               -250         0       250       500
day =  7 subtracted from:

day    Lower   Center   Upper  -----+---------+---------+---------+----
10   -294.68   -68.99  156.70     (--------*--------)
14   -290.04   -64.35  161.34     (--------*--------)
18   -251.34   -25.65  200.04       (--------*--------)
21   -369.98  -144.29   81.40  (--------*--------)
24   -289.84   -64.15  161.54     (--------*--------)
28   -368.07  -142.38   83.31  (--------*--------)
                              -----+---------+---------+---------+----
                                -250         0       250       500
day = 10 subtracted from:

day    Lower  Center   Upper  -----+---------+---------+---------+----
14   -221.05    4.64  230.33        (--------*--------)
18   -182.35   43.34  269.03          (--------*--------)
21   -300.99  -75.30  150.39     (--------*--------)
24   -220.86    4.83  230.52        (--------*--------)
28   -299.09  -73.40  152.30     (--------*--------)
                             -----+---------+---------+---------+----
                               -250         0       250       500


day = 14 subtracted from:

day    Lower  Center   Upper  -----+---------+---------+---------+----
18   -186.99   38.70  264.39          (--------*--------)
21   -305.63  -79.94  145.75     (--------*--------)
24   -225.50    0.19  225.88        (--------*--------)
28   -303.73  -78.04  147.65     (--------*--------)
                             -----+---------+---------+---------+----
                               -250         0       250       500
```

```
day = 18 subtracted from:

day    Lower   Center   Upper  -----+---------+---------+---------+----
21   -344.33  -118.64  107.05   (--------*--------)
24   -264.20   -38.51  187.18      (--------*--------)
28   -342.43  -116.74  108.95   (--------*--------)
                               -----+---------+---------+---------+----
                                 -250         0       250       500


day = 21 subtracted from:

day    Lower  Center   Upper  -----+---------+---------+---------+----
24   -145.56   80.13  305.82           (--------*--------)
28   -223.79    1.90  227.59        (--------*--------)
                              -----+---------+---------+---------+----
                                -250         0       250       500


day = 24 subtracted from:

day    Lower  Center   Upper  -----+---------+---------+---------+----
28   -303.92  -78.23  147.46     (--------*--------)
                              -----+---------+---------+---------+----
                                -250         0       250       500
```

General Linear Model: Time 1, Time 2, Time 3, Area 1, Area 2, Area 3 versus Day (for green tea extract loaded liposomes prepared in acetate buffer by microfluidization.)

```
Factor  Type   Levels  Values
Day     fixed      10  0, 3, 5, 7, 10, 14, 18, 21, 25, 28


Analysis of Variance for Time 1, using Adjusted SS for Tests

Source  DF     Seq SS     Adj SS     Adj MS     F      P
Day      9  0.0002566  0.0002566  0.0000285  1.24  0.325
Error   20  0.0004588  0.0004588  0.0000229
Total   29  0.0007154


S = 0.00478942   R-Sq = 35.87%   R-Sq(adj) = 7.01%


Unusual Observations for Time 1

Obs    Time 1       Fit    SE Fit  Residual  St Resid
 19  0.018000  0.008267  0.002765  0.009733      2.49 R

R denotes an observation with a large standardized residual.
```

Analysis of Variance for Time 2, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Day	9	0.010687	0.010687	0.001187	0.35	0.945
Error	20	0.067435	0.067435	0.003372		
Total	29	0.078121				

S = 0.0580666 R-Sq = 13.68% R-Sq(adj) = 0.00%

Unusual Observations for Time 2

Obs	Time 2	Fit	SE Fit	Residual	St Resid
7	0.220000	0.107333	0.033525	0.112667	2.38 R

R denotes an observation with a large standardized residual.

Analysis of Variance for Time 3, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Day	9	0.0622700	0.0622700	0.0069189	14.93	0.000
Error	20	0.0092667	0.0092667	0.0004633		
Total	29	0.0715367				

S = 0.0215252 R-Sq = 87.05% R-Sq(adj) = 81.22%

Unusual Observations for Time 3

Obs	Time 3	Fit	SE Fit	Residual	St Resid
16	1.00000	1.05000	0.01243	-0.05000	-2.84 R
17	1.10000	1.05000	0.01243	0.05000	2.84 R
25	0.92000	0.97333	0.01243	-0.05333	-3.03 R

R denotes an observation with a large standardized residual.

Analysis of Variance for Area1, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Day	9	0.0032653	0.0032653	0.0003628	2.95	0.021
Error	20	0.0024633	0.0024633	0.0001232		
Total	29	0.0057286				

S = 0.0110981 R-Sq = 57.00% R-Sq(adj) = 37.65%

Unusual Observations for Area1

Obs	Area1	Fit	SE Fit	Residual	St Resid
23	0.001781	0.025661	0.006407	-0.023880	-2.64 R

R denotes an observation with a large standardized residual.

Analysis of Variance for Area2, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Day	9	0.0007108	0.0007108	0.0000790	1.66	0.166
Error	20	0.0009530	0.0009530	0.0000477		
Total	29	0.0016639				

S = 0.00690297 R-Sq = 42.72% R-Sq(adj) = 16.95%

Unusual Observations for Area2

Obs	Area2	Fit	SE Fit	Residual	St Resid
12	0.032550	0.020269	0.003985	0.012280	2.18 R
23	0.033398	0.017112	0.003985	0.016286	2.89 R

R denotes an observation with a large standardized residual.

Analysis of Variance for Area3, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Day	9	0.027953	0.027953	0.003106	1.61	0.178
Error	20	0.038491	0.038491	0.001925		
Total	29	0.066445				

S = 0.0438699 R-Sq = 42.07% R-Sq(adj) = 16.00%

Unusual Observations for Area3

Obs	Area3	Fit	SE Fit	Residual	St Resid
17	0.961959	0.867585	0.025328	0.094374	2.63 R
18	0.773196	0.867585	0.025328	-0.094389	-2.64 R
29	0.812660	0.907883	0.025328	-0.095222	-2.66 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95.0% Confidence for Time 1

Day	N	Mean	Grouping
10	3	0.0	A
14	3	0.0	A
3	3	0.0	A
21	3	0.0	A
28	3	0.0	A
25	3	0.0	A
5	3	0.0	A
0	3	0.0	A
7	3	0.0	A
18	3	0.0	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for Time 2

Day	N	Mean	Grouping
10	3	0.1	A
5	3	0.1	A
25	3	0.1	A
14	3	0.1	A
3	3	0.1	A
7	3	0.1	A
0	3	0.1	A
21	3	0.1	A
28	3	0.1	A
18	3	0.1	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for Time 3

Day	N	Mean	Grouping
14	3	1.1	A
18	3	1.0	A B
10	3	1.0	A B
25	3	1.0	B C
28	3	0.9	C
21	3	0.9	C
5	3	0.9	C
3	3	0.9	C
0	3	0.9	C
7	3	0.9	C

Means that do not share a letter are significantly different.
Grouping Information Using Tukey Method and 95.0% Confidence for Area1

Day	N	Mean	Grouping
25	3	0.0	A
5	3	0.0	A B
0	3	0.0	A B
28	3	0.0	A B
3	3	0.0	A B
14	3	0.0	A B
7	3	0.0	A B
21	3	0.0	A B
18	3	0.0	A B
10	3	0.0	B

Means that do not share a letter are significantly different.
Grouping Information Using Tukey Method and 95.0% Confidence for Area2

Day	N	Mean	Grouping
18	3	0.0	A
7	3	0.0	A
0	3	0.0	A
21	3	0.0	A
10	3	0.0	A
25	3	0.0	A
5	3	0.0	A
28	3	0.0	A
3	3	0.0	A
14	3	0.0	A

Means that do not share a letter are significantly different.
Grouping Information Using Tukey Method and 95.0% Confidence for Area3

Day	N	Mean	Grouping
10	3	1.0	A
7	3	1.0	A
21	3	1.0	A
3	3	1.0	A
25	3	0.9	A
0	3	0.9	A
5	3	0.9	A
28	3	0.9	A
18	3	0.9	A
14	3	0.9	A

Means that do not share a letter are significantly different.

General Linear Model: Time 1, Time 2, Time 3, Area 1, Area 2, Area 3 versus Day (for green tea extract loaded liposomes prepared in distilled water by microfluidization.)

```
Factor  Type   Levels  Values
Day     fixed      10  0, 3, 5, 7, 10, 14, 18, 21, 25, 28


Analysis of Variance for Time 1, using Adjusted SS for Tests

Source  DF     Seq SS     Adj SS     Adj MS     F      P
Day      9  0.0009009  0.0009009  0.0001001  1.35  0.272
Error   20  0.0014787  0.0014787  0.0000739
Total   29  0.0023796


S = 0.00859853   R-Sq = 37.86%   R-Sq(adj) = 9.90%


Unusual Observations for Time 1

Obs    Time 1       Fit    SE Fit   Residual  St Resid
 13  0.043000  0.024567  0.004964   0.018433      2.63 R
 15  0.004700  0.024567  0.004964  -0.019867     -2.83 R

R denotes an observation with a large standardized residual.



Analysis of Variance for Time 2, using Adjusted SS for Tests

Source  DF    Seq SS    Adj SS    Adj MS     F      P
Day      9  0.057813  0.057813  0.006424  1.30  0.298
Error   20  0.099043  0.099043  0.004952
Total   29  0.156855


S = 0.0703714   R-Sq = 36.86%   R-Sq(adj) = 8.44%
Unusual Observations for Time 2

Obs    Time 2       Fit    SE Fit   Residual  St Resid
 10  0.043000  0.174333  0.040629  -0.131333     -2.29 R
 24  0.280000  0.134000  0.040629   0.146000      2.54 R

R denotes an observation with a large standardized residual.


Analysis of Variance for Time 3, using Adjusted SS for Tests

Source  DF     Seq SS     Adj SS     Adj MS      F      P
Day      9  0.0513200  0.0513200  0.0057022  26.73  0.000
Error   20  0.0042667  0.0042667  0.0002133
Total   29  0.0555867


S = 0.0146059   R-Sq = 92.32%   R-Sq(adj) = 88.87%
```

```
Unusual Observations for Time 3
Obs    Time 3      Fit   SE Fit  Residual  St Resid
  7  0.92000  0.97333  0.00843  -0.05333     -4.47 R
  8  1.00000  0.97333  0.00843   0.02667      2.24 R
  9  1.00000  0.97333  0.00843   0.02667      2.24 R

R denotes an observation with a large standardized residual.


Analysis of Variance for Area1, using Adjusted SS for Tests

Source  DF     Seq SS     Adj SS     Adj MS     F      P
Day      9  0.0024888  0.0024888  0.0002765  1.70  0.155
Error   20  0.0032532  0.0032532  0.0001627
Total   29  0.0057420


S = 0.0127538   R-Sq = 43.34%   R-Sq(adj) = 17.85%


Unusual Observations for Area1

Obs     Area1       Fit    SE Fit  Residual  St Resid
 15  0.055918  0.031574  0.007363  0.024344      2.34 R

R denotes an observation with a large standardized residual.


Analysis of Variance for Area2, using Adjusted SS for Tests

Source  DF     Seq SS     Adj SS     Adj MS     F      P
Day      9  0.0053693  0.0053693  0.0005966  5.30  0.001
Error   20  0.0022533  0.0022533  0.0001127
Total   29  0.0076226


S = 0.0106143   R-Sq = 70.44%   R-Sq(adj) = 57.14%


Unusual Observations for Area2

Obs     Area2       Fit    SE Fit   Residual  St Resid
 10  0.023182  0.057175  0.006128  -0.033994     -3.92 R
 12  0.078449  0.057175  0.006128   0.021274      2.45 R

R denotes an observation with a large standardized residual.

Analysis of Variance for Area3, using Adjusted SS for Tests

Source  DF     Seq SS     Adj SS     Adj MS     F      P
Day      9  0.0088709  0.0088709  0.0009857  3.02  0.019
Error   20  0.0065335  0.0065335  0.0003267
Total   29  0.0154043


S = 0.0180741   R-Sq = 57.59%   R-Sq(adj) = 38.50%
```

Unusual Observations for Area3

Obs	Area3	Fit	SE Fit	Residual	St Resid
10	0.954545	0.909995	0.010435	0.044550	3.02 R
12	0.876788	0.909995	0.010435	-0.033207	-2.25 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95.0% Confidence for Time 1

Day	N	Mean	Grouping
10	3	0.0	A
25	3	0.0	A
0	3	0.0	A
5	3	0.0	A
21	3	0.0	A
3	3	0.0	A
7	3	0.0	A
28	3	0.0	A
14	3	0.0	A
18	3	0.0	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for Time 2

Day	N	Mean	Grouping
7	3	0.2	A
3	3	0.2	A
10	3	0.2	A
21	3	0.1	A
0	3	0.1	A
25	3	0.1	A
14	3	0.1	A
5	3	0.1	A
28	3	0.1	A
18	3	0.1	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for Time 3

Day	N	Mean	Grouping
14	3	1.1	A
28	3	1.0	B
25	3	1.0	B
21	3	1.0	B
18	3	1.0	B
10	3	1.0	B
3	3	1.0	B
0	3	1.0	B
5	3	1.0	B
7	3	0.9	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for Area1

Day	N	Mean	Grouping
14	3	0.0	A
28	3	0.0	A
7	3	0.0	A
10	3	0.0	A
3	3	0.0	A
18	3	0.0	A
25	3	0.0	A
21	3	0.0	A
0	3	0.0	A
5	3	0.0	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for Area2

Day	N	Mean	Grouping
7	3	0.1	A
0	3	0.0	B
21	3	0.0	B
3	3	0.0	B
18	3	0.0	B
14	3	0.0	B
25	3	0.0	B
28	3	0.0	B
10	3	0.0	B
5	3	0.0	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for Area3

Day	N	Mean	Grouping
5	3	1.0	A
0	3	1.0	A B
25	3	1.0	A B
10	3	1.0	A B
21	3	1.0	A B
18	3	1.0	A B
3	3	1.0	A B
28	3	0.9	A B
14	3	0.9	A B
7	3	0.9	B

Means that do not share a letter are significantly different.

General Linear Model: Time 1, Time 2, Time 3, Area 1, Area 2, Area 3 versus Day (for unloaded liposomes prepared in acetate buffer by microfluidization.)

```
Factor  Type   Levels  Values
Day     fixed      10  0, 3, 5, 7, 10, 14, 18, 21, 25, 28


Analysis of Variance for Time 1, using Adjusted SS for Tests

Source  DF     Seq SS     Adj SS     Adj MS     F      P
Day      9  0.0004571  0.0004571  0.0000508  2.77  0.028
Error   20  0.0003665  0.0003665  0.0000183
Total   29  0.0008235


S = 0.00428050   R-Sq = 55.50%   R-Sq(adj) = 35.48%


Unusual Observations for Time 1

Obs    Time 1       Fit    SE Fit   Residual  St Resid
 20  0.033000  0.024000  0.002471   0.009000      2.58 R
 21  0.017000  0.024000  0.002471  -0.007000     -2.00 R
 25  0.009300  0.016433  0.002471  -0.007133     -2.04 R

R denotes an observation with a large standardized residual.


Analysis of Variance for Time 2, using Adjusted SS for Tests

Source  DF    Seq SS    Adj SS    Adj MS     F      P
Day      9  0.058987  0.058987  0.006554  2.60  0.036
Error   20  0.050400  0.050400  0.002520
Total   29  0.109387


S = 0.0501996   R-Sq = 53.92%   R-Sq(adj) = 33.19%


Unusual Observations for Time 2

Obs    Time 2       Fit    SE Fit   Residual  St Resid
  2  0.510000  0.363333  0.028983   0.146667      3.58 R
  3  0.280000  0.363333  0.028983  -0.083333     -2.03 R

R denotes an observation with a large standardized residual.


Analysis of Variance for Time 3, using Adjusted SS for Tests

Source  DF    Seq SS    Adj SS    Adj MS     F      P
Day      9  0.067000  0.067000  0.007444  1.86  0.119
Error   20  0.080000  0.080000  0.004000
Total   29  0.147000


S = 0.0632456   R-Sq = 45.58%   R-Sq(adj) = 21.09%
```

Unusual Observations for Time 3

Obs	Time 3	Fit	SE Fit	Residual	St Resid
7	1.50000	1.63333	0.03651	-0.13333	-2.58 R
10	1.50000	1.63333	0.03651	-0.13333	-2.58 R
28	1.50000	1.63333	0.03651	-0.13333	-2.58 R

R denotes an observation with a large standardized residual.

Analysis of Variance for Area1, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Day	9	0.0041208	0.0041208	0.0004579	2.42	0.048
Error	20	0.0037901	0.0037901	0.0001895		
Total	29	0.0079108				

S = 0.0137660 R-Sq = 52.09% R-Sq(adj) = 30.53%

Unusual Observations for Area1

Obs	Area1	Fit	SE Fit	Residual	St Resid
2	0.085526	0.057798	0.007948	0.027728	2.47 R

R denotes an observation with a large standardized residual.

Analysis of Variance for Area2, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Day	9	0.0008982	0.0008982	0.0000998	0.95	0.508
Error	20	0.0021071	0.0021071	0.0001054		
Total	29	0.0030054				

S = 0.0102643 R-Sq = 29.89% R-Sq(adj) = 0.00%

Unusual Observations for Area2

Obs	Area2	Fit	SE Fit	Residual	St Resid
10	0.074866	0.042769	0.005926	0.032097	3.83 R
12	0.025786	0.042769	0.005926	-0.016983	-2.03 R

R denotes an observation with a large standardized residual.

Analysis of Variance for Area3, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Day	9	0.0126764	0.0126764	0.0014085	1.58	0.187
Error	20	0.0177927	0.0177927	0.0008896		
Total	29	0.0304691				

S = 0.0298267 R-Sq = 41.60% R-Sq(adj) = 15.33%

Unusual Observations for Area3

Obs	Area3	Fit	SE Fit	Residual	St Resid
2	0.789474	0.875180	0.017220	-0.085706	-3.52 R
10	0.855615	0.908065	0.017220	-0.052450	-2.15 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95.0% Confidence for Time 1

Day	N	Mean	Grouping
18	3	0.0	A
14	3	0.0	A B
28	3	0.0	A B
10	3	0.0	A B
5	3	0.0	A B
25	3	0.0	A B
21	3	0.0	A B
7	3	0.0	A B
3	3	0.0	A B
0	3	0.0	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for Time 2

Day	N	Mean	Grouping
0	3	0.4	A
14	3	0.3	A B
7	3	0.3	A B
28	3	0.3	A B
21	3	0.2	A B
10	3	0.2	A B
5	3	0.2	A B
18	3	0.2	A B
25	3	0.2	B
3	3	0.2	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for Time 3

Day	N	Mean	Grouping
14	3	1.8	A
25	3	1.7	A
21	3	1.7	A
18	3	1.7	A
10	3	1.7	A
3	3	1.7	A
0	3	1.7	A
28	3	1.6	A
7	3	1.6	A
5	3	1.6	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for Area1

Day	N	Mean	Grouping
0	3	0.1	A
21	3	0.1	A
18	3	0.1	A
7	3	0.0	A
25	3	0.0	A
3	3	0.0	A
28	3	0.0	A
10	3	0.0	A
5	3	0.0	A
14	3	0.0	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for Area2

Day	N	Mean	Grouping
7	3	0.0	A
0	3	0.0	A
14	3	0.0	A
25	3	0.0	A
28	3	0.0	A
3	3	0.0	A
10	3	0.0	A
18	3	0.0	A
21	3	0.0	A
5	3	0.0	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for Area3

Day	N	Mean	Grouping
5	3	1.0	A
14	3	0.9	A
10	3	0.9	A
28	3	0.9	A
3	3	0.9	A
25	3	0.9	A
18	3	0.9	A
21	3	0.9	A
7	3	0.9	A
0	3	0.9	A

Means that do not share a letter are significantly different.

General Linear Model: Time 1, Time 2, Time 3, Area 1, Area 2, Area 3 versus Day (for unloaded liposomes prepared in distilled water by microfluidization.)

