FORMATION AND CHARACTERIZATION OF FOOD GRADE LIPOSOME SYSTEMS

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ABSTRACT

FORMATION AND CHARACTERIZATION OF FOOD GRADE LIPOSOME SYSTEMS

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Liposomes are double-layered spherical vesicles made up of polar lipids and could be used in pharmaceutical, personal care, chemical and food industries to encapsulate both hydrophobic and hydrophilic compounds. Being biodegradable, biocompatible not having any toxic effects and having the ability to release the active agents when desired make these systems advantageous for many applications. The main natural sources used for liposome formation are egg and soy. In this study, egg and soy lecithin with higher purity (>70%) were used to determine the effect of different mechanical formation methods on the characterization of liposomes. Microfluidization and ultrasonic probe methods were used. Effects of microfluidization pressure, ultrasound time, and solvent type on particle size, and zeta potential were investigated. Trials were conducted between 400-1300 bars for microfluidization, 2-8 minutes for ultrasound in phosphate buffer (pH≈7.2), distilled water (pH≈6.5), and acetate buffer (pH≈3.8) solvents. Effect of solvent type on particle size was found significant ($p<0.05$) while phosphate buffer samples were inefficient to form liposome since presence of ions drastically decreased zeta potential of the particles. When microfluidization and ultrasonication methods were compared, microfluidization was better to decrease particle size. Increasing ultrasonication time did not significantly affect particle size after 5 minutes ($p\geq0.05$). For the long term stability, the samples were stored at refrigerator (4°C) and room temperatures (25°C) and NMR T_2 Relaxometry experiments were conducted during storage. Storing at room temperature adversely affected liposome stability which were detected by both particle size and T_2 (spin spin relaxation time) values. Increase in T_2 (spin spin relaxation time) values after some time during storage is validated to be a sign for liposome degradation with time. In addition to high correlation with particle size results, T_2 values were promising for further characterization of

liposome system, where chemical changes like lipid oxidation cannot be detected by particle size measurement.

Keywords: Liposome, mechanical methods, particle size, characterization, NMR relaxometry

ÖZ

GIDADA KULLANILABİLİR LİPOZOM SİSTEMLERİNİN OLUŞTURULMASI VE KARAKTERİZASYONU

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Polar lipidlerden çift katmanlı, küresel kesecikler oluşturma özelliğine sahip lipozomlar farmasötik, kişisel bakım, kimya ve gıda endüstrilerince hem hidrofilik hem de hidrofobik bileşenleri kapsüllemek amacıyla kullanılabilir. Biyobozunur, biyouyumlu olması ve toksik özellik göstermeyişinin yanında kapsüllediği aktif ajanların istenilen şekilde kontrollü olarak salınımını sağlayabilmesi, lipozomları birçok uygulamada avantajlı kılmaktadır. Lipozomların üretildiği fosfolipidlerin doğadaki ana kaynağı yumurta ve soya fasulyesidir. Bu çalışmada, iki farklı mekanik metod, mikrofludizasyon ve direkt uç tipi ultrason, metodları kullanılarak oluşturulan yüksek konsantrasyonlu (>70%) soya ve yumurta lipozomlarının karakterizasyonu amaçlanmıştır. Bu amaçla mikrofludizasyon basıncı, ultrasonikasyon zamanı ve kullanılan solvent tiplerinin parçacık boyutu üzerine etkisi incelenmiştir. Denemeler mikrofludizasyon basıncı, ultrason zamanı ve kullanılan solvent tipleri için sırasıyla 400-1300 bar, 2-8 dakika, son olarak ise fosfat tampon çözeltisi (pH≈7.2), asetat tampon çözeltisi (pH≈3.8) ve distile su (pH≈6.5) kullanılarak yürütülmüştür. Kullanılan solvent tipinin parçacık boyutunda kayda değer miktarda farklılığa neden olduğu bulunmuştur ($p<0.05$). Bu solvent tipleri arasında fosfat tampon çözeltisi en yüksek parçacık boyutu sonuçlarını vermiş ve sistemin instabil durumu, tampon çözeltideki iyonların lipozom keseciklerinin zeta potansiyelini düşürmesi ile ilişkilendirilmiştir. Mikrofludizasyon ve ultrasonikasyon metodları karşılaştırıldığında mikrofludizasyonun parçacık boyutu üzerinde daha etkili olduğu bulunmuştur. Diğer yandan 5 dakikadan fazla ultrasonikasyon uygulamanın parçacık boyutu üzerinde etkisi olmamıştır ($p\geq0.05$). Uzun vadede stabilite çalışmaları için örnekler buzdolabı (4°C) ve oda sıcaklıklarında (25°C) saklanmış ve bu süre boyunca NMR T_2 Relaksometre ölçümleri yapılmıştır. Oda sıcaklığında

depolamanın numuneler üzerindeki olumsuz etkisi hem parçacık boyutu, hem de T_2 (spin spin relaksasyon zamanı) ölçümleriyle doğrulanmıştır. Saklma süresince çeşitli aralıklarda görülen T_2 değerlerindeki artışın lipozom degradayonuna bağlı olduğu, parçacık boyutu değerleriyle yapılan korelasyon analiziyle onaylanmıştır. Buna ek olarak T_2 relaksasyon ölçümlerinin lipozom karakterizasyonunda lipid oksidayon gibi parçacık boyutunun ölçemeyeceği kimyasal değişimleri saptamada geçerli bir yöntem olduğu yönünde güçlü bulgular elde edilmiştir.

Anahtar Kelimeler: Lipozom, mekanik metotlar, parçacık boyutu, karakterizasyon, NMR Relaksometre

To my devoted family, without whom I could have never come so far...

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CHAPTER 1

INTRODUCTION

1.1 Encapsulation

Protection of sensitive bioactive agents against processing operations and digestion by encapsulation is a favorable means to increase bioavailability (Akbari & Wu, 2016).

Food ingredients are generally encapsulated to enhance the functional properties of the active agents like flavors, colorings, antimicrobials and nutrients. In food industry, encapsulation is used for various purposes (Ray, Raychaudhuri, & Chakraborty, 2015):

1. Sensitive compounds can be protected from degradation or its reactivity can be reduced to prevent interaction with the environment.
2. Volatile substances can be kept longer due to decrease of transfer rate by this permeability barrier.
3. Physical properties like solubility, viscosity can be enhanced for functional use.
4. They can enable targeted release.
5. Undesirable attributes like flavor can be masked.
6. It would be easier to disperse traces of material more homogeneously.
7. A shield can be formed between substances in the same mixture.

Encapsulation materials and methods vary for each substance to be coated since every compound has its unique molecular characteristics. Encapsulating materials can be hydrophilic or hydrophobic depending on the nature of agent. For foods, starch (derivatives), proteins, gums, lipids and combination of these are generally used for shielding against stresses, which can be chemical (presence of enzymes, light, oxygen… etc.) or environmental (change in ion concentration, pH, temperature… etc.) (Taylor, Gaysinsky, Davidson, Bruce, & Weiss, 2007).

In the literature, encapsulation is used for various kinds of active agent and coating materials. These include food protein hydrolysates and peptides, which have undesirable attributes like bitter taste and hygroscopicity (Mohan, Rajendran, He, & Udenigwe, 2015). Entrapment in a matrix repress these properties and increase bioavailability while providing their interaction with the food matrix (Mohan et al., 2015). Vitamins and orange oil emulsions are efficiently encapsulated by spray drying to prevent oxidation and loss during storage (Y. D. Kim, Morr, & Schenz, 1996). Fats, waxes, polyethylene glycol, fatty acids and fatty alcohols are used for coating purposes in spray chilling applications. In their study, Turchiuli et al. (2005) and Phoungchandang & Sanchai (2009) used maltodextrin to encapsulate vegetable oil and ginger powder, respectively.

One of the most challenging encapsulation is experienced in hydrophobic active agents. Since these agents do not solubilize in the aqueous environment, hydrophobic compounds are far more difficult to cope with. Dispersing a hydrophobic material into aqueous medium undoubtly needs an emulsifier. These lipophilic compounds are either encapsulated by synthetic polymers (like polylactic acid, polyglycolic acid, polylactide-co-glycolide, polyethylene oxide or polyethylene glycol... etc.) or natural polymers as well as lipids (Dima, Dima, & Iordăchescu, 2015). Being biocompatible, biodegradable and non-toxic (Lu et al., 2014), natural capsulation materials are mostly preferred in food and pharmaceutical applications.

1.2 Emulsifiers

Emulsifiers have amphiphilic characteristics that can mix two substances that naturally tend to repel each other. An emulsifier has both at least one hydrophilic (water soluble) and one hydrophobic (oil soluble) compartments, which make it suitable for habilitating in the contact surface. With this significant behavior, emulsifiers participate in many branches of the industry including pharmaceutical, chemical and food industries.

Two liquids with different natural tendencies to form bonds are dispersed in each other by the help of emulsifiers, and the resulting solutions are called emulsions. Emulsions can be categorized with respect to size range of their droplets as conventional or micro/nanoemulsions. Conventional emulsion particle size range varies between 100 nm and 1000 μm while micro/nanoemulsions are much smaller (varies between 10 to 100 nm) (Dima et al., 2015). To compare in appearance, conventional emulsions have an opaque nature. On the other hand, micro/nanoemulsions look more transparent due to their small particle size. Incorporation of micro/nanoemulsions in the applications provide some advantages like:

- Enhanced penetration due to increased surface area, which proportionally influence the drug efficiency (Tadros, Izquierdo, Esquena, & Solans, 2004).
- More effective when prepared at the same concentration with the same emulsion with larger particles in terms of absorption and skin penetration (Hoeller, Sperger, & Valenta, 2009).
- Increased area and free energy makes it possible to deliver active agents to the location of interest (Anton & Vandamme, 2009).
- They are very elastic and deformable (Lovelyn, 2011).
- They are resistant to aggregation and separation (McClements, 2012).

To define the mechanism and stability of emulsions, further knowledge of microemulsion and nanoemulsion concepts should be well understood.

1.3 Microemulsions and Nanoemulsion Concepts

Micro and nano words are very prone to misunderstanding in emulsion systems since they also represent the unit of metric scale. In metric scale, nano size is thousand times smaller than micro size however it is not the case in literature development of microemulsion and nanoemulsion concepts.

The basic and most important difference between micro and nanoemulsions is that microemulsions are thermodynamically stable whereas nanoemulsions are not. For a system to be thermodynamically stable, the formed emulsion, named as "product" should have a more favorable energetic state that would prevent the reaction going backwards to first ingredients. Although nanoemulsions are not stable thermodynamically, they are described as kinetically stable, since the reaction that converts the products into reactants is very slow. On the other hand, microemulsions are formed spontaneously and need energy to form reactants back from products since their products are in a state that is energetically more favorable than reactants, unlike nanoemulsions (Ozery, n.d.).

These two emulsion types also response differently against some environmental alterations so their applications should also be considered carefully. For example, microemulsions are susceptible to temperature changes and dilution (Anton & Vandamme, 2011). In applications like introducing the emulsion in blood stream or water (dilution) as well as storing the samples at elevated/lowered temperatures during storage directly affect the microemulsion stability while nanoemulsions resist (Anton & Vandamme, 2011).

Microemulsions are also nano sized dispersions, exhibiting a transparent behavior just like nanoemulsions. Nanoemulsion needs energy to form and this energy can be high or low. That is why it is better to make differentiation of these two type of

emulsions by the preparation technique. Nanoemulsions are formed by mixing first the hydrophobic phase with the emulsifier and then addition of hydrophilic phase takes place. This order is very important in the formation of nanoemulsions since they cannot be formed otherwise (Anton & Vandamme, 2011). The opposite condition also applies for microemulsions, they cannot be formed by following the order of nanoemulsion formation.

Polydispersity index (PDI) is another sign of detecting nanoemulsions. While microemulsions have PDI with a slim particle size distribution peak, nanoemulsions may have high or low PDI with multiple particle size distribution peaks (McClements, 2012).

Shape of the emulsion droplet can also indicate the type of the emulsion. Nanoemulsions generally form spherical droplets to minimize their energy and contact area (McClements, 2012) however microemulsions can form many structures like cane, hexagonal or spherical (Anton & Vandamme, 2011).

Another difference between nano and microemulsion is the emulsifier amount used to form. Microemulsions need considerable amount of emulsifier to form while nanoemulsion can be formed in dilute concentrations (McClements, 2012).

For the food industry, lipid sourced food grade emulsifiers can be categorized in 3 main groups which are (van Nieuwenhuyzen & Szuhaj, 1998);

1. Lecithins or phospholipids,

2. Mono- and diglycerides of edible oils and fatty acids and their diacetyl, lactic acid, citric acid and ethoxylated esters,

3. Sucroglycerides and their esters and polysorbates.

Among these emulsifiers, lecithin has a spectacular enclosure mechanism that makes it favorable to use for delivery and controlled release in addition to emulsifying properties.

1.4 Phospholipids

As the name implies, the lipid structure of the phospholipids consists of a phosphoric head group which add these hydrophobic molecules another functional property, hydrophilicity. This characteristic distinguishes phospholipids from their counterparts, hydrophobic lipids.

Natural phospholipids mostly used in researches are phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylethanolamine (PE) and phosphatidylglycerol (PG). They are mostly found in egg, soy bean, sunflower and rapeseed. In Figure 1, the chemical structure of phosphatidylcholine (PC), also known as lecithin is shown. PC is the most abundant phospholipid in nature (Taylor, Weiss, Davidson, & Bruce, 2005) and remarked for its similarity to cell membrane since it provides many advantages for delivery and release of active agents in the body.

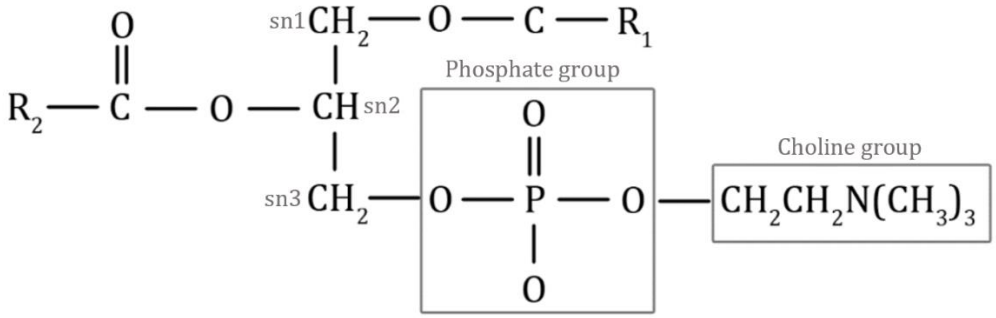

Figure 1. 1 Chemical structure of phosphatidylcholine (Kırtıl & Öztop, 2014).

In the literature, soy lecithin is preferred instead of egg lecithin since soy lecithin can mimic the properties of egg lecithin successfully and it is cheaper (Bryła, Lewandowicz, & Juzwa, 2015; van Nieuwenhuyzen & Szuhaj, 1998). The phase transition temperature of soy and egg lecithins vary between -5 to -15 and -20 to -30 ^{o}C, respectively. Some of the synthetic phospholipids mainly studied in the literature and their phase transition temperatures are given in Table 1.1.

Table 1. 1 Phase transition temperature and carbon number of some synthetic phospholipids (Kırtıl & Öztop, 2014).

Carbon number	**Synthetic Phospholipid**	**Transition Temperature**
12	Dilaurylphosphatidylcholine	0
14	Dimyristoylphosphatidylcholine	23
16	Dipalmitoylphosphatidylcholine	41
18	Distearoylphosphatidylcholine	58

Most significant advantage of phospholipids is that they provide targeted release, which leads them one step ahead from the competitors. Natively being a vesicle former, another significant functionality of phospholipids is that they ensure encapsulation of both lipophilic and hydrophilic compounds. Thanks to their dual character, phospholipids are one of the uncommon mechanisms to be used for protection of hydrophobic substances until consumption.

When enclosed, phospholipids are called “liposome”, and liposomes can be used to protect compounds against enzymes or reactive chemicals as well as environmental stresses like extreme pH, temperature and high ion concentrations. The type and composition of liposome affect the efficiency of encapsulation (Taylor et al., 2007).

1.5 Liposome Formation Mechanism

In its structure, phospholipids consist of phosphorous head with choline group attached to sn-3 position and hydrocarbon chains bound with ester linkages at sn-1 and sn-2 positions of glycerol backbone. In the aqueous environment, hydrophilic choline group covalently make bonds with the surrounding atoms while hydrophobic tails favor interaction with each other (Bozzuto & Molinari, 2015). Such tendency leads to the formation of a flat bilayer which is the first step of liposome formation. Binding of nonpolar chains results in the exposure of phosphorous heads to the aqueous environment, maintaining hydrogen bonds and polar interactions on the surface. At the inner side, hydrophobic interactions along with van der Waals forces keep this structure intact and act as a permeability barrier both inward and outward (Bozzuto & Molinari, 2015).

Second step of liposome formation is the enclosure, which bends the properly aligned bilayer to form vesicles (Antonietti & Förster, 2003). The reason for this further formation is the instability resulting from the contact of hydrophobic tails with hydrophilic solution at the edges of sandwich like flat bilayer.

This enclosure mechanism can be explained by the effort of molecules to minimize their elastic free energy (μ_E). When they are forced, the lipid assemblies, also known as "hate edges" (Frolov, Shnyrova, & Zimmerberg, 2011) tend to curl. This attempt is to reach intrinsic or equilibrium radius of curvature (R_o), where the lipid particle has minimum possible free energy (Gruner, 1985). The theory explained by Kirk et al. (1984) relates the elastic free energy and radius of curvature by the formula (Gruner, 1985):

$$\mu_E = k\left({}^{1}/_{R} - {}^{1}/_{R_o}\right)^2$$

(Equation 1.1)

Since lipids do not have one rigid shape, their flexibility leads to formation of structures with different volumes, depending on the phase transitions. The final shape of the lipid molecules at the equilibrium radius of curvature can be predicted by the packing diameter given by (Frolov et al., 2011):

$$P = {}^{v}/_{al}$$

(Equation 1.2)

where *V* is the specific volume occupied by the tails, *a* is the area per lipid molecule in the dividing surface, and *l* is the effective length of the hydrophobic region. P values for different shapes of molecules are given in Table 2.

Table 1. 2 Packing diameter of common molecule shapes (Frolov et al., 2011).

P value	**Shape**	**Example**
< 1	Inverse cone	Lysophosphocholine
≈ 1	Cylinder	Dioleoylphosphocholine
> 1	Cone	Dioleoylphosphotidylethanolamine

1.6 Liposome Formation Methods

Lamellar structures of phospholipids can be transformed into closed, spherical vesicles -liposomes- only by supplying energy to the system. The liposomes can be differentiated according to arrangement of embedded vesicles and size, which has a range of 20 nm to several micrometers with a thickness of approximately 3.5 nm (Sabín, Ruso, González-Pérez, Prieto, & Sarmiento, 2006). Liposomes can be prepared in many ways to produce lipid droplets in the desired size, depending on the purpose of use.

Most abundant phospholipid, phosphatidylcholine forms ordered lamellar structures and the liposomes formed from these phospholipids can be mainly grouped in 4 different categories; small unilamellar vesicles (SUVs), large unilamellar vesicles (LUVs), multilamellar vesicles (MLVs) and multivesicular vesicles (MVVs), also seen in Figure 1.2. The dispersions of LUVs having

diameter around 300 nm look cloudy white while this color evolves to be transparent as particle size is decreased to form SUVs (Taylor et al., 2005).

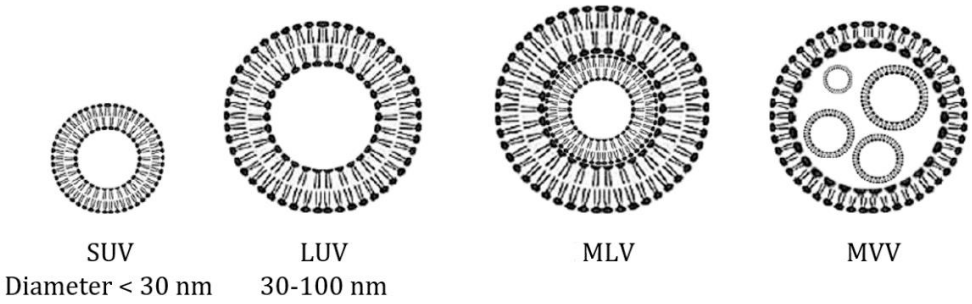

Figure 1. 2 Liposomal vesicles in different sizes (Kırtıl & Öztop, 2014)

Liposomes are not thermodynamically stable which means they do not form spontaneously in nature (Taylor et al., 2005). Since energy is a necessity to form liposome, change in applied energy also change the type of vesicle formed. Some of the liposome types and preparation techniques are summarized with significant characteristics in the Table 1.3.

Table 1. 3 Method of preparation and characteristic properties of liposomes with different size (Kırtıl & Öztop, 2014).

Liposome type	Method of Preparation	Characteristic Properties
MLV	Thin film hydration/Hand shaken vesicles	Low energy, high encapsulation efficiency, ease of preparation, low cost, maximum storage stability.
LUV	Membrane extrusion, ultrasonication, high pressure homogenization of MLVs	Moderate energy, high encapsulation efficiency, higher storage stability than SUVs.
SUV	Membrane extrusion and Ultrasonication of MLVs nd LUVs; dialysis and high pressure homogenization of MLVs	High energy, low encapsulation efficiency, clear solution.

There are many preparation techniques for liposome vesicles which can be summoned under two titles; mechanical and non-mechanical techniques.

1.6.1 Mechanical Methods

1.6.1.1 Hand shaken vesicles

The simplest way of MLV formation is hand shaken, also known as thin film hydration method. As the name implies, the liposome formation with this method includes the formation of thin film of phospholipids and subsequently the dispersion of this film in an aqueous medium. For this purpose, dry lipids are first dispersed in an organic solvent (ex. chloroform, ethanol...) and rapidly dried under vacuum by a rotary evaporator to form a thin sheet (Rickwood & Hames, 1990). Second step takes place when an aqueous solution (water) is added to the lipid. Mixture of the lipid sheet with water leads to swelling and formation of tubular structures of the phospholipids only, so further energy is applied by mixing or bath sonication to form proper MLVs (Torchilin, & Weissig, 2003). The final particle size of dispersion still do not have a uniform behavior (Laouini et al., 2012) but the low cost and ease of the method makes it worthy to apply for primary and secondary liposome preparations.