```
Factor  Type   Levels  Values
Day     fixed      10  0, 3, 5, 7, 10, 14, 18, 21, 25, 28


Analysis of Variance for Time 1, using Adjusted SS for Tests

Source  DF     Seq SS     Adj SS     Adj MS     F      P
Day      9  0.0005082  0.0005082  0.0000565  1.50  0.213
Error   20  0.0007506  0.0007506  0.0000375
Total   29  0.0012588


S = 0.00612634   R-Sq = 40.37%   R-Sq(adj) = 13.53%


Unusual Observations for Time 1

Obs    Time 1       Fit    SE Fit  Residual  St Resid
 13  0.039000  0.024333  0.003537  0.014667      2.93 R
 27  0.031000  0.020667  0.003537  0.010333      2.07 R

R denotes an observation with a large standardized residual.

Analysis of Variance for Time 2, using Adjusted SS for Tests

Source  DF    Seq SS    Adj SS    Adj MS     F      P
Day      9  0.063697  0.063697  0.007077  5.09  0.001
Error   20  0.027800  0.027800  0.001390
Total   29  0.091497


S = 0.0372827   R-Sq = 69.62%   R-Sq(adj) = 55.94%


Unusual Observations for Time 2

Obs    Time 2       Fit    SE Fit  Residual  St Resid
  4  0.360000  0.293333  0.021525  0.066667      2.19 R

R denotes an observation with a large standardized residual.


Analysis of Variance for Time 3, using Adjusted SS for Tests

Source  DF    Seq SS    Adj SS    Adj MS      F      P
Day      9  0.740333  0.740333  0.082259  15.42  0.000
Error   20  0.106667  0.106667  0.005333
Total   29  0.847000


S = 0.0730297   R-Sq = 87.41%   R-Sq(adj) = 81.74%
```

Unusual Observations for Time 3

Obs	Time 3	Fit	SE Fit	Residual	St Resid
11	1.50000	1.63333	0.04216	-0.13333	-2.24 R
13	2.20000	2.06667	0.04216	0.13333	2.24 R
25	2.20000	2.06667	0.04216	0.13333	2.24 R
29	1.80000	1.93333	0.04216	-0.13333	-2.24 R

R denotes an observation with a large standardized residual.

Analysis of Variance for Area1, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Day	9	0.0015770	0.0015770	0.0001752	0.70	0.698
Error	20	0.0049712	0.0049712	0.0002486		
Total	29	0.0065482				

S = 0.0157658 R-Sq = 24.08% R-Sq(adj) = 0.00%

Unusual Observations for Area1

Obs	Area1	Fit	SE Fit	Residual	St Resid
5	0.082961	0.050172	0.009102	0.032789	2.55 R

R denotes an observation with a large standardized residual.

Analysis of Variance for Area2, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Day	9	0.0006428	0.0006428	0.0000714	0.50	0.856
Error	20	0.0028478	0.0028478	0.0001424		
Total	29	0.0034907				

S = 0.0119328 R-Sq = 18.42% R-Sq(adj) = 0.00%

Unusual Observations for Area2

Obs	Area2	Fit	SE Fit	Residual	St Resid
11	0.060664	0.034949	0.006889	0.025715	2.64 R

R denotes an observation with a large standardized residual.

Analysis of Variance for Area3, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Day	9	0.0031066	0.0031066	0.0003452	0.95	0.506
Error	20	0.0072598	0.0072598	0.0003630		
Total	29	0.0103665				

S = 0.0190524 R-Sq = 29.97% R-Sq(adj) = 0.00%

Unusual Observations for Area3

Obs	Area3	Fit	SE Fit	Residual	St Resid
11	0.893997	0.928867	0.011000	-0.034870	-2.24 R
19	0.880162	0.913556	0.011000	-0.033393	-2.15 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95.0% Confidence for Time 1

Day	N	Mean	Grouping
10	3	0.0	A
18	3	0.0	A
25	3	0.0	A
21	3	0.0	A
28	3	0.0	A
7	3	0.0	A
14	3	0.0	A
3	3	0.0	A
5	3	0.0	A
0	3	0.0	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for Time 2

Day	N	Mean	Grouping
18	3	0.4	A
28	3	0.3	A B
21	3	0.3	A B
25	3	0.3	A B
10	3	0.3	A B
14	3	0.3	A B C
3	3	0.3	A B C
0	3	0.3	A B C
7	3	0.3	B C
5	3	0.2	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for Time 3

Day	N	Mean	Grouping
25	3	2.1	A
10	3	2.1	A
18	3	2.0	A B
21	3	2.0	A B
14	3	2.0	A B
28	3	1.9	A B
0	3	1.8	B C
5	3	1.7	C
3	3	1.7	C
7	3	1.6	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for Area1

Day	N	Mean	Grouping
0	3	0.1	A
18	3	0.1	A
3	3	0.1	A
28	3	0.0	A
14	3	0.0	A
25	3	0.0	A
7	3	0.0	A
21	3	0.0	A
10	3	0.0	A
5	3	0.0	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for Area2

Day	N	Mean	Grouping
28	3	0.0	A
10	3	0.0	A
0	3	0.0	A
3	3	0.0	A
18	3	0.0	A
7	3	0.0	A
14	3	0.0	A
5	3	0.0	A
21	3	0.0	A
25	3	0.0	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for Area3

Day	N	Mean	Grouping
5	3	0.9	A
21	3	0.9	A
25	3	0.9	A
7	3	0.9	A
10	3	0.9	A
14	3	0.9	A
28	3	0.9	A
3	3	0.9	A
18	3	0.9	A
0	3	0.9	A

Means that do not share a letter are significantly different.

One-way ANOVA: Time 1 versus Liposome Type (at day 0)

```
Source  DF         SS         MS     F      P
Type     3  0.0001842  0.0000614  3.58  0.066
Error    8  0.0001372  0.0000172
Total   11  0.0003215

S = 0.004142   R-Sq = 57.31%   R-Sq(adj) = 41.30%


                              Individual 95% CIs For Mean Based on
                              Pooled StDev
Level  N      Mean     StDev  ----+---------+---------+---------+-----
A      3  0.009267  0.004110  (--------*---------)
B      3  0.019333  0.005033                   (--------*--------)
C      3  0.010567  0.005105    (---------*--------)
D      3  0.011667  0.000577      (--------*---------)
                              ----+---------+---------+---------+-----
                                0.0060    0.0120    0.0180    0.0240

Pooled StDev = 0.004142


Grouping Information Using Tukey Method

Type  N      Mean  Grouping
B     3  0.019333  A
D     3  0.011667  A
C     3  0.010567  A
A     3  0.009267  A

Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%

Type = A subtracted from:

Type      Lower    Center     Upper  ------+---------+---------+---------+--
-
B     -0.000766  0.010067  0.020900                (--------*--------)
C     -0.009533  0.001300  0.012133          (--------*--------)
D     -0.008433  0.002400  0.013233           (--------*--------)
                                     ------+---------+---------+---------+--
-
                                        -0.012     0.000     0.012     0.024

Type = B subtracted from:

Type      Lower     Center     Upper  ------+---------+---------+---------+-
--
C     -0.019600  -0.008767  0.002066  (--------*--------)
D     -0.018500  -0.007667  0.003166   (--------*--------)
                                      ------+---------+---------+---------+-
--
                                         -0.012     0.000     0.012
0.024
```

```
Type = C subtracted from:

Type      Lower    Center     Upper  ------+---------+---------+---------+--
-
D     -0.009733  0.001100  0.011933          (--------*--------)
                                     ------+---------+---------+---------+--
-
                                        -0.012     0.000     0.012     0.024
```

One-way ANOVA: Time 2 versus Liposome Type (at day 0)

```
Source  DF       SS       MS      F      P
Type     3  0.16460  0.05487  11.17  0.003
Error    8  0.03930  0.00491
Total   11  0.20391

S = 0.07009   R-Sq = 80.73%   R-Sq(adj) = 73.50%


                           Individual 95% CIs For Mean Based on
                           Pooled StDev
Level  N     Mean    StDev   -+---------+---------+---------+--------
A      3  0.07700  0.03351   (------*-------)
B      3  0.12733  0.04649       (-------*------)
C      3  0.36333  0.12741                          (------*-------)
D      3  0.29333  0.01155                     (------*-------)
                            -+---------+---------+---------+--------
                           0.00      0.12      0.24      0.36

Pooled StDev = 0.07009


Grouping Information Using Tukey Method

Type  N     Mean  Grouping
C     3  0.36333  A
D     3  0.29333  A B
B     3  0.12733    B C
A     3  0.07700      C

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%


Type = A subtracted from:

Type      Lower   Center    Upper  -------+---------+---------+---------+--
B     -0.13298  0.05033  0.23365              (------*------)
C      0.10302  0.28633  0.46965                       (------*-------)
D      0.03302  0.21633  0.39965                  (-------*------)
                                  -------+---------+---------+---------+--
                                      -0.25      0.00      0.25      0.50
```

```
Type = B subtracted from:

Type     Lower   Center    Upper  -------+---------+---------+---------+--
C      0.05268  0.23600  0.41932                    (------*-------)
D     -0.01732  0.16600  0.34932                 (-------*------)
                                  -------+---------+---------+---------+--
                                      -0.25      0.00      0.25      0.50


Type = C subtracted from:

Type     Lower    Center    Upper  -------+---------+---------+---------+--
D     -0.25332  -0.07000  0.11332         (------*-------)
                                   -------+---------+---------+---------+--
                                       -0.25      0.00      0.25      0.50
```

One-way ANOVA: Time 3 versus Liposome Type (at day 0)

```
Source  DF        SS        MS  F  P
Type     3  1.896900  0.632300  *  *
Error    8  0.000000  0.000000
Total   11  1.896900

S = 0   R-Sq = 100.00%   R-Sq(adj) = 100.00%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N     Mean    StDev  ---+---------+---------+---------+------
A      3  0.92000  0.00000  *
B      3  1.00000  0.00000    *
C      3  1.70000  0.00000                              *
D      3  1.80000  0.00000                                  *
                          ---+---------+---------+---------+------
                           1.00      1.25      1.50      1.75

Pooled StDev = 0.00000


Grouping Information Using Tukey Method

Type  N      Mean  Grouping
D     3  1.800000  A
C     3  1.700000    B
B     3  1.000000      C
A     3  0.920000        D

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%
```

```
Type = A subtracted from:

Type     Lower    Center     Upper  ------+---------+---------+---------+---
B     0.080000  0.080000  0.080000                  *
C     0.780000  0.780000  0.780000                              *
D     0.880000  0.880000  0.880000                                *
                                   ------+---------+---------+---------+---
                                      -0.50      0.00      0.50      1.00


Type = B subtracted from:

Type     Lower    Center     Upper  ------+---------+---------+---------+---
C     0.700000  0.700000  0.700000                            *
D     0.800000  0.800000  0.800000                              *
                                   ------+---------+---------+---------+---
                                      -0.50      0.00      0.50      1.00


Type = C subtracted from:

Type     Lower    Center     Upper  ------+---------+---------+---------+---
D     0.100000  0.100000  0.100000                  *
                                   ------+---------+---------+---------+---
                                      -0.50      0.00      0.50      1.00
```

One-way ANOVA: Area 1 versus Liposome Type (at day 0)

```
Source  DF        SS        MS     F      P
Type     3  0.002851  0.000950  4.29  0.044
Error    8  0.001774  0.000222
Total   11  0.004625

S = 0.01489   R-Sq = 61.65%   R-Sq(adj) = 47.27%


                           Individual 95% CIs For Mean Based on Pooled
StDev
Level  N     Mean    StDev   -+---------+---------+---------+--------
A      3  0.04044  0.01321             (---------*---------)
B      3  0.01757  0.00764   (---------*---------)
C      3  0.05780  0.02401                       (---------*---------)
D      3  0.05207  0.00879                    (---------*---------)
                            -+---------+---------+---------+--------
                           0.000     0.020     0.040     0.060

Pooled StDev = 0.01489


Grouping Information Using Tukey Method

Type  N     Mean  Grouping
C     3  0.05780  A
D     3  0.05207  A B
A     3  0.04044  A B
B     3  0.01757    B

Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals
```

```
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%

Type = A subtracted from:

Type     Lower    Center    Upper     +---------+---------+---------+-------
--
B     -0.06181  -0.02287  0.01607          (--------*---------)
C     -0.02158   0.01736  0.05630                   (--------*---------)
D     -0.02731   0.01164  0.05058                 (---------*---------)
                                     +---------+---------+---------+-------
--
                                   -0.080    -0.040     0.000     0.040

Type = B subtracted from:

Type     Lower   Center    Upper     +---------+---------+---------+--------
-
C      0.00129  0.04023  0.07917                          (---------*--------
-)
D     -0.00444  0.03450  0.07344                         (---------*--------)
                                  +---------+---------+---------+---------
-
                                -0.080    -0.040     0.000     0.040

Type = C subtracted from:

Type     Lower    Center    Upper     +---------+---------+---------+-------
--
D     -0.04467  -0.00573  0.03321              (---------*--------)
                                     +---------+---------+---------+-------
--
                                   -0.080    -0.040     0.000     0.040
```

One-way ANOVA: Area 2 versus Liposome Type (at day 0)

```
Source  DF         SS         MS      F      P
Type     3  0.0009943  0.0003314  17.12  0.001
Error    8  0.0001549  0.0000194
Total   11  0.0011492

S = 0.004401   R-Sq = 86.52%   R-Sq(adj) = 81.46%


                            Individual 95% CIs For Mean Based on
                            Pooled StDev
Level  N      Mean     StDev  --+---------+---------+---------+-------
A      3  0.019872  0.006610  (------*------)
B      3  0.020367  0.004684  (------*-------)
C      3  0.038055  0.000632                       (-------*------)
D      3  0.038580  0.003380                        (------*-------)
                            --+---------+---------+---------+-------
                            0.0160    0.0240    0.0320    0.0400

Pooled StDev = 0.004401
```

```
Grouping Information Using Tukey Method

Type  N      Mean  Grouping
D     3  0.038580  A
C     3  0.038055  A
B     3  0.020367    B
A     3  0.019872    B

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%


Type = A subtracted from:

Type      Lower    Center     Upper
B     -0.011014  0.000495  0.012005
C      0.006674  0.018183  0.029692
D      0.007199  0.018709  0.030218

Type     +---------+---------+---------+---------
B                     (------*-------)
C                               (-------*-------)
D                                (------*-------)
         +---------+---------+---------+---------
      -0.030    -0.015     0.000     0.015



Type = B subtracted from:

Type     Lower    Center     Upper
C     0.006178  0.017688  0.029197
D     0.006704  0.018213  0.029723

Type     +---------+---------+---------+---------
C                               (-------*------)
D                               (-------*-------)
         +---------+---------+---------+---------
      -0.030    -0.015     0.000     0.015

Type = C subtracted from:

Type      Lower    Center     Upper
D     -0.010984  0.000526  0.012035

Type     +---------+---------+---------+---------
D                     (------*-------)
         +---------+---------+---------+---------
      -0.030    -0.015     0.000     0.015
```

One-way ANOVA: Area 3 versus Liposome Type (at day 0)

```
Source  DF       SS       MS     F      P
Type     3  0.01281  0.00427  2.98  0.096
Error    8  0.01147  0.00143
Total   11  0.02428

S = 0.03786   R-Sq = 52.76%   R-Sq(adj) = 35.05%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean   StDev  -----+---------+---------+---------+----
A      3  0.9397  0.0130              (---------*---------)
B      3  0.9621  0.0051                  (---------*---------)
C      3  0.8752  0.0742  (---------*---------)
D      3  0.9093  0.0054        (---------*---------)
                          -----+---------+---------+---------+----
                             0.850     0.900     0.950     1.000

Pooled StDev = 0.0379

Grouping Information Using Tukey Method

Type  N     Mean  Grouping
B     3  0.96207  A
A     3  0.93969  A
D     3  0.90935  A
C     3  0.87518  A

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%

Type = A subtracted from:

Type     Lower    Center    Upper  ---------+---------+---------+---------+
B     -0.07665   0.02238  0.12141            (---------*---------)
C     -0.16354  -0.06451  0.03452     (---------*--------)
D     -0.12938  -0.03034  0.06869        (---------*---------)
                                   ---------+---------+---------+---------+
                                         -0.10      0.00      0.10      0.20

Type = B subtracted from:

Type     Lower    Center    Upper  ---------+---------+---------+---------+
C     -0.18592  -0.08689  0.01214  (---------*---------)
D     -0.15175  -0.05272  0.04631      (---------*---------)
                                   ---------+---------+---------+---------+
                                         -0.10      0.00      0.10      0.20
Type = C subtracted from:

Type     Lower   Center    Upper  ---------+---------+---------+---------+
D     -0.06486  0.03417  0.13320              (--------*---------)
                                  ---------+---------+---------+---------+
                                        -0.10      0.00      0.10      0.20
```

One-way ANOVA: Time 1 versus Liposome Type (day 3)

```
Source  DF         SS         MS     F      P
Type     3  0.0000115  0.0000038  0.22  0.881
Error    8  0.0001404  0.0000175
Total   11  0.0001518

S = 0.004189   R-Sq = 7.55%   R-Sq(adj) = 0.00%


                              Individual 95% CIs For Mean Based on Pooled
StDev
Level  N      Mean     StDev   -+---------+---------+---------+--------
A      3  0.012400  0.004503   (---------------*---------------)
B      3  0.015100  0.006421           (---------------*---------------)
C      3  0.013333  0.000577      (---------------*---------------)
D      3  0.013333  0.002887      (---------------*---------------)
                               -+---------+---------+---------+--------
                              0.0070    0.0105    0.0140    0.0175

Pooled StDev = 0.004189

Grouping Information Using Tukey Method

Type  N      Mean  Grouping
B     3  0.015100  A
D     3  0.013333  A
C     3  0.013333  A
A     3  0.012400  A

Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%

Type = A subtracted from:

Type      Lower    Center     Upper  --------+---------+---------+---------
+-
B     -0.008255  0.002700  0.013655        (---------------*---------------)
C     -0.010021  0.000933  0.011888      (--------------*---------------)
D     -0.010021  0.000933  0.011888      (--------------*---------------)
                                       --------+---------+---------+---------
+-
                                            -0.0070    0.0000    0.0070
0.0140

Type = B subtracted from:

Type      Lower     Center     Upper
C     -0.012721  -0.001767  0.009188
D     -0.012721  -0.001767  0.009188

Type  --------+---------+---------+---------+-
C     (--------------*---------------)
D     (--------------*---------------)
      --------+---------+---------+---------+-
           -0.0070    0.0000    0.0070    0.0140
```

```
Type = C subtracted from:

Type      Lower    Center     Upper  --------+---------+---------+---------
+-
D     -0.010955  0.000000  0.010955    (---------------*---------------)
                                     --------+---------+---------+---------
+-
                                         -0.0070    0.0000    0.0070
0.0140
```

One-way ANOVA: Time 2 versus Liposome Type (day 3)