1.6.1.2 Ultrasonication

Sonication is one of the first and easy methods to form liposomes. Sonication power can be applied by either probe or bath type homogenization. In bath type homogenization, dispersions are placed in a liquid (water) which acts as the transfer medium and then ultrasound is applied through this medium. Such type of homogenization is used to prepare MLVs since energy, applied indirectly from transfer medium to the wall of dispersion vessel and vessel walls through the sample, remains low.

On the other hand, probe type ultrasonication consists of a probe, chosen properly according to the sample type, amount and concentration, which transmits the energy directly to the sample. The frequency of the sound applied is just above the

human hearing level (Frydenberg, Hammershøj, Andersen, Greve, & Wiking, 2016), changing from 16 to several hundred kHz. Resulting dispersions to form LUVs or SUVs, depend on the given energy.

When direct ultrasonication is applied to an environment, formation as well as collapse of cavities are triggered (Isailović et al., 2013). This process increases temperature and exposes the sample to high pressure, resulting in turbulent flow. When applied to MLVs, this flow behavior results in disruption of previous structure to form SUVs (Taylor et al., 2005).

Ultrasonication provides high energy to the system and localization of this energy causes overheating of the sample. It transfers ultrasound waves, which causes rapid change in pressure. This pressure gradient results in physical vibration as well as cavities. Formation and deformation of cavities is one of the reasons of ultrasound power localization. Generated pressure leads to compression of the processed liquid and cavities form because of this liquid trying to keep up with the elevated pressure (Kentish & Feng, 2014). Presence of these cavities prevent homogeneous wave transfer and their explosion cause sudden energy formation. By dissipation of this intense energy into heat, temperature of the sample rises. This problem is commonly solved by immersing the sample container in ice bath during application.

1.6.1.3 High pressure homogenization

Pressurizing the aqueous dispersion through a nozzle by applying high pressure is another technique for liposome formation. Industrial production of liposomes should meet the regulatory requirements which forbid the nonfood-grade solvent and detergent residuals in the final product (Chaudhry et al., 2008). High pressure homogenization (HPH) is one of the methods that does not require involvement of any toxic solvent (Jafari, He, & Bhandari, 2006; H. Y. Kim & Baianu, 1991; Vemuri, Yu, Wangsatorntanakun, & Roosdorp, 1990). Another significant

advantage of HPH is that it is reproducible and can be adapted to large scale production (Taylor et al., 2005). With this property, HPH is widely used in the pharmaceutical industry (Mozafari, Johnson, Hatziantoniou, & Demetzos, 2008). For microfluidization purposes, lipids are first pre-homogenized in a regular mixing instrument and formed MLVs are further pressurized in a nanodisperser to form particles with desired size range. Microfluidization can decrease particle size considerably to prepare SUVs by forcing the fluid to pass from two hair thin nozzles at the same time and allowing their collision at the exit (Taylor et al., 2005). One can face some troubles in high pressure application like overheating of the interaction chamber where the nozzle is located. This problem can be tackled with a cooling unit attached to instrument. Another difficulty of such a system is that it does not offer a homogeneous dispersion in just one cycle. For HPH applications, the samples are re-pressurized many times (at least 3 passes) to decrease heterogeneity. With this method, final vesicle size can be decreased up to 20 nm (Torchilin, & Weissig, 2003) and both SUVs and LUVs can be produced.

1.6.2 Nonmechanical Techniques

1.6.2.1 Freeze dried rehydration vesicles

Integrating a freezing and thawing process into encapsulation process is generally required to increase the encapsulation efficiency (Costa, Xu, & Burgess, 2014). The samples are freezed by means of liquid nitrogen (-196°C) and thawed below phase transition temperature and this cycle can be repeated up to 10 times to finally reach an equilibrium concentration of encapsulated material. In liposome studies, this repetition breaks the layers of MLVs and promote the penetration of active agent, also decreasing polydispersity (Costa et al., 2014).

1.6.2.2 Reverse phase evaporation

The idea of reverse phase evaporation is the formation of inverted micelles, which expose the hydrophobic tails to the surrounding while hydrophilic heads remain at the periphery of inner core. The phospholipid is dispersed in an organic solvent and a water in oil emulsion is prepared by mixing it with the aqueous buffer. Liposomes are formed by sonication and when the organic solvent is evaporated by vacuum, emulsion is formed following a viscous gel (J.S. Dua, Prof. A. C. Rana, 2012). This method yields high encapsulation efficiency however it is challenging to eradicate organic solvent completely. It is reported by Laouini et al. (2012) that reverse phase evaporation yields 30 and 4 times higher efficiency when compared to sonicated vesicles and multilamellar vesicles, respectively. On the other hand, the most problematic step of the method is removal of the solvent traces which affects the vesicle stability as well as being harmful for human consumption.

1.6.2.3 Detergent depletion

The method includes mixing the lipid in an organic solvent, preferably chloroform/ethanol (50:50, v/v) in the presence of a detergent. The recommended ratios and detergents used are 1:1.6 for sodium cholate, 1:5 for octylglucoside, and 1:3 for CHAPS (Torchilin, & Weissig, 2003). The method is convenient for even sensitive proteins and formed liposomes are very homogeneous (Torchilin, & Weissig, 2003). Characteristics of phospholipids prepared by different detergents are given in Table 1.4. Among others, detergent depletion method is the one which ensures the highest stability (Gregoriadis, 2007).

Table 1. 4 Characteristics of the liposomes produced by different detergents and removal techniques (Torchilin, & Weissig, 2003).

[a] 3-(3-Cholamidopropyl)-dimethylammonio-1propanesultfonate, [b] Egg Yolk Lecithin, [c] Octylglucoside, [d] Egg Yolk Phosphatidic Acid, [e] Glycocholate, [f] Dimyristoyl Phosphatidyl Choline, [g] n-Dodecyl nonaethylene glycol monoether, [h] Sodium cholate

Detergent	**Phospholipid**	**Diameter (A°)**	**Detergent Removal**
CHAPS [a]	EYL [b]	2900	Very slow (dialysis)
OG [c]	EYL	2400	Very slow
CHAPS	EYL	2080	Slow (gel chromatography)
Triton X-100	EYL-EPA [d] (9:1)	1500	Slow (adsorbent beads)
GCh [e]	DMPC [f]	1300/380	Fast (dilution): gel state/liquid crystalline state
C12 E8 [g]	EYL	600	Medium (adsorbent beads)
OG	EYL	610	Fast (dilution)
C12 E8	EYL	560	Fast (adsorption)
OG	EYL	300	Extremely fast (dilution)
SCh [h]	EYL	300	Medium (gel chromatography)

1.7 Liposome Characterization

Stability of the liposomes are of great concern for the quality attributes of the product during storage. Main analysis conducted to quantify liposome stability are particle size and polydispersity index (PDI) measurements, encapsulation efficiency, zeta potential, differential scanning calorimetry (DSC) and gas chromatography (GC) (Laouini et al., 2012).

The analyses to be made also depend on the liposome formation technique. Liposomes are thermodynamically unstable systems so they have a tendency to fuse or break with time. This behavior can be measured by particle size and PDI analysis and it is adaptable to all liposomes prepared by different methods. Disintegration of particles from the solvent can also be measured by visual analysis as well as optical microscopy. Being a lipid, unsaturated fatty acids of liposome phospholipids are also prone to oxidation which in turn may affect the permeability characteristics of the structure (Laouini et al., 2012). That is why lipid oxidation can also be checked as a criteria at different temperature-time combinations to get information about liposome stability. DSC is another adaptable method to predict the behavior of lipid in question at different temperatures and processes. On the contrary, GC is specifically performed to determine residuals for the techniques using organic solvents. For the charged particles, zeta potential is an important parameter since the initial surface charge and its change directly affects the dispersion stability. For further investigation, morphology and shape of the particles can be investigated by microscopic techniques, depending on the purpose of use. In the following parts, some of the methods associated with stability are explained in detail.

1.7.1 Particle size measurement

Particle size measurement is a contributing factor to indicate quality and stability for various applications. The technique of size measurement depends on sample to be examined.

1.7.1.2 Microscopy

Microscopy can be defined as magnification of the images for further detail, making it possible to study up to 0.1 mm with the naked eye (William, 1996). Microscopic analysis of particle size is generally used to study 2D morphology of the particles however the mechanism as well as sample preparation varies for each method. There are various microscopic techniques including, Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM) and optical microscopy.

TEM is applied to study samples in atomic level. As the name implies, TEM imaging is based on the transmitted electrons and provides information on the internal structure of the sample. The theory stands upon the exposure of the sample to electron beam, which is described as a type of "ionizing radiation". Such a radiation has the capability to pull out a strongly bound electron from the nucleus of the sample (William, 1996). Collection of secondary signals coming from these removed electrons enables examination of the characteristics in atomic range. TEM equipment applies vacuum during studies to exclude oxygen in the environment to prevent any interpretation of air gases. To not to limit transmission of electrons, TEM samples should be prepared thin. Although it is really advantageous and efficient to use, TEM equipment is really expensive and electron removal mechanism can be detrimental (beam damage) for sensitive samples (Kuntsche, Horst, & Bunjes, 2011) although resolution is very high. In the literature, TEM is widely applied to study morphological changes of liposomes to observe phase transitions at different pH values, effect of drying, freeze fracture…

etc. (Chiu et al., 2005; Duangjit, Opanasopit, Rojanarata, & Ngawhirunpat, 2011; Ickenstein, Arfvidsson, Needham, Mayer, & Edwards, 2003; Lu et al., 2014; Rashidinejad, Birch, Sun-Waterhouse, & Everett, 2014; M. J. Taylor, Tanna, & Sahota, 2010; Wessman, Edwards, & Mahlin, 2010).

SEM is also a method based on materials' response to electron beam but unlike TEM, SEM provides the information on surface properties only, that is why the thickness of the sample is out of concern (Karcz, 2009). SEM system is equipped with a vacuum chamber like TEM, so samples should be dry. For foods, conductivity decreases with the loss of water so generally coating is applied before SEM imaging, since the sample must also be conductive (Karcz, 2009). In literature, SEM is also widely used to investigate internal structure, morphology and freezing characteristics of liposome systems (Costa et al., 2014; Rashidinejad et al., 2014).

1.7.1.3 Light scattering

The size of 3D structures are hard to define by just one quantity since there are several dimensions to consider. For the ease of calculation and reporting, the principle of particle size measurements lie on "the equivalent sphere theory" (Rawle, n.d.), to describe size of the particles by one unique quantity in light scattering measurements. Generally volume or surface areas of particles are reported in the literature. As the name of the theory implies, the results reported as the diameter of the sphere, which has the equivalent volume and/or surface area with the particle of concern.

There are two types of measurement techniques by light scattering; laser diffraction and dynamic light scattering.

1.7.1.3.1 Laser Diffraction (Low Angle Laser Light Scattering)

When a particle is exposed to a beam of light, they reflect, refract, absorb and reradiate or diffract the light. Low Angle Laser Light Scattering (LALLS), commonly known as Laser Diffraction technique is based upon the angle of light diffracted from a particle. For small particle diameters ($d < \lambda/10$), scattering is assumed to obey Rayleigh approximation, which states that scattering is equal in all directions (isotropic) and intensity is proportional to $1/\lambda^4$ (λ: wavelength). The theory suggests that as the particles get smaller, they break and spread the light in wider angle. LALLS is quick and user friendly method to apply. The solution containing particles are diluted in an appropriate medium (liquid or gas) and light (usually laser) is passed through the media (Kahkashan, n.d.). Detectors collect the scattered light at different angles from particles and report them as mathematical value. Schematic representation of the method setup is given in Figure 1.3. This method is applicable for particles sizes of very wide range, 0.1 to 3000 µm, according to ISO13320 criteria (Rawle, n.d.).

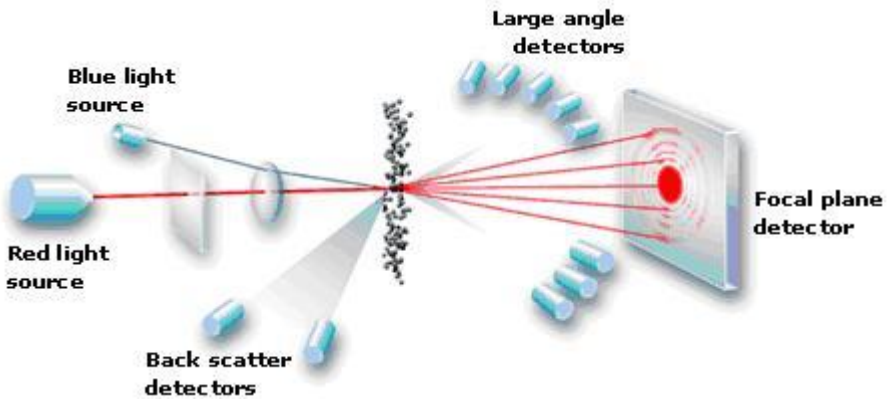

Figure 1. 3 Mechanism of LALLS (Analytical, 2016).

The instrument used are upgraded from low-angle (LALLS) to multi-angle (MALLS) by further improvements (Allen, 2003). Mathematical interpretation of the data collected by these detectors can be processed by Lorenz-Mie or Fraunhofer diffraction theories. Both of them assumes to measure particle size of a two phase system. The behavior of scattering changes when particle sizes change below or above tenth of laser wavelength (ʎ/10). Mie theory can explain the minima or maxima of the intensity with respect to angle that is why it is applicable for a wide range of particle sizes. However, refractive index and absorbance values should be provided for the calculations and the results are very sensitive to changes in these parameters. For this theory, particles are assumed to be spherical and scattering behavior is presumed to obey Rayleigh.

Apart from Mie theory, Fraunhofer diffraction theory assumes that particles are opaque and disk shaped, with small scattering angle. However, ISO 13320-1 (1999) regulations report that Mie theory is recommended for particle size smaller than 50 μm (Jones, 2003).

1.7.1.3.2 Dynamic Light Scattering (Photon Correlation Spectroscopy)

Dynamic light scattering technique mechanism is based on the Brownian motion theory, which states that particles in a solution are constantly in motion. This motion is the result of collisions between neighboring molecules. The laser light send to such solutions enables the measurement of intensity fluctuations of scattered light, which can be used to find translational diffusion coefficient. The relationship that quantifies particle size by Brownian motion is Stokes-Einstein equation given by (Frolov et al., 2011):

$$d_H = \frac{k\,T}{3\pi\eta D}$$

(Equation 1.3)

where k, T, η, D and d_H are Boltzmann's constant, absolute temperature, viscosity, translational diffusion coefficient and hydrodynamic diameter, respectively. PCS measurements report two main results: hydrodynamic diameter (Z_{av}) and polydispersity index (PDI). Hydrodynamic diameter is the actual diameter of the particle plus the electrical double layer that adheres at its surface. Z_{av} of a particle does not give the actual size which can be observed via microscopic techniques. However the significance of hydrodynamic stability comes from the motion behavior of a particle inside a suspension. As described earlier, Z_{av} is measured from the random movement of particles, meaning that even if the particle has a smaller particle size when measured directly from the borders, the actual behavior in the solution demonstrate the characteristics of a particle having Z_{av}. Hydrodynamic diameter gives how the particle choose to act in a solution. That is why it is considered as the stability criteria for most of the applications instead of real core diameter. Presence of ions remarkably affects the thickness of the double layer, which can be suppressed in high concentrations.

PDI is another significant outcome of PCS measurement, which gives an idea of the uniformity of particle distribution in the solution. It is a contributing factor for determination of physicochemical properties, biodistribution, bioavailability and potential biological reactions in the body (Akbari & Wu, 2016). Although polydispersity changes according to processes and area of use, smaller PDI values are desirable for the stability of two phase systems. PDI values below 0.1 is considered as monodisperse while higher PDI describes polydispersity of the liposome system (Karn, Cho, Park, Park, & Hwang, 2013).

Particle sizing techniques for various mediums and measured property are given in Table 1.5.

Table 1. 5 Method of measurement for different mediums and measured property/principle (Merkus, 2009).

Method		**Medium**	**Size Range (μm)**	**Measured Property/Principle**
Microscopy	Optical	Liq/gas	0.3 - 500	Usually for 2D/3D representation of the particles
	Electron	Vacuum	0.001 - 500	
Sedimentation	Gravity	Liq	0.3 – 200	Settling rate
	Centrifuge	Liq	0.02 – 10	Stokes Law
Gas absorption		Gas	0.001 – 100	Surface Area
Light Scattering	DLS (PCS)	Liq	0.005 – 1	Brownian motion
	MIE (Diffraction)	Liq/Gas	0.04 - 2000	Equivalent Scatterers

1.7.2 Zeta potential

The surface of particles are generally covered by an electrically charged layer in a solution. The closest layer adhering the particle consists of dense molecules of opposite charge, and this density diffuses as the distance from the surface increase. Within these layers, the thickness that the particle act as an individual and demonstrate stable characteristics is called hydrodynamic diameter, as mentioned earlier. The charge at this layer is referred as zeta potential (ζ), which enables an indirect measurement of colloid stability (Carvalho et al., 2015). A schematic representation of zeta potential is shown in Figure 1.4.

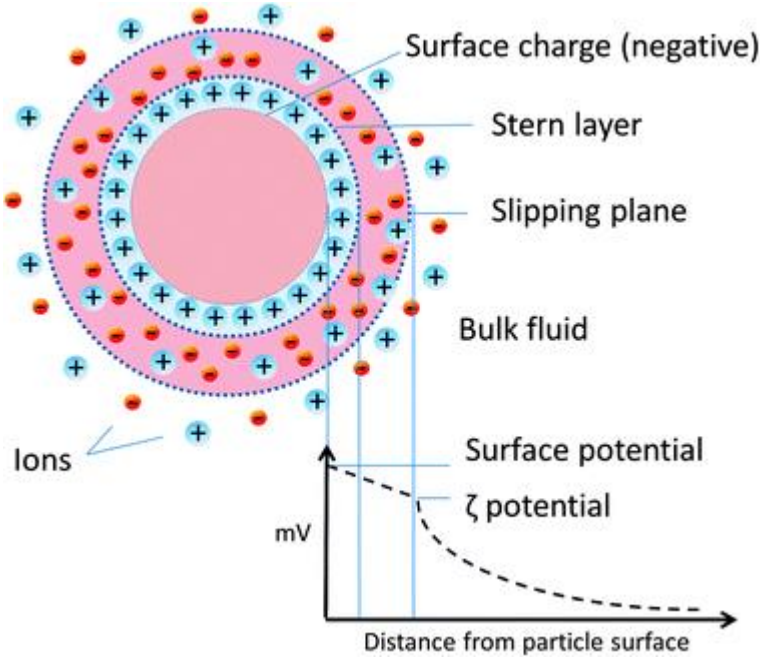

Figure 1. 4 Schematic representation of zeta potential (Liese & Hilterhaus, 2013).

Zeta potential is affected from change of pH, ionic strength and concentration of the components (Vallar, Houivet, El Fallah, Kervadec, & Haussonne, 1999).

1.7.3 Lipid Oxidation in Liposomes

Oxidation is one of the major causes of food deterioration and being part of the natural components of foods, dietary lipids are responsible for quality changes due to lipid oxidation. Oxidation of lipids are influenced from the oxygen present in the environment, water activity, presence of antioxidant materials, irradiation, composition of the lipid, medium temperature and pH (Decker & McClements, 2000; Wang & Wang, 2008).

In food systems, lipids are generally present in the form of emulsion, not as bulk lipids (Nuchi, Julian McClements, & Decker, 2001) so the mechanism of oxidation differs from largely oil dominated systems. Presence of the emulsifier as well as a second phase influence the rate of reactions (Decker & McClements, 2000), that is why oxidation of lipids significantly depends on where the lipid is located in the emulsion system and the environment of the particles (Wedzicha, 1988). For example, when a volatile substance is to be distributed in the bulk oil, it should pass through only one phase while in an emulsion it has to overcome at least three possible phases; aqueous, oil and interface (Jacobsen, Meyer, & Adler-Nissen, 1999). This difference in mechanism makes liposome or vesicle systems suitable for mimicking the lipid oxidation of heterogeneous structures like tissue or muscles for the medical studies and raw foods as they natively occur in the nature (Coupland & McClements, 1996).

Oxidation of the bilayer systems are mostly due to fatty acid tails and the hydrophilic heads are rarely oxidized although they may contribute to oxidation in the presence of transition metals or pro-oxidants (Schnitzer, Pinchuk, & Lichtenberg, 2007).

1.7.4 NMR Relaxometry and MR Imaging

Nuclear Magnetic Resonance (NMR) Relaxometry is a promising, novel technology to investigate biological systems without spoiling or disrupting the samples. In mechanism, NMR is used to detect the changes in proton alignment, produced by radiofrequency (RF) pulses. The resulting signal is obtained from the entire sample regardless of orientation, although it enables the detection of separate entities (Kirtil & Oztop, 2015). Parameters acquired from NMR experiments are T_1 and T_2 relaxation times. T_1 is the longitudinal relaxation time and related to spin-lattice interaction, giving information on the ability of the protons to convey energy across or absorb energy from the surrounding lattice (Hashemi, Bradley Jr, & Lisanti, 2010). On the other hand, T_2, transverse

relaxation time, gives information on spin-spin relaxation, at when the effect of applied magnetization approaches to zero (Kirtil & Oztop, 2015). Longer T_2 values are obtained when the sample has high water content while increase in oil content considerably decreases the T_2 value. The lowest T_2 results are obtained in solid samples since spin-spin energy transfer is very rapid in solid molecules (Hashemi et al., 2010). T_1 behavior of some foods can be organized in decreasing order as H_2O, proteinaceous food and fats while solid tissue gives an average T_1 (Hashemi et al., 2010).

Magnetic Resonance (MR) imaging has come to be known for its applications in medical industry for diagnostic purposes (Kirtil & Oztop, 2015). In the recent years, MR imaging is also studied in the field of engineering for biological as well as non-biological entities (d'Avila et al., 2005) since it is handy in use and provides reliable results. Radiofrequency pulses sent via magnetic coils enables to create an image detecting the contrast change within the sample in three axes and provides spatial information of the sample in contrast to NMR relaxometry. Visual interpretation of this contrast generally relies on the proton content of the compartments (i.e.: water, oil content) of the sample and sample orientation is very important unlike NMR relaxometry.