```
Source  DF       SS       MS     F      P
Type     3  0.05851  0.01950  4.86  0.033
Error    8  0.03208  0.00401
Total   11  0.09059

S = 0.06333   R-Sq = 64.59%   R-Sq(adj) = 51.31%


                           Individual 95% CIs For Mean Based on
                           Pooled StDev
Level  N     Mean    StDev  ---------+---------+---------+---------+
A      3  0.09900  0.04747  (--------*-------)
B      3  0.17267  0.09122          (-------*--------)
C      3  0.20667  0.04619             (--------*-------)
D      3  0.29333  0.05774                      (-------*--------)
                           ---------+---------+---------+---------+
                                  0.10      0.20      0.30      0.40

Pooled StDev = 0.06333


Grouping Information Using Tukey Method

Type  N     Mean  Grouping
D     3  0.29333  A
C     3  0.20667  A B
B     3  0.17267  A B
A     3  0.09900    B

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%

Type = A subtracted from:

Type     Lower   Center    Upper  ----+---------+---------+---------+-----
B     -0.09196  0.07367  0.23929           (--------*-------)
C     -0.05796  0.10767  0.27329             (-------*--------)
D      0.02871  0.19433  0.35996                 (--------*-------)
                                 ----+---------+---------+---------+-----
                                  -0.20      0.00      0.20      0.40
```

```
Type = B subtracted from:

Type     Lower   Center    Upper  ----+---------+---------+---------+-----
C     -0.13162  0.03400  0.19962         (--------*-------)
D     -0.04496  0.12067  0.28629              (-------*-------)
                                  ----+---------+---------+---------+-----
                                   -0.20      0.00      0.20      0.40

Type = C subtracted from:

Type     Lower   Center    Upper  ----+---------+---------+---------+-----
D     -0.07896  0.08667  0.25229           (--------*--------)
                                  ----+---------+---------+---------+-----
                                   -0.20      0.00      0.20      0.40
```

One-way ANOVA: Time 3 versus Liposome Type (day 3)

```
Source  DF        SS        MS  F  P
Type     3  1.652400  0.550800  *  *
Error    8  0.000000  0.000000
Total   11  1.652400

S = 0   R-Sq = 100.00%   R-Sq(adj) = 100.00%


                           Individual 95% CIs For Mean Based on
                           Pooled StDev
Level  N     Mean    StDev  ----+---------+---------+---------+-----
A      3  0.92000  0.00000  *
B      3  1.00000  0.00000      *
C      3  1.70000  0.00000                                        *
D      3  1.70000  0.00000                                        *
                           ----+---------+---------+---------+-----
                             1.00      1.20      1.40      1.60

Pooled StDev = 0.00000

Grouping Information Using Tukey Method

Type  N      Mean  Grouping
D     3  1.700000  A
C     3  1.700000    B
B     3  1.000000      C
A     3  0.920000        D

Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%

Type = A subtracted from:

Type     Lower    Center     Upper  -------+---------+---------+---------+--
B     0.080000  0.080000  0.080000                    *
C     0.780000  0.780000  0.780000                                       *
D     0.780000  0.780000  0.780000                                       *
                                   -------+---------+---------+---------+--
                                       -0.40      0.00      0.40      0.80
```

```
Type = B subtracted from:

Type     Lower    Center     Upper  -------+---------+---------+---------+--
C     0.700000  0.700000  0.700000                                      *
D     0.700000  0.700000  0.700000                                      *
                                   -------+---------+---------+---------+--
                                       -0.40      0.00      0.40      0.80

Type = C subtracted from:

Type     Lower    Center     Upper  -------+---------+---------+---------+--
D     0.000000  0.000000  0.000000                  *
                                   -------+---------+---------+---------+--
                                       -0.40      0.00      0.40      0.80
```

One-way ANOVA: Area 1 versus Liposome Type (day 3)

```
Source  DF        SS        MS     F      P
Type     3  0.000630  0.000210  0.73  0.562
Error    8  0.002303  0.000288
Total   11  0.002933

S = 0.01697   R-Sq = 21.49%   R-Sq(adj) = 0.00%


                           Individual 95% CIs For Mean Based on
                           Pooled StDev
Level  N     Mean    StDev  ------+---------+---------+---------+---
A      3  0.03518  0.01013    (-----------*----------)
B      3  0.03061  0.01169  (----------*-----------)
C      3  0.03962  0.00711       (----------*----------)
D      3  0.05017  0.02935            (----------*----------)
                           ------+---------+---------+---------+---
                               0.020     0.040     0.060     0.080

Pooled StDev = 0.01697

Grouping Information Using Tukey Method

Type  N     Mean  Grouping
D     3  0.05017  A
C     3  0.03962  A
A     3  0.03518  A
B     3  0.03061  A

Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%

Type = A subtracted from:

Type     Lower    Center    Upper  --------+---------+---------+---------+-
B     -0.04895  -0.00457  0.03980      (-----------*-----------)
C     -0.03994   0.00443  0.04880         (-----------*-----------)
D     -0.02938   0.01499  0.05936            (-----------*-----------)
                                   --------+---------+---------+---------+-
                                        -0.035     0.000     0.035     0.070
```

```
Type = B subtracted from:

Type     Lower   Center    Upper  --------+---------+---------+---------+-
C     -0.03536  0.00901  0.05338          (------------*-----------)
D     -0.02481  0.01956  0.06393             (------------*-----------)
                                  --------+---------+---------+---------+-
                                       -0.035     0.000     0.035     0.070

Type = C subtracted from:

Type     Lower   Center    Upper  --------+---------+---------+---------+-
D     -0.03382  0.01055  0.05493          (------------*------------)
                                  --------+---------+---------+---------+-
                                       -0.035     0.000     0.035     0.070
```

One-way ANOVA: Time 3 versus Liposome Type (day 3)

```
Source  DF        SS        MS  F  P
Type     3  1.652400  0.550800  *  *
Error    8  0.000000  0.000000
Total   11  1.652400

S = 0   R-Sq = 100.00%   R-Sq(adj) = 100.00%


                           Individual 95% CIs For Mean Based on
                           Pooled StDev
Level  N     Mean    StDev  ----+---------+---------+---------+-----
A      3  0.92000  0.00000  *
B      3  1.00000  0.00000      *
C      3  1.70000  0.00000                                        *
D      3  1.70000  0.00000                                        *
                           ----+---------+---------+---------+-----
                             1.00      1.20      1.40      1.60

Pooled StDev = 0.00000



Grouping Information Using Tukey Method

Type  N      Mean  Grouping
D     3  1.700000  A
C     3  1.700000     B
B     3  1.000000       C
A     3  0.920000         D

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%
```

```
Type = A subtracted from:

Type     Lower    Center     Upper  -------+---------+---------+---------+--
B     0.080000  0.080000  0.080000                   *
C     0.780000  0.780000  0.780000                                       *
D     0.780000  0.780000  0.780000                                       *
                                   -------+---------+---------+---------+--
                                       -0.40      0.00      0.40      0.80


Type = B subtracted from:

Type     Lower    Center     Upper  -------+---------+---------+---------+--
C     0.700000  0.700000  0.700000                                     *
D     0.700000  0.700000  0.700000                                     *
                                   -------+---------+---------+---------+--
                                       -0.40      0.00      0.40      0.80


Type = C subtracted from:

Type     Lower    Center     Upper  -------+---------+---------+---------+--
D     0.000000  0.000000  0.000000                  *
                                   -------+---------+---------+---------+--
                                       -0.40      0.00      0.40      0.80
```

One-way ANOVA: Area 2 versus Liposome Type (day 3)

```
Source  DF         SS         MS     F      P
Type     3  0.0012651  0.0004217  6.07  0.019
Error    8  0.0005558  0.0000695
Total   11  0.0018209

S = 0.008335   R-Sq = 69.48%   R-Sq(adj) = 58.03%


                              Individual 95% CIs For Mean Based on Pooled
StDev
Level  N      Mean     StDev   -+---------+---------+---------+--------
A      3  0.010329  0.001534   (---------*--------)
B      3  0.017459  0.002813         (---------*--------)
C      3  0.030769  0.001495                   (---------*--------)
D      3  0.036109  0.016291                        (--------*--------)
                               -+---------+---------+---------+--------
                              0.000     0.012     0.024     0.036

Pooled StDev = 0.008335


Grouping Information Using Tukey Method

Type  N      Mean  Grouping
D     3  0.036109  A
C     3  0.030769  A B
B     3  0.017459  A B
A     3  0.010329    B

Means that do not share a letter are significantly different.
```

```
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%

Type = A subtracted from:

Type      Lower    Center     Upper  ------+---------+---------+---------+--
-
B     -0.014670  0.007130  0.028931            (--------*--------)
C     -0.001359  0.020441  0.042241                 (--------*--------)
D      0.003980  0.025780  0.047580                    (-------*--------)
                                    ------+---------+---------+---------+--
-
                                       -0.025     0.000     0.025     0.050


Type = B subtracted from:

Type      Lower    Center     Upper  ------+---------+---------+---------+--
-
C     -0.008490  0.013311  0.035111               (-------*--------)
D     -0.003150  0.018650  0.040450                 (-------*--------)
                                    ------+---------+---------+---------+--
-
                                       -0.025     0.000     0.025     0.050


Type = C subtracted from:

Type      Lower    Center     Upper  ------+---------+---------+---------+--
-
D     -0.016461  0.005339  0.027139           (--------*--------)
                                    ------+---------+---------+---------+--
-
                                       -0.025     0.000     0.025     0.050
```

One-way ANOVA: Area 3 versus Liposome Type (day 3)

```
Source  DF        SS        MS     F      P
Type     3  0.003374  0.001125  7.20  0.012
Error    8  0.001249  0.000156
Total   11  0.004623

S = 0.01250   R-Sq = 72.98%   R-Sq(adj) = 62.85%


                           Individual 95% CIs For Mean Based on
                           Pooled StDev
Level  N     Mean    StDev   -+---------+---------+---------+--------
A      3  0.95449  0.01068                      (-------*--------)
B      3  0.95193  0.01026                     (-------*-------)
C      3  0.92961  0.00860            (--------*-------)
D      3  0.91372  0.01820   (-------*-------)
                            -+---------+---------+---------+--------
                           0.900     0.920     0.940     0.960

Pooled StDev = 0.01250
```

```
Grouping Information Using Tukey Method

Type  N     Mean  Grouping
A     3  0.95449  A
B     3  0.95193  A
C     3  0.92961  A B
D     3  0.91372    B

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%


Type = A subtracted from:

Type     Lower    Center     Upper  --------+---------+---------+---------+-
B     -0.03524  -0.00256   0.03013           (-------*--------)
C     -0.05756  -0.02488   0.00781      (-------*-------)
D     -0.07345  -0.04077  -0.00809  (-------*-------)
                                    --------+---------+---------+---------+-
                                         -0.040     0.000     0.040
0.080


Type = B subtracted from:

Type     Lower    Center     Upper  --------+---------+---------+---------+-
C     -0.05500  -0.02232   0.01036      (-------*--------)
D     -0.07090  -0.03821  -0.00553  (-------*--------)
                                    --------+---------+---------+---------+-
                                         -0.040     0.000     0.040
0.080


Type = C subtracted from:

Type     Lower    Center    Upper  --------+---------+---------+---------+-
D     -0.04858  -0.01589  0.01679        (-------*-------)
                                   --------+---------+---------+---------+-
                                        -0.040     0.000     0.040     0.080
```

One-way ANOVA: Time 1 versus Liposome Type (day 5)

```
Source  DF         SS         MS     F      P
Type     3  0.0000115  0.0000038  0.22  0.881
Error    8  0.0001404  0.0000175
Total   11  0.0001518

S = 0.004189   R-Sq = 7.55%   R-Sq(adj) = 0.00%
```

```
                              Individual 95% CIs For Mean Based on Pooled
StDev
Level  N      Mean     StDev   -+---------+---------+---------+--------
A      3  0.012400  0.004503   (---------------*---------------)
B      3  0.015100  0.006421            (---------------*---------------)
C      3  0.013333  0.000577      (---------------*---------------)
D      3  0.013333  0.002887      (---------------*---------------)
                               -+---------+---------+---------+--------
                              0.0070    0.0105    0.0140    0.0175

Pooled StDev = 0.004189


Grouping Information Using Tukey Method

Type  N      Mean  Grouping
B     3  0.015100  A
D     3  0.013333  A
C     3  0.013333  A
A     3  0.012400  A

Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%

Type = A subtracted from:

Type      Lower    Center     Upper  --------+---------+---------+---------
+-
B     -0.008255  0.002700  0.013655         (---------------*---------------)
C     -0.010021  0.000933  0.011888       (---------------*---------------)
D     -0.010021  0.000933  0.011888       (---------------*---------------)
                                       --------+---------+---------+---------
+-
                                            -0.0070    0.0000    0.0070
0.0140

Type = B subtracted from:

Type      Lower     Center     Upper
C     -0.012721  -0.001767  0.009188
D     -0.012721  -0.001767  0.009188

Type  --------+---------+---------+---------+-
C     (---------------*---------------)
D     (---------------*---------------)
      --------+---------+---------+---------+-
           -0.0070    0.0000    0.0070    0.0140

Type = C subtracted from:

Type      Lower    Center     Upper  --------+---------+---------+---------
+-
D     -0.010955  0.000000  0.010955    (---------------*---------------)
                                       --------+---------+---------+---------
+-
                                            -0.0070    0.0000    0.0070
0.0140
```

One-way ANOVA: Time 2 versus Liposome Type (day 5)

```
Source  DF       SS       MS     F      P
Type     3  0.05851  0.01950  4.86  0.033
Error    8  0.03208  0.00401
Total   11  0.09059

S = 0.06333   R-Sq = 64.59%   R-Sq(adj) = 51.31%


                           Individual 95% CIs For Mean Based on
                           Pooled StDev
Level  N     Mean    StDev  ---------+---------+---------+---------+
A      3  0.09900  0.04747  (--------*-------)
B      3  0.17267  0.09122          (-------*--------)
C      3  0.20667  0.04619             (--------*-------)
D      3  0.29333  0.05774                      (-------*--------)
                           ---------+---------+---------+---------+
                                  0.10      0.20      0.30      0.40

Pooled StDev = 0.06333


Grouping Information Using Tukey Method

Type  N     Mean  Grouping
D     3  0.29333  A
C     3  0.20667  A B
B     3  0.17267  A B
A     3  0.09900    B

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%


Type = A subtracted from:

Type     Lower   Center    Upper  ----+---------+---------+---------+-----
B     -0.09196  0.07367  0.23929           (--------*-------)
C     -0.05796  0.10767  0.27329             (-------*--------)
D      0.02871  0.19433  0.35996                 (--------*-------)
                                 ----+---------+---------+---------+-----
                                  -0.20      0.00      0.20      0.40


Type = B subtracted from:

Type     Lower   Center    Upper  ----+---------+---------+---------+-----
C     -0.13162  0.03400  0.19962         (--------*-------)
D     -0.04496  0.12067  0.28629              (-------*-------)
                                 ----+---------+---------+---------+-----
                                  -0.20      0.00      0.20      0.40
```

```
Type = C subtracted from:

Type     Lower   Center    Upper  ----+---------+---------+---------+-----
D     -0.07896  0.08667  0.25229            (-------*--------)
                                 ----+---------+---------+---------+-----
                                  -0.20      0.00      0.20      0.40
```

One-way ANOVA: Time 3 versus Liposome Type (day 5)

```
Source  DF        SS        MS  F  P
Type     3  1.652400  0.550800  *  *
Error    8  0.000000  0.000000
Total   11  1.652400

S = 0   R-Sq = 100.00%   R-Sq(adj) = 100.00%


                           Individual 95% CIs For Mean Based on
                           Pooled StDev
Level  N     Mean    StDev  ----+---------+---------+---------+-----
A      3  0.92000  0.00000  *
B      3  1.00000  0.00000      *
C      3  1.70000  0.00000                                        *
D      3  1.70000  0.00000                                        *
                           ----+---------+---------+---------+-----
                             1.00      1.20      1.40      1.60

Pooled StDev = 0.00000



Grouping Information Using Tukey Method

Type  N      Mean  Grouping
D     3  1.700000  A
C     3  1.700000    B
B     3  1.000000      C
A     3  0.920000        D

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%


Type = A subtracted from:

Type     Lower    Center     Upper  -------+---------+---------+---------+--
B     0.080000  0.080000  0.080000                    *
C     0.780000  0.780000  0.780000                                      *
D     0.780000  0.780000  0.780000                                      *
                                   -------+---------+---------+---------+--
                                       -0.40      0.00      0.40      0.80
```

```
Type = B subtracted from:

Type     Lower    Center     Upper  -------+---------+---------+---------+--
C     0.700000  0.700000  0.700000                                     *
D     0.700000  0.700000  0.700000                                     *
                                   -------+---------+---------+---------+--
                                       -0.40      0.00      0.40      0.80


Type = C subtracted from:

Type     Lower    Center     Upper  -------+---------+---------+---------+--
D     0.000000  0.000000  0.000000                   *
                                   -------+---------+---------+---------+--
                                       -0.40      0.00      0.40      0.80
```

One-way ANOVA: Area 1 versus Liposome Type (day 5)

```
Source  DF        SS        MS     F      P
Type     3  0.000630  0.000210  0.73  0.562
Error    8  0.002303  0.000288
Total   11  0.002933

S = 0.01697   R-Sq = 21.49%   R-Sq(adj) = 0.00%


                            Individual 95% CIs For Mean Based on
                            Pooled StDev
Level  N     Mean    StDev  ------+---------+---------+---------+---
A      3  0.03518  0.01013    (-----------*----------)
B      3  0.03061  0.01169  (----------*-----------)
C      3  0.03962  0.00711       (----------*----------)
D      3  0.05017  0.02935             (----------*----------)
                            ------+---------+---------+---------+---
                                0.020     0.040     0.060     0.080

Pooled StDev = 0.01697


Grouping Information Using Tukey Method

Type  N     Mean  Grouping
D     3  0.05017  A
C     3  0.03962  A
A     3  0.03518  A
B     3  0.03061  A

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%
```

```
Type = A subtracted from:

Type     Lower    Center    Upper  --------+---------+---------+---------+-
B     -0.04895  -0.00457  0.03980       (-----------*-----------)
C     -0.03994   0.00443  0.04880          (-----------*-----------)
D     -0.02938   0.01499  0.05936             (-----------*-----------)
                                  --------+---------+---------+---------+-
                                       -0.035     0.000     0.035     0.070


Type = B subtracted from:

Type     Lower   Center    Upper  --------+---------+---------+---------+-
C     -0.03536  0.00901  0.05338          (-----------*-----------)
D     -0.02481  0.01956  0.06393             (-----------*-----------)
                                 --------+---------+---------+---------+-
                                      -0.035     0.000     0.035     0.070


Type = C subtracted from:

Type     Lower   Center    Upper  --------+---------+---------+---------+-
D     -0.03382  0.01055  0.05493          (-----------*-----------)
                                 --------+---------+---------+---------+-
                                      -0.035     0.000     0.035     0.070
```

One-way ANOVA: Time 3 versus Liposome Type (day 5)

```
Source  DF        SS        MS  F  P
Type     3  1.652400  0.550800  *  *
Error    8  0.000000  0.000000
Total   11  1.652400