MRI is used to characterize drug release from liposomes in vivo in the literature. However there have not been conducted any study to design food grade liposome systems by using MRI and NMR relaxometry up until today.

1.8 Objectives of the Study

This study was funded by National and Scientific Technological Council of Turkey, Career Development Program (3501) with proposal number 113O442. The project aims to formulate green tea extract loaded liposomes for preparing a spray product to prevent lipid oxidation in oil rich foods. The first part of the project deals with the formulation and characterization of unloaded liposome

systems using different homogenization techniques: microfluidization and ultrasonication. In this thesis, the main objective was;

- To produce stable unloaded liposome systems using high pressure homogenization and ultrasonication,
- To characterize the liposome systems in term of particle size, zeta potential, electron microscopy, and time domain low resolution NMR relaxometry,
- To investigate the time dependent stability of these liposome systems.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

Phospholipid from two different sources with different phosphatidylcholine compositions were used in the study. Soy phospholipid, coded as S75 by the manufacturer was purchased from Lipoid GmbH (Ludwigshafen, Germany). Egg phospholipid, E80 was kindly provided from Lipoid GmbH (Ludwigshafen, Germany). Number of the codes on the products denoted the phosphatidylcholine purity.

Two of the three solvents, acetate and phosphate buffers used in the study were prepared manually. For acidic buffer, glacial acetic acid was supplied from Merck KGaA (Darmstadt, Germany) and analytical grade sodium acetate trihydrate was supplied from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). 0.2 M solutions of these chemicals were mixed to achieve a final pH of 3.8 for the acetate buffer. For phosphate buffer, analytical grade disodium hydrogen phosphate dihyrate and potassium dihydrogen phosphate were purchased from Merck KGaA. (Darmstadt, Germany) and their aqueous solutions were mixed properly to give a final pH of 7.2. As the third solvent, distilled water was used and all the water used in the experiments were obtained using 0.2 μS/cm purity mpMinipure Dest system supplied from mpMinipure Ultrapure Water Systems (Ankara, Turkey).

2.2 Methods

2.2.1 Preparation of Liposomes

2.2.1.1 Primary Processing by High Speed Homogenization

In the scope of this study, all liposomes were prepared by two step homogenization. For primary homogenization, 1% (w/v) phospholipid was added to the buffer/distilled water and hydration of lipids was achieved by using high speed homogenizer (IKA T25 digital Ultra-Turrax, Selangor, Malaysia) at 20000 rpm for 2 min. This initial homogenization led to formation of multilamellar vesicles which could easily be detected by an optical microscope.

As the secondary homogenization, liposomes were prepared by means of two different mechanical methods, which were high pressure homogenization (microfluidization) and high intensity ultrasound homogenization (direct probe ultrasonication). This further process led to formation of small unilamellar vesicles of higher stability.

2.2.1.2 Secondary Processing by High Pressure Homogenization

After primary processing, pre-homogenized samples were fed to the inlet chamber of ISA-N-10M Nano Disperser (Ilshin Autoclave, South Korea) equipment. Each sample was subjected to 5 pass pressurization through 75 μm diameter nozzle of the interaction chamber at 400, 900, and 1300 bars separately. The equipment was installed with a cooling unit which provided temperature control maintained at the range of 20-25°C, depending on the applied pressure. Both E80 and S75 liposomes were prepared by this method in all three solvents (acetate and phosphate buffers and distilled water) for all three pressures in triplicate and stored at 4°C and 25°C separately, wrapped in aluminum foil for further analysis.

2.2.1.3 Secondary Processing by High Intensity Ultrasound Homogenization

Following pre-homogenization, samples were divided in 10 ml portions and subjected to ultrasonication at 100% amplitude at frequency of 20 kHz (Sonoplus Ultrasonic Homogenizer, BANDELIN Electronic GmbH & Co. KG, Berlin, Germany). TT13 flat tip probe was used for all the applications at nonstop mode for 2, 5, and 8 minutes. Since E80 phospholipids were foaming at ultrasonication and microfluidization results of the liposomes showed unstable liposomes, only S75 phospholipid was used for ultrasound experiments in all three solvents (acetate and phosphate buffers and distilled water) for all three ultrasonication times. Each sample was prepared in triplicate and stored at 4°C and 25°C separately, wrapped in aluminum foil for further analysis.

2.2.2 Characterization of Liposomal Systems

For characterization of prepared liposome systems during storage; particle size and Nuclear Magnetic Resonance (NMR) Relaxometry experiments were conducted with respect to time. To confirm and verify the particle sizes measured by Photon Correlation Spectroscopy, Transmission Electron Microscopy (TEM) images were taken and zeta potentials were measured to explain stability of dispersions prepared in different solvents.

2.2.2.1 Particle Size Measurements

The change of the particle sizes with time was measured by using Malvern Zetasizer instrument (Malvern Nano ZS90, Malvern Instruments Ltd., Worcestershire, UK) present in METU Central Laboratory. Measurement parameters; refractive index and absorption values are set to 1.56 and 0.01, respectively. Before each measurement, samples were diluted in 1/10 ratio and particle sizes were recorded at 1st, 7th, 14th and 30th days for each sample.

Polydispersity index (PDI) were also recorded to check the change in width of particle size distribution with time.

2.2.2.2 Transmission Electron Microscopy (TEM) Experiments

To confirm the particle size and define the state of liposomes during storage, bright field illumination was conducted with conventional Transmission Electron Microscopy (TEM). The samples were imaged at METU Central Laboratory by using High Contrast Transmission Electron Microscope (Tecnai G^2 Spirit Biotwin, FEI Company) with Lantanhexaborid (LaB_6) electron gun at 120 kV [2]. To eliminate possible interference of the buffer salts, TEM images were taken for samples prepared in distilled water only.

2.2.2.3 Zeta Potential Measurement

Zeta potentials of the liposomes were measured by photon correlation spectroscopy (PCS) using Malvern Zetasizer instrument (Malvern Nano ZS90, Malvern Instruments Ltd., Worcestershire, UK) present in METU Central Laboratory. The electrophoretic mobility of the particles were measured and substituted into Henry's equation (Equation 2.1) by assuming Smoluchowski approximation f(κa)=1.5 to obtain zeta potential:

$$U_E = \frac{2\,\mathcal{E}\,Z\,f(\kappa a)}{3\,\eta}$$

(Equation 2.1)

where $U_E, Z, \mathcal{E}, \eta$ and f(κa) are electrophoretic mobility, zeta potential, dielectric constant, viscosity and Henry's function, respectively.

All the samples were diluted in 1/10 ratio before experiment.

2.2.2.4 Nuclear Magnetic Resonance (NMR) Relaxometry

The NMR Relaxation measurements were carried out by using a 0.5T (22.40 MHz) low resolution NMR Spectrometer system (SpinCore Inc,, Gainesville, U.S.A). Spin-spin relaxation times (T_2) were measured using a Carr-Purcell-Meiboom-Gill (CPMG) sequence. CPMG sequence was performed with an echo time (TE) of 1000 μs, repetition delay of 3s, 5000 echoes and 24 scans. Obtained T_2 signals were analyzed with MATLAB and T_2 relaxation curves were obtained. Mono and biexponential fitting was conducted on relaxation curves. The NMR samples were prepared by loading 0.8 ml of sample into a 10 mm NMR tube. All T_2 measurements were carried out after samples equilibrate to room temperature. The T_2 times were measured for every three days during one month storage period. All measurements were carried out in replicates of 3.

2.2.2.5 Statistical Analysis

All the experimental results were analyzed by analysis of variance (ANOVA) general linear model tool of Minitab (Version 16.2.0.0, Minitab Inc., Coventry, UK) at 5% significance level. For comparisons, Tukey's comparison test was applied in 95% confidence interval. Assumptions necessary to conduct a true ANOVA were checked before analysis. Anderson Darling test for normality and Barnett test for constant variance were checked. If the assumptions fail to be satisfied, transformation (Square root, logarithmic, Box-Cox, i.e..) was conducted on the data set.

2.3 Experimental Design

Table 2.1 Experimental Design Parameters

UNLOADED LIPOSOME CHARACTERIZATION				
#	**FACTORS**	**LEVELS**		
1	Lecithin type	Soya lecithin (S75),	Egg lecithin (E80)	
2	Solvent type	Acetate buffer (pH≈3.8),	Distilled water (pH≈6.5),	Phosphate buffer (pH≈7.2)
3	Storage temperature	Refrigeration temperature (4°C),	Room temperature (25°C)	
4	Microfluidization pressure	400 bar,	900 bar,	1300 bar
5	Ultrasonication time	2 min,	5 min,	8 min
6	Storage time*	1st, 4th, 7th, 10th, 14th, 21th, 28th, 30th, 35th days		

* Measurements are conducted at different time periods for different experiments during storage.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Mean Particle Size of Liposomes

Liposomes which are on the form of multilamellar vesicles (MLVs) are not preferred for utilization since their multiple compartments harden to define and control the system and they are inhomogeneous (Hope, Bally, Mayer, Janoff, & Cullis, 1986). Liposomes that are in the form of small unilamellar vesicles (SUVs) are efficient tools that help to characterize lipid bilayers, biological membranes in general (Woodle & Papahadjopoulos, 1989). On the other hand, liposomes in the form of large unilamellar vesicles (LUVs) are preferred for their larger inner volume and stability for encapsulation purposes (Hope et al., 1986). Two non-thermal methods, microfluidization and ultrasonication, are generally used to form SUVs and LUVs and this purpose was successfully fulfilled in this study.

Particle sizes of both microfluidized and ultrasonicated liposomes are measured by Photon Correlation Spectrophotometry (PCS) and mean particle size of the liposomes were reported as Z_{av} values.

3.1.1 Preliminary Experiments for Determination of Microfluidization and Ultrasound Parameters for Liposome Formation

As explained in materials and methods section, lecithins dispersed in different solutions were first exposed to a primary homogenization before ultrasonication and microfluidization. Primary homogenization throughout the study was

achieved by using high speed homogenizer at 20,000 rpm. This speed level was determined based on the preliminary trials. Effect of primary homogenization on final particle size of pre-homogenized lecithins was investigated by increasing the speed from 20,000 to 24,000 rpm (max. limit of the equipment). It was observed that initial homogenization speed did not have any significant effect on final particle size of the liposomes ($p \geq 0.05$) (Figure 3.1).

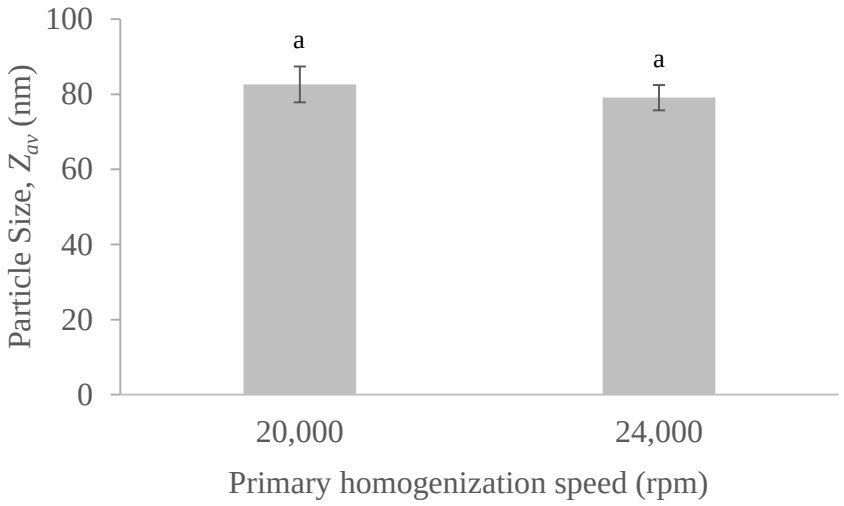

Figure 3. 1 Effect of primary homogenization speed on final particle size.

3.1.1.1 Microfluidization

Increasing microfluidization pass number on particle size is reported to cause a gradual decrease in final particle size in the literature (Zhang, Peppard, & Reineccius, 2015). Providing multiple passes enhances homogeneity and avoids multiple peaks on the distribution plot. Resulting peak on the particle distribution plot has a finer shoulder towards the right hand side and become narrower and symmetrical as pass number is increased. In microfluidization studies, 5 pass is generally sufficient for the uniform distribution of unilamellar vesicles (Ahmad, 2012). In this study, a preliminary trial on pass number was also conducted. One example of the effect of pass number on particle size and size distribution is shown on Figure 3.2. Mean particle size of the liposomes for different pass numbers were found to be significantly different ($p \leq 0.05$). However still the distributions and particles sizes obtained were reasonable at 5 passes. Thus in the rest of the study considering the feasibility of the experiments, number of passes was kept constant as 5.

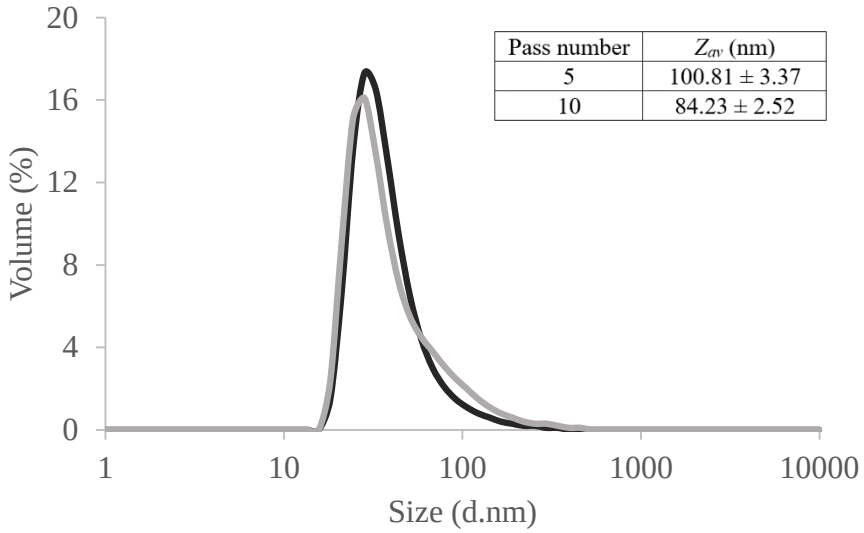

Figure 3. 2 Change in size distribution of commercial lecithin prepared in acetate buffer and pressurized at 900 bars by increasing pass number from 5 (—) to 10 (—).

Another parameter to be tested on microfluidization was the pressure. Based on literature and the limits of the equipment, microfluidization pressures were chosen to represent a low (400 bars), medium (900bars) and a high pressure (1300 bars) respectively (Torchilin & Weissig, 2003). It was confirmed in the experiments that microfluidization pressure would have significant effect ($p \leq 0.05$) on the particle size as shown in Figure 3.3. Although 900 and 1300 bar pressurized samples did not show any significant difference when analyzed with other pressure levels, they were found to show significant difference ($p \leq 0.05$) when only these two levels were individually analysed (Table A.2). Since distilled water (DW) as the solvent type was added to the study at a later time; for liposomes prepared in DW, only pressures of low (400 bars) and high (1300 bars) were tested.

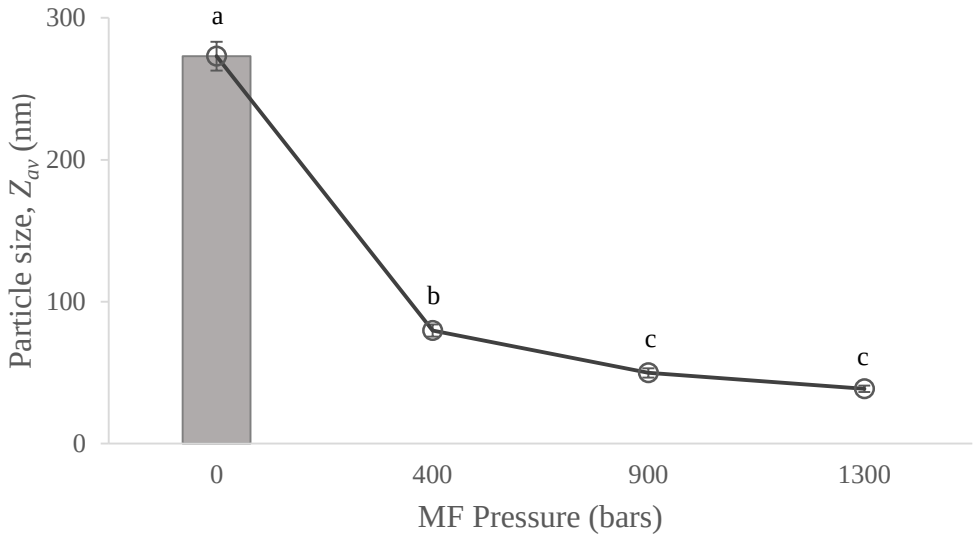

Figure 3. 3 Mean particle sizes of S75* liposomes dispersed in acetate buffer at different pressure levels (—o—) at first day of preparation, compared with the non-pressurized control (■).

*Effect of the pass number on liposomes was tested on another type of lecithin which was named as the 'Commercial Lecithin' throughout the text. So the particle sizes at the same pressures was expected to be different from Figure 3.2.

3.1.1.2 Ultrasonication

Ultrasonication time to prepare liposomes was also determined by preliminary experiments. The results revealed that the mean particle size of liposomes was not

affected from homogenization time after 5 minutes ($p \geq 0.05$). The change of particle size with respect to ultrasonication time is shown in Figure 3.4.

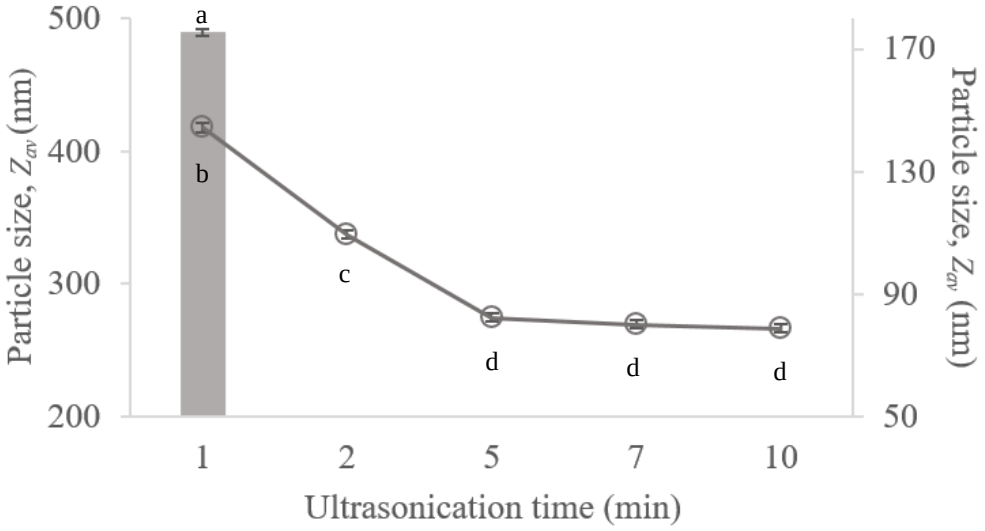

Figure 3. 4 Particle size results of preliminary experiments conducted by commercial lecithin prepared in acetate buffer to observe the effect of applied ultrasonication time (—o—) on particle size, comparing with the only pre-homogenized control (■).

The particle sizes did not show significant difference after 5 minutes of operation ($p \geq 0.05$). However, to find out if there will be any differences on the long term

stability of liposomes, ultrasonication was decided to be conducted at 3 different homogenization times: 2, 5 and 8 minutes.

3.1.2 Effect of microfluidization pressure on mean particle size

Effect of pressure was already discussed in the previous section (Figure 3.3) while discussing the parameters of MF that could be used in the study. For all samples, the change in mean particle size with respect to microfluidization pressure was clearly detected by the PCS measurements without exception. In literature, the MF pressure effect on particle size was reported to be parallel with our results (Taylor et al., 2005).

The change in particle size was also validated by visual observation of the samples. Regardless of the solvent or lecithin type, transparency of all samples increased as the MF pressure was increased. When mean particle size decreased, light penetrates into the sample more easily and cloudy white appearance turned to be clearer (Figure 3.5).

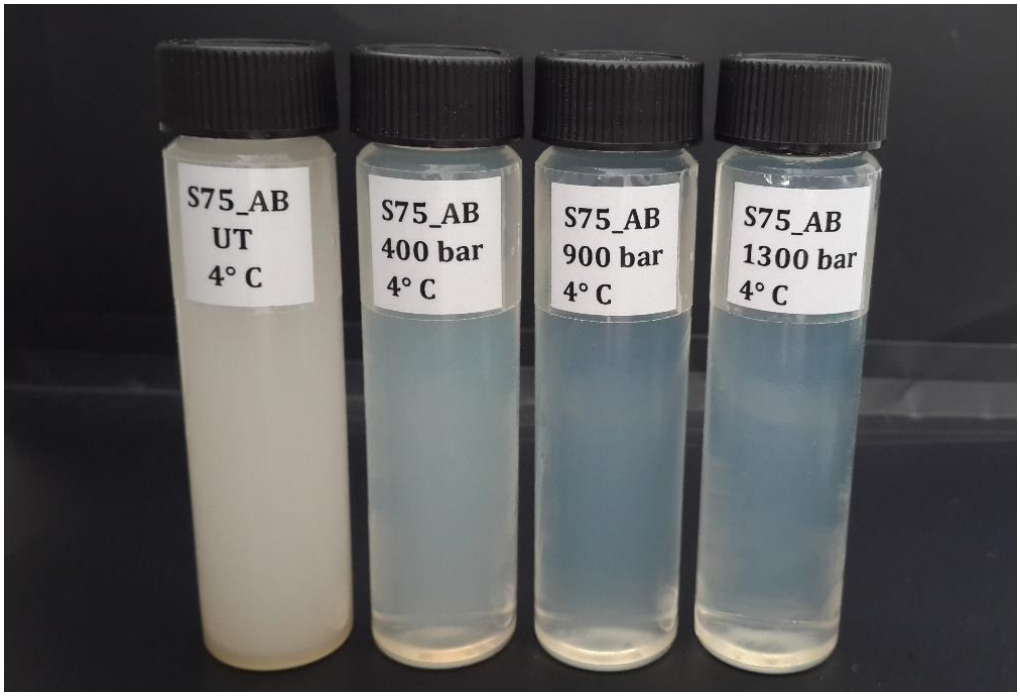

Figure 3. 5 Change in transparency of S75 liposomes prepared in acetate buffer with respect to microfluidization pressure. (S75_AB denotes liposomes prepared using soy lecithin and acetate buffer as the solution; UT denotes samples that were not exposed to microfluidization).

3.1.3 Effect of ultrasonication time on mean particle size

As stated before, ultrasonication is another method to prepare SUVs. In this study, effect of three different ultrasonication times; 2, 5 and 8 min were investigated on the mean particle size of the liposomes.

Ultrasonication experiments were only conducted for S75 type lecithin since E80 lecithin was creating significant amount of foam during homogenization which

could make the latter measurements difficult. Moreover, as will be explained later liposomes prepared from E80 type lecithin did not result in stable liposomes.

Both solvent type and ultrasonication time were found to be significant on the mean particle size ($p \leq 0.05$) (Table A.4). Liposomes prepared in phosphate buffer had the highest particle size whereas distilled water ones had the lowest. Except the samples prepared in distilled water, 2 min ultrasonicated samples' particle sizes were found to be higher than 5 and 8 min samples ($p \leq 0.05$) whereas 5 and 8 min did not show a significant difference ($p \geq 0.05$) (Figure 3.6).

Ultrasonication can affect an emulsion both in positive or negative ways; it may break large particles into smaller ones or it may induce instability by interfering between water and lipid interface (Li & Fogler, 1978). In our case, there did not seem any negative effect of the ultrasound, however increasing the ultrasonication time after 5 min did not significantly affect the mean hydrodynamic diameter in buffers ($p \geq 0.05$). The photos to observe the change in transparency of liposomes with respect to ultrasonication time is shown in Figure 3.7.

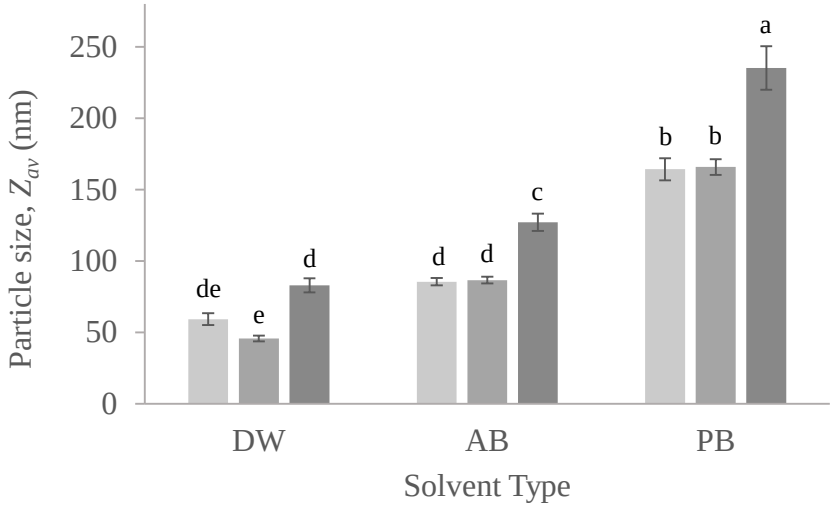

Figure 3. 6 Particle size change of soy lecithin (S75) liposomes prepared in distilled water (DW), acetate buffer (AB) and phosphate buffer (PB) at different ultrasonication times of 8 min ( ), 5 min ( ) and 2 min ( ).

There might be two reasons of this trend. In emulsion systems, the minimum particle size that can be achieved via ultrasonication was reported to be dependent on the surfactant amount of the dispersion (Kentish & Feng, 2014). Although liposomes could not exactly be classified as emulsions, phospholipid concentration for liposome formation could be a reason for this trend. It was possible that 1% phospholipid ratio was capable of producing minimum particle size around 5 minute ultrasonication operating at 100 % amplitude and extending the duration of ultrasonication did not contribute to further reduction in particle size. For distilled water samples that show very close particle sizes at different ultrasonication times, the same mechanism should have applied. Another reason might be the particle size measurement technique. By looking at the difference between particle sizes with respect to solvents, the only important difference is the

insignificant effect of ultrasonication time on distilled water samples ($p \geq 0.05$). Moreover, when 2 Way ANOVA results were examined, it was seen that distilled water samples had the lowest particle size ($p \leq 0.05$) (Table A.4). This situation might be explained due to the measurement technique of PCS. As pointed before, PCS measures hydrodynamic diameter, not actual particle size of the droplets. The ions in buffers might have contributed to electrical double layer and might have increased the particle size compared to distilled water samples. The loss of this layer might be able to reveal itself in distilled water samples (Figure 3.6). This difference could further have been detected by an advanced microscopic technique however it was not applied in this study since the most appropriate technique to observe liposomes is TEM and it does not work at high ionic strengths.

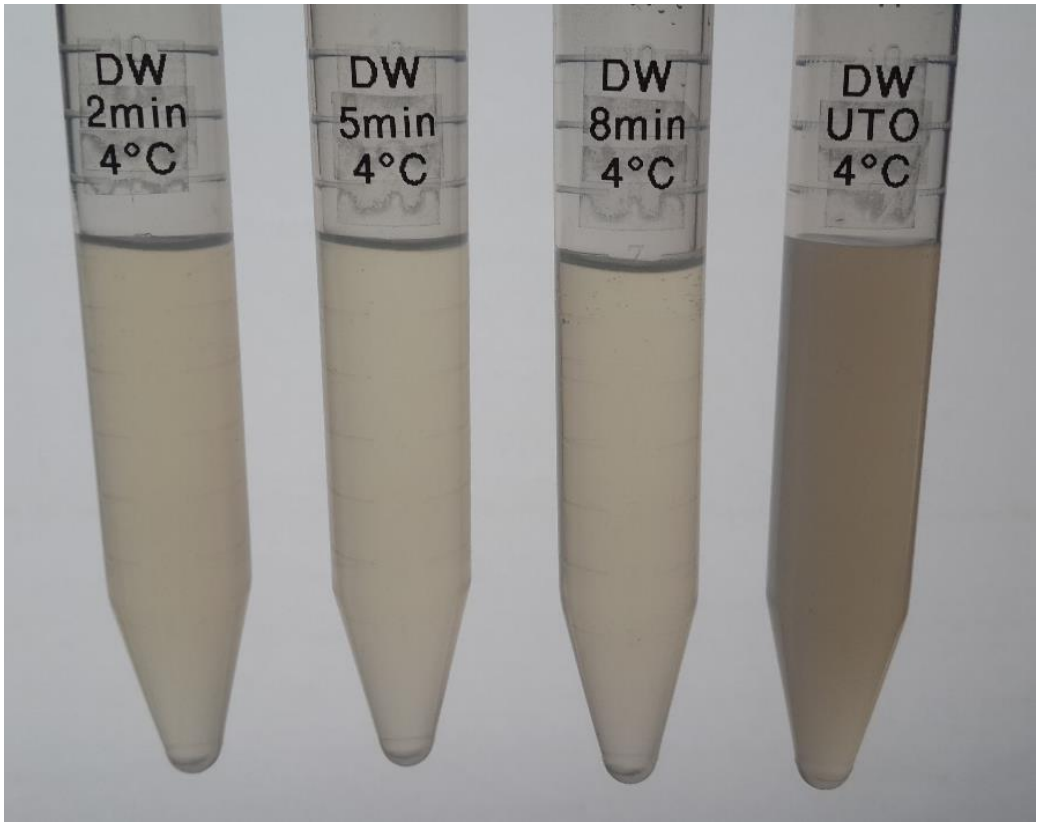

Figure 3. 7 Change in transparency of S75 liposomes prepared in distilled water with respect to ultrasonication time. The visuals are taken from the samples stored at 4°C and ultrasonication time increases from 2 to 8 min from left to right. UT denotes only pre-homogenized sample.

3.1.4 Effect of phospholipid and solvent type

In the scope of this study, two types of phospholipids, referred as S75 and E80 for soy and egg lecithin respectively, were dispersed in three different solvents which were distilled water (DW), acetate buffer (AB) and phosphate buffer (PB). Egg phospholipid is the traditional source of lecithin however it is more expensive and

less stable than soy lecithin, which was confirmed by experiments in this study. Soy lecithin accepted to be an efficient alternative for egg lecithin. Since the instability of egg lecithin in microfluidized samples and excess foaming problem was confirmed, it was not further studied in ultrasound experiments. Reasons and results of E80 experiments will be discussed in a later section.

Selection of the solvents studied were based on the availability and functionality. Water is easy to access for all kind of purposes however it does not resist pH change of the environment which may result in fluctuations in the measurements. For this purpose, a buffer near physiological pH to mimic water (PB) was also used. The purpose of using a medium with acidic pH was to diversify the use of liposome system for different purposes, which can be effective for acid soluble compounds as well as layer by layer coating with a polymeric material to increase the stability of the liposome. Rashidinejad et al. (2014) reported polyphenols to be resistant to oxidation at 3.8 pH and formed liposomes at that pH for further use, which indicated functionality of this study for multiple purposes.

The change in particle size of S75 dispersions prepared in aforementioned solvents by ultrasonication had been shown in Figure 3.6. Final particle size of the solutions was significantly affected from the solvent type ($p \leq 0.05$). PB yielded the highest liposome size while it got smaller for AB and DW, respectively. A similar trend was also observed for microfluidized samples, data shown in Figure 3.8. Considering the increase in particle size as a disturbance of the stability, the stability of PB can be said to fail to catch up with AB and DW. Furthermore, DW samples had the lowest particle size while AB was in between but much closer to DW rather than PB.

The difference due to solvent types was explained based on the contribution of electrical double layer to the hydrodynamic diameter. This trend could also be associated with zeta potential. Liposomes lose their stability as their zeta potential decreases in the presence of charged compounds. As will be explained and shown

later, the overall charge of liposomes reaches to zero in PB samples, which directly leads to instability. The same interference is valid for AB samples as well, however the decrease in zeta potential was much less than PB samples that was why those liposomes were chemically more resistant.

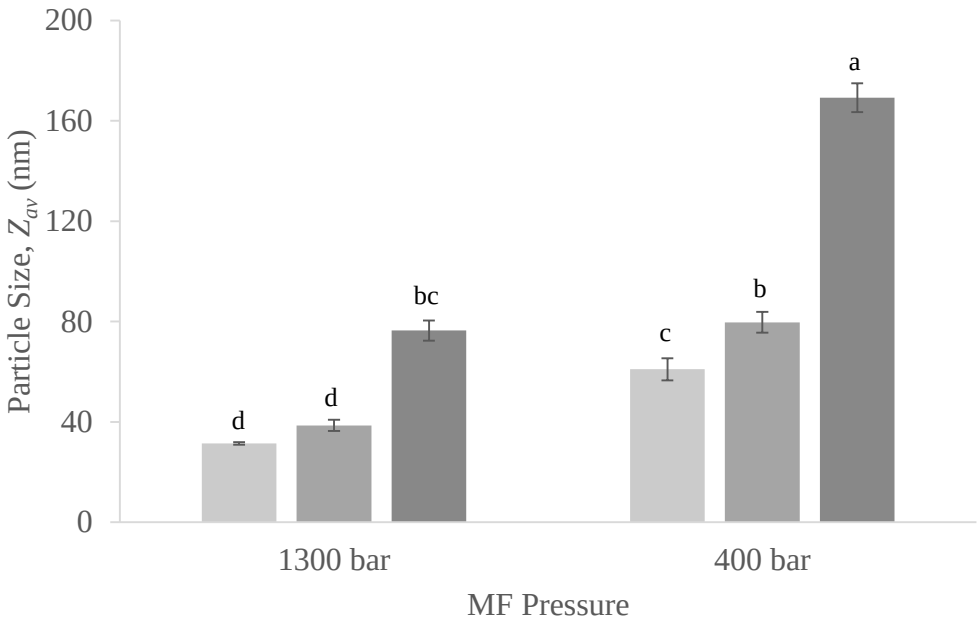

Figure 3. 8 Particle size change of soy lecithin (S75) liposomes prepared in distilled water* ( ), acetate buffer ( ), and phosphate buffer ( ) at different microfluidization pressures.

*MF experiments were not conducted for DW samples at 900 bar.

S75 liposomes prepared by using PB buffer showed precipitates after one-day, indicating instability of those liposomes. Zeta potentials of these samples approaching zero also confirmed the instable behavior. When compared with their counterparts PB samples had the highest particles size both in MF and ultrasonication. That's why, to see the effect of phospholipid type on particle size Figure 3.8 was re-drawn excluding the PB (Figure 3.9).

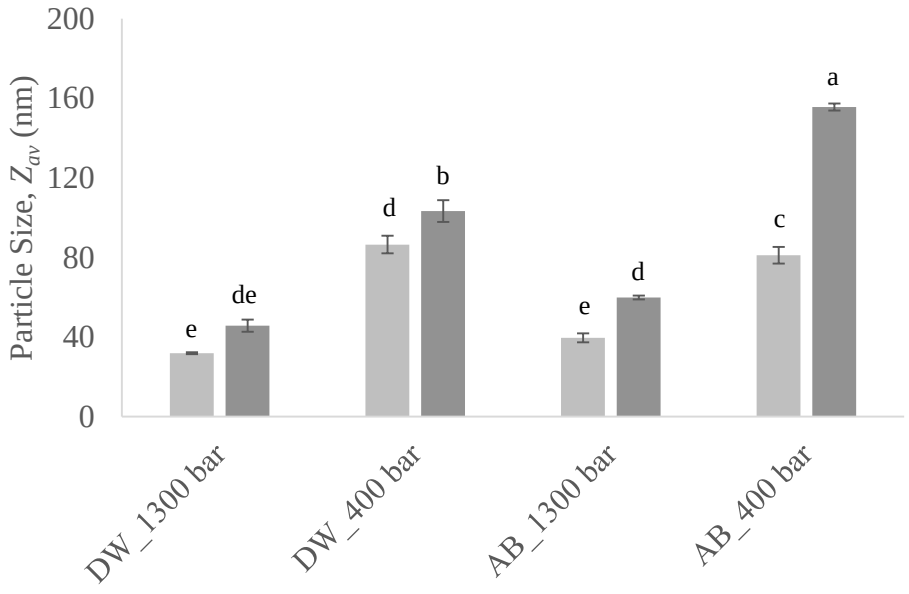

Figure 3. 9 Effect of S75 ( ) and E80 ( ) type phospholipid on final particle size of solutions prepared in different solvents at different MF pressures.

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As seen on Figure 3.9, liposomes made from E80 lecithin had always higher mean particle size than S75 samples regardless of the solvent type and pressure. The instability of E80 samples also revealed themselves visually as color change, agglomeration and flocculation, as would be discussed later in 'stability' section.

3.1.5 Effect of homogenization type

Microfluidization and ultrasonication were both successful to decrease the particle size of liposome dispersions. Microfluidization yielded lower results in a wide size range, which can be adjusted according to purpose of use by changing pressure. On the other hand, ultrasonication was also successful for SUV formation, however the particle size could not be decreased after an extent.

For ultrasonication, 3 homogenization levels were evaluated whereas for microfluidization 3 pressures were tested except DW. Moreover, ultrasonication was not conducted on E80 samples due to instability and foaming problems. That's why a complete 3 Way ANOVA (including the effect of solvent, lecithin and homogenization type) could not be conducted to test the effect of homogenization clearly. But still, to obtain a balanced experimental design matrix for ANOVA, 900 bars data and E80 data for microfluidization were excluded and ultrasonication times and microfluidization pressures were treated analogous of each other and ANOVA was conducted. Results showed that homogenization type was significant on mean particle size of liposomes ($p<0.05$) with MF samples (82 nm) having lower mean partice size compared to US ones (127 nm) (Table A.7).

When the particle sizes achieved by these two methods were compared for different solvent types in detail, it was obvious that microfluidization was more efficient to decrease the average particle size, which is shown in Figure 3.10. The trend was similar for DW and PB samples (Table A.9 & 10).

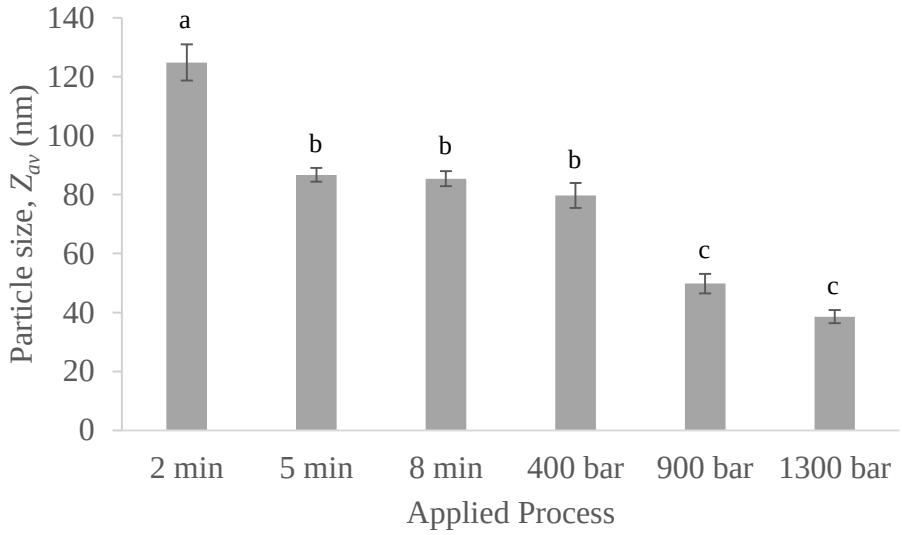

Figure 3. 10 Particle size of S75 liposomes prepared in AB by different applications, measured at the first day of preparation.

3.1.6 Effect of Storage Time and Temperature

Liposome samples of soy lecithin (S75) and egg lecithin (E80) were stored at 2 different temperatures and stability of the samples were checked based on their particle size for 30 days.

For samples prepared by ultrasonication, effect of storage time and temperature were statistically analyzed and the results showed that these two parameters did not significantly affect the particle size of the most samples. Data are summarized in Table 3.1. A similar observation was also detected in the literature for liposome samples stored at refrigeration temperature (Lu et al., 2014) where the particle size of allicin loaded liposomes stored at 4°C are monitored for 30 days.

An irregular trend was observed at samples prepared in PB. Mean particles size tend to increase with storage time which was a clear indication of the instability.

On the other hand, for microfluidized samples, all the samples did not follow the same trend as well. Some exceptions were detected, exhibiting steep increases at different time periods. There were also samples demonstrating a decrease in particle size with time when stored at 4°C. These samples were; 400 bar pressurized and non-pressurized E80 lecithin in water, non-pressurized S75 lecithin in water. The statistical analysis results of these samples are summarized in Table 3.2-3.5.

Table 3.1 Mean particle size analysis of ultrasonicated S75 samples prepared in acetate buffer (AB), phosphate buffer (PB) and distilled water at different ultrasonication times (2, 5, 8 minutes) and stored at different temperatures (4 & 25°C).

Solvent Ultrasonication Time	Temperature	Mean Particle Size, Z_{av} (nm)			
		1st day	7th day	14th day	30th day
AB_2 min	4 °C	124.80^{a} ± 10.61	124.20^{a} ± 5.67	125.60^{a} ± 6.30	123.20^{a} ± 8.58
	25 °C	128.40^{a} ± 3.80	12360^{a} ± 5.89	125.20^{a} ± 9.17	128.40^{a} ± 5.02
AB_5 min	4 °C	86.70^{a} ± 4.11	8500^{a} ± 7.47	85.90^{a} ± 3.81	91.60^{a} ± 3.36
	25 °C	85.00^{a} ± 2.32	92.00^{a} ± 6.93	88.50^{a} ± 5.98	94.00^{a} ± 3.75
AB_8 min	4 °C	85.40^{a} ± 4.41	83.20^{a} ± 7.90	85.70^{a} ± 1.12	85.60^{a} ± 2.97
	25 °C	84.70^{a} ± 8.41	90.20^{a} ± 3.91	89.30^{a} ± 3.91	91.40^{a} ± 6.63
DW_2 min	4 °C	83.60^{a} ± 8.49	76.00^{a} ± 2.39	80.80^{a} ± 5.40	76.00^{a} ± 1.06
	25 °C	83.60^{a} ± 3.50	81.50^{a} ± 6.33	79.80^{a} ± 4.66	74.70^{a} ± 4.37
DW_5 min	4 °C	45.50^{b} ± 3.52	56.60^{ab} ± 5.07	55.10^{ab} ± 6.43	55.30^{ab} ± 2.14
	25 °C	46.10^{ab} ± 3.33	58.00^{ab} ± 6.64	59.60^{a} ± 5.96	55.70^{ab} ± 4.31
DW_8 min	4 °C	59.30^{a} ± 8.19	54.60^{a} ± 4.16	57.10^{a} ± 6.44	55.40^{a} ± 3.71
	25 °C	59.30^{a} ± 8.20	64.50^{a} ± 8.24	61.80^{a} ± 3.90	57.10^{a} ± 1.43
PB_2 min	4 °C	234.40^{b} ± 26.38	292.90^{ab} ± 27.04	291.60^{ab} ± 28.04	295.90^{a} ± 5.96
	25 °C	233.70^{b} ± 30.50	299.80^{a} ± 9.53	303.40^{a} ± 8.69	306.20^{a} ± 10.17
PB_5 min	4 °C	164.00^{a} ± 9.46	173.20^{a} ± 9.48	173.00^{a} ± 10.10	175.10^{a} ± 2.86
	25 °C	166.60^{a} ± 18.31	179.20^{a} ± 13.53	183.60^{a} ± 13.10	185.90^{a} ± 9.89
PB_8 min	4 °C	164.40^{a} ± 9.46	158.60^{a} ± 9.52	170.60^{a} ± 3.95	169.00^{a} ± 7.02
	25 °C	166.40^{a} ± 31.99	166.30^{a} ± 12.25	172.50^{a} ± 7.32	184.90^{a} ± 11.55

*Each sample is analyzed individually by one way ANOVA using Tukey's comparison test.