S = 0   R-Sq = 100.00%   R-Sq(adj) = 100.00%


                           Individual 95% CIs For Mean Based on
                           Pooled StDev
Level  N     Mean    StDev  ----+---------+---------+---------+-----
A      3  0.92000  0.00000  *
B      3  1.00000  0.00000      *
C      3  1.70000  0.00000                                        *
D      3  1.70000  0.00000                                        *
                           ----+---------+---------+---------+-----
                             1.00      1.20      1.40      1.60

Pooled StDev = 0.00000


Grouping Information Using Tukey Method

Type  N      Mean  Grouping
D     3  1.700000  A
C     3  1.700000     B
B     3  1.000000        C
A     3  0.920000           D

Means that do not share a letter are significantly different.
Tukey 95% Simultaneous Confidence Intervals
```

```
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%


Type = A subtracted from:

Type     Lower    Center     Upper  -------+---------+---------+---------+--
B     0.080000  0.080000  0.080000                  *
C     0.780000  0.780000  0.780000                              *
D     0.780000  0.780000  0.780000                              *
                                   -------+---------+---------+---------+--
                                       -0.40      0.00      0.40      0.80


Type = B subtracted from:

Type     Lower    Center     Upper  -------+---------+---------+---------+--
C     0.700000  0.700000  0.700000                            *
D     0.700000  0.700000  0.700000                            *
                                   -------+---------+---------+---------+--
                                       -0.40      0.00      0.40      0.80

Type = C subtracted from:

Type     Lower    Center     Upper  -------+---------+---------+---------+--
D     0.000000  0.000000  0.000000                 *
                                   -------+---------+---------+---------+--
                                       -0.40      0.00      0.40      0.80
```

One-way ANOVA: Area 2 versus Liposome Type (day 5)

```
Source  DF         SS         MS     F      P
Type     3  0.0012651  0.0004217  6.07  0.019
Error    8  0.0005558  0.0000695
Total   11  0.0018209

S = 0.008335   R-Sq = 69.48%   R-Sq(adj) = 58.03%


                             Individual 95% CIs For Mean Based on Pooled
StDev
Level  N      Mean     StDev   -+---------+---------+---------+--------
A      3  0.010329  0.001534   (---------*---------)
B      3  0.017459  0.002813         (---------*---------)
C      3  0.030769  0.001495                   (---------*---------)
D      3  0.036109  0.016291                        (---------*---------)
                              -+---------+---------+---------+--------
                             0.000     0.012     0.024     0.036

Pooled StDev = 0.008335
Grouping Information Using Tukey Method

Type  N      Mean  Grouping
D     3  0.036109  A
C     3  0.030769  A B
B     3  0.017459  A B
A     3  0.010329    B

Means that do not share a letter are significantly different.
```

```
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%


Type = A subtracted from:

Type      Lower    Center     Upper  ------+---------+---------+---------+--
-
B     -0.014670  0.007130  0.028931            (--------*--------)
C     -0.001359  0.020441  0.042241                 (--------*--------)
D      0.003980  0.025780  0.047580                    (-------*--------)
                                    ------+---------+---------+---------+--
-
                                       -0.025     0.000     0.025     0.050


Type = B subtracted from:

Type      Lower    Center     Upper  ------+---------+---------+---------+--
-
C     -0.008490  0.013311  0.035111               (-------*--------)
D     -0.003150  0.018650  0.040450                 (-------*--------)
                                    ------+---------+---------+---------+--
-
                                       -0.025     0.000     0.025     0.050


Type = C subtracted from:

Type      Lower    Center     Upper  ------+---------+---------+---------+--
-
D     -0.016461  0.005339  0.027139           (--------*--------)
                                    ------+---------+---------+---------+--
-
                                       -0.025     0.000     0.025     0.050
```

One-way ANOVA: Area 3 versus Liposome Type (day 5)

```
Source  DF        SS        MS     F      P
Type     3  0.003374  0.001125  7.20  0.012
Error    8  0.001249  0.000156
Total   11  0.004623

S = 0.01250   R-Sq = 72.98%   R-Sq(adj) = 62.85%

                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N     Mean    StDev   -+---------+---------+---------+--------
A      3  0.95449  0.01068                      (-------*--------)
B      3  0.95193  0.01026                     (-------*-------)
C      3  0.92961  0.00860            (--------*-------)
D      3  0.91372  0.01820   (-------*-------)
                            -+---------+---------+---------+--------
                          0.900     0.920     0.940     0.960
Pooled StDev = 0.01250
```

```
Grouping Information Using Tukey Method

Type  N     Mean  Grouping
A     3  0.95449  A
B     3  0.95193  A
C     3  0.92961  A B
D     3  0.91372    B

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%


Type = A subtracted from:

Type     Lower    Center     Upper  --------+---------+---------+---------+-
B     -0.03524  -0.00256   0.03013           (-------*--------)
C     -0.05756  -0.02488   0.00781      (-------*-------)
D     -0.07345  -0.04077  -0.00809  (-------*-------)
                                    --------+---------+---------+---------+-
                                         -0.040     0.000     0.040
0.080


Type = B subtracted from:

Type     Lower    Center     Upper  --------+---------+---------+---------+-
C     -0.05500  -0.02232   0.01036      (-------*--------)
D     -0.07090  -0.03821  -0.00553  (-------*--------)
                                    --------+---------+---------+---------+-
                                         -0.040     0.000     0.040
0.080



Type = C subtracted from:

Type     Lower    Center    Upper  --------+---------+---------+---------+-
D     -0.04858  -0.01589  0.01679        (-------*-------)
                                   --------+---------+---------+---------+-
                                        -0.040     0.000     0.040     0.080
```

One-way ANOVA: Time 1 versus Liposome Type (day 7)

```
Source  DF         SS         MS     F      P
Type     3  0.0000978  0.0000326  1.39  0.314
Error    8  0.0001875  0.0000234
Total   11  0.0002853

S = 0.004841   R-Sq = 34.27%   R-Sq(adj) = 9.62%
```

```
                                Individual 95% CIs For Mean Based on
                                Pooled StDev
Level  N      Mean     StDev  ------+---------+---------+---------+---
A      3  0.008550  0.002450  (---------*----------)
B      3  0.014167  0.007006           (----------*---------)
C      3  0.013667  0.003786          (----------*----------)
D      3  0.016333  0.004933              (----------*----------)
                                ------+---------+---------+---------+---
                                    0.0060    0.0120    0.0180    0.0240
Pooled StDev = 0.004841


Grouping Information Using Tukey Method

Type  N      Mean  Grouping
D     3  0.016333  A
B     3  0.014167  A
C     3  0.013667  A
A     3  0.008550  A

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%


Type = A subtracted from:

Type      Lower    Center     Upper  -----+---------+---------+---------+---
-
B     -0.007045  0.005617  0.018279           (------------*-----------)
C     -0.007545  0.005117  0.017779          (------------*------------)
D     -0.004879  0.007783  0.020445             (------------*-----------)
                                    -----+---------+---------+---------+---
-
                                      -0.010     0.000     0.010     0.020


Type = B subtracted from:

Type      Lower     Center     Upper  -----+---------+---------+---------+--
--
C     -0.013162  -0.000500  0.012162    (-----------*------------)
D     -0.010495   0.002167  0.014829       (-----------*------------)
                                     -----+---------+---------+---------+--
--
                                       -0.010     0.000     0.010     0.020


Type = C subtracted from:

Type      Lower    Center     Upper  -----+---------+---------+---------+---
-
D     -0.009995  0.002667  0.015329       (------------*-----------)
                                    -----+---------+---------+---------+---
-
                                      -0.010     0.000     0.010     0.020
```

One-way ANOVA: Time 2 versus Liposome Type (day 7)

```
Source  DF       SS       MS     F      P
Type     3  0.07180  0.02393  3.77  0.059
Error    8  0.05079  0.00635
Total   11  0.12259

S = 0.07968   R-Sq = 58.57%   R-Sq(adj) = 43.03%


                           Individual 95% CIs For Mean Based on
                           Pooled StDev
Level  N     Mean    StDev  --+---------+---------+---------+-------
A      3  0.08633  0.08119  (--------*--------)
B      3  0.17433  0.11374          (--------*-------)
C      3  0.28667  0.06429                   (--------*--------)
D      3  0.25333  0.04163                (--------*--------)
                           --+---------+---------+---------+-------
                           0.00      0.12      0.24      0.36

Pooled StDev = 0.07968


Grouping Information Using Tukey Method

Type  N     Mean  Grouping
C     3  0.28667  A
D     3  0.25333  A
B     3  0.17433  A
A     3  0.08633  A

Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%

Type = A subtracted from:

Type     Lower   Center    Upper  ------+---------+---------+---------+---
B     -0.12039  0.08800  0.29639            (---------*----------)
C     -0.00806  0.20033  0.40873                  (---------*---------)
D     -0.04139  0.16700  0.37539                (---------*----------)
                                  ------+---------+---------+---------+---
                                     -0.20      0.00      0.20      0.40

Type = B subtracted from:

Type     Lower   Center    Upper  ------+---------+---------+---------+---
C     -0.09606  0.11233  0.32073             (----------*---------)
D     -0.12939  0.07900  0.28739            (----------*---------)
                                  ------+---------+---------+---------+---
                                     -0.20      0.00      0.20      0.40

Type = C subtracted from:

Type     Lower    Center    Upper  ------+---------+---------+---------+---
D     -0.24173  -0.03333  0.17506       (---------*----------)
                                   ------+---------+---------+---------+---
                                      -0.20      0.00      0.20      0.40
```

One-way ANOVA: Time 3 versus Liposome Type (day 7)

```
Source  DF       SS       MS      F      P
Type     3  1.52653  0.50884  76.33  0.000
Error    8  0.05333  0.00667
Total   11  1.57987

S = 0.08165   R-Sq = 96.62%   R-Sq(adj) = 95.36%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean   StDev  --------+---------+---------+---------+-
A      3  0.9200  0.0000  (----*---)
B      3  0.9200  0.0000  (----*---)
C      3  1.6333  0.1155                            (---*----)
D      3  1.6333  0.1155                            (---*----)
                          --------+---------+---------+---------+-
                                1.00      1.25      1.50      1.75

Pooled StDev = 0.0816


Grouping Information Using Tukey Method

Type  N    Mean  Grouping
D     3  1.6333  A
C     3  1.6333  A
B     3  0.9200    B
A     3  0.9200    B

Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%

Type = A subtracted from:

Type    Lower  Center   Upper  ---------+---------+---------+---------+
B     -0.2135  0.0000  0.2135                (---*---)
C      0.4998  0.7133  0.9269                          (---*----)
D      0.4998  0.7133  0.9269                          (---*----)
                           ---------+---------+---------+---------+
                                 -0.50      0.00      0.50      1.00

Type = B subtracted from:

Type    Lower  Center   Upper  ---------+---------+---------+---------+
C      0.4998  0.7133  0.9269                          (---*----)
D      0.4998  0.7133  0.9269                          (---*----)
                           ---------+---------+---------+---------+
                                 -0.50      0.00      0.50      1.00
Type = C subtracted from:

Type    Lower  Center   Upper  ---------+---------+---------+---------+
D     -0.2135  0.0000  0.2135                (---*---)
                           ---------+---------+---------+---------+
                                 -0.50      0.00      0.50      1.00
```

One-way ANOVA: Area 1 versus Liposome Type (day 7)

```
Source  DF        SS        MS     F      P
Type     3  0.000845  0.000282  1.68  0.247
Error    8  0.001337  0.000167
Total   11  0.002182

S = 0.01293   R-Sq = 38.71%   R-Sq(adj) = 15.73%


                           Individual 95% CIs For Mean Based on
                           Pooled StDev
Level  N     Mean    StDev  ----+---------+---------+---------+-----
A      3  0.02610  0.00801  (----------*-----------)
B      3  0.03283  0.01131      (-----------*----------)
C      3  0.04917  0.01893                (-----------*----------)
D      3  0.03618  0.01087         (----------*-----------)
                           ----+---------+---------+---------+-----
                             0.015     0.030     0.045     0.060

Pooled StDev = 0.01293


Grouping Information Using Tukey Method

Type  N     Mean  Grouping
C     3  0.04917  A
D     3  0.03618  A
B     3  0.03283  A
A     3  0.02610  A

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%


Type = A subtracted from:

Type     Lower   Center    Upper  -------+---------+---------+---------+--
B     -0.02708  0.00673  0.04055          (----------*-----------)
C     -0.01074  0.02307  0.05688               (-----------*----------)
D     -0.02372  0.01009  0.04390           (----------*-----------)
                                  -------+---------+---------+---------+--
                                      -0.030     0.000     0.030     0.060



Type = B subtracted from:

Type     Lower   Center    Upper  -------+---------+---------+---------+--
C     -0.01748  0.01634  0.05015             (----------*-----------)
D     -0.03046  0.00335  0.03717          (----------*----------)
                                  -------+---------+---------+---------+--
                                      -0.030     0.000     0.030     0.060
```

```
Type = C subtracted from:

Type     Lower    Center    Upper  -------+---------+---------+---------+--
D     -0.04680  -0.01298  0.02083   (-----------*----------)
                                   -------+---------+---------+---------+--
                                       -0.030     0.000     0.030     0.060
```

One-way ANOVA: Area 2 versus Liposome Type (day 7)

```
Source  DF        SS        MS     F      P
Type     3  0.002135  0.000712  1.25  0.354
Error    8  0.004544  0.000568
Total   11  0.006679

S = 0.02383   R-Sq = 31.96%   R-Sq(adj) = 6.45%


                           Individual 95% CIs For Mean Based on
                           Pooled StDev
Level  N     Mean    StDev  ----+---------+---------+---------+-----
A      3  0.02027  0.01069  (----------*---------)
B      3  0.05718  0.02975            (----------*----------)
C      3  0.04277  0.02781         (---------*----------)
D      3  0.03495  0.02235      (----------*---------)
                           ----+---------+---------+---------+-----
                             0.000     0.030     0.060     0.090

Pooled StDev = 0.02383

Grouping Information Using Tukey Method

Type  N     Mean  Grouping
B     3  0.05718  A
C     3  0.04277  A
D     3  0.03495  A
A     3  0.02027  A

Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%

Type = A subtracted from:

Type     Lower   Center    Upper  -------+---------+---------+---------+--
B     -0.02543  0.03691  0.09924             (-----------*-----------)
C     -0.03984  0.02250  0.08483          (-----------*-----------)
D     -0.04766  0.01468  0.07701        (-----------*-----------)
                                   -------+---------+---------+---------+--
                                       -0.050     0.000     0.050     0.100

Type = B subtracted from:

Type     Lower    Center    Upper  -------+---------+---------+---------+--
C     -0.07674  -0.01441  0.04793    (-----------*-----------)
D     -0.08456  -0.02223  0.04011  (-----------*-----------)
                                   -------+---------+---------+---------+--
                                       -0.050     0.000     0.050     0.100
```

```
Type = C subtracted from:

Type     Lower    Center    Upper  -------+---------+---------+---------+--
D     -0.07016  -0.00782  0.05451     (-----------*-----------)
                                   -------+---------+---------+---------+--
                                       -0.050     0.000     0.050     0.100
```

One-way ANOVA: Area 3 versus Liposome Type (day 7)

```
Source  DF       SS       MS     F      P
Type     3  0.00570  0.00190  1.49  0.288
Error    8  0.01017  0.00127
Total   11  0.01587

S = 0.03566   R-Sq = 35.90%   R-Sq(adj) = 11.87%

                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level  N    Mean   StDev  -----+---------+---------+---------+----
A      3  0.9623  0.0195               (-----------*----------)
B      3  0.9100  0.0401   (----------*-----------)
C      3  0.9081  0.0458  (-----------*-----------)
D      3  0.9289  0.0316       (-----------*-----------)
                         -----+---------+---------+---------+----
                            0.880     0.920     0.960     1.000
Pooled StDev = 0.0357

Grouping Information Using Tukey Method

Type  N     Mean  Grouping
A     3  0.96234  A
D     3  0.92887  A
B     3  0.91000  A
C     3  0.90806  A

Means that do not share a letter are significantly different.
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%

Type = A subtracted from:

Type     Lower    Center    Upper    -+---------+---------+---------+-------
-
B     -0.14560  -0.05234  0.04092    (-------------*------------)
C     -0.14753  -0.05427  0.03899    (------------*-------------)
D     -0.12673  -0.03347  0.05979       (------------*-------------)
                                     -+---------+---------+---------+-------
-
                                   -0.140    -0.070     0.000     0.070

Type = B subtracted from:

Type     Lower    Center    Upper    -+---------+---------+---------+-------
-
C     -0.09519  -0.00193  0.09133           (-------------*------------)
D     -0.07439   0.01887  0.11213              (-------------*------------)
                                     -+---------+---------+---------+-------
-
                                   -0.140    -0.070     0.000     0.070
```

```
Type = C subtracted from:

Type     Lower   Center    Upper    -+---------+---------+---------+--------
D     -0.07246  0.02080  0.11406               (------------*------------)
                                   -+---------+---------+---------+--------
                                 -0.140    -0.070     0.000     0.070
```

One-way ANOVA: Time 1 versus Liposome Type (day 10)

```
Source  DF         SS         MS     F      P
Type     3  0.0000978  0.0000326  1.39  0.314
Error    8  0.0001875  0.0000234
Total   11  0.0002853

S = 0.004841   R-Sq = 34.27%   R-Sq(adj) = 9.62%


                              Individual 95% CIs For Mean Based on
                              Pooled StDev
Level  N      Mean     StDev  ------+---------+---------+---------+---
A      3  0.008550  0.002450  (---------*----------)
B      3  0.014167  0.007006           (----------*---------)
C      3  0.013667  0.003786          (----------*----------)
D      3  0.016333  0.004933              (----------*----------)
                              ------+---------+---------+---------+---
                                  0.0060    0.0120    0.0180    0.0240

Pooled StDev = 0.004841

Grouping Information Using Tukey Method

Type  N      Mean  Grouping
D     3  0.016333  A
B     3  0.014167  A
C     3  0.013667  A
A     3  0.008550  A

Means that do not share a letter are significantly different.
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type
Individual confidence level = 98.74%

Type = A subtracted from:

Type      Lower    Center     Upper  -----+---------+---------+---------+---
-
B     -0.007045  0.005617  0.018279          (-----------*-----------)
C     -0.007545  0.005117  0.017779         (-----------*-----------)
D     -0.004879  0.007783  0.020445            (-----------*-----------)
                                    -----+---------+---------+---------+---
-
                                       -0.010     0.000     0.010     0.020
Type = B subtracted from:

Type      Lower     Center     Upper  -----+---------+---------+---------+--
--
C     -0.013162  -0.000500  0.012162    (-----------*-----------)
D     -0.010495   0.002167  0.014829       (-----------*-----------)
                                     -----+---------+---------+---------+--
--
                                       -0.010     0.000     0.010     0.020
```

```
Type = C subtracted from:

Type      Lower    Center     Upper  -----+---------+---------+---------+---
-
D     -0.009995  0.002667  0.015329       (------------*-----------)
                                    -----+---------+---------+---------+---
-
                                      -0.010     0.000     0.010     0.020
```

One-way ANOVA: Time 2 versus Liposome Type (day 10)

```
Source  DF       SS       MS     F      P
Type     3  0.07180  0.02393  3.77  0.059
Error    8  0.05079  0.00635
Total   11  0.12259