Table 3.2 Mean particle size analysis of S75 samples prepared in acetate buffer (AB), phosphate buffer (PB) and distilled water at different microfluidization pressures (0, 400, 900, 1300 bars) and stored at 4°C.

Solvent MF Pressure	MEAN PARTICLE SIZE, Z_{av} (nm)			
	1st day	7th day	14th day	30th day
AB_1300 bar	$38.59^{a} \pm 3.85$	$48.86^{b} \pm 0.39$	$46.90^{b} \pm 3.85$	$65.26^{a} \pm 10.84$
AB_900 bar	$49.82^{b} \pm 5.74$	$70.64^{a} \pm 2.59$	$53.16^{b} \pm 4.47$	$54.09^{b} \pm 3.30$
AB_400 bar	$79.71^{a} \pm 7.27$	$86.67^{a} \pm 2.07$	$84.51^{a} \pm 5.78$	$86.70^{a} \pm 4.68$
PB_1300 bar	$76.44^{a} \pm 6.92$	$84.09^{a} \pm 4.17$	$83.00^{a} \pm 4.06$	$80.71^{a} \pm 6.77$
PB_900 bar	$106.83^{a} \pm 5.05$	$111.02^{a} \pm 3.88$	$113.19^{a} \pm 3.17$	$114.76^{a} \pm 5.05$
PB_400 bar	$169.17^{a} \pm 9.99$	$177.36^{a} \pm 4.57$	$174.08^{a} \pm 8.07$	$160.16^{a} \pm 9.55$
DW_1300 bar	$31.44^{a} \pm 0.82$	$31.80^{a} \pm 0.98$	$34.47^{a} \pm 3.36$	$31.64^{a} \pm 1.95$
DW_400 bar	$60.96^{b} \pm 7.64$	$76.55^{ab} \pm 8.03$	$81.55^{a} \pm 6.43$	$74.35^{ab} \pm 6.64$
DW_UTO	$355.19^{a} \pm 23.51$	$340.12^{ab} \pm 13.73$	$297.66^{b} \pm 11.99$	$295.37^{b} \pm 23.45$

*Each sample is analyzed individually by one way ANOVA using Tukey's comparison test.

Table 3.3 Mean particle size analysis of S75 samples prepared in acetate buffer (AB), phosphate buffer (PB) and distilled water at different microfluidization pressures (0, 400, 900, 1300 bars) and stored at 25°C.

Solvent MF Pressure	MEAN PARTICLE SIZE, Z_{av} (nm)			
	1st day	7th day	14th day	30th day
AB_1300 bar	$38.59^{a} \pm 3.85$	$47.96^{a} \pm 2.28$	$46.49^{a} \pm 2.36$	$47.12^{a} \pm 5.24$
AB_900 bar	$49.82^{b} \pm 5.74$	$51.36^{b} \pm 1.23$	$50.18^{b} \pm 0.65$	$212.69^{a} \pm 34.43$
AB_400 bar	$79.71^{a} \pm 7.27$	$88.26^{a} \pm 2.27$	$81.19^{a} \pm 4.73$	$87.58^{a} \pm 2.53$
PB_1300 bar	$76.44^{b} \pm 6.92$	$120.32^{a} \pm 13.00$	$118.5^{a} \pm 7.13$	$119.38^{a} \pm 9.33$
PB_900 bar	$106.83^{b} \pm 5.05$	$136.54^{a} \pm 5.77$	$138.05^{a} \pm 3.48$	$137.89^{a} \pm 3.46$
PB_400 bar	$169.17^{a} \pm 9.99$	$162.93^{a} \pm 9.72$	$165.56^{a} \pm 6.99$	$164.40^{a} \pm 7.89$
DW_1300 bar	$31.44^{b} \pm 0.82$	$44.97^{a} \pm 3.40$	$45.92^{a} \pm 1.30$	$46.13^{a} \pm 5.59$
DW_400 bar	$60.96^{b} \pm 7.64$	$105.31^{a} \pm 4.45$	$102.73^{a} \pm 4.91$	$103.07^{a} \pm 10.48$
DW_UTO	$355.19^{c} \pm 23.51$	$343.97^{c} \pm 13.50$	$757.56^{a} \pm 11.35$	$564.63^{b} \pm 38.63$

*Each sample is analyzed individually by one way ANOVA using Tukey's comparison test.

Table 3.4 Mean particle size analysis of E80 samples prepared in acetate buffer (AB), phosphate buffer (PB) and distilled water at different microfluidization pressures (0, 400, 900, 1300 bars) and stored at 4°C.

Solvent MF Pressure	MEAN PARTICLE SIZE, Z_{av} (nm)			
	1st day	7th day	14th day	30th day
AB_1300 bar	$60.06^{b} \pm 1.66$	$64.83^{ab} \pm 3.05$	$67.52^{a} \pm 2.78$	$66.11^{ab} \pm 1.30$
AB_900 bar	$93.65^{a} \pm 1.57$	$99.94^{a} \pm 9.98$	$91.38^{a} \pm 2.37$	$94.12^{a} \pm 5.45$
AB_400 bar	$154.75^{a} \pm 3.14$	$162.82^{a} \pm 15.22$	$159.25^{a} \pm 1.60$	$152.17^{a} \pm 5.83$
PB_1300 bar	$81.75^{a} \pm 2.63$	$87.07^{a} \pm 8.02$	$78.70^{a} \pm 2.84$	$86.09^{a} \pm 9.68$
PB_900 bar	$112.47^{a} \pm 4.61$	$118.75^{a} \pm 6.57$	$115.22^{a} \pm 15.66$	$113.48^{a} \pm 3.86$
PB_400 bar	$185.04^{a} \pm 11.07$	$179.60^{a} \pm 10.51$	$184.87^{a} \pm 12.67$	$178.89^{a} \pm 10.49$
DW_1300 bar	$45.97^{a} \pm 5.23$	$45.69^{a} \pm 4.88$	$36.55^{a} \pm 1.61$	$42.99^{a} \pm 6.23$
DW_400 bar	$103.63^{a} \pm 9.50$	$85.88^{b} \pm 5.50$	$86.90^{ab} \pm 7.48$	$82.02^{b} \pm 3.48$
DW_UTO	$677.94^{a} \pm 26.77$	$335.41^{b} \pm 4.94$	$333.55^{b} \pm 8.97$	$328.18^{b} \pm 19.13$

*Each sample is analyzed individually by one way ANOVA using Tukey's comparison test.

Table 3.5 Mean particle size analysis of E80 samples prepared in acetate buffer (AB), phosphate buffer (PB) and distilled water at different microfluidization pressures (0, 400, 900, 1300 bars) and stored at 25°C.

Solvent MF Pressure	MEAN PARTICLE SIZE, Z_{av} (nm)			
	1st day	7th day	14th day	30th day
AB_1300 bar	$60.06^{ab} \pm 1.66$	$64.18^{a} \pm 4.55$	$61.81^{ab} \pm 2.01$	$55.82^{b} \pm 1.40$
AB_900 bar	$93.65^{a} \pm 1.57$	$103.70^{a} \pm 12.71$	$89.84^{a} \pm 8.53$	$89.25^{a} \pm 1.91$
AB_400 bar	$154.75^{a} \pm 3.14$	$154.62^{a} \pm 7.30$	$143.92^{ab} \pm 3.14$	$131.28^{b} \pm 8.51$
PB_1300 bar	$81.75^{a} \pm 2.63$	$76.55^{a} \pm 6.40$	$79.04^{a} \pm 11.37$	$88.99^{a} \pm 6.13$
PB_900 bar	$112.47^{a} \pm 4.61$	$112.89^{a} \pm 12.96$	$110.78^{a} \pm 4.68$	$110.26^{a} \pm 8.92$
PB_400 bar	$185.04^{a} \pm 11.07$	$185.04^{a} \pm 11.07$	$186.92^{a} \pm 11.44$	$181.44^{a} \pm 10.87$
DW_1300 bar	$45.97^{a} \pm 5.23$	$48.24^{a} \pm 4.88$	$48.72^{a} \pm 1.61$	$48.95^{a} \pm 6.23$
DW_400 bar	$103.63^{a} \pm 9.50$	$103.08^{a} \pm 5.50$	$109.96^{a} \pm 7.48$	$109.25^{a} \pm 3.48$
DW_UTO	$677.94^{a} \pm 26.77$	$642.88^{a} \pm 4.94$	$556.61^{b} \pm 8.97$	$379.23^{c} \pm 19.13$

*Each sample is analyzed individually by one way ANOVA using Tukey's comparison test.

The reason for the changes in particle size will be explained by emulsion kinetics. In our study, liposomes formed as a result of ultrasonication and microfluidization had particle sizes changing between 30-300 nm. Discussion regarding micro and nanoemulsions have already been provided in Chapter 1. It should be reminded that, in contrast to their names ‘micro’ emulsions are smaller in particles size compared to ‘nano’ emulsions. Since liposomes resemble emulsions in an aspect bearing dispersed particles on a continuous phase, it seems reasonable to make interpretation based on emulsions systems. And to highlight the changes on the particle size, the difference between these two emulsions systems: ‘micro and ‘nano’ would be discussed. So, to fully understand and interpret the particle size changes during storage, one should determine what kind of emulsion is formed after different homogenization procedures. There are several reasons to conclude that the liposomes formed in this study resemble nanoemulsions. First of all, the mean particle size of the liposome systems did not change with concentration. In other words, dilution was applied prior to all particle size measurements, and the concentrated system (1%) was also checked for any fluctuations to eliminate any possible misunderstanding. Nanoemulsions mean particle size do not change with dilution (Anton & Vandamme, 2011).

The second reason was the concentration of the phospholipid in the mixture. All the samples were prepared at 1%, which was far below to form a microemulsion. Microemulsions can only be formed at high concentrations (Zhong et al., 2009). The minimum surfactant concentration used in the study of Abbasi and Radi (2016) was 10%. The other indicator to check if the prepared solution is categorized as nanoemulsion or microemulsion was that the formed particles were spherical in shape. The images of TEM, which will be discussed further under another title revealed that liposomes formed mostly exhibited a spherical shape. Being not the exact cursor, sphericity of the particles resulted from the need of decreasing surface area and contact energy, could be regarded as a pointer. Another feature is polydispersity index (PDI). The PDI range might also give an information about the emulsion nature. Although there are very clear liposomes

among the prepared samples, their PDI values resulted in high ranges. The PDIs acquired from particle size measurements fluctuated between 0.2 and 0.5, ignoring a couple of exceptions. Last and most important sign of producing nanoemulsion in this study was the liposome formation techniques. High pressure and direct probe ultrasound techniques were mechanical methods that would supply high energy to the system, which in turn formed the liposomes. As discussed earlier, the need of energy for formation was one of the significant signs of kinetically stable nanoemulsion formation.

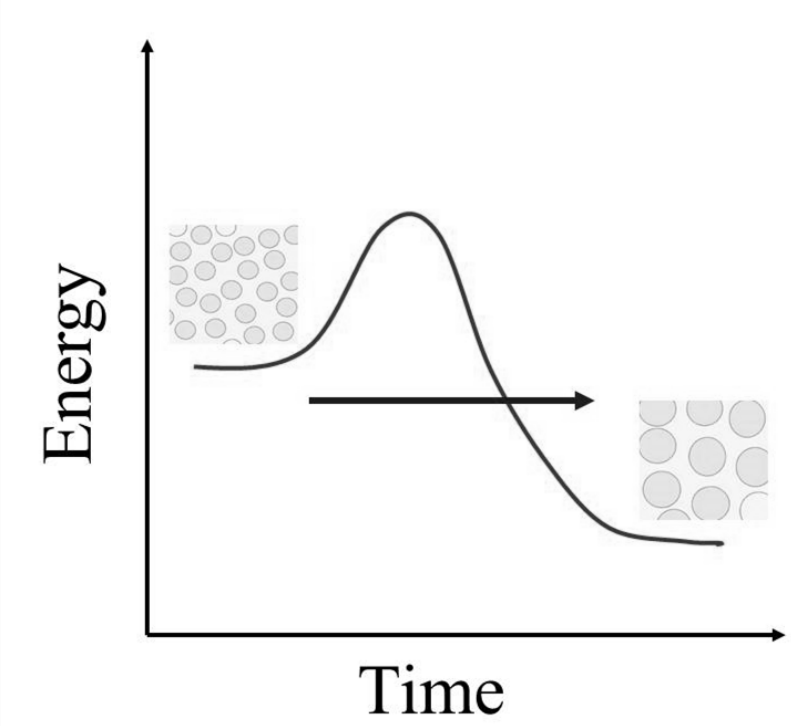

Figure 3. 11 Energy necessary for a kinetically stable system to reach its minimum energy level.

As these factors point out, the system that should be dealt with in this study had an only kinetically stable nature. Figure 3.11 represents the energy penalty of a kinetically stable system. When this critical energy was achieved, the particles rearranged themselves to a more favorable state, which was generally followed by an increase in particle size.

For the exceptional cases mentioned before and as listed in Table 3.2-3.5, it could be said that the energy barrier needed to be overcome in these cases were encouraged by the elevated temperature. When compared with their equivalent samples stored at refrigerator temperature, which did not demonstrate any change with neither time nor temperature, these samples should have collected the energy necessary to minimize their free energy. This mechanism in the end resulted in a sudden increase in particle size, which was shown in Figure 3.12. The same trend also applies for 400 bar pressurized S75 lecithin prepared in water and stored at 25°C. These steep increases in particle sizes are observed at first week for all three samples, meaning that the prepared liposomes are degraded at following days of preparation. This disintegration of the liposomes were due to insufficient energy given to system for the samples in question, indicating instability.

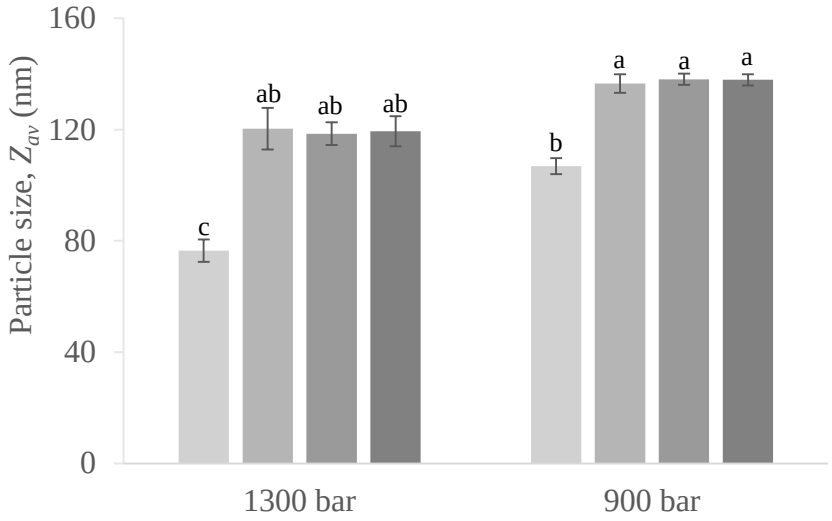

Figure 3. 12 Change in particle size of soy lecithin prepared at 1300 and 900 bars in phosphate buffer and stored at 25°C for day 1 ( ), day 7 ( ), day 14 ( ) and day 30 ( ).

On the other hand, there were also some samples with decreasing in particle size with time. This trend was not expected, however there may be some reasons that could make the situation more rational. Since this trend was not observed in all the samples, that should be beneficial to detect the property in common for the suspected samples. Those samples were the ones that only a little or no pressure was applied. Normally for the microfluidized samples, the phospholipid particles' ability to form smaller, more stable products increased as the pressure applied to the environment was increased because of the energy barrier to form kinetically stable liposomes. These questionable systems were probably in an energetically favorable state since the energy applied was not sufficient, so even a little change in mechanical energy could have resulted in a decrease in the particle size. In most

of the visuals of low pressurized samples including these suspected ones, forming of a cream layer or agglomeration was detected. In such a case, the concept of depletion flocculation might have acted on the particle size of the dispersions. Depletion forces act on the particles of an emulsion or foam when small sized nanoparticles are present in the solution are removed between macromolecules. These small sized nanoparticles are called depletants, which can be polymers or micelle like entities (Trokhymchuk & Henderson, 2015). The change in osmotic pressure results from the ionic or nonionic micelles are the main source of the interaction (Kralchevsky, Danov, & Anachkov, 2015). Depletion flocculation is known to be easily destroyed by mechanical mixing (Chang & McClements, 2015). When the cream layer due to depletion flocculation was broken by vortex or hand shaking before sampling of the measurements, the diluted particles might have reformed and they may scatter light different than the cream layer itself.

Another clear feature that was observable on the results was shown on liposomes prepared with E80. E80 lecithin, has the ability to form big multilamellar vesicles when subjected to low energy (Laouini et al., 2012). The E80 samples of non-pressurized and 400 bar pressurized ones prepared in DW and stored at 4°C deformed in the same manner, exhibiting a steep and significant decrease ($p \leq 0.05$) in particle size after the first week (Table 3.2-3.5). This can be due to decomposing of the outer layer of the multivesicular system. The resulting particles after the first week remained at the same range, keeping their stability. On the other hand, only Ultraturrax applied E80 sample stored at 25°C exhibited a gradual decrease with time unlike its replicates at refrigeration, which decomposed drastically at the first week. This gradual decrease might be due to loss of the layer in the structure, which may be the result of oxidation. The color change of E80 samples stored at 25°C during storage were already exceedingly prominent.

3.2 Transmission Electron Microscopy

Transmission Electron Microscopy (TEM) analysis was incorporated into this study to check the validity of measured particle size by PCS and to obtain information about the morphological changes of liposomes during storage.

TEM imaging was not performed for all samples. For the instrument used at METU Central Laboratory, ions could have interfered with the results that is why imaging was only conducted for the samples prepared in DW. S75, E80 samples that were microfluidized at 1300 bar and S75 samples ultrasonicated for 5 min were imaged for 30 days. Since there were not detected any difference between 15th and 30th days images, only 15th day images were reported. Images were given on Figures 3.13-3.15.

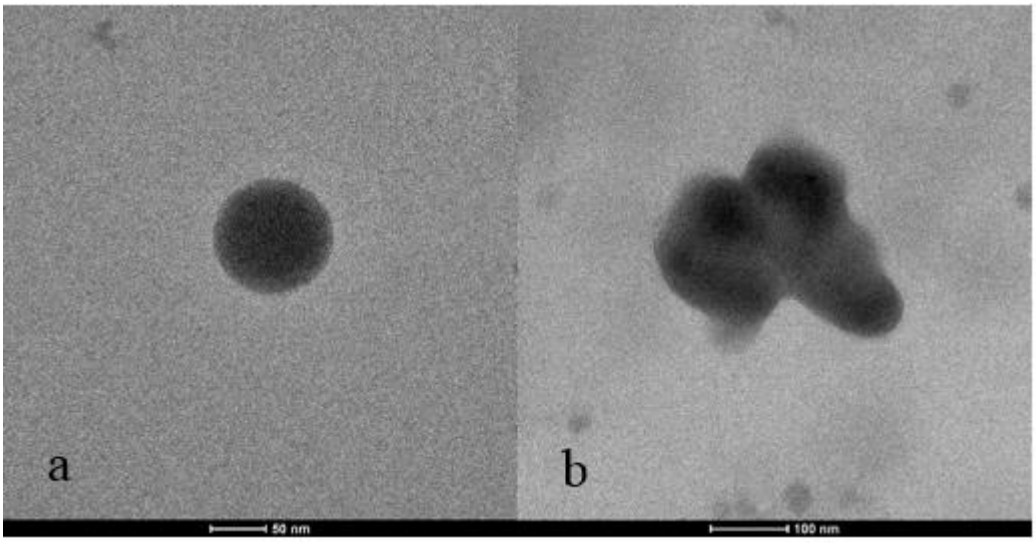

Figure 3. 13 TEM images of S75 liposomes prepared in DW by 5 minutes ultrasonication at 1st (a), and 15th (b) days of storage at 4°C.

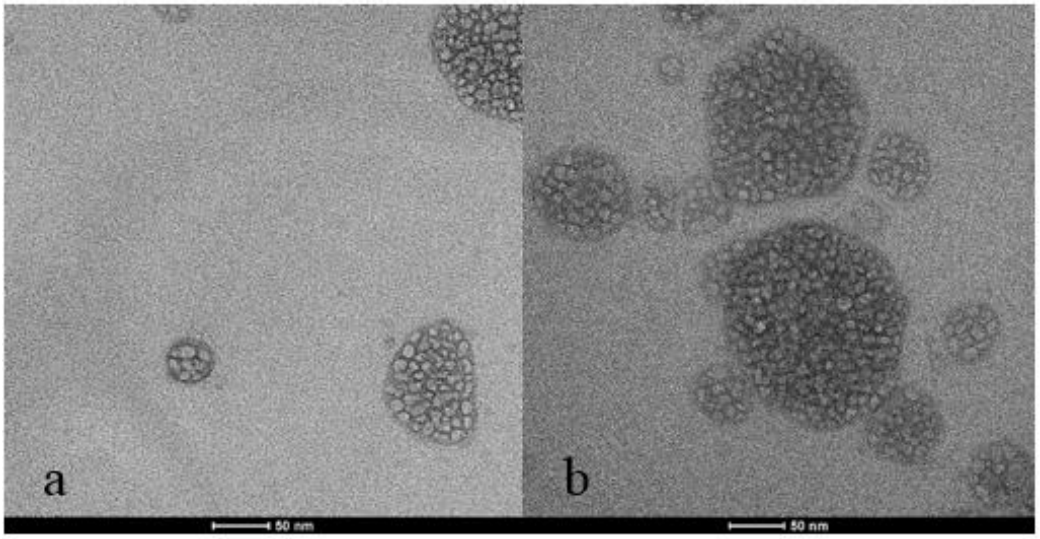

Figure 3. 14 TEM images of S75 liposomes prepared in DW by 1300 bar 5 pass microfluidization at 1st (a), and 15th (b) days of storage at 25°C.