S = 0.07968   R-Sq = 58.57%   R-Sq(adj) = 43.03%

                           Individual 95% CIs For Mean Based on
                           Pooled StDev
Level  N     Mean    StDev  --+---------+---------+---------+-------
A      3  0.08633  0.08119  (--------*--------)
B      3  0.17433  0.11374          (--------*-------)
C      3  0.28667  0.06429                   (--------*--------)
D      3  0.25333  0.04163                 (--------*--------)
                           --+---------+---------+---------+-------
                           0.00      0.12      0.24      0.36

Pooled StDev = 0.07968

Grouping Information Using Tukey Method

Type  N     Mean  Grouping
C     3  0.28667  A
D     3  0.25333  A
B     3  0.17433  A
A     3  0.08633  A

Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type
Individual confidence level = 98.74%

Type = A subtracted from:

Type     Lower   Center    Upper  ------+---------+---------+---------+---
B     -0.12039  0.08800  0.29639            (---------*----------)
C     -0.00806  0.20033  0.40873                  (---------*---------)
D     -0.04139  0.16700  0.37539               (---------*----------)
                                  ------+---------+---------+---------+---
                                     -0.20      0.00      0.20      0.40
Type = B subtracted from:

Type     Lower   Center    Upper  ------+---------+---------+---------+---
C     -0.09606  0.11233  0.32073             (----------*---------)
D     -0.12939  0.07900  0.28739            (---------*---------)
                                  ------+---------+---------+---------+---
                                     -0.20      0.00      0.20      0.40
```

```
Type = C subtracted from:

Type     Lower    Center    Upper  ------+---------+---------+---------+---
D     -0.24173  -0.03333  0.17506      (---------*----------)
                                   ------+---------+---------+---------+---
                                      -0.20      0.00      0.20      0.40
```

One-way ANOVA: Time 3 versus Liposome Type (day 10)

```
Source  DF       SS       MS      F      P
Type     3  1.52653  0.50884  76.33  0.000
Error    8  0.05333  0.00667
Total   11  1.57987

S = 0.08165   R-Sq = 96.62%   R-Sq(adj) = 95.36%

                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean   StDev  --------+---------+---------+---------+-
A      3  0.9200  0.0000  (----*---)
B      3  0.9200  0.0000  (----*---)
C      3  1.6333  0.1155                            (---*----)
D      3  1.6333  0.1155                            (---*----)
                          --------+---------+---------+---------+-
                                1.00      1.25      1.50      1.75
Pooled StDev = 0.0816


Grouping Information Using Tukey Method

Type  N    Mean  Grouping
D     3  1.6333  A
C     3  1.6333  A
B     3  0.9200    B
A     3  0.9200    B

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%

Type = A subtracted from:

Type    Lower  Center   Upper  ---------+---------+---------+---------+
B     -0.2135  0.0000  0.2135               (---*---)
C      0.4998  0.7133  0.9269                         (---*----)
D      0.4998  0.7133  0.9269                         (---*----)
                                ---------+---------+---------+---------+
                                      -0.50      0.00      0.50      1.00

Type = B subtracted from:

Type   Lower  Center   Upper  ---------+---------+---------+---------+
C     0.4998  0.7133  0.9269                         (---*----)
D     0.4998  0.7133  0.9269                         (---*----)
                              ---------+---------+---------+---------+
                                   -0.50      0.00      0.50      1.00
```

```
Type = C subtracted from:

Type    Lower  Center   Upper  ---------+---------+---------+---------+
D     -0.2135  0.0000  0.2135               (---*---)
                             ---------+---------+---------+---------+
                                   -0.50      0.00      0.50      1.00
```

One-way ANOVA: Area 1 versus Liposome Type (day 10)

```
Source  DF        SS        MS     F      P
Type     3  0.000845  0.000282  1.68  0.247
Error    8  0.001337  0.000167
Total   11  0.002182

S = 0.01293   R-Sq = 38.71%   R-Sq(adj) = 15.73%


                           Individual 95% CIs For Mean Based on
                           Pooled StDev
Level  N     Mean    StDev  ----+---------+---------+---------+-----
A      3  0.02610  0.00801  (-----------*-----------)
B      3  0.03283  0.01131      (-----------*----------)
C      3  0.04917  0.01893                 (-----------*----------)
D      3  0.03618  0.01087          (----------*-----------)
                           ----+---------+---------+---------+-----
                             0.015     0.030     0.045     0.060

Pooled StDev = 0.01293
Grouping Information Using Tukey Method

Type  N     Mean  Grouping
C     3  0.04917  A
D     3  0.03618  A
B     3  0.03283  A
A     3  0.02610  A

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%


Type = A subtracted from:

Type     Lower   Center    Upper  -------+---------+---------+---------+--
B     -0.02708  0.00673  0.04055           (----------*-----------)
C     -0.01074  0.02307  0.05688                (-----------*----------)
D     -0.02372  0.01009  0.04390           (----------*-----------)
                                 -------+---------+---------+---------+--
                                     -0.030     0.000     0.030     0.060

Type = B subtracted from:

Type     Lower   Center    Upper  -------+---------+---------+---------+--
C     -0.01748  0.01634  0.05015             (----------*-----------)
D     -0.03046  0.00335  0.03717          (----------*----------)
                                 -------+---------+---------+---------+--
                                     -0.030     0.000     0.030     0.060
```

```
Type = C subtracted from:

Type     Lower    Center    Upper  -------+---------+---------+---------+--
D     -0.04680  -0.01298  0.02083   (-----------*----------)
                                   -------+---------+---------+---------+--
                                       -0.030     0.000     0.030     0.060
```

One-way ANOVA: Area 2 versus Liposome Type (day 10)

```
Source  DF        SS        MS     F      P
Type     3  0.002135  0.000712  1.25  0.354
Error    8  0.004544  0.000568
Total   11  0.006679

S = 0.02383   R-Sq = 31.96%   R-Sq(adj) = 6.45%


                           Individual 95% CIs For Mean Based on
                           Pooled StDev
Level  N     Mean    StDev  ----+---------+---------+---------+-----
A      3  0.02027  0.01069  (----------*---------)
B      3  0.05718  0.02975             (----------*----------)
C      3  0.04277  0.02781          (---------*----------)
D      3  0.03495  0.02235       (----------*---------)
                           ----+---------+---------+---------+-----
                             0.000     0.030     0.060     0.090

Pooled StDev = 0.02383


Grouping Information Using Tukey Method

Type  N     Mean  Grouping
B     3  0.05718  A
C     3  0.04277  A
D     3  0.03495  A
A     3  0.02027  A

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type
Individual confidence level = 98.74%

Type = A subtracted from:

Type     Lower   Center    Upper  -------+---------+---------+---------+--
B     -0.02543  0.03691  0.09924             (-----------*-----------)
C     -0.03984  0.02250  0.08483          (-----------*-----------)
D     -0.04766  0.01468  0.07701        (-----------*-----------)
                                   -------+---------+---------+---------+--
                                      -0.050     0.000     0.050     0.100
Type = B subtracted from:

Type     Lower    Center    Upper  -------+---------+---------+---------+--
C     -0.07674  -0.01441  0.04793    (-----------*-----------)
D     -0.08456  -0.02223  0.04011  (-----------*-----------)
                                   -------+---------+---------+---------+--
                                      -0.050     0.000     0.050     0.100
```

```
Type = C subtracted from:

Type     Lower    Center    Upper  -------+---------+---------+---------+--
D     -0.07016  -0.00782  0.05451     (-----------*-----------)
                                   -------+---------+---------+---------+--
                                       -0.050     0.000     0.050     0.100
```

One-way ANOVA: Area 3 versus Liposome Type (day 10)

```
Source  DF       SS       MS     F      P
Type     3  0.00570  0.00190  1.49  0.288
Error    8  0.01017  0.00127
Total   11  0.01587

S = 0.03566   R-Sq = 35.90%   R-Sq(adj) = 11.87%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean   StDev  -----+---------+---------+---------+----
A      3  0.9623  0.0195               (-----------*----------)
B      3  0.9100  0.0401   (----------*-----------)
C      3  0.9081  0.0458  (-----------*-----------)
D      3  0.9289  0.0316       (-----------*-----------)
                          -----+---------+---------+---------+----
                             0.880     0.920     0.960     1.000

Pooled StDev = 0.0357

Grouping Information Using Tukey Method

Type  N     Mean  Grouping
A     3  0.96234  A
D     3  0.92887  A
B     3  0.91000  A
C     3  0.90806  A

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%


Type = A subtracted from:

Type     Lower    Center    Upper    -+---------+---------+---------+-------
-
B     -0.14560  -0.05234  0.04092    (-------------*------------)
C     -0.14753  -0.05427  0.03899    (------------*-------------)
D     -0.12673  -0.03347  0.05979       (------------*-------------)
                                     -+---------+---------+---------+-------
-
                                  -0.140    -0.070     0.000     0.070
```

```
Type = B subtracted from:

Type     Lower    Center    Upper    -+---------+---------+---------+-------
-
C     -0.09519  -0.00193  0.09133           (-------------*------------)
D     -0.07439   0.01887  0.11213              (-------------*------------)
                                    -+---------+---------+---------+-------
-
                                  -0.140    -0.070     0.000     0.070


Type = C subtracted from:

Type     Lower   Center    Upper    -+---------+---------+---------+--------
D     -0.07246  0.02080  0.11406              (------------*------------)
                                   -+---------+---------+---------+--------
                                 -0.140    -0.070     0.000     0.070
```

One-way ANOVA: Time 1 versus Liposome Type (day 14)

```
Source  DF         SS         MS     F      P
Type     3  0.0001900  0.0000633  7.84  0.009
Error    8  0.0000647  0.0000081
Total   11  0.0002547

S = 0.002843   R-Sq = 74.61%   R-Sq(adj) = 65.09%


                              Individual 95% CIs For Mean Based on
                              Pooled StDev
Level  N      Mean     StDev  ------+---------+---------+---------+---
A      3  0.015000  0.000000         (-------*-------)
B      3  0.011000  0.000000  (-------*-------)
C      3  0.022000  0.005292                       (-------*-------)
D      3  0.014667  0.002082         (------*-------)
                              ------+---------+---------+---------+---
                                  0.0100    0.0150    0.0200    0.0250

Pooled StDev = 0.002843

Grouping Information Using Tukey Method

Type  N      Mean  Grouping
C     3  0.022000  A
A     3  0.015000  A B
D     3  0.014667  A B
B     3  0.011000    B

Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%
```

```
Type = A subtracted from:

Type      Lower     Center     Upper  --------+---------+---------+---------
+-
B     -0.011436  -0.004000  0.003436         (------*------)
C     -0.000436   0.007000  0.014436                   (------*------)
D     -0.007769  -0.000333  0.007103            (-------*------)
                                     --------+---------+---------+---------
+-
                                          -0.010     0.000     0.010
0.020
Type = B subtracted from:

Type      Lower    Center     Upper  --------+---------+---------+---------
+-
C      0.003564  0.011000  0.018436                       (------*------)
D     -0.003769  0.003667  0.011103               (-------*------)
                                    --------+---------+---------+---------
+-
                                         -0.010     0.000     0.010
0.020

Type = C subtracted from:

Type      Lower     Center     Upper  --------+---------+---------+---------
+-
D     -0.014769  -0.007333  0.000103     (-------*------)
                                     --------+---------+---------+---------
+-
                                          -0.010     0.000     0.010
0.020
```

One-way ANOVA: Time 2 versus Liposome Type (day 14)

```
Source  DF        SS        MS      F      P
Type     3  0.144942  0.048314  62.03  0.000
Error    8  0.006231  0.000779
Total   11  0.151173

S = 0.02791   R-Sq = 95.88%   R-Sq(adj) = 94.33%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N     Mean    StDev  ---+---------+---------+---------+------
A      3  0.10100  0.00900   (----*---)
B      3  0.09533  0.03002  (----*----)
C      3  0.30000  0.00000                          (----*---)
D      3  0.33333  0.04619                             (----*---)
                          ---+---------+---------+---------+------
                           0.080     0.160     0.240     0.320
Pooled StDev = 0.02791

Grouping Information Using Tukey Method

Type  N     Mean  Grouping
D     3  0.33333  A
C     3  0.30000  A
A     3  0.10100    B
B     3  0.09533    B
Means that do not share a letter are significantly different.
```

```
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%

Type = A subtracted from:

Type     Lower    Center    Upper  ---------+---------+---------+---------+
B     -0.07866  -0.00567  0.06733               (----*---)
C      0.12601   0.19900  0.27199                         (---*----)
D      0.15934   0.23233  0.30533                           (----*---)
                                  ---------+---------+---------+---------+
                                        -0.16      0.00      0.16      0.32
Type = B subtracted from:

Type    Lower   Center    Upper  ---------+---------+---------+---------+
C     0.13167  0.20467  0.27766                        (----*---)
D     0.16501  0.23800  0.31099                          (----*---)
                                 ---------+---------+---------+---------+
                                       -0.16      0.00      0.16      0.32


Type = C subtracted from:

Type     Lower   Center    Upper  ---------+---------+---------+---------+
D     -0.03966  0.03333  0.10633                  (---*----)
                                  ---------+---------+---------+---------+
                                        -0.16      0.00      0.16      0.32
```

One-way ANOVA: Time 3 versus Liposome Type (day 14)

```
Source  DF        SS        MS        F      P
Type     3  2.105625  0.701875  1123.00  0.000
Error    8  0.005000  0.000625
Total   11  2.110625

S = 0.025   R-Sq = 99.76%   R-Sq(adj) = 99.67%


                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level  N    Mean   StDev  ------+---------+---------+---------+---
A      3  1.0500  0.0500  (*)
B      3  1.1000  0.0000    (*)
C      3  1.8000  0.0000                        (*)
D      3  2.0000  0.0000                              (*)
                         ------+---------+---------+---------+---
                             1.20      1.50      1.80      2.10

Pooled StDev = 0.0250
```

```
Grouping Information Using Tukey Method

Type  N    Mean  Grouping
D     3  2.0000  A
C     3  1.8000    B
B     3  1.1000      C
A     3  1.0500      C

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%


Type = A subtracted from:

Type    Lower  Center   Upper  ---------+---------+---------+---------+
B     -0.0154  0.0500  0.1154                    (*)
C      0.6846  0.7500  0.8154                              (*)
D      0.8846  0.9500  1.0154                                  (*)
                            ---------+---------+---------+---------+
                                  -0.50      0.00      0.50      1.00


Type = B subtracted from:

Type   Lower  Center   Upper  ---------+---------+---------+---------+
C     0.6346  0.7000  0.7654                               (*)
D     0.8346  0.9000  0.9654                                   (*)
                            ---------+---------+---------+---------+
                                  -0.50      0.00      0.50      1.00

Type = C subtracted from:

Type   Lower  Center   Upper  ---------+---------+---------+---------+
D     0.1346  0.2000  0.2654                      (*)
                            ---------+---------+---------+---------+
                                  -0.50      0.00      0.50      1.00
```

One-way ANOVA: Area 1 versus Liposome Type (day 14)

```
Source  DF        SS        MS     F      P
Type     3  0.001182  0.000394  3.11  0.089
Error    8  0.001014  0.000127
Total   11  0.002196

S = 0.01126   R-Sq = 53.82%   R-Sq(adj) = 36.50%

                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N     Mean    StDev  ---+---------+---------+---------+------
A      3  0.02710  0.00001   (---------*---------)
B      3  0.04800  0.00118                (---------*---------)
C      3  0.02488  0.01259  (---------*---------)
D      3  0.04288  0.01863             (---------*---------)
                          ---+---------+---------+---------+------
                           0.015     0.030     0.045     0.060
Pooled StDev = 0.01126
```

```
Grouping Information Using Tukey Method

Type  N     Mean  Grouping
B     3  0.04800  A
D     3  0.04288  A
A     3  0.02710  A
C     3  0.02488  A

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%


Type = A subtracted from:

Type     Lower    Center    Upper  --------+---------+---------+---------+-
B     -0.00855   0.02090  0.05035                (---------*---------)
C     -0.03167  -0.00222  0.02723        (---------*---------)
D     -0.01367   0.01578  0.04523              (---------*---------)
                                   --------+---------+---------+---------+-
                                        -0.030     0.000     0.030     0.060

Type = B subtracted from:

Type     Lower    Center    Upper  --------+---------+---------+---------+-
C     -0.05257  -0.02312  0.00633  (---------*---------)
D     -0.03457  -0.00512  0.02433       (---------*---------)
                                   --------+---------+---------+---------+-
                                        -0.030     0.000     0.030     0.060
Type = C subtracted from:

Type     Lower   Center    Upper  --------+---------+---------+---------+-
D     -0.01145  0.01800  0.04745               (---------*---------)
                                  --------+---------+---------+---------+-
                                       -0.030     0.000     0.030     0.060
```

One-way ANOVA: Area 2 versus Liposome Type (day 14)

```
Source  DF         SS         MS      F      P
Type     3  0.0010605  0.0003535  16.50  0.001
Error    8  0.0001714  0.0000214
Total   11  0.0012320

S = 0.004629   R-Sq = 86.08%   R-Sq(adj) = 80.87%

                            Individual 95% CIs For Mean Based on
                            Pooled StDev
Level  N      Mean     StDev  ------+---------+---------+---------+---
A      3  0.010268  0.000680  (-----*-----)
B      3  0.016313  0.000081       (-----*-----)
C      3  0.032109  0.002561                    (-----*-----)
D      3  0.031062  0.008871                   (-----*-----)
                            ------+---------+---------+---------+---
                                0.010     0.020     0.030     0.040

Pooled StDev = 0.004629
```

```
Grouping Information Using Tukey Method

Type  N      Mean  Grouping
C     3  0.032109  A
D     3  0.031062  A
B     3  0.016313    B
A     3  0.010268    B

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%


Type = A subtracted from:

Type      Lower    Center     Upper  -------+---------+---------+---------+-
-
B     -0.006062  0.006045  0.018152               (-------*------)
C      0.009734  0.021841  0.033948                         (-------*------)
D      0.008686  0.020794  0.032901                       (-------*-------)
                                   -------+---------+---------+---------+-
-
                                       -0.016     0.000     0.016
0.032
Type = B subtracted from:

Type     Lower    Center     Upper  -------+---------+---------+---------+--
C     0.003688  0.015795  0.027902                    (-------*------)
D     0.002641  0.014748  0.026855                    (------*-------)
                                  -------+---------+---------+---------+--
                                      -0.016     0.000     0.016     0.032


Type = C subtracted from:

Type      Lower     Center     Upper  -------+---------+---------+---------
+--
D     -0.013154  -0.001047  0.011060           (------*-------)
                                   -------+---------+---------+---------
+--
                                       -0.016     0.000     0.016
0.032
```

One-way ANOVA: Area 3 versus Liposome Type (day 14)

```
Source  DF       SS       MS     F      P
Type     3  0.01063  0.00354  1.55  0.275
Error    8  0.01829  0.00229
Total   11  0.02892

S = 0.04782   R-Sq = 36.76%   R-Sq(adj) = 13.05%
```

```
                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean   StDev  ------+---------+---------+---------+---
A      3  0.8676  0.0944  (----------*---------)
B      3  0.9357  0.0011            (----------*----------)
C      3  0.9430  0.0114              (---------*----------)
D      3  0.9261  0.0103           (---------*----------)
                          ------+---------+---------+---------+---
                              0.840     0.900     0.960     1.020

Pooled StDev = 0.0478

Grouping Information Using Tukey Method

Type  N     Mean  Grouping
C     3  0.94301  A
B     3  0.93568  A
D     3  0.92605  A
A     3  0.86758  A

Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%


Type = A subtracted from:

Type     Lower   Center    Upper  ----+---------+---------+---------+-----
B     -0.05696  0.06810  0.19315          (------------*-----------)
C     -0.04963  0.07542  0.20048           (------------*-----------)
D     -0.06659  0.05847  0.18353         (------------*-----------)
                                  ----+---------+---------+---------+-----
                                   -0.10      0.00      0.10      0.20
Type = B subtracted from:

Type     Lower    Center    Upper  ----+---------+---------+---------+-----
C     -0.11773   0.00732  0.13238    (------------*-----------)
D     -0.13469  -0.00963  0.11543   (-----------*------------)
                                   ----+---------+---------+---------+-----
                                    -0.10      0.00      0.10      0.20

Type = C subtracted from:

Type     Lower    Center    Upper  ----+---------+---------+---------+-----
D     -0.14201  -0.01695  0.10810  (-----------*------------)
                                   ----+---------+---------+---------+-----
                                    -0.10      0.00      0.10      0.20
```

One-way ANOVA: Time 1 versus Liposome Type (day 18)

```
Source  DF         SS         MS     F      P
Type     3  0.0008245  0.0002748  6.30  0.017
Error    8  0.0003492  0.0000436
Total   11  0.0011737
```

```
S = 0.006607   R-Sq = 70.25%   R-Sq(adj) = 59.09%
                               Individual 95% CIs For Mean Based on
                               Pooled StDev
Level  N      Mean     StDev  ----+---------+---------+---------+-----
A      3  0.008267  0.008451     (--------*--------)
B      3  0.005267  0.003721  (--------*--------)
C      3  0.024000  0.008185                    (--------*--------)
D      3  0.022333  0.004726                  (-------*--------)
                               ----+---------+---------+---------+-----
                                 0.000     0.010     0.020     0.030

Pooled StDev = 0.006607


Grouping Information Using Tukey Method

Type  N      Mean  Grouping
C     3  0.024000  A
D     3  0.022333  A B
A     3  0.008267  A B
B     3  0.005267    B

Means that do not share a letter are significantly different.
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%


Type = A subtracted from:

Type      Lower     Center     Upper  --------+---------+---------+---------
+-
B     -0.020279  -0.003000  0.014279          (--------*-------)
C     -0.001546   0.015733  0.033012                   (--------*--------)
D     -0.003212   0.014067  0.031346                 (--------*--------)
                                     --------+---------+---------+---------
+-
                                          -0.020     0.000     0.020
0.040
Type = B subtracted from:

Type      Lower    Center     Upper  --------+---------+---------+---------
+-
C      0.001454  0.018733  0.036012                     (-------*--------)
D     -0.000212  0.017067  0.034346                   (--------*-------)
                                    --------+---------+---------+---------
+-
                                        -0.020     0.000     0.020
0.040

Type = C subtracted from:

Type      Lower     Center     Upper  --------+---------+---------+---------
+-
D     -0.018946  -0.001667  0.015612           (-------*--------)
                                     --------+---------+---------+---------
+-
                                          -0.020     0.000     0.020
0.040
```

One-way ANOVA: Time 2 versus Liposome Type (day 18)

```
Source  DF       SS       MS      F      P
Type     3  0.22617  0.07539  51.22  0.000
Error    8  0.01177  0.00147
Total   11  0.23794

S = 0.03836   R-Sq = 95.05%   R-Sq(adj) = 93.20%


                            Individual 95% CIs For Mean Based on Pooled
StDev
Level  N     Mean    StDev    +---------+---------+---------+---------
A      3  0.06000  0.05231     (---*---)
B      3  0.05033  0.02201    (---*---)
C      3  0.23333  0.01155                 (---*----)
D      3  0.38333  0.05033                           (---*---)
                              +---------+---------+---------+---------
                            0.00      0.12      0.24      0.36
Pooled StDev = 0.03836


Grouping Information Using Tukey Method

Type  N     Mean  Grouping
D     3  0.38333  A
C     3  0.23333    B
A     3  0.06000      C
B     3  0.05033      C

Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%

Type = A subtracted from:

Type     Lower    Center    Upper  -------+---------+---------+---------+--
B     -0.11000  -0.00967  0.09067              (---*---)
C      0.07300   0.17333  0.27367                      (---*---)
D      0.22300   0.32333  0.42367                            (---*---)
                                  -------+---------+---------+---------+--
                                      -0.25      0.00      0.25      0.50

Type = B subtracted from:

Type    Lower   Center    Upper  -------+---------+---------+---------+--
C     0.08266  0.18300  0.28334                    (---*---)
D     0.23266  0.33300  0.43334                          (---*---)
                                -------+---------+---------+---------+--
                                    -0.25      0.00      0.25      0.50

Type = C subtracted from:

Type    Lower   Center    Upper  -------+---------+---------+---------+--
D     0.04966  0.15000  0.25034                   (---*---)
                                -------+---------+---------+---------+--
                                    -0.25      0.00      0.25      0.50
```

One-way ANOVA: Time 3 versus Liposome Type (day 18)

```
Source  DF        SS        MS  F  P
Type     3  2.302500  0.767500  *  *
Error    8  0.000000  0.000000
Total   11  2.302500

S = 0   R-Sq = 100.00%   R-Sq(adj) = 100.00%


                           Individual 95% CIs For Mean Based on Pooled
StDev
Level  N     Mean    StDev    +---------+---------+---------+---------
A      3  1.00000  0.00000    *
B      3  1.00000  0.00000    *
C      3  1.70000  0.00000                              *
D      3  2.00000  0.00000                                           *
                              +---------+---------+---------+---------
                             1.00      1.25      1.50      1.75

Pooled StDev = 0.00000

Grouping Information Using Tukey Method

Type  N     Mean  Grouping
D     3  2.00000  A
C     3  1.70000    B
B     3  1.00000      C
A     3  1.00000        D

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%


Type = A subtracted from:

Type    Lower   Center    Upper     +---------+---------+---------+---------
B     0.00000  0.00000  0.00000                        *
C     0.70000  0.70000  0.70000                                    *
D     1.00000  1.00000  1.00000
*
                                   +---------+---------+---------+---------
                                -1.00     -0.50      0.00      0.50

Type = B subtracted from:

Type    Lower   Center    Upper     +---------+---------+---------+---------
C     0.70000  0.70000  0.70000                                    *
D     1.00000  1.00000  1.00000
*
                                   +---------+---------+---------+---------
                                -1.00     -0.50      0.00      0.50
```

```
Type = C subtracted from:

Type    Lower   Center    Upper     +---------+---------+---------+---------
D     0.30000  0.30000  0.30000                           *
                                   +---------+---------+---------+---------
                                -1.00     -0.50      0.00      0.50
```

One-way ANOVA: Area 1 versus Liposome Type (day 18)

```
Source  DF        SS        MS     F      P
Type     3  0.002369  0.000790  4.05  0.050
Error    8  0.001559  0.000195
Total   11  0.003928

S = 0.01396   R-Sq = 60.32%   R-Sq(adj) = 45.44%


                            Individual 95% CIs For Mean Based on Pooled
StDev
Level  N     Mean    StDev  ---------+---------+---------+---------+
A      3  0.02080  0.00971  (--------*---------)
B      3  0.03040  0.01868       (--------*--------)
C      3  0.05447  0.01092                 (--------*---------)
D      3  0.05106  0.01473              (---------*--------)
                            ---------+---------+---------+---------+
                                   0.020     0.040     0.060     0.080

Pooled StDev = 0.01396
Grouping Information Using Tukey Method

Type  N     Mean  Grouping
C     3  0.05447  A
D     3  0.05106  A
B     3  0.03040  A
A     3  0.02080  A

Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%

Type = A subtracted from:

Type     Lower   Center    Upper  -------+---------+---------+---------+--
B     -0.02691  0.00960  0.04610           (----------*---------)
C     -0.00284  0.03367  0.07018                  (----------*---------)
D     -0.00625  0.03026  0.06677                (----------*---------)
                                -------+---------+---------+---------+--
                                    -0.035     0.000     0.035     0.070

Type = B subtracted from:

Type     Lower   Center    Upper  -------+---------+---------+---------+--
C     -0.01244  0.02407  0.06058              (----------*---------)
D     -0.01585  0.02066  0.05717            (----------*---------)
                                -------+---------+---------+---------+--
                                    -0.035     0.000     0.035     0.070
```

```
Type = C subtracted from:

Type     Lower    Center    Upper  -------+---------+---------+---------+--
D     -0.03992  -0.00341  0.03310        (---------*---------)
                                  -------+---------+---------+---------+--
                                      -0.035     0.000     0.035     0.070
```

One-way ANOVA: Area 2 versus Liposome Type (day 18)

```
Source  DF         SS         MS     F      P
Type     3  0.0005157  0.0001719  2.08  0.181
Error    8  0.0006607  0.0000826
Total   11  0.0011764

S = 0.009088   R-Sq = 43.84%   R-Sq(adj) = 22.78%


                            Individual 95% CIs For Mean Based on
                            Pooled StDev
Level  N      Mean     StDev  ------+---------+---------+---------+---
A      3  0.025335  0.005434        (---------*---------)
B      3  0.016880  0.004911  (---------*---------)
C      3  0.025248  0.006956        (---------*---------)
D      3  0.035383  0.015110                  (---------*----------)
                            ------+---------+---------+---------+---
                                0.012     0.024     0.036     0.048

Pooled StDev = 0.009088

Grouping Information Using Tukey Method

Type  N      Mean  Grouping
D     3  0.035383  A
A     3  0.025335  A
C     3  0.025248  A
B     3  0.016880  A

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%

Type = A subtracted from:

Type      Lower     Center     Upper  -------+---------+---------+---------
+--
B     -0.032223  -0.008455  0.015313      (---------*--------)
C     -0.023854  -0.000086  0.023681         (---------*--------)
D     -0.013719   0.010048  0.033816              (--------*---------)
                                       -------+---------+---------+---------
+--
                                           -0.025     0.000     0.025
0.050
```

```
Type = B subtracted from:

Type      Lower    Center     Upper  -------+---------+---------+---------+-
-
C     -0.015399  0.008369  0.032137             (--------*---------)
D     -0.005264  0.018504  0.042271                 (--------*---------)
                                    -------+---------+---------+---------+-
-
                                        -0.025     0.000     0.025
0.050

Type = C subtracted from:

Type      Lower    Center     Upper  -------+---------+---------+---------+-
-
D     -0.013633  0.010135  0.033903              (--------*---------)
                                    -------+---------+---------+---------+-
-
                                        -0.025     0.000     0.025
0.050
```

One-way ANOVA: Area 3 versus Liposome Type (day 18)

```
Source  DF        SS        MS     F      P
Type     3  0.003830  0.001277  1.49  0.289
Error    8  0.006841  0.000855
Total   11  0.010670

S = 0.02924   R-Sq = 35.89%   R-Sq(adj) = 11.85%


                           Individual 95% CIs For Mean Based on
                           Pooled StDev
Level  N     Mean    StDev  --+---------+---------+---------+-------
A      3  0.90578  0.04578  (----------*----------)
B      3  0.95272  0.01398               (----------*----------)
C      3  0.92028  0.01656      (----------*----------)
D      3  0.91356  0.02924    (----------*----------)
                           --+---------+---------+---------+-------
                           0.875     0.910     0.945     0.980

Pooled StDev = 0.02924


Grouping Information Using Tukey Method

Type  N     Mean  Grouping
B     3  0.95272  A
C     3  0.92028  A
D     3  0.91356  A
A     3  0.90578  A

Means that do not share a letter are significantly different.
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%
```

```
Type = A subtracted from:

Type     Lower   Center    Upper  ---------+---------+---------+---------+
B     -0.02953  0.04694  0.12342               (------------*------------)
C     -0.06197  0.01450  0.09098           (-----------*------------)
D     -0.06870  0.00778  0.08426          (-----------*------------)
                                  ---------+---------+---------+---------+
                                        -0.060     0.000     0.060     0.120

Type = B subtracted from:

Type     Lower    Center    Upper  ---------+---------+---------+---------+
C     -0.10892  -0.03244  0.04404   (------------*-----------)
D     -0.11564  -0.03917  0.03731  (-----------*------------)
                                   ---------+---------+---------+---------+
                                         -0.060     0.000     0.060
0.120

Type = C subtracted from:

Type     Lower    Center    Upper  ---------+---------+---------+---------+
D     -0.08320  -0.00673  0.06975       (------------*------------)
                                   ---------+---------+---------+---------+
                                         -0.060     0.000     0.060
0.120
```

One-way ANOVA: Time 1 versus Liposome Type (day 21)

```
Source  DF         SS         MS     F      P
Type     3  0.0001448  0.0000483  2.53  0.131
Error    8  0.0001529  0.0000191
Total   11  0.0002977

S = 0.004372   R-Sq = 48.64%   R-Sq(adj) = 29.38%

                            Individual 95% CIs For Mean Based on
                            Pooled StDev
Level  N      Mean     StDev   -+---------+---------+---------+--------
A      3  0.011200  0.004257   (---------*--------)
B      3  0.015400  0.007529          (---------*--------)
C      3  0.013667  0.000577       (---------*--------)
D      3  0.020667  0.001155                   (--------*---------)
                             -+---------+---------+---------+--------
                            0.0060    0.0120    0.0180    0.0240

Pooled StDev = 0.004372


Grouping Information Using Tukey Method

Type  N      Mean  Grouping
D     3  0.020667  A
B     3  0.015400  A
C     3  0.013667  A
A     3  0.011200  A

Means that do not share a letter are significantly different.
```

```
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%


Type = A subtracted from:

Type      Lower    Center     Upper  --------+---------+---------+---------
+-
B     -0.007235  0.004200  0.015635             (----------*----------)
C     -0.008969  0.002467  0.013902           (----------*----------)
D     -0.001969  0.009467  0.020902                  (----------*----------
)
                                     --------+---------+---------+---------
+-
                                          -0.010     0.000     0.010
0.020


Type = B subtracted from:

Type      Lower     Center     Upper  --------+---------+---------+---------
+-
C     -0.013169  -0.001733  0.009702       (----------*----------)
D     -0.006169   0.005267  0.016702              (----------*----------)
                                      --------+---------+---------+---------
+-
                                           -0.010     0.000     0.010
0.020

Type = C subtracted from:

Type      Lower    Center     Upper  --------+---------+---------+---------
+-
D     -0.004435  0.007000  0.018435               (----------*----------)
                                     --------+---------+---------+---------
+-
                                          -0.010     0.000     0.010
0.020
```

One-way ANOVA: Time 2 versus Liposome Type (day 21)

```
Source  DF       SS       MS     F      P
Type     3  0.13702  0.04567  8.36  0.008
Error    8  0.04371  0.00546
Total   11  0.18073

S = 0.07392   R-Sq = 75.82%   R-Sq(adj) = 66.75%

                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N     Mean    StDev  --+---------+---------+---------+-------
A      3  0.07100  0.06255  (-------*-------)
B      3  0.13400  0.13232       (-------*-------)
C      3  0.24667  0.01155                (--------*-------)
D      3  0.35000  0.01732                         (-------*-------)
                          --+---------+---------+---------+-------
                          0.00      0.12      0.24      0.36

Pooled StDev = 0.07392
```

```
Grouping Information Using Tukey Method

Type  N     Mean  Grouping
D     3  0.35000  A
C     3  0.24667  A B
B     3  0.13400    B
A     3  0.07100    B

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%


Type = A subtracted from:

Type     Lower   Center    Upper  ------+---------+---------+---------+---
B     -0.13032  0.06300  0.25632             (-------*------)
C     -0.01765  0.17567  0.36899                 (-------*-------)
D      0.08568  0.27900  0.47232                     (-------*-------)
                                  ------+---------+---------+---------+---
                                     -0.25      0.00      0.25      0.50


Type = B subtracted from:

Type     Lower   Center    Upper  ------+---------+---------+---------+---
C     -0.08065  0.11267  0.30599               (-------*------)
D      0.02268  0.21600  0.40932                   (-------*------)
                                  ------+---------+---------+---------+---
                                     -0.25      0.00      0.25      0.50


Type = C subtracted from:

Type     Lower   Center    Upper  ------+---------+---------+---------+---
D     -0.08999  0.10333  0.29665             (-------*-------)
                                  ------+---------+---------+---------+---
                                     -0.25      0.00      0.25      0.50
```

One-way ANOVA: Time 3 versus Liposome Type (day 21)

```
Source  DF        SS        MS  F  P
Type     3  2.520900  0.840300  *  *
Error    8  0.000000  0.000000
Total   11  2.520900

S = 0   R-Sq = 100.00%   R-Sq(adj) = 100.00%
```

```
                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N     Mean    StDev ---------+---------+---------+---------+
A      3  0.92000  0.00000 *
B      3  1.00000  0.00000   *
C      3  1.70000  0.00000                     *
D      3  2.00000  0.00000                               *
                          ---------+---------+---------+---------+
                                1.20      1.50      1.80      2.10

Pooled StDev = 0.00000


Grouping Information Using Tukey Method

Type  N     Mean  Grouping
D     3  2.00000  A
C     3  1.70000    B
B     3  1.00000      C
A     3  0.92000        D

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%


Type = A subtracted from:

Type    Lower   Center    Upper  -------+---------+---------+---------+--
B     0.08000  0.08000  0.08000                *
C     0.78000  0.78000  0.78000                          *
D     1.08000  1.08000  1.08000                                   *
                                -------+---------+---------+---------+--
                                    -0.60      0.00      0.60      1.20


Type = B subtracted from:

Type    Lower   Center    Upper  -------+---------+---------+---------+--
C     0.70000  0.70000  0.70000                     *
D     1.00000  1.00000  1.00000                               *
                                -------+---------+---------+---------+--
                                    -0.60      0.00      0.60      1.20


Type = C subtracted from:

Type    Lower   Center    Upper  -------+---------+---------+---------+--
D     0.30000  0.30000  0.30000                  *
                                -------+---------+---------+---------+--
                                    -0.60      0.00      0.60      1.20
```

One-way ANOVA: Area 1 versus Liposome Type (day 21)

```
Source  DF        SS        MS     F      P
Type     3  0.001714  0.000571  4.49  0.040
Error    8  0.001019  0.000127
Total   11  0.002733

S = 0.01129   R-Sq = 62.72%   R-Sq(adj) = 48.75%


                           Individual 95% CIs For Mean Based on
                           Pooled StDev
Level  N     Mean    StDev  ---+---------+---------+---------+------
A      3  0.02566  0.02070  (---------*---------)
B      3  0.02700  0.00322   (---------*---------)
C      3  0.05558  0.00840                      (---------*---------)
D      3  0.03601  0.00001         (---------*---------)
                           ---+---------+---------+---------+------
                            0.015     0.030     0.045     0.060

Pooled StDev = 0.01129



Grouping Information Using Tukey Method

Type  N     Mean  Grouping
C     3  0.05558  A
D     3  0.03601  A B
B     3  0.02700  A B
A     3  0.02566    B

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%


Type = A subtracted from:

Type     Lower   Center    Upper  ---------+---------+---------+---------+
B     -0.02818  0.00134  0.03085            (--------*---------)
C      0.00041  0.02992  0.05944                     (---------*---------)
D     -0.01917  0.01035  0.03986               (--------*---------)
                                  ---------+---------+---------+---------+
                                        -0.030     0.000     0.030     0.060


Type = B subtracted from:

Type     Lower   Center    Upper  ---------+---------+---------+---------+
C     -0.00093  0.02859  0.05810                     (---------*--------)
D     -0.02050  0.00901  0.03853              (---------*---------)
                                  ---------+---------+---------+---------+
                                        -0.030     0.000     0.030     0.060
```

```
Type = C subtracted from:

Type     Lower    Center    Upper  ---------+---------+---------+---------+
D     -0.04909  -0.01957  0.00994     (--------*---------)
                                   ---------+---------+---------+---------+
                                         -0.030     0.000     0.030
0.060
```

One-way ANOVA: Area 2 versus Liposome Type (day 21)

```
Source  DF        SS        MS     F      P
Type     3  0.000234  0.000078  0.76  0.546
Error    8  0.000817  0.000102
Total   11  0.001051

S = 0.01011   R-Sq = 22.25%   R-Sq(adj) = 0.00%


                           Individual 95% CIs For Mean Based on
                           Pooled StDev
Level  N     Mean    StDev  ------+---------+---------+---------+---
A      3  0.01711  0.01410  (------------*------------)
B      3  0.01808  0.01031   (------------*------------)
C      3  0.02507  0.00892          (------------*------------)
D      3  0.02742  0.00488            (------------*------------)
                           ------+---------+---------+---------+---
                               0.010     0.020     0.030     0.040

Pooled StDev = 0.01011


Grouping Information Using Tukey Method

Type  N     Mean  Grouping
D     3  0.02742  A
C     3  0.02507  A
B     3  0.01808  A
A     3  0.01711  A

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%


Type = A subtracted from:

Type     Lower   Center    Upper  --------+---------+---------+---------+-
B     -0.02547  0.00097  0.02741       (------------*------------)
C     -0.01848  0.00796  0.03440           (------------*------------)
D     -0.01613  0.01031  0.03674            (------------*------------)
                                  --------+---------+---------+---------+-
                                       -0.020     0.000     0.020     0.040
```

```
Type = B subtracted from:

Type     Lower   Center    Upper  --------+---------+---------+---------+-
C     -0.01945  0.00699  0.03343          (------------*-------------)
D     -0.01710  0.00933  0.03577           (-------------*------------)
                                  --------+---------+---------+---------+-
                                       -0.020     0.000     0.020     0.040


Type = C subtracted from:

Type     Lower   Center    Upper  --------+---------+---------+---------+-
D     -0.02409  0.00235  0.02878        (------------*------------)
                                  --------+---------+---------+---------+-
                                       -0.020     0.000     0.020     0.040
```

One-way ANOVA: Area 3 versus Liposome Type (day 21)

```
Source  DF         SS         MS      F      P
Type     3  0.0028235  0.0009412  21.58  0.000
Error    8  0.0003488  0.0000436
Total   11  0.0031723

S = 0.006603   R-Sq = 89.00%   R-Sq(adj) = 84.88%


                           Individual 95% CIs For Mean Based on
                           Pooled StDev
Level  N     Mean    StDev  ---+---------+---------+---------+------
A      3  0.95723  0.00666                           (-----*-----)
B      3  0.95492  0.01026                         (-----*----)
C      3  0.91935  0.00116  (-----*-----)
D      3  0.93660  0.00485             (----*-----)
                           ---+---------+---------+---------+------
                            0.915     0.930     0.945     0.960

Pooled StDev = 0.00660



Grouping Information Using Tukey Method

Type  N      Mean  Grouping
A     3  0.957227  A
B     3  0.954919  A
D     3  0.936600    B
C     3  0.919345    B

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%
```

```
Type = A subtracted from:

Type      Lower     Center      Upper
B     -0.019578  -0.002308   0.014963
C     -0.055152  -0.037882  -0.020611
D     -0.037897  -0.020627  -0.003356

Type  --------+---------+---------+---------+-
B               (-----*-----)
C     (----*-----)
D          (-----*-----)
      --------+---------+---------+---------+-
           -0.030     0.000     0.030     0.060


Type = B subtracted from:

Type      Lower     Center      Upper
C     -0.052845  -0.035574  -0.018304
D     -0.035590  -0.018319  -0.001049

Type  --------+---------+---------+---------+-
C     (-----*-----)
D           (-----*-----)
      --------+---------+---------+---------+-
           -0.030     0.000     0.030     0.060

Type = C subtracted from:

Type      Lower    Center     Upper  --------+---------+---------+---------
+-
D     -0.000016  0.017255  0.034525                    (-----*-----)
                                    --------+---------+---------+---------
+-
                                         -0.030     0.000     0.030
0.060
```

One-way ANOVA: Time 1 versus Liposome Type (day 24)

```
Source  DF         SS         MS     F      P
Type     3  0.0002811  0.0000937  1.69  0.245
Error    8  0.0004425  0.0000553
Total   11  0.0007236

S = 0.007437   R-Sq = 38.85%   R-Sq(adj) = 15.91%

                            Individual 95% CIs For Mean Based on Pooled
StDev
Level  N      Mean     StDev    +---------+---------+---------+---------
A      3  0.010267  0.002401    (---------*---------)
B      3  0.023000  0.008544                (---------*---------)
C      3  0.016433  0.006493           (--------*---------)
D      3  0.020667  0.010017               (---------*---------)
                              +---------+---------+---------+---------
                            0.000     0.010     0.020     0.030

Pooled StDev = 0.007437
```

```
Grouping Information Using Tukey Method

Type  N      Mean  Grouping
B     3  0.023000  A
D     3  0.020667  A
C     3  0.016433  A
A     3  0.010267  A

Means that do not share a letter are significantly different.
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%


Type = A subtracted from:

Type      Lower    Center     Upper  -------+---------+---------+---------+-
-
B     -0.006718  0.012733  0.032185               (-----------*-----------)
C     -0.013285  0.006167  0.025618          (------------*------------)
D     -0.009052  0.010400  0.029852             (------------*------------)
                                     -------+---------+---------+---------+-
-
                                         -0.015     0.000     0.015
0.030


Type = B subtracted from:

Type      Lower     Center     Upper  -------+---------+---------+---------
+--
C     -0.026018  -0.006567  0.012885  (------------*------------)
D     -0.021785  -0.002333  0.017118    (------------*------------)
                                      -------+---------+---------+---------
+--
                                          -0.015     0.000     0.015
0.030


Type = C subtracted from:

Type      Lower    Center     Upper  -------+---------+---------+---------+-
-
D     -0.015218  0.004233  0.023685         (------------*------------)
                                     -------+---------+---------+---------+-
-
                                         -0.015     0.000     0.015
0.030
```

One-way ANOVA: Time 2 versus Liposome Type (day 24)

```
Source  DF       SS       MS      F      P
Type     3  0.10644  0.03548  16.38  0.001
Error    8  0.01733  0.00217
Total   11  0.12377

S = 0.04655   R-Sq = 86.00%   R-Sq(adj) = 80.74%
```

```
                           Individual 95% CIs For Mean Based on
                           Pooled StDev
Level  N     Mean    StDev  -----+---------+---------+---------+----
A      3  0.10700  0.06601  (-----*-----)
B      3  0.11600  0.02400  (------*-----)
C      3  0.21667  0.04041           (------*-----)
D      3  0.34000  0.04583                     (-----*-----)
                           -----+---------+---------+---------+----
                              0.10      0.20      0.30      0.40

Pooled StDev = 0.04655

Grouping Information Using Tukey Method

Type  N     Mean  Grouping
D     3  0.34000  A
C     3  0.21667    B
B     3  0.11600    B
A     3  0.10700    B

Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%

Type = A subtracted from:

Type     Lower   Center    Upper  -------+---------+---------+---------+--
B     -0.11274  0.00900  0.13074             (-----*------)
C     -0.01207  0.10967  0.23140                  (-----*------)
D      0.11126  0.23300  0.35474                       (-----*-----)
                                  -------+---------+---------+---------+--
                                      -0.20      0.00      0.20      0.40
Type = B subtracted from:

Type     Lower   Center    Upper  -------+---------+---------+---------+--
C     -0.02107  0.10067  0.22240                  (-----*-----)
D      0.10226  0.22400  0.34574                       (-----*-----)
                                  -------+---------+---------+---------+--
                                      -0.20      0.00      0.20      0.40


Type = C subtracted from:

Type    Lower   Center    Upper  -------+---------+---------+---------+--
D     0.00160  0.12333  0.24507                  (-----*-----)
                                 -------+---------+---------+---------+--
                                     -0.20      0.00      0.20      0.40
```

One-way ANOVA: Time 3 versus Liposome Type (day 24)

```
Source  DF       SS       MS       F      P
Type     3  2.61477  0.87159  225.41  0.000
Error    8  0.03093  0.00387
Total   11  2.64570

S = 0.06218   R-Sq = 98.83%   R-Sq(adj) = 98.39%
```

```
                          Individual 95% CIs For Mean Based on
                           Pooled StDev
Level  N    Mean   StDev  -----+---------+---------+---------+----
A      3  0.9733  0.0462  (--*-)
B      3  1.0000  0.0000   (--*-)
C      3  1.7000  0.0000                      (--*-)
D      3  2.0667  0.1155                                (-*-)
                          -----+---------+---------+---------+----
                             1.05      1.40      1.75      2.10

Pooled StDev = 0.0622

Grouping Information Using Tukey Method

Type  N    Mean  Grouping
D     3  2.0667  A
C     3  1.7000     B
B     3  1.0000       C
A     3  0.9733       C

Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%

Type = A subtracted from:

Type    Lower  Center   Upper  --------+---------+---------+---------+-
B     -0.1360  0.0267  0.1893                  (-*--)
C      0.5640  0.7267  0.8893                            (-*--)
D      0.9307  1.0933  1.2560                                 (--*-)
                            --------+---------+---------+---------+-
                                 -0.70      0.00      0.70      1.40

Type = B subtracted from:

Type   Lower  Center   Upper  --------+---------+---------+---------+-
C     0.5374  0.7000  0.8626                           (-*-)
D     0.9040  1.0667  1.2293                                (-*--)
                           --------+---------+---------+---------+-
                                -0.70      0.00      0.70      1.40
Type = C subtracted from:

Type   Lower  Center   Upper  --------+---------+---------+---------+-
D     0.2040  0.3667  0.5293                      (-*--)
                           --------+---------+---------+---------+-
                                -0.70      0.00      0.70      1.40
```

One-way ANOVA: Area 1 versus Liposome Type (day 24)

```
Source  DF        SS        MS     F      P
Type     3  0.000546  0.000182  0.69  0.584
Error    8  0.002113  0.000264
Total   11  0.002659

S = 0.01625   R-Sq = 20.54%   R-Sq(adj) = 0.00%
```

```
                           Individual 95% CIs For Mean Based on
                           Pooled StDev
Level  N     Mean    StDev -----+---------+---------+---------+----
A      3  0.04725  0.01467            (-------------*------------)
B      3  0.02971  0.01121  (-------------*------------)
C      3  0.04487  0.01881           (------------*-------------)
D      3  0.03946  0.01902        (-------------*------------)
                           -----+---------+---------+---------+----
                              0.016     0.032     0.048     0.064

Pooled StDev = 0.01625

Grouping Information Using Tukey Method

Type  N     Mean  Grouping
A     3  0.04725  A
C     3  0.04487  A
D     3  0.03946  A
B     3  0.02971  A

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%

Type = A subtracted from:

Type     Lower    Center    Upper     +---------+---------+---------+-------
--
B     -0.06004  -0.01754  0.02496     (-------------*-------------)
C     -0.04488  -0.00238  0.04013          (-------------*-------------)
D     -0.05029  -0.00779  0.03471        (-------------*-------------)
                                      +---------+---------+---------+-------
--
                                   -0.060    -0.030     0.000     0.030
Type = B subtracted from:

Type     Lower   Center    Upper     +---------+---------+---------+--------
-
C     -0.02734  0.01516  0.05767               (-------------*-------------
)
D     -0.03275  0.00975  0.05226             (-------------*-------------)
                                     +---------+---------+---------+--------
-
                                  -0.060    -0.030     0.000     0.030
Type = C subtracted from:

Type     Lower    Center    Upper     +---------+---------+---------+-------
--
D     -0.04791  -0.00541  0.03709          (-------------*-------------)
                                      +---------+---------+---------+-------
--
                                   -0.060    -0.030     0.000     0.030
```

One-way ANOVA: Area 2 versus Liposome Type (day 24)

```
Source  DF         SS         MS     F      P
Type     3  0.0007854  0.0002618  7.66  0.010
Error    8  0.0002733  0.0000342
Total   11  0.0010587

S = 0.005845   R-Sq = 74.19%   R-Sq(adj) = 64.51%


                              Individual 95% CIs For Mean Based on
                              Pooled StDev
Level  N      Mean     StDev  -----+---------+---------+---------+----
A      3  0.012328  0.006363  (------*-------)
B      3  0.014102  0.005955   (-------*-------)
C      3  0.031825  0.005487                    (-------*-------)
D      3  0.025674  0.005530              (-------*------)
                              -----+---------+---------+---------+----
                                 0.010     0.020     0.030     0.040
Pooled StDev = 0.005845


Grouping Information Using Tukey Method

Type  N      Mean  Grouping
C     3  0.031825  A
D     3  0.025674  A B
B     3  0.014102    B
A     3  0.012328    B

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%


Type = A subtracted from:

Type      Lower    Center     Upper  -------+---------+---------+---------+-
-
B     -0.013512  0.001774  0.017060            (-------*-------)
C      0.004211  0.019497  0.034783                     (-------*------)
D     -0.001941  0.013345  0.028632                  (-------*------)
                                    -------+---------+---------+---------+-
-
                                        -0.020     0.000     0.020
0.040
Type = B subtracted from:

Type      Lower    Center     Upper  -------+---------+---------+---------+-
-
C      0.002437  0.017723  0.033009                    (-------*-------)
D     -0.003715  0.011572  0.026858                 (-------*------)
                                    -------+---------+---------+---------+-
-
                                        -0.020     0.000     0.020
0.040
```

```
Type = C subtracted from:

Type      Lower     Center     Upper  -------+---------+---------+---------
+--
D     -0.021438  -0.006151  0.009135        (-------*-------)
                                     -------+---------+---------+---------
+--
                                         -0.020     0.000     0.020
0.040
```

One-way ANOVA: Area 3 versus Liposome Type (day 24)

```
Source  DF        SS        MS     F      P
Type     3  0.002225  0.000742  2.66  0.119
Error    8  0.002228  0.000278
Total   11  0.004453

S = 0.01669   R-Sq = 49.96%   R-Sq(adj) = 31.20%


                           Individual 95% CIs For Mean Based on Pooled
StDev
Level  N     Mean    StDev    +---------+---------+---------+---------
A      3  0.94042  0.00951           (--------*--------)
B      3  0.96089  0.01011                    (--------*--------)
C      3  0.92330  0.01854    (--------*--------)
D      3  0.93487  0.02403         (--------*--------)
                              +---------+---------+---------+---------
                           0.900     0.925     0.950     0.975

Pooled StDev = 0.01669


Grouping Information Using Tukey Method

Type  N     Mean  Grouping
B     3  0.96089  A
A     3  0.94042  A
D     3  0.93487  A
C     3  0.92330  A

Means that do not share a letter are significantly different.
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%

Type = A subtracted from:

Type     Lower    Center    Upper  ------+---------+---------+---------+---
B     -0.02318   0.02047  0.06411             (--------*--------)
C     -0.06076  -0.01712  0.02653      (--------*-------)
D     -0.04920  -0.00556  0.03809        (--------*--------)
                                   ------+---------+---------+---------+---
                                      -0.050     0.000     0.050     0.100
```

```
Type = B subtracted from:

Type     Lower    Center    Upper  ------+---------+---------+---------+---
C     -0.08123  -0.03758  0.00606  (-------*--------)
D     -0.06967  -0.02602  0.01762    (--------*--------)
                                  ------+---------+---------+---------+---
                                     -0.050     0.000     0.050     0.100

Type = C subtracted from:

Type     Lower   Center    Upper  ------+---------+---------+---------+---
D     -0.03208  0.01156  0.05521            (-------*--------)
                                  ------+---------+---------+---------+---
                                    -0.050     0.000     0.050     0.100
```

One-way ANOVA: Time 1 versus Liposome Type (day 28)

```
Source  DF         SS         MS     F      P
Type     3  0.0001490  0.0000497  1.78  0.228
Error    8  0.0002228  0.0000279
Total   11  0.0003719

S = 0.005278   R-Sq = 40.08%   R-Sq(adj) = 17.60%


                              Individual 95% CIs For Mean Based on
                              Pooled StDev
Level  N      Mean     StDev  -----+---------+---------+---------+----
A      3  0.010300  0.007252  (-----------*-----------)
B      3  0.011433  0.006868    (-----------*-----------)
C      3  0.018333  0.001528                (-----------*----------)
D      3  0.017333  0.003055              (-----------*-----------)
                              -----+---------+---------+---------+----
                                 0.0060    0.0120    0.0180    0.0240

Pooled StDev = 0.005278


Grouping Information Using Tukey Method

Type  N      Mean  Grouping
C     3  0.018333  A
D     3  0.017333  A
B     3  0.011433  A
A     3  0.010300  A

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%
```

```
Type = A subtracted from:

Type      Lower    Center     Upper  -------+---------+---------+---------+-
-
B     -0.012670  0.001133  0.014937        (-----------*----------)
C     -0.005770  0.008033  0.021837              (-----------*----------)
D     -0.006770  0.007033  0.020837             (-----------*----------)
                                     -------+---------+---------+---------+-
-
                                         -0.012     0.000     0.012
0.024


Type = B subtracted from:

Type      Lower    Center     Upper  -------+---------+---------+---------+-
-
C     -0.006903  0.006900  0.020703             (-----------*----------)
D     -0.007903  0.005900  0.019703            (-----------*----------)
                                     -------+---------+---------+---------+-
-
                                         -0.012     0.000     0.012
0.024


Type = C subtracted from:

Type      Lower     Center     Upper  -------+---------+---------+---------
+--
D     -0.014803  -0.001000  0.012803       (----------*-----------)
                                      -------+---------+---------+---------
+--
                                          -0.012     0.000     0.012
0.024
```

One-way ANOVA: Time 2 versus Type Liposome Type (day 28)

```
Source  DF        SS        MS       F      P
Type     3  0.199641  0.066547  149.12  0.000
Error    8  0.003570  0.000446
Total   11  0.203211

S = 0.02112   R-Sq = 98.24%   R-Sq(adj) = 97.58%

                           Individual 95% CIs For Mean Based on
                           Pooled StDev
Level  N     Mean    StDev  -------+---------+---------+---------+--
A      3  0.06267  0.02810  (--*--)
B      3  0.05633  0.02371  (--*-)
C      3  0.27333  0.01155                      (-*--)
D      3  0.35000  0.01732                             (--*--)
                           -------+---------+---------+---------+--
                                0.10      0.20      0.30      0.40
Pooled StDev = 0.02112
```

```
Grouping Information Using Tukey Method

Type  N     Mean  Grouping
D     3  0.35000  A
C     3  0.27333    B
A     3  0.06267      C
B     3  0.05633      C

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%


Type = A subtracted from:

Type     Lower    Center    Upper  -------+---------+---------+---------+--
B     -0.06158  -0.00633  0.04892                (--*-)
C      0.15542   0.21067  0.26592                          (--*-)
D      0.23208   0.28733  0.34258                              (-*--)
                                  -------+---------+---------+---------+--
                                      -0.20      0.00      0.20      0.40

Type = B subtracted from:

Type     Lower    Center    Upper  -------+---------+---------+---------+--
C      0.16175  0.21700  0.27225                          (--*--)
D      0.23842  0.29367  0.34892                              (--*-)
                                  -------+---------+---------+---------+--
                                      -0.20      0.00      0.20      0.40

Type = C subtracted from:

Type     Lower    Center    Upper  -------+---------+---------+---------+--
D      0.02142  0.07667  0.13192                   (--*--)
                                  -------+---------+---------+---------+--
                                      -0.20      0.00      0.20      0.40
```

One-way ANOVA: Time 3 versus Liposome Type (day 28)

```
Source  DF       SS       MS       F      P
Type     3  2.17823  0.72608  108.91  0.000
Error    8  0.05333  0.00667
Total   11  2.23157

S = 0.08165   R-Sq = 97.61%   R-Sq(adj) = 96.71%

                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean   StDev  -------+---------+---------+---------+--
A      3  0.9200  0.0000  (--*--)
B      3  1.0000  0.0000    (---*--)
C      3  1.6333  0.1155                      (--*--)
D      3  1.9333  0.1155                               (--*--)
                        -------+---------+---------+---------+--
                             1.05      1.40      1.75      2.10

Pooled StDev = 0.0816
```

```
Grouping Information Using Tukey Method

Type  N    Mean  Grouping
D     3  1.9333  A
C     3  1.6333    B
B     3  1.0000      C
A     3  0.9200      C

Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%

Type = A subtracted from:

Type    Lower  Center   Upper  ---------+---------+---------+---------+
B     -0.1335  0.0800  0.2935                   (--*---)
C      0.4998  0.7133  0.9269                            (---*--)
D      0.7998  1.0133  1.2269                                 (---*--)
                              ---------+---------+---------+---------+
                                    -0.60      0.00      0.60      1.20

Type = B subtracted from:

Type   Lower  Center   Upper  ---------+---------+---------+---------+
C     0.4198  0.6333  0.8469                         (---*--)
D     0.7198  0.9333  1.1469                              (---*--)
                              ---------+---------+---------+---------+
                                    -0.60      0.00      0.60      1.20

Type = C subtracted from:

Type   Lower  Center   Upper  ---------+---------+---------+---------+
D     0.0865  0.3000  0.5135                   (---*---)
                              ---------+---------+---------+---------+
                                    -0.60      0.00      0.60      1.20
```

One-way ANOVA: Area 1 versus Liposome Type (day 28)

```
Source  DF        SS        MS     F      P
Type     3  0.000277  0.000092  0.52  0.683
Error    8  0.001434  0.000179
Total   11  0.001711

S = 0.01339   R-Sq = 16.20%   R-Sq(adj) = 0.00%

                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N     Mean    StDev  -------+---------+---------+---------+--
A      3  0.03566  0.00762    (--------------*--------------)
B      3  0.04174  0.01719         (--------------*--------------)
C      3  0.03377  0.01286  (--------------*--------------)
D      3  0.04584  0.01408            (--------------*--------------)
                          -------+---------+---------+---------+--
                               0.024     0.036     0.048     0.060

Pooled StDev = 0.01339
```

```
Grouping Information Using Tukey Method

Type  N     Mean  Grouping
D     3  0.04584  A
B     3  0.04174  A
A     3  0.03566  A
C     3  0.03377  A

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%


Type = A subtracted from:

Type     Lower    Center    Upper  ---------+---------+---------+---------+
B     -0.02894   0.00608  0.04110         (-------------*-------------)
C     -0.03690  -0.00189  0.03313      (-------------*-------------)
D     -0.02484   0.01017  0.04519           (-------------*-------------)
                                   ---------+---------+---------+---------+
                                         -0.025     0.000     0.025
0.050


Type = B subtracted from:

Type     Lower    Center    Upper  ---------+---------+---------+---------+
C     -0.04298  -0.00796  0.02705    (-------------*-------------)
D     -0.03092   0.00410  0.03911         (-------------*-------------)
                                   ---------+---------+---------+---------+
                                         -0.025     0.000     0.025
0.050

Type = C subtracted from:

Type     Lower   Center    Upper  ---------+---------+---------+---------+
D     -0.02296  0.01206  0.04708           (-------------*-------------)
                                  ---------+---------+---------+---------+
                                        -0.025     0.000     0.025     0.050
```

One-way ANOVA: Area 2 versus Liposome Type (day 28)

```
Source  DF         SS         MS      F      P
Type     3  0.0017912  0.0005971  20.04  0.000
Error    8  0.0002384  0.0000298
Total   11  0.0020295

S = 0.005458   R-Sq = 88.26%   R-Sq(adj) = 83.85%

                           Individual 95% CIs For Mean Based on
                           Pooled StDev
Level  N      Mean     StDev  -------+---------+---------+---------+--
A      3  0.011328  0.005254  (-----*-----)
B      3  0.011617  0.004763   (-----*-----)
C      3  0.031679  0.001982                (-----*-----)
D      3  0.039019  0.008060                      (------*-----)
                           -------+---------+---------+---------+--
                                0.012     0.024     0.036     0.048
```

```
Pooled StDev = 0.005458

Grouping Information Using Tukey Method

Type  N      Mean  Grouping
D     3  0.039019  A
C     3  0.031679  A
B     3  0.011617    B
A     3  0.011328    B

Means that do not share a letter are significantly different.
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%

Type = A subtracted from:

Type      Lower    Center     Upper  -------+---------+---------+---------+-
-
B     -0.013987  0.000288  0.014564             (-----*-----)
C      0.006075  0.020351  0.034627                      (-----*-----)
D      0.013415  0.027690  0.041966                         (-----*-----)
                                    -------+---------+---------+---------+-
-
                                        -0.025     0.000     0.025
0.050

Type = B subtracted from:

Type     Lower    Center     Upper  -------+---------+---------+---------+--
C     0.005786  0.020062  0.034338                    (-----*-----)
D     0.013126  0.027402  0.041678                       (-----*-----)
                                   -------+---------+---------+---------+--
                                       -0.025     0.000     0.025     0.050
Type = C subtracted from:

Type      Lower    Center     Upper  -------+---------+---------+---------+-
-
D     -0.006936  0.007340  0.021616               (-----*-----)
                                    -------+---------+---------+---------+-
-
                                        -0.025     0.000     0.025
0.050
```

One-way ANOVA: Area 3 versus Liposome Type (day 28)

```
Source  DF       SS       MS     F      P
Type     3  0.00284  0.00095  0.47  0.711
Error    8  0.01606  0.00201
Total   11  0.01890

S = 0.04481   R-Sq = 15.01%   R-Sq(adj) = 0.00%
```

```
                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean   StDev  --------+---------+---------+---------+-
A      3  0.9079  0.0832  (--------------*--------------)
B      3  0.9466  0.0219            (--------------*--------------)
C      3  0.9345  0.0144          (--------------*--------------)
D      3  0.9151  0.0207    (--------------*--------------)
                          --------+---------+---------+---------+-
                                0.880     0.920     0.960     1.000

Pooled StDev = 0.0448
Grouping Information Using Tukey Method

Type  N     Mean  Grouping
B     3  0.94664  A
C     3  0.93455  A
D     3  0.91515  A
A     3  0.90788  A

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 98.74%


Type = A subtracted from:

Type     Lower   Center    Upper  ---------+---------+---------+---------+
B     -0.07843  0.03876  0.15595            (--------------*-------------)
C     -0.09052  0.02666  0.14385          (-------------*--------------)
D     -0.10992  0.00726  0.12445       (--------------*--------------)
                                   ---------+---------+---------+---------+
                                         -0.080     0.000     0.080     0.160


Type = B subtracted from:

Type     Lower    Center    Upper  ---------+---------+---------+---------+
C     -0.12929  -0.01210  0.10509     (-------------*--------------)
D     -0.14869  -0.03150  0.08569  (--------------*--------------)
                                   ---------+---------+---------+---------+
                                         -0.080     0.000     0.080
0.160

Type = C subtracted from:

Type     Lower    Center    Upper  ---------+---------+---------+---------+
D     -0.13659  -0.01940  0.09779    (--------------*-------------)
                                   ---------+---------+---------+---------+
                                         -0.080     0.000     0.080
0.160
```

Table E.2 Analysis of Variance for lysozyme coated liposomes produced by microfluidization in distilled water. Effect of chitosan coating on zeta potential, particle size, antioxidant activity and total phenolic content using Adjusted SS for Tests.

General Linear Model: Particle Size; Zeta Potential; Antioxidant Activity; Total Phenolic Content versus Day

```
Factor  Type   Levels  Values
Day     fixed       5  0; 7; 14; 21; 28


Analysis of Variance for Particle Size, using Adjusted SS for Tests

Source  DF  Seq SS  Adj SS  Adj MS     F      P
Day      4   88.51   88.51   22.13  1.19  0.372
Error   10  185.67  185.67   18.57
Total   14  274.18


S = 4.30894   R-Sq = 32.28%   R-Sq(adj) = 5.19%


Unusual Observations for Particle Size

     Particle
Obs      Size      Fit  SE Fit  Residual  St Resid
  7   72.4600  63.8800  2.4878    8.5800      2.44 R

R denotes an observation with a large standardized residual.




Analysis of Variance for Zeta Potential, using Adjusted SS for Tests

Source  DF  Seq SS  Adj SS  Adj MS      F      P
Day      4  497.24  497.24  124.31  38.30  0.000
Error   10   32.45   32.45    3.25
Total   14  529.69


S = 1.80148   R-Sq = 93.87%   R-Sq(adj) = 91.42%


Unusual Observations for Zeta Potential

          Zeta
Obs  Potential       Fit  SE Fit  Residual  St Resid
 13   -40.5000  -37.0333  1.0401   -3.4667     -2.36 R
 15   -33.8000  -37.0333  1.0401    3.2333      2.20 R

R denotes an observation with a large standardized residual.
```

Analysis of Variance for Total Phenolic Content, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Day	4	6623.9	6623.9	1656.0	29.73	0.000
Error	10	557.1	557.1	55.7		
Total	14	7180.9				

S = 7.46359 R-Sq = 92.24% R-Sq(adj) = 89.14%

Analysis of Variance for Antioxidant Activity, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Day	4	287.789	287.789	71.947	52.31	0.000
Error	10	13.753	13.753	1.375		
Total	14	301.542				

S = 1.17273 R-Sq = 95.44% R-Sq(adj) = 93.61%

Grouping Information Using Tukey Method and 95.0% Confidence for Particle Size

Day	N	Mean	Grouping
7	3	65.5	A
21	3	64.4	A
28	3	64.2	A
14	3	63.9	A
0	3	58.6	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for Zeta Potential

Day	N	Mean	Grouping
0	3	-20.4	A
14	3	-23.5	A B
7	3	-23.5	A B
21	3	-25.6	B
28	3	-37.0	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for Total Phenolic Content

Day	N	Mean	Grouping
14	3	271.1	A
28	3	270.0	A
7	3	251.5	A B
21	3	238.4	B
0	3	215.0	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for Antioxidant Activity

Day	N	Mean	Grouping
0	3	40.4	A
14	3	33.9	B
21	3	30.6	C
7	3	28.8	C
28	3	28.6	C

Means that do not share a letter are significantly different.

Table E.3 Analysis of Variance for lysozyme coated liposomes produced by microfluidization in distilled water. Effect of chitosan coating on zeta potential, particle size, antioxidant activity and total phenolic content using Adjusted SS for Tests.

General Linear Model: Particle Size; Zeta Potential; Antioxidant Activity; Total Phenolic Content versus Day

Factor	Type	Levels	Values
Day	fixed	5	0; 7; 14; 21; 28

Analysis of Variance for Particle Size, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Day	4	87.265	87.265	21.816	9.54	0.002
Error	10	22.858	22.858	2.286		
Total	14	110.124				

S = 1.51190 R-Sq = 79.24% R-Sq(adj) = 70.94%

Unusual Observations for Particle Size

Obs	Particle Size	Fit	SE Fit	Residual	St Resid
13	48.5900	45.9067	0.8729	2.6833	2.17 R

R denotes an observation with a large standardized residual.

Analysis of Variance for Zeta Potential, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Day	4	161.471	161.471	40.368	9.99	0.002
Error	10	40.427	40.427	4.043		
Total	14	201.897				

S = 2.01064 R-Sq = 79.98% R-Sq(adj) = 71.97%

Analysis of Variance for Total Phenolic Content, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Day	4	640.40	640.40	160.10	2.76	0.088
Error	10	580.30	580.30	58.03		
Total	14	1220.71				

S = 7.61775 R-Sq = 52.46% R-Sq(adj) = 33.45%

Unusual Observations for Total Phenolic Content

Obs	Total Phenolic Content	Fit	SE Fit	Residual	St Resid
13	250.639	264.608	4.398	-13.968	-2.25 R

R denotes an observation with a large standardized residual.

Analysis of Variance for Antioxidant Activity, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Day	4	304.657	304.657	76.164	47.56	0.000
Error	10	16.014	16.014	1.601		
Total	14	320.671				

S = 1.26548 R-Sq = 95.01% R-Sq(adj) = 93.01%

Unusual Observations for Antioxidant Activity

Obs	Antioxidant Activity	Fit	SE Fit	Residual	St Resid
4	29.5628	32.3535	0.7306	-2.7907	-2.70 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95.0% Confidence for Particle Size

Day	N	Mean	Grouping
7	3	49.5	A
14	3	48.4	A
28	3	45.9	A B
21	3	45.9	A B
0	3	42.5	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for Zeta Potential

Day	N	Mean	Grouping
0	3	-22.1	A
7	3	-25.1	A
21	3	-26.6	A
14	3	-26.6	A
28	3	-32.2	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for Total Phenolic Content

Day	N	Mean	Grouping
14	3	268.9	A
28	3	264.6	A
21	3	259.5	A
0	3	256.1	A
7	3	250.1	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for Antioxidant Activity

Day	N	Mean	Grouping
0	3	42.6	A
14	3	34.9	B
7	3	32.4	B C
21	3	31.5	B C
28	3	29.8	C

Means that do not share a letter are significantly different.

Table E.4 Analysis of Variance for whey protein coated liposomes produced by microfluidization in distilled water. Effect of chitosan coating on zeta potential, particle size, antioxidant activity and total phenolic content using Adjusted SS for Tests.

General Linear Model: Particle Size; Zeta Potential; Antioxidant Activity; Total Phenolic Content versus Day

Factor	Type	Levels	Values
Day	fixed	5	0; 7; 14; 21; 28

Analysis of Variance for Particle Size, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Day	4	12.292	12.292	3.073	1.58	0.254
Error	10	19.466	19.466	1.947		
Total	14	31.758				

S = 1.39521 R-Sq = 38.70% R-Sq(adj) = 14.19%

Analysis of Variance for Zeta Potential, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Day	4	225.040	225.040	56.260	7.68	0.004
Error	10	73.220	73.220	7.322		
Total	14	298.260				

S = 2.70592 R-Sq = 75.45% R-Sq(adj) = 65.63%

Analysis of Variance for Total Phenolic Content, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Day	4	4234.0	4234.0	1058.5	23.91	0.000
Error	10	442.6	442.6	44.3		
Total	14	4676.6				

S = 6.65305 R-Sq = 90.54% R-Sq(adj) = 86.75%

Unusual Observations for Total Phenolic Content

Obs	Total Phenolic Content	Fit	SE Fit	Residual	St Resid
4	189.143	204.562	3.841	-15.420	-2.84 R

R denotes an observation with a large standardized residual.

Analysis of Variance for Antioxidant Activity, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Day	4	382.902	382.902	95.725	84.49	0.000
Error	10	11.330	11.330	1.133		
Total	14	394.232				

S = 1.06443 R-Sq = 97.13% R-Sq(adj) = 95.98%

Grouping Information Using Tukey Method and 95.0% Confidence for Particle Size

Day	N	Mean	Grouping
28	3	46.7	A
0	3	45.9	A
14	3	45.6	A
7	3	45.1	A
21	3	44.0	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for Zeta Potential

Day	N	Mean	Grouping
14	3	-24.4	A
21	3	-25.8	A
0	3	-26.3	A
7	3	-26.6	A
28	3	-35.3	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for Total Phenolic Content

Day	N	Mean	Grouping
14	3	246.6	A
21	3	222.3	B
28	3	215.4	B C
7	3	204.6	B C
0	3	198.2	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for Antioxidant Activity

Day	N	Mean	Grouping
0	3	41.7	A
14	3	36.7	B
21	3	32.2	C
28	3	29.3	D
7	3	28.0	D

Means that do not share a letter are significantly different.

Table E.5 Analysis of Variance for chitosan coated liposomes produced by microfluidization in acetate buffer. Effect of chitosan coating on zeta potential, particle size, antioxidant activity and total phenolic content using Adjusted SS for Tests.

General Linear Model: Particle Size; Zeta Potential; Antioxidant Activity; Total Phenolic Content versus Day

Factor	Type	Levels	Values
Day	fixed	5	0; 7; 14; 21; 28

Analysis of Variance for Particle Size, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Day	4	953149	953149	238287	39.28	0.000
Error	10	60659	60659	6066		
Total	14	1013808				

S = 77.8842 R-Sq = 94.02% R-Sq(adj) = 91.62%

Unusual Observations for Particle Size

Obs	Particle Size	Fit	SE Fit	Residual	St Resid
6	447.100	278.767	44.966	168.333	2.65 R

R denotes an observation with a large standardized residual.

Analysis of Variance for Zeta Potential, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Day	4	2.4560	2.4560	0.6140	0.90	0.500
Error	10	6.8333	6.8333	0.6833		
Total	14	9.2893				

S = 0.826640 R-Sq = 26.44% R-Sq(adj) = 0.00%

Unusual Observations for Zeta Potential

Obs	Zeta Potential	Fit	SE Fit	Residual	St Resid
12	23.2000	24.6333	0.4773	-1.4333	-2.12 R

R denotes an observation with a large standardized residual.

Analysis of Variance for Total Phenolic Content, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Day	4	5979.5	5979.5	1494.9	5.53	0.013
Error	10	2701.7	2701.7	270.2		
Total	14	8681.2				

S = 16.4369 R-Sq = 68.88% R-Sq(adj) = 56.43%

Unusual Observations for Total Phenolic Content

Obs	Total Phenolic Content	Fit	SE Fit	Residual	St Resid
6	82.707	118.200	9.490	-35.492	-2.64 R

R denotes an observation with a large standardized residual.

Analysis of Variance for Antioxidant Activity, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Day	4	324.548	324.548	81.137	203.25	0.000
Error	10	3.992	3.992	0.399		
Total	14	328.540				

S = 0.631828 R-Sq = 98.78% R-Sq(adj) = 98.30%

Grouping Information Using Tukey Method and 95.0% Confidence for Particle Size

Day	N	Mean	Grouping
21	3	772.9	A
14	3	749.6	A
0	3	356.0	B
7	3	278.8	B
28	3	156.2	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for Zeta Potential

Day	N	Mean	Grouping
14	3	25.2	A
21	3	24.6	A
0	3	24.4	A
28	3	24.2	A
7	3	24.1	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for Total Phenolic Content

Day	N	Mean	Grouping
14	3	158.4	A
21	3	141.1	A B
0	3	124.4	A B
7	3	118.2	A B
28	3	99.9	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for Antioxidant Activity

Day	N	Mean	Grouping
0	3	39.2	A
14	3	34.5	B
21	3	30.4	C
28	3	27.5	D
7	3	26.8	D

Means that do not share a letter are significantly different.