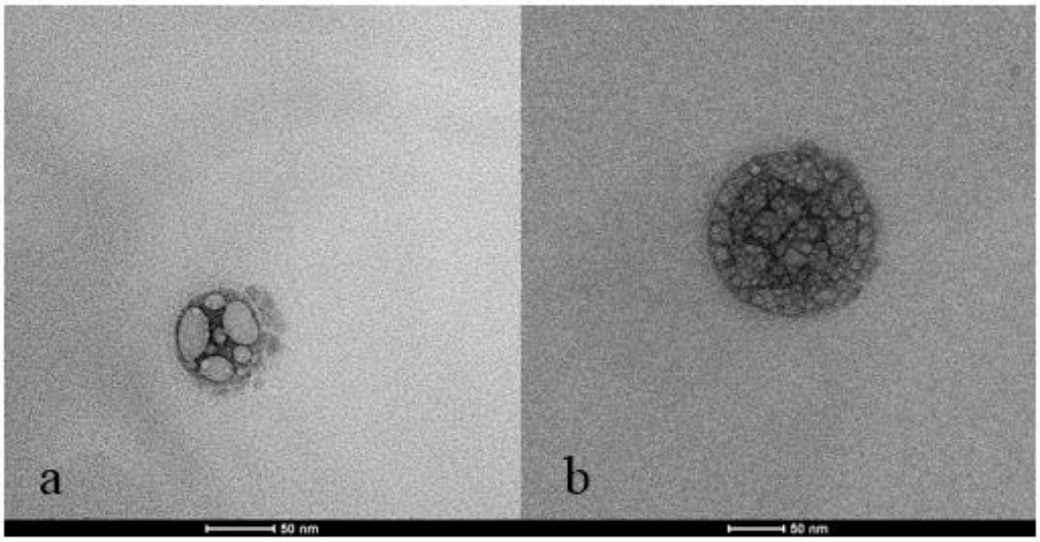

Figure 3. 15 TEM images of E80 liposomes prepared in DW by 1300 bar 5 pass microfluidization at 1st (a), and 15th (b) days of storage at 25°C.

Loss of shape for S75 liposomes prepared in DW ultrasonicated for 5 minutes was shown on Figure 3.13. This merging can be shown as an indicator of the increase in particle size results, which demonstrated a significant change after 14 days of storage at 25°C (Table 3.3). Since TEM measurement requires very low sampling capacity, the overall trend may not be gathered easily but it gives an idea about the morphology in any way. The shape loss and merging detected at the sample's replicate stored at 4°C might have increased at 25°C to an extend that to reveal itself as particle size change.

The sensitivity of E80 lecithin also revealed itself in TEM images in the form of bubbling (Kuntsche et al., 2011), after exposure to the electron beams during imaging (Figure 3.16).

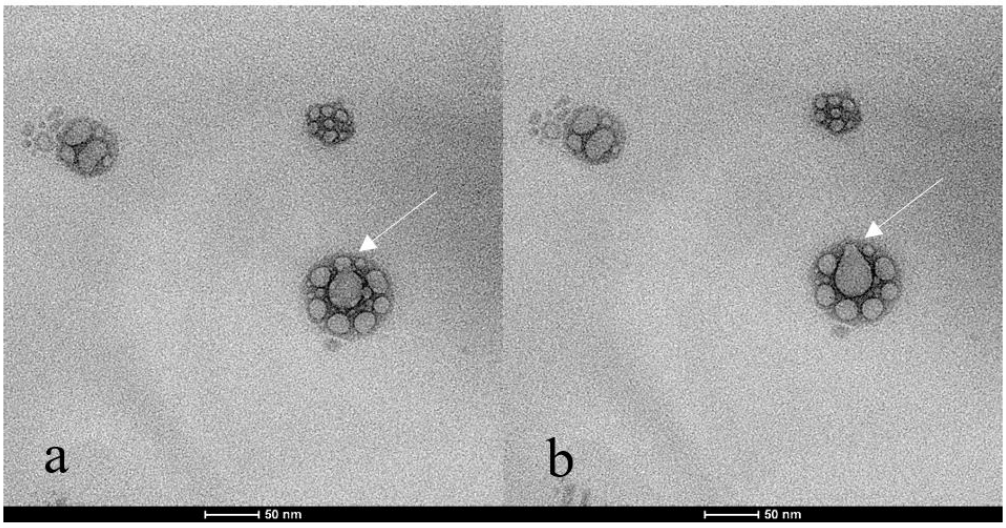

Figure 3. 16 Expansion of beam damage (bubbling) from left (a) to right (b). The images were consecutively taken from 1300 bar 5 pass pressurized E80 liposomes in DW, at the first day of preparation.

An interesting finding of finger-print like behavior was observed in the TEM image of S75 lecithin in water, stored at 4°C for 15 days (Figure 3.17). This behavior did not spread all over the sample but it was demonstrating an ordered pattern. Similar visuals from CTEM experiments taken from phosphatidylethanolamine are available in the literature (Siegel & Epand, 1997).

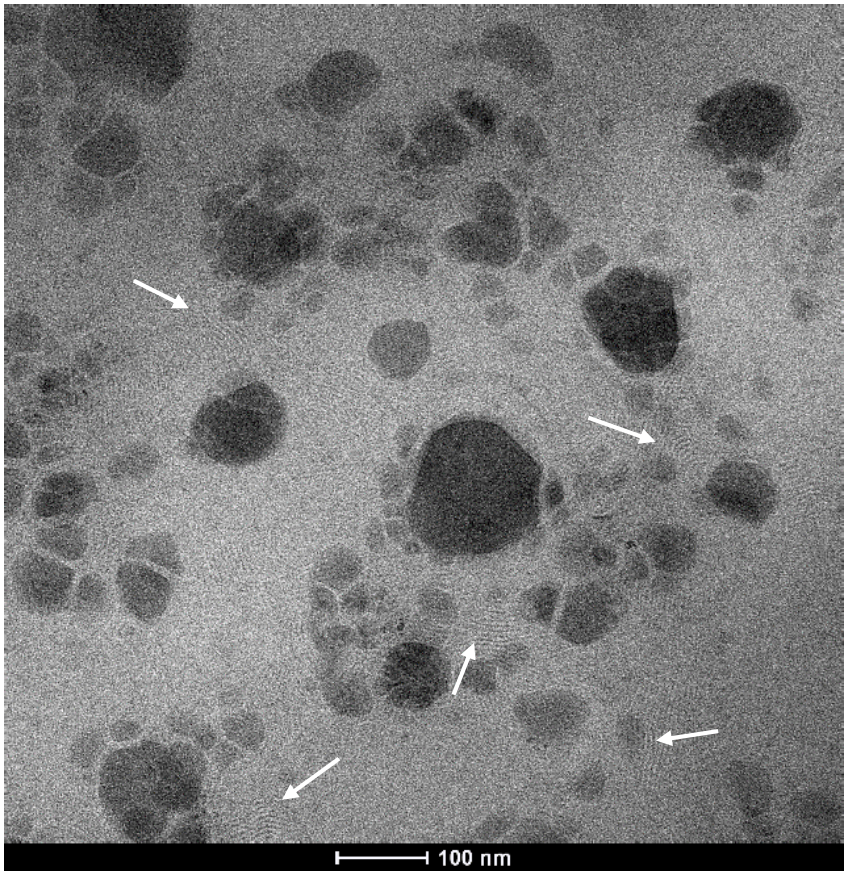

Figure 3. 17 TEM image of 1300 bar 5 pass pressurized soy lecithin sample prepared in water and stored at 4°C for 15 days. Arrows shows the wave like hexagonal packings.

Phosphatidylethanolamine (PE) is an uncharged phospholipid which may undergo transition from L_α to H_{II} at equilibrium phase transition temperature, (T_H) which was also measured by Siegel et al. (1997) to be 43°C. L_α and H_{II} denote lamellar and reverse hexagonal phases, respectively (Figure 3.18). At this temperature, PEs may transform from lamellar structure into invert hexagonal phase. The visuals of the cited article were taken from samples stored at 5°C, very close to the storage temperature of this study.

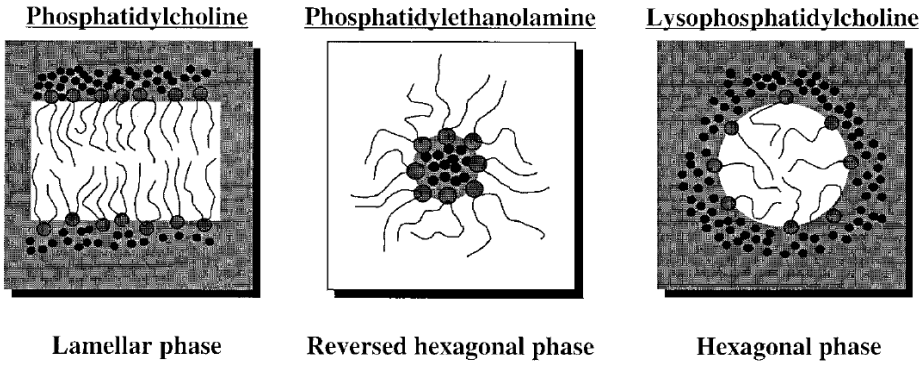

Figure 3. 18 The forms of phospholipids that may exist in an emulsion (van Nieuwenhuyzen & Szuhaj, 1998).

Manufacturer's product data sheet for S75 soy lecithin showed the presence of 7% to 14% phosphatidylethanolamine, which might have shown itself in the connection points of hexagonally packed particles. The question in hand was how

the level of temperature might have increased from refrigerator temperature to 43°C to cause this behavior. The reason can be explained by the preprocesses applied to the sample before imaging, since it was cited that lipid morphologies can change due to different processes applied (Siegel & Epand, 1997). TEM imaging was conducted under vacuum so the samples were dried before experiment. One reason that might have altered the morphology may be the accelerated drying under light focus while another may be the rapid removal of water from sample when inserted in the equipment. If the sample was not fully dry, sudden vacuum exerted might have caused the transformation. The wave like pattern normally spreads circular away from the liposomes like the water drops on a waterhole, when transition occurs in proper phase transition temperature. The irregularity of these lines was another indication of further process, which forced transformation below T_H (Siegel & Epand, 1997).

The merging of E80 egg lecithin after 15 days was also validated by TEM (Figure 3.19.a). On the other hand, another pattern was noticed in S75 samples which looked angular in shape (Figure 3.19.b). This behavior was explained by the size of rigid double layer of the lipids in literature. When they were smaller in size, the particles might result in angular shapes under microscope (Chiu et al., 2005; Kuntsche et al., 2011).

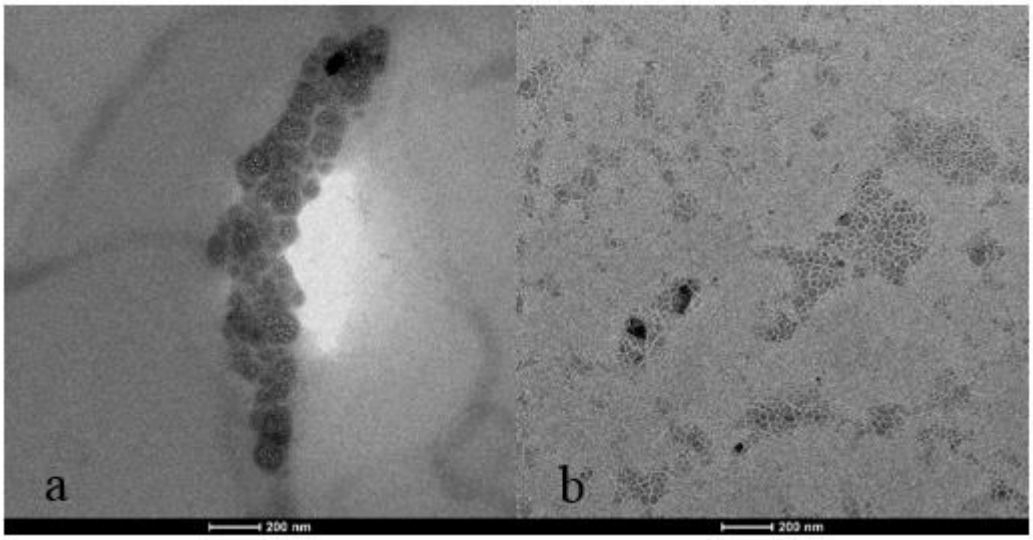

Figure 3. 19 Merged image of 1300 bar 5 pass pressurized E80 liposomes prepared in DW and stored for 15 days at 25°C (a), image of angular appearance of the small sized liposomes subjected to1300 bar 5 pass pressure in DW and stored at 4°C for 15 days (b).

In summary TEM results showed that; particle size measurements conducted by PCS were reliable and the morphology of the liposomes were spherical in shape at first day of preparation, as expected. Both phospholipids, but especially E80 was affected from electron beams so the time of imaging was not prolonged too much after first day. Although it was not the exact cursor of instability, TEM images gave an idea about the time dependent behavior of liposomes, changing with time which are detected as merging of the particles on the images.

3.3 Zeta Potential of Liposomes

Zeta potential is an important parameter to study stability of colloidal suspensions. In this study, zeta potentials were measured for both microfluidized and ultrasonicated samples at three different solvents stabilizing pH at 7.2, 6.5 and 3.8 for PB, DW, and AB, respectively. Normally, phospholipids used in this study are negatively charged. The change in zeta potential with respect to microfluidization pressures and solvent type is given in Figure 3.20 and Figure 3.21. Presence of ions alter the zeta potential which in turn affects the stability of particles in the solution. By stability, agglomeration or any kind of physical change is referred, as mentioned in previous sections for PB samples.

The way how the particle charge affects the stability relies on the Brownian motion of the particles. When the particles having high repulsive forces in each other present in the solution, they do not tend to agglomerate when they came close due to Brownian motion. However the situation reverses if the particles do not have the enough degree of net charge to repel each other. This degree of repulsion can be categorized as excellent for zeta potential more than 61 mV, moderate stability between 31 to 40 mV and instability between 10 to 30 mV (Duplessis, Ramachandran, Weiner, & Muller, 1996). The results showed that the surface charge of the particles decreased as the pH is set to 7.2. Zeta potential range of -30 to +30 mV indicates instability for the emulsion and the degree of instability increases as the value approaches to zero. As clearly seen from Figure 3.20, the samples prepared in PB have zeta potential around zero, highly instable. This results also complies with the results found in particle size section.

In principle, zeta potential of the system should not change with particle size. This is also confirmed by these results, yielding no significant change with changing pressure, which is inversely proportional with particle size (Table A.13).

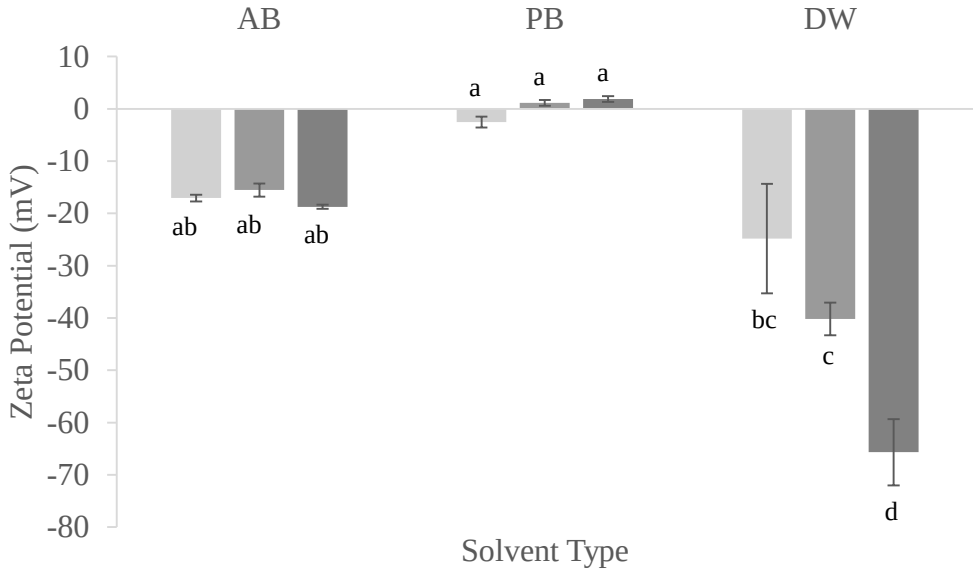

Figure 3. 20 Change in zeta potential of liposomes with respect to solvent types AB, PB, DW and microfluidization pressures of 1300 bar ( ), 400 bar ( ), control ( ).

For ultrasound samples, the effect of solvent type on zeta potential was shown in Figure 3.21. It was also clearly seen that change in particle size by the increase in ultrasonication time does not affect zeta potential. Pearson correlation between particle size and zeta potential of S75 samples prepared in different solvents by ultrasonication was found to be 0.812 (Table A.13). On the other hand no such correlation is detected for microfluidized samples, most probably due to chemical changes like lipid oxidation that might have been triggered by incorporation of air during 5 pass operation and exposure of light, which altered zeta potential but did not affect particle size.

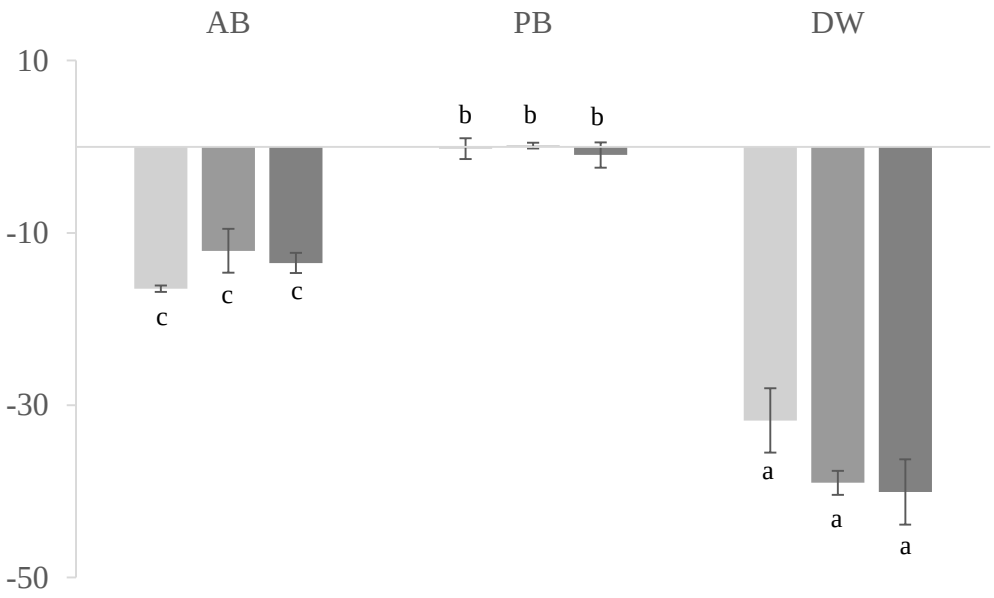

Figure 3. 21 Change in zeta potential of liposomes with respect to solvent types AB, PB, DW and ultrasonication times of 2 min ( ), 5 min ( ), 8 min ( ).

3.4 Characterization by NMR Relaxometry

NMR is a noninvasive, nondestructive method for characterizing biological and non-biological systems. In this study, it was used to track the changes of liposomes prepared from different phospholipid types in different solvents at different storage temperatures with respect to time. Since temperature was a contributing factor affecting NMR relaxometry measurements, all the samples were allowed to rest for sufficient time at room temperature prior to each measurement to achieve equilibrium. As NMR relaxometry experiments, T_2 relaxation times were measured. NMR experiments were conducted for S75, E80 type lecithins at acetate, phosphate buffer for the 2 storage temperature at 1st, 4th, 7th, 10th, 14th,

28th, and 35th days. NMR relaxometry experiments were not conducted for ultrasound samples since microfluidized samples resulted in much smaller liposomes in the form of SUVs. Moreover, our aim was to use the NMR relaxometry for the first time as a characterization tool for liposomes. On the scope of the study ultrasound samples and DW samples of microfluidization were eliminated due to timing issues.

The change in pressure, solvent type and phospholipid type were analyzed all together to check if there exists any difference between the samples (Table 3.6). Changes in T_2 values at different pressure with respect to storage type are also provided on Table 3.7-3.9. Since introduction of the storage time as a 4th factor would complicate the ANOVA results and make interpretation harder, ANOVA was conducted for each lecithin type solvent combination for a specific temperature and microfluidization pressure. The correlation between particle size and T_2 values of S75 lecithin in acetate and phosphate buffers; E80 lecithin in acetate and phosphate buffers at first day of preparation were found to be -0.958 and -0.955; -0.985 and -0.861, respectively (Table A.14). The negative correlation was explained by the increase in free water as the particle size decreases.

When 3-Way ANOVA was conducted on T_2 values for freshly made liposomes, solvent and lecithin type and microfluidization pressure were all found to be significant ($p \leq 0.05$) (Table A.14). T_2 values increased with increasing pressure. Increase in pressure also resulted in decrease in particle size as stated before. Thus, when particle size of the liposomes decreased by high pressure, the encapsulated volume of the aqueous environment decreased, which meant less water being entrapped inside the liposomes indicating the formation of SUVs. Expelling of the water inside the liposome core resulted in an increase in T_2 times, which was associated with non-capsulated, bulk water.

Solvent and lecithin types were also found to be significant ($p<0.05$). AB liposomes had longer T_2 times which was consistent with the smaller particle sizes

of these samples. On the other hand, E80 samples had slightly longer T_2 times compared to S75 ones whereas the particle size of E80 liposomes were significantly higher than S75 samples ($p<0.05$). This was the point where the perfect correlation between T_2 and particle size halted. The reason of the difference in T_2 between those two lecithin types could be explained based on the difference water entrapping ability of the liposomes. It is hypothesized that, even though E80 results in bigger liposomes, the entrapping efficiency was not sufficient enough to include bulk water to the core of the liposome thus resulting in smaller changes in T_2.

T_2 relaxation times were also measured with respect to storage time and relationship between particle size and relaxation times were sought. Particle size measurement by PCS is widely accepted and used for the characterization purposes. One of the greatest result that was achieved in this study was the validation of the results measured in PCS technique by NMR relaxometry. The results of the statistical analysis were able to detect any change due to microfluidization pressure in particle size and free water as explained before. Both particle size and T_2 had the same increasing trend at around one week of storage for S75 lecithin prepared in PB and stored at 25°C (Figure 3.22). Change in particle size was explained by kinetic stability of the particles which tended to decrease their free energy and merge. T_2 values could also be explained by a similar way since NMR relaxometry could detect the compartmental changes on proton distribution at molecular level.

Merging of the liposomes resulted in an increase in particle size as well as increasing the amount of water released to the system. Decomposition of nanocapsules broke the encapsulated aqueous volume free to the surrounding, which in turn increased overall T_2. When compared with water, lipids in general have much lower T_2 values so the incorporation of lipids in an aqueous system was expected to decrease overall T_2 time.

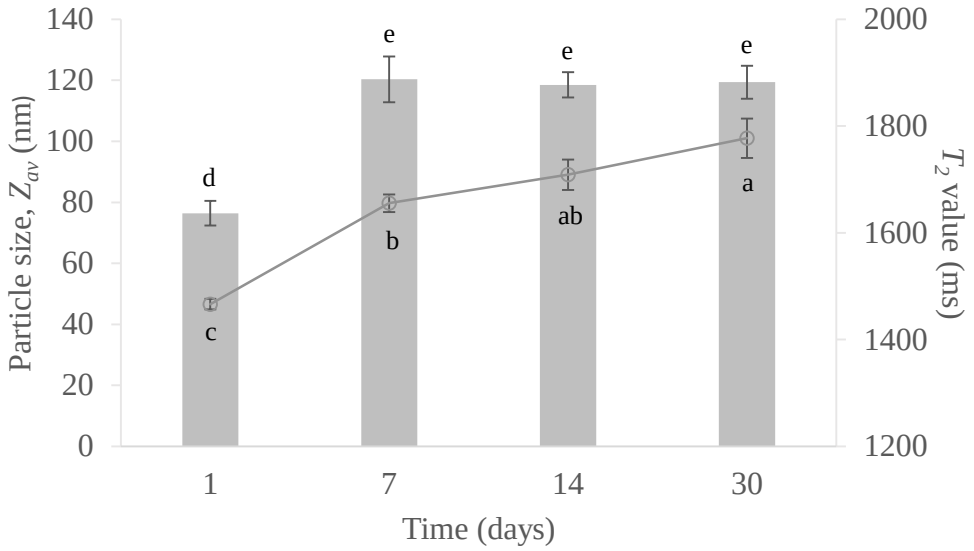

Figure 3. 22 Change in particle size (■) and T_2 values (—○—) of 1300 bar 5 pass pressurized S75 lecithin prepared in PB and stored at 25°C with time.

According to our results, another significance of NMR relaxometry results was that it was possible to trace the slight changes on liposomes by the T_2 values. Figure 3.22 shows the particle size and T_2 value changes of S75 liposomes prepared in PB with time. T_2 values followed the same trend with particle size at the beginning where the characteristic change was most significant. Later, the trend was mostly similar however the particle size results failed to detect any change during later stages of storage which might be due to degradation or lipid oxidation which were chemical reactions that could be slowed down but cannot be stopped. The possibility of a change was more reasonable in this perspective, making NMR relaxometry results more favorable. It was important to state that any differences due to MF pressure change measured by PCS technique in this study could also be detected by T_2 times.

It was obvious that the liposomes prepared in PB underwent degradation with time, which could also easily be detected from the visuals of the samples. However, the particle size changes did not result in any change. At this point, NMR might have been able to explain the full representative character of the system. Figure 3.23 showed the change in T_2 with time for 400 bar pressurized E80 samples kept at 4°C and 25°C. The persistent increase in T_2 was due to breaking of the liposomes, as described before. The interesting point is that storage temperature did not affect the degradation trend. They both increased at the same time interval for 10 days and somehow decreased later.

Another probable instability behavior was observed in 900 bar pressurized E80 samples prepared again in PB. The temperature effect was significant in these data ($p \leq 0.05$) (Figure 3.24). 4°C samples degraded gradually with time while the degradation at 25°C sample was seen at first 3 days of storage with a steep increase in T_2. These changes could be a sign for chemical changes that did not reveal itself as particle size changed. One of the most probable chemical reaction that might result in such a change was lipid oxidation, which was expected to be faster at 25°C. Figure 3.25 shows the similar trend for T_2 change of 1300 bar pressurized E80 samples. It is important to notice that the increase in T_2 for 4°C lasted shorter at 1300 bars pressurized sample than the same sample pressurized at 900 bars. This was also an indication of chemical change which happened faster at 1300 bar pressurized sample. As the particle size decreased by pressure, the surface area increased which lead to an increase in lipid oxidation. The increasing value of T_2 which was faster at 1300 bars than 900 bars could also be hypothesized to be due to the increase in lipid oxidation with time. With the same hypothesis, one should expect that 400 bar pressurized samples should take longer to oxidize but the T_2 change seemed to end around 10 days while the others were longer, however the T_2 times in this case was lower than 1300 and 900 bar pressurized samples, which also supported the hypothesis.

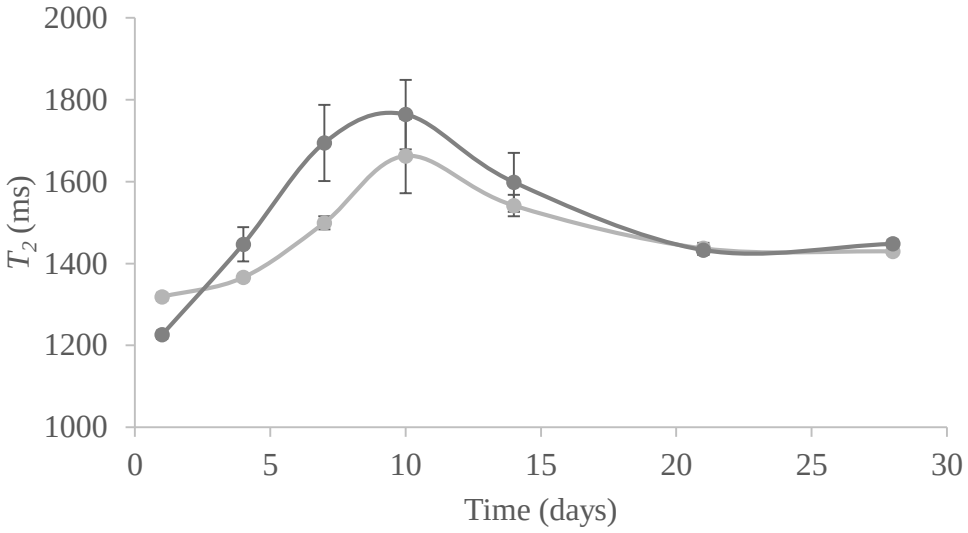

Figure 3. 23 T_2 change of 400 bar pressurized E80 lecithin prepared in PB and stored at 4°C ( ) and 25°C ( ).

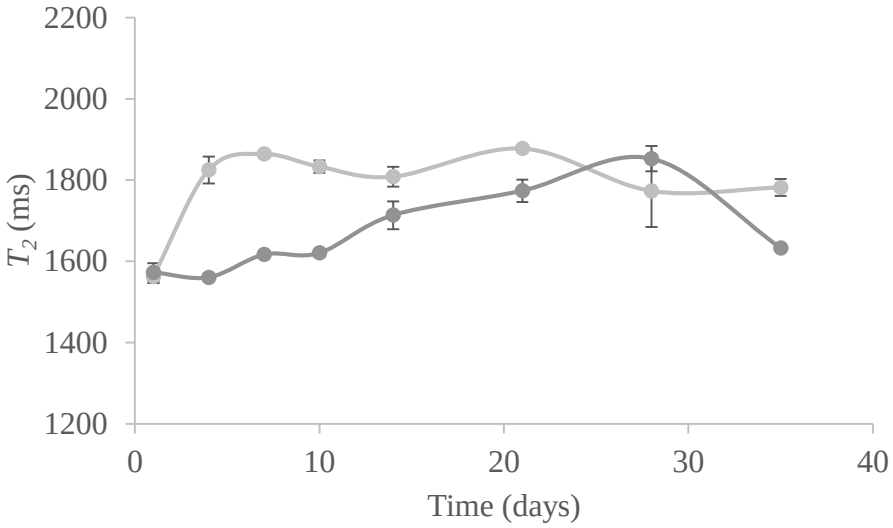

Figure 3. 24 T_2 change of 900 bar pressurized E80 lecithin prepared in PB and stored at 4°C ( ) and 25°C ( ).

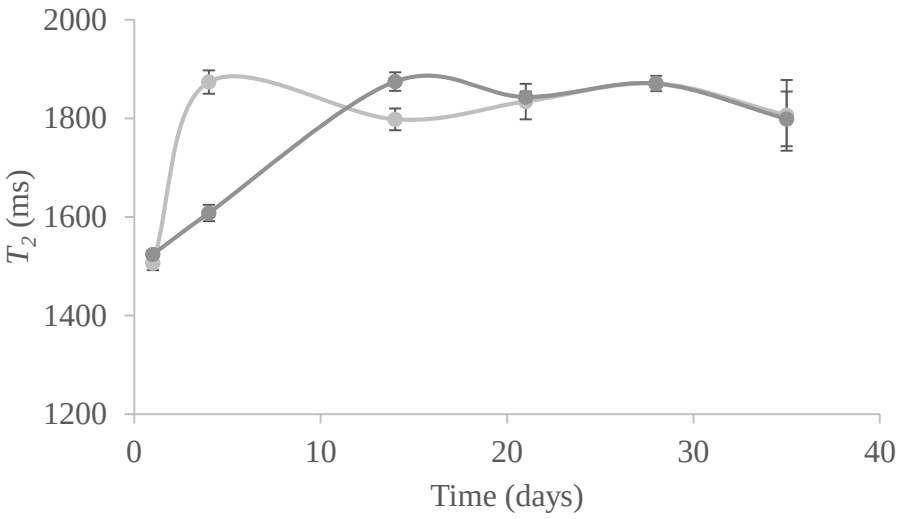

Figure 3. 25 T_2 change of 1300 bar pressurized E80 lecithin prepared in PB and stored at 4°C ( ) and 25°C ( ).

Table 3.6 T_2 values of liposomes prepared by E80 and S75 type liposomes in acetate buffer (AB) and phosphate buffer (PB), analyzed by 3-Way ANOVA.

	AB			**PB**		
	400 BAR	**900 BAR**	**1300 BAR**	**400 BAR**	**900 BAR**	**1300 BAR**
E80	$1227.05^{g} \pm 1.78$	$1593.28^{b} \pm 27.99$	$1741.83^{a} \pm 56.73$	$1318.67^{f} \pm 16.96$	$1572.94^{b} \pm 39.05$	$1523.95^{bc} \pm 15.32$
S75	$960.37^{h} \pm 11.55$	$1399.67^{e} \pm 3.85$	$1694.40^{a} \pm 19.86$	$1165.17^{g} \pm 17.95$	$1416.79d^{e} \pm 19.52$	$1479.94^{cd} \pm 21.39$

Table 3.7 T_2 changes of 400 bar 5 pass microfluidized liposomes at different phospholipid and buffer combinations with time, analyzed by 1-Way ANOVA.

400 BAR	Time (days)	T_2 (ms)			
		S75_AB	S75_PB	E80_AB	E80_PB
4°C	1	$960.37^{d} \pm 11.55$	$1165.17^{e} \pm 17.95$	$1227.05^{bc} \pm 1.78$	$1318.67^{d} \pm 16.96$
	4	$1019.80^{c} \pm 16.01$	$1210.10^{de} \pm 17.70$	$1251.65^{b} \pm 30.82$	$1365.72^{cd} \pm 35.75$
	7	$1046.27^{c} \pm 15.64$	$1292.12^{cd} \pm 8.46$	$1260.50^{b} \pm 5.06$	$1499.27^{abc} \pm 15.11$
	10	$1038.23^{c} \pm 9.14$	$1338.28^{c} \pm 47.34$	$1253.14^{b} \pm 3.80$	$1471.72^{bcd} \pm 27.78$
	14	$1089.93^{b} \pm 13.90$	$1715.06^{a} \pm 64.61$	$1372.13^{a} \pm 23.55$	$1662.59^{a} \pm 157.19$
	21	$1111.20^{b} \pm 7.68$	$1468.65^{b} \pm 11.68$	$1193.31^{cd} \pm 23.09$	$1541.52^{ab} \pm 45.47$
	28	$1109.55^{b} \pm 2.72$	$1384.74^{bc} \pm 13.16$	$1156.11^{de} \pm 10.59$	$1436.81^{bcd} \pm 23.48$
	35	$1173.56^{a} \pm 13.10$	$1359.38^{c} \pm 38.54$	$1134.67^{e} \pm 17.96$	$1429.42^{bcd} \pm 3.83$
25°C	1	$1133.39^{c} \pm 6.76$	$1234.39^{d} \pm 15.40$	$930.15^{a} \pm 3.80$	$1225.83^{c} \pm 17.40$
	4	$1234.93^{ab} \pm 7.31$	$1443.41^{abc} \pm 22.92$	$953.85^{a} \pm 14.77$	$1446.93^{bc} \pm 28.16$
	7	$1244.02^{ab} \pm 6.71$	$1535.55^{a} \pm 69.65$	$950.52^{a} \pm 28.24$	$1694.41^{ab} \pm 72.05$
	10	$1260.62^{a} \pm 7.93$	$1426.25^{bc} \pm 26.21$	$878.57^{b} \pm 11.41$	$1610.21^{ab} \pm 161.75$
	14	$1269.67^{a} \pm 9.67$	$1520.08^{ab} \pm 26.12$	$876.91^{b} \pm 4.86$	$1763.66^{a} \pm 146.56$
	21	$1220.56^{b} \pm 3.85$	$1377.92^{c} \pm 32.14$	$775.83^{c} \pm 6.95$	$1598.57^{ab} \pm 124.59$
	28	$1221.49^{b} \pm 15.36$	$1379.01^{c} \pm 25.68$	$771.39^{c} \pm 3.89$	$1432.87^{bc} \pm 20.53$
	35	$1156.71^{c} \pm 27.77$	$1478.83^{ab} \pm 25.96$	$784.62^{c} \pm 14.61$	$1447.87^{bc} \pm 18.23$

Table 3.8 T_2 changes of 900 bar 5 pass microfluidized liposomes at different phospholipid and acetate buffer combinations with time, analyzed by 1-Way ANOVA.

900 BAR	Time (days)	T_2 (ms)			
		S75_AB	S75_PB	E80_AB	E80_PB
4°C	1	$1399.67^{b} \pm 3.85$	$1416.79^{d} \pm 19.52$	$1593.28^{b} \pm 27.99$	$1572.94^{d} \pm 39.05$
	4	$1408.15^{b} \pm 21.97$	$1405.33^{d} \pm 12.62$	$1743.21^{a} \pm 8.06$	$1560.41^{d} \pm 11.04$
	7	$1422.52^{ab} \pm 7.04$	$1454.62^{cd} \pm 14.22$	$1695.37^{ab} \pm 23.70$	$1616.63^{cd} \pm 14.46$
	10	$1426.41^{ab} \pm 7.76$	$1507.88^{bc} \pm 9.30$	$1719.50^{a} \pm 50.96$	$1620.44^{cd} \pm 15.69$
	14	$1401.39^{b} \pm 27.32$	$1523.57^{b} \pm 33.68$	$1678.71^{ab} \pm 30.07$	$1713.58^{bc} \pm 59.37$
	21	$1441.05^{ab} \pm 7.78$	$1684.41^{a} \pm 29.43$	$1747.93^{a} \pm 73.19$	$1773.68^{ab} \pm 47.88$
	28	$1460.24^{a} \pm 18.46$	$1666.57^{a} \pm 7.32$	$1758.09^{a} \pm 35.02$	$1852.64^{a} \pm 53.91$
	35	$1404.41^{b} \pm 10.10$	$1522.56^{b} \pm 5.83$	$1663.35^{ab} \pm 45.16$	$1632.84^{cd} \pm 10.11$
25°C	1	$1412.69^{e} \pm 10.16$	$1544.02^{b} \pm 26.24$	$1601.56^{de} \pm 27.76$	$1562.71^{b} \pm 27.91$
	4	$1519.09^{cd} \pm 31.23$	$1539.42^{b} \pm 48.88$	$1699.53^{abc} \pm 17.33$	$1824.68^{a} \pm 56.97$
	7	$1573.56^{bc} \pm 18.81$	$1642.37^{ab} \pm 74.02$	$1712.11^{ab} \pm 11.05$	$1863.96^{a} \pm 15.86$
	10	$1622.31^{ab} \pm 25.80$	$1699.39^{a} \pm 26.87$	$1740.36^{a} \pm 54.82$	$1832.95^{a} \pm 26.56$
	14	$1607.92^{b} \pm 9.95$	$1658.08^{ab} \pm 52.70$	$1676.05^{abc} \pm 20.08$	$1808.11^{a} \pm 41.75$
	21	$1629.98^{ab} \pm 19.37$	$1709.91^{a} \pm 15.91$	$1656.14^{bcd} \pm 20.58$	$1877.47^{a} \pm 7.28$
	28	$1671.39^{a} \pm 10.48$	$1745.69^{a} \pm 84.55$	$1637.22^{cd} \pm 15.97$	$1773.01^{a} \pm 152.71$
	35	$1507.98^{d} \pm 25.98$	$1611.69^{ab} \pm 48.96$	$1540.36^{e} \pm 11.39$	$1781.71^{a} \pm 36.58$

Table 3.9 T_2 changes of 1300 bar 5 pass microfluidized liposomes at different phospholipid and acetate buffer combinations with time, analyzed by 1-Way ANOVA.

1300 BAR	Time (days)	T_2 (ms)			
		S75_AB	S75_PB	E80_AB	E80_PB
4 °C	1	$1694.40^{a} \pm 19.86$	$1479.94^{d} \pm 21.39$	$1741.83^{b} \pm 56.73$	$1523.95^{c} \pm 15.32$
	4	$1720.55^{a} \pm 48.25$	$1499.23^{cd} \pm 19.12$	$1798.63^{b} \pm 27.13$	$1607.87^{bc} \pm 28.65$
	7	$1651.83^{a} \pm 12.91$	$1504.61^{cd} \pm 24.14$	$1734.04^{b} \pm 9.31$	$1543.96^{bc} \pm 32.34$
	10	$1684.41^{a} \pm 38.21$	$1563.37^{bcd} \pm 17.08$	$1796.42^{b} \pm 55.21$	$1651.99^{b} \pm 20.12$
	14	$1709.81^{a} \pm 28.37$	$1658.40^{b} \pm 84.55$	$1802.84^{b} \pm 12.27$	$1874.59^{a} \pm 27.05$
	21	$1680.20^{a} \pm 14.52$	$1808.58^{a} \pm 70.49$	$2051.67^{a} \pm 67.31$	$1843.19^{a} \pm 95.78$
	28	$1711.37^{a} \pm 36.05$	$1823.79^{a} \pm 41.47$	$1989.06^{a} \pm 52.95$	$1870.53^{a} \pm 42.57$
	35	$1723.37^{a} \pm 10.94$	$1626.93^{bc} \pm 53.17$	$1821.96^{b} \pm 56.73$	$1799.01^{a} \pm 38.64$
25 °C	1	$1698.67^{a} \pm 9.21$	$1466.50^{c} \pm 17.53$	$1728.24^{d} \pm 39.24$	$1506.14^{b} \pm 24.57$
	4	$1708.42^{a} \pm 136.35$	$1685.58^{ab} \pm 45.01$	$1939.88^{abc} \pm 51.87$	$1873.78^{a} \pm 41.00$
	7	$1721.34^{a} \pm 21.10$	$1655.47^{b} \pm 28.40$	$1960.44^{ab} \pm 63.38$	$1812.23^{a} \pm 38.49$
	10	$1769.48^{a} \pm 21.37$	$1678.11^{ab} \pm 35.96$	$1991.25^{a} \pm 78.44$	$1864.61^{a} \pm 62.25$
	14	$1727.44^{a} \pm 29.02$	$1709.06^{ab} \pm 49.35$	$1853.65^{bcd} \pm 12.61$	$1797.93^{a} \pm 9.05$
	21	$1830.36^{a} \pm 20.83$	$1711.03^{ab} \pm 31.05$	$1931.03^{abc} \pm 37.69$	$1834.23^{a} \pm 124.33$
	28	$1761.60^{a} \pm 1.13$	$1777.18^{a} \pm 63.73$	$1830.27^{cd} \pm 5.37$	$1870.87^{a} \pm 40.88$
	35	$1794.93^{a} \pm 35.89$	$1657.24^{b} \pm 32.31$	$1955.08^{abc} \pm 15.48$	$1806.45^{a} \pm 60.01$

CHAPTER 4

CONCLUSION AND RECOMMENDATIONS

In the scope of this study, liposomes were formed by using two sources of phospholipid (egg and soy) in three different solvents (acetate buffer, phosphate buffer, and distilled water) by using high energy techniques, which were ultrasonication and microfluidization. For characterization of the liposomes, samples were stored at refrigeration and room temperatures. To explain time dependent behavior of the liposome particles, particle size and time domain low resolution NMR relaxometry experiments were conducted during 30 days storage. Zeta potential were also measured as a stability indicator and TEM images were teken to confirm the structure of liposomes formed.

When the particle size results of microfluidization and ultrasonication were compared, microfluidization was found to be more efficient to decrease liposome size. Best parameters for these methods decreased particle size 87% in ultrasonication while 91% reduction is achieved by microfluidization. Moreover, increasing microfluidization pressure resulted in a gradual decrease in particle size, however increasing ultrasonication time did not change particle size after 5 minutes processing. The influence of solvent and phospholipid type on particle size followed the same trend for both methods. The samples prepared by using either E80 lecithin or phosphate buffer yielded highest particle sizes. The lowest mean average was achieved in samples prepared in distilled water by using S75 as the phospholipid. Particle size distribution of acetate buffer samples remained

betweeen those of distilled water and phosphate buffer samples, demonstrating average stability.

Time dependency of particle sizes tend to remain unchanged with temperature for one month storage, however the samples prepared in phosphate buffer were more unstable, giving steep increases during storage especially at room temperature. Instability of liposomes prepared in different solvents were also validated by zeta potential measurements. Liposome charges were around -45, -15 and +1 for distilled water, acetate buffer and phosphate buffer samples, respectively.

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 which increses the bulk water in the environment.

This study suggested that the most stable liposomes were formed by using soy lecitihin (S75) prepared in distilled water while egg lecithin (E80) and phosphate buffer (pH≈7.2) were not efficient to use in liposome formation. Microfluidization level of 1300 bar (5 pass) were more stable over all other parameters of ultrasonication. In conclusion, it was decided to proceed throughout the project with microfluidization of S75 lecitihin prepared in distilled water and stored at refrigeration temperature.

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APPENDIX

A. ANOVA TABLES

Table A.1 Analysis of Variance for Primary Homogenization Speed and Pass Number, using Adjusted SS for Tests.

```
Source  DF     SS    MS     F      P
rpm      1   18,8  18,8  0,50  0,519
Error    4  150,8  37,7
Total    5  169,7

S = 6,141   R-Sq = 11,10%   R-Sq(adj) = 0,00%


                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level  N    Mean  StDev  -----+---------+---------+---------+----
20     3  82,643  8,290        (----------------*---------------)
24     3  79,100  2,588  (----------------*---------------)
                         -----+---------+---------+---------+----
                           72,0      78,0      84,0      90,0

Pooled StDev = 6,141


Grouping Information Using Tukey Method

rpm  N    Mean  Grouping
20   3  82,643  A
24   3  79,100  A

Means that do not share a letter are significantly different.


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

Individual confidence level = 95,00%
```

One-way ANOVA: Psize versus pass no

```
Source   DF      SS      MS      F      P
pass no   1  274,90  274,90  30,96  0,031
Error     2   17,76    8,88
Total     3  292,66

S = 2,980   R-Sq = 93,93%   R-Sq(adj) = 90,90%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev  -----+---------+---------+---------+----
 5     2  100,81   3,38                  (--------*--------)
10     2   84,23   2,52  (--------*--------)
                          -----+---------+---------+---------+----
                              80        90       100       110

Pooled StDev = 2,98


Grouping Information Using Tukey Method

pass
no    N     Mean  Grouping
 5    2  100,810  A
10    2   84,230    B

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of pass no

Individual confidence level = 95,00%


pass no =  5 subtracted from:

pass
no      Lower   Center   Upper  -----+---------+---------+---------+----
10    -29,402  -16,580  -3,758  (----------*----------)
                                -----+---------+---------+---------+----
                                   -24       -12         0        12
```

Table A.2 Analysis of Variance for the Effect of Microfluidizer Pressure on Particle Size, using Adjusted SS for Tests.

```
Source       DF        SS       MS       F      P
MF Pressure   3  108584,2  36194,7  371,98  0,000
Error         8     778,4     97,3
Total        11  109362,6

S = 9,864   R-Sq = 99,29%   R-Sq(adj) = 99,02%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev  ------+---------+---------+---------+---
   0   3  272,96  17,53                                    (-*-)
 400   3   79,71   7,27        (*-)
 900   3   49,82   3,76   (-*-)
1300   3   38,59   3,85  (-*)
                          ------+---------+---------+---------+---
                               70       140       210       280

Pooled StDev = 9,86


Grouping Information Using Tukey Method

MF
Pressure  N    Mean  Grouping
   0      3  272,96  A
 400      3   79,71    B
 900      3   49,82      C
1300      3   38,59      C

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


MF Pressure =    0 subtracted from:

MF
Pressure    Lower   Center    Upper  ------+---------+---------+--------
-+---
 400      -219,05  -193,25  -167,46      (--*-)
 900      -248,94  -223,14  -197,34   (--*-)
1300      -260,17  -234,37  -208,57  (--*-)
                                     ------+---------+---------+--------
-+---
                                        -200      -100         0
100


MF Pressure =  400 subtracted from:
```

```
MF
Pressure   Lower  Center   Upper  ------+---------+---------+---------+-
--
 900      -55,68  -29,89   -4,09                    (--*--)
1300      -66,92  -41,12  -15,32                  (--*-)
                                 ------+---------+---------+---------+-
--
                                    -200      -100         0       100
```

```
MF Pressure =  900 subtracted from:

MF
Pressure   Lower  Center  Upper  ------+---------+---------+---------+--
-
1300      -37,03  -11,23  14,57                      (--*-)
                                 ------+---------+---------+---------+--
-
                                   -200      -100         0       100
```

One-way ANOVA: S75_AB_4°C_Day1 versus 900 and 1300 bar Pressure

```
Source    DF     SS     MS     F      P
Pressure   1  189,2  189,2  7,92  0,048
Error      4   95,6   23,9
Total      5  284,8

S = 4,888   R-Sq = 66,44%   R-Sq(adj) = 58,05%


                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean  StDev  ------+---------+---------+---------+---
 900   3  49,822  5,738                (----------*----------)
1300   3  38,590  3,855  (----------*----------)
                        ------+---------+---------+---------+---
                           35,0      42,0      49,0      56,0

Pooled StDev = 4,888


Grouping Information Using Tukey Method

Pressure  N    Mean  Grouping
 900      3  49,822  A
1300      3  38,590    B

Means that do not share a letter are significantly different.


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

Individual confidence level = 95,00%
```

```
Pressure =  900 subtracted from:

Pressure    Lower   Center   Upper  --+---------+---------+---------+---
----
1300      -22,313  -11,232  -0,151  (----------*----------)
                                    --+---------+---------+---------+---
----
                                    -20       -10         0        10
```

Table A.3 Analysis of Variance for the Effect of Ultrasonication Time on Particle Size, using Adjusted SS for Tests.

```
Source   DF        SS       MS         F      P
US time   5  260535,2  52107,0  11529,34  0,000
Error     6      27,1      4,5
Total    11  260562,3

S = 2,126   R-Sq = 99,99%   R-Sq(adj) = 99,98%


                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level  N    Mean  StDev  ----+---------+---------+---------+-----
 0     2  489,45   3,32                                    (*
 1     2  144,30   1,98        *
 2     2  109,28   1,87     *
 5     2   80,11   1,74  (*
 7     2   82,33   1,68   *
10     2   78,78   1,70  (*
                         ----+---------+---------+---------+-----
                           120       240       360       480

Pooled StDev = 2,13


Grouping Information Using Tukey Method

US
time  N    Mean  Grouping
 0    2  489,45  A
 1    2  144,30     B
 2    2  109,28       C
 7    2   82,33         D
 5    2   80,11         D
10    2   78,78         D

Means that do not share a letter are significantly different.


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

Individual confidence level = 99,27%


US time =  0 subtracted from:

US
time    Lower   Center    Upper  --------+---------+---------+---------
+-
 1    -353,61  -345,15  -336,68       (*)
 2    -388,63  -380,17  -371,70     (*
 5    -417,80  -409,34  -400,87   (*
 7    -415,58  -407,12  -398,66   (*
10    -419,13  -410,67  -402,20   (*
```

```
                                  --------+---------+---------+---------
+-
                                       -300      -150         0       150


US time =  1 subtracted from:

US
time   Lower  Center   Upper  --------+---------+---------+---------+-
 2    -43,48  -35,02  -26,56                          (*
 5    -72,65  -64,19  -55,73                        (*
 7    -70,44  -61,97  -53,51                        (*
10    -73,98  -65,52  -57,06                        (*
                             --------+---------+---------+---------+-
                                  -300      -150         0       150


US time =  2 subtracted from:

US
time   Lower  Center   Upper  --------+---------+---------+---------+-
 5    -37,63  -29,17  -20,71                         (*)
 7    -35,42  -26,95  -18,49                          *)
10    -38,96  -30,50  -22,04                         (*)
                             --------+---------+---------+---------+-
                                  -300      -150         0       150


US time =  5 subtracted from:

US
time  Lower  Center  Upper  --------+---------+---------+---------+-
 7    -6,25    2,22  10,68                           *)
10    -9,79   -1,33   7,13                          (*
                           --------+---------+---------+---------+-
                                -300      -150         0       150


US time =  7 subtracted from:

US
time   Lower  Center  Upper  --------+---------+---------+---------+-
10    -12,01   -3,55   4,92                          (*
                           --------+---------+---------+---------+-
                                -300      -150         0       150
```

Table A.4 Analysis of Variance for the Effect of Solvent Type on Particle Size, using Adjusted SS for Tests.

```
Factor   Type   Levels  Values
Solvent  fixed       3  AB; DW; PB
US Time  fixed       3  2; 5; 8


Analysis of Variance for US Particle size, using Adjusted SS for Tests

Source           DF  Seq SS  Adj SS  Adj MS       F      P
Solvent           2   74193   74193   37096  266,27  0,000
US Time           2   13187   13187    6593   47,33  0,000
Solvent*US Time   4    1921    1921     480    3,45  0,029
Error            18    2508    2508     139
Total            26   91808


S = 11,8033   R-Sq = 97,27%   R-Sq(adj) = 96,05%


Grouping Information Using Tukey Method and 95,0% Confidence

Solvent  N   Mean  Grouping
PB       9  187,6  A
AB       9   99,0    B
DW       9   62,8      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

US
Time  N   Mean  Grouping
2     9  147,6  A
8     9  103,0    B
5     9   98,7    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

         US
Solvent  Time  N   Mean  Grouping
PB       2     3  234,4  A
PB       8     3  164,4    B
PB       5     3  164,0    B
AB       2     3  124,8      C
AB       5     3   86,7        D
AB       8     3   85,4        D
DW       2     3   83,6        D
DW       8     3   59,3        D E
DW       5     3   45,5          E

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

Table A.5 Analysis of Variance for the Effect of Solvent Type and Micofluidization Pressure on Particle Size, using Adjusted SS for Tests.

Factor	Type	Levels	Values
Pressure	fixed	2	400; 1300
Solvent	fixed	3	AB; DW; PB

Analysis of Variance for MFS75 Parçacık, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pressure	1	13343,7	13343,7	13343,7	291,83	0,000
Solvent	2	20174,7	20174,7	10087,4	220,61	0,000
Pressure*Solvent	2	3396,7	3396,7	1698,4	37,14	0,000
Error	12	548,7	548,7	45,7		
Total	17	37463,9				

S = 6,76199 R-Sq = 98,54% R-Sq(adj) = 97,93%

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	N	Mean	Grouping
400	9	103,3	A
1300	9	48,8	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Solvent	N	Mean	Grouping
PB	6	122,8	A
AB	6	59,1	B
DW	6	46,2	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Solvent	N	Mean	Grouping
400	PB	3	169,2	A
400	AB	3	79,7	B
1300	PB	3	76,4	B C
400	DW	3	61,0	C
1300	AB	3	38,6	D
1300	DW	3	31,4	D

Means that do not share a letter are significantly different.

Table A.6 Analysis of Variance for the Effect of Solvent and Phospholipid Type on Particle Size-Excluding Phosphate Buffer, using Adjusted SS for Tests.

```
Factor            Type   Levels  Values
Lecithin          fixed       2  E80; S75
Solvent_Pressure  fixed       4  AB_1300 bar; AB_400 bar; DW_1300 bar;
DW_400
                                 bar


Analysis of Variance for ParticleSize, using Adjusted SS for Tests

Source                      DF   Seq SS   Adj SS  Adj MS       F      P
Lecithin                     1   8858,7   8858,7  8858,7  275,85  0,000
Solvent_Pressure             3  22644,7  22644,7  7548,2  235,04  0,000
Lecithin*Solvent_Pressure    3   3325,8   3325,8  1108,6   34,52  0,000
Error                       16    513,8    513,8    32,1
Total                       23  35343,1


S = 5,66700   R-Sq = 98,55%   R-Sq(adj) = 97,91%


Grouping Information Using Tukey Method and 95,0% Confidence

Lecithin   N  Mean  Grouping
E80       12  91,1  A
S75       12  52,7    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Solvent_Pressure  N   Mean  Grouping
AB_400 bar        6  117,2  A
DW_400 bar        6   82,3    B
AB_1300 bar       6   49,3      C
DW_1300 bar       6   38,7        D

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Lecithin  Solvent_Pressure  N   Mean  Grouping
E80       AB_400 bar        3  154,7  A
E80       DW_400 bar        3  103,6    B
S75       AB_400 bar        3   79,7      C
S75       DW_400 bar        3   61,0        D
E80       AB_1300 bar       3   60,1        D
E80       DW_1300 bar       3   46,0        D E
S75       AB_1300 bar       3   38,6          E
S75       DW_1300 bar       3   31,4          E

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

Table A.7 Analysis of Variance for the Ultrasound and Microfluidization Particle Sizes, using Adjusted SS for Tests.

```
Factor   Type   Levels  Values
htYPE    fixed       2  mf; US
USTime   fixed       2  2; 8
Solvent  fixed       3  AB; DW; PB


Analysis of Variance for USParçacık, using Adjusted SS for Tests

Source   DF  Seq SS  Adj SS  Adj MS      F      P
htYPE     1    7278   16140   16140  28,14  0,000
USTime    1   18514   15412   15412  26,87  0,000
Solvent   2   81364   81364   40682  70,92  0,000
Error    31   17782   17782     574
Total    35  124938


S = 23,9499   R-Sq = 85,77%   R-Sq(adj) = 83,93%


Grouping Information Using Tukey Method and 95,0% Confidence

htYPE   N   Mean  Grouping
US     15  126,9  A
mf     21   82,0    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Solvent   N   Mean  Grouping
PB       12  172,3  A
AB       12   82,1    B
DW       12   58,8    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

USTime   N   Mean  Grouping
2       18  125,4  A
8       18   83,4    B

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

Table A.8 Analysis of Variance to Compare the Effect of Applied Process on Particle Size of Samples in Acetate Buffer, using Adjusted SS for Tests.

```
Source           DF       SS      MS      F      P
Applied Process   5  14020,6  2804,1  67,43  0,000
Error            12    499,0    41,6
Total            17  14519,6

S = 6,449   R-Sq = 96,56%   R-Sq(adj) = 95,13%


                            Individual 95% CIs For Mean Based on
                            Pooled StDev
Level     N    Mean  StDev   +---------+---------+---------+---------
1300 bar  3   38,59   3,85   (--*--)
2 min     3  124,84  10,61                                  (--*-)
400 bar   3   79,71   7,27                (--*-)
5 min     3   86,72   4,11                  (--*--)
8 min     3   85,40   4,41                  (-*--)
900 bar   3   49,82   5,74       (--*-)
                             +---------+---------+---------+---------
                            30        60        90       120

Pooled StDev = 6,45


Grouping Information Using Tukey Method

Applied
Process   N    Mean  Grouping
2 min     3  124,84  A
5 min     3   86,72    B
8 min     3   85,40    B
400 bar   3   79,71    B
900 bar   3   49,82      C
1300 bar  3   38,59      C

Means that do not share a letter are significantly different.


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

Individual confidence level = 99,43%


Applied Process = 1300 bar subtracted from:

Applied
Process  Lower  Center   Upper  ---------+---------+---------+---------+
2 min    68,57   86,25  103,94                                    (--*---
)
400 bar  23,43   41,12   58,80                          (--*---)
5 min    30,44   48,13   65,81                           (---*--)
8 min    29,13   46,81   64,50                           (--*---)
900 bar  -6,45   11,23   28,92                  (--*---)
                        ---------+---------+---------+---------+
                               -50         0        50       100
```

Applied Process = 2 min subtracted from:

```
Applied
Process   Lower  Center   Upper  ---------+---------+---------+---------
+
400 bar  -62,82  -45,13  -27,45        (---*---)
5 min    -55,81  -38,13  -20,44          (--*---)
8 min    -57,12  -39,44  -21,76          (--*---)
900 bar  -92,70  -75,02  -57,33  (---*---)
                                 ---------+---------+---------+---------
+
                                        -50         0        50
100
```

Applied Process = 400 bar subtracted from:

```
Applied
Process   Lower  Center   Upper  ---------+---------+---------+---------
+
5 min    -10,68    7,01   24,69                   (--*---)
8 min    -11,99    5,69   23,38                   (--*---)
900 bar  -47,57  -29,89  -12,20           (---*---)
                                 ---------+---------+---------+---------
+
                                        -50         0        50
100
```

Applied Process = 5 min subtracted from:

```
Applied
Process   Lower  Center   Upper  ---------+---------+---------+---------
+
8 min    -19,00   -1,31   16,37                 (---*--)
900 bar  -54,58  -36,89  -19,21          (---*--)
                                 ---------+---------+---------+---------
+
                                        -50         0        50
100
```

Applied Process = 8 min subtracted from:

```
Applied
Process   Lower  Center   Upper  ---------+---------+---------+---------
+
900 bar  -53,26  -35,58  -17,89          (---*--)
                                 ---------+---------+---------+---------
+
                                        -50         0        50
100
```

Table A.9 Analysis of Variance to Compare the Effect of Applied Process on Particle Size of Samples in Phosphate Buffer, using Adjusted SS for Tests.

```
Source           DF     SS    MS      F      P
Applied Process   5  45391  9078  47,70  0,000
Error            12   2284   190
Total            17  47675

S = 13,80   R-Sq = 95,21%   R-Sq(adj) = 93,21%


                            Individual 95% CIs For Mean Based on
                            Pooled StDev
Level     N    Mean  StDev  --------+---------+---------+---------+-
1300 bar  3   76,44   6,92  (--*---)
2 min     3  234,41  26,38                                (---*--)
400 bar   3  169,17   9,99                     (---*--)
5 min     3  163,95   9,46                    (---*--)
8 min     3  164,40  13,53                    (---*--)
900 bar   3  106,83   5,05         (--*---)
                            --------+---------+---------+---------+-
                                  100       150       200       250

Pooled StDev = 13,80


Grouping Information Using Tukey Method

Applied
Process   N    Mean  Grouping
2 min     3  234,41  A
400 bar   3  169,17    B
8 min     3  164,40    B
5 min     3  163,95    B
900 bar   3  106,83      C
1300 bar  3   76,44      C

Means that do not share a letter are significantly different.


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

Individual confidence level = 99,43%


Applied Process = 1300 bar subtracted from:

Applied
Process   Lower  Center   Upper  -------+---------+---------+---------+-
-
2 min    120,13  157,97  195,80                                (---*---)
400 bar   54,89   92,73  130,56                          (---*---)
5 min     49,68   87,51  125,35                          (---*---)
8 min     50,13   87,96  125,80                          (---*---)
900 bar   -7,45   30,39   68,22                  (---*---)
                         -------+---------+---------+---------+-
-
```

```
                                    -100         0       100       200

Applied Process = 2 min subtracted from:

Applied
Process    Lower   Center   Upper  -------+---------+---------+---------
+--
400 bar  -103,07   -65,24  -27,40          (--*---)
5 min    -108,29   -70,45  -32,62        (---*---)
8 min    -107,84   -70,00  -32,17        (---*---)
900 bar  -165,41  -127,58  -89,74  (---*---)
                                  -------+---------+---------+---------
+--
                                      -100         0       100
200


Applied Process = 400 bar subtracted from:

Applied
Process    Lower  Center   Upper  -------+---------+---------+---------
+--
5 min     -43,05   -5,22   32,62               (--*---)
8 min     -42,60   -4,77   33,07               (---*--)
900 bar  -100,17  -62,34  -24,51         (---*---)
                                 -------+---------+---------+---------
+--
                                    -100         0       100       200


Applied Process = 5 min subtracted from:

Applied
Process   Lower  Center   Upper  -------+---------+---------+---------+-
-
8 min    -37,38    0,45   38,28              (---*---)
900 bar  -94,96  -57,12  -19,29          (--*---)
                                -------+---------+---------+---------+-
-
                                   -100         0       100       200


Applied Process = 8 min subtracted from:

Applied
Process   Lower  Center   Upper  -------+---------+---------+---------+-
-
900 bar  -95,41  -57,57  -19,74         (---*---)
                               -------+---------+---------+---------+-
-
                                   -100         0       100       200
```

Table A.10 Analysis of Variance to Compare the Effect of Applied Process on Particle Size of Samples in Distilled Water, using Adjusted SS for Tests.

```
Source           DF      SS      MS      F      P
Applied Process   4  4531,0  1132,7  29,02  0,000
Error            10   390,3    39,0
Total            14  4921,3

S = 6,248   R-Sq = 92,07%   R-Sq(adj) = 88,90%


                            Individual 95% CIs For Mean Based on
                            Pooled StDev
Level     N    Mean  StDev  --------+---------+---------+---------+-
1300 bar  3  31,444  0,823  (---*---)
2 min     3  83,587  8,489                              (---*---)
400 bar   3  60,962  7,642                 (---*---)
5 min     3  45,480  3,517          (---*---)
8 min     3  59,334  7,188                 (---*---)
                            --------+---------+---------+---------+-
                                   40        60        80       100

Pooled StDev = 6,248


Grouping Information Using Tukey Method

Applied
Process   N    Mean  Grouping
2 min     3  83,587  A
400 bar   3  60,962    B
8 min     3  59,334    B
5 min     3  45,480    B C
1300 bar  3  31,444      C

Means that do not share a letter are significantly different.


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

Individual confidence level = 99,18%


Applied Process = 1300 bar subtracted from:

Applied
Process   Lower  Center   Upper  ------+---------+---------+---------+--
-
2 min    35,370  52,143  68,917                              (----*----)
400 bar  12,744  29,518  46,291                        (---*----)
5 min    -2,737  14,036  30,810                   (----*----)
8 min    11,117  27,890  44,664                       (----*----)
                                 ------+---------+---------+---------+--
-
                                    -35         0        35        70


Applied Process = 2 min subtracted from:
```

```
Applied
Process    Lower   Center    Upper  ------+---------+---------+---------
+---
400 bar  -39,399  -22,626   -5,852       (----*---)
5 min    -54,880  -38,107  -21,334  (----*----)
8 min    -41,026  -24,253   -7,480      (----*----)
                                  ------+---------+---------+---------
+---
                                      -35         0        35
70
```

Applied Process = 400 bar subtracted from:

```
Applied
Process    Lower   Center   Upper  ------+---------+---------+---------
+---
5 min    -32,255  -15,481   1,292         (----*---)
8 min    -18,401   -1,627  15,146             (----*---)
                                 ------+---------+---------+---------
+---
                                    -35         0        35        70
```

Applied Process = 5 min subtracted from:

```
Applied
Process   Lower  Center   Upper  ------+---------+---------+---------+--
-
8 min    -2,919  13,854  30,627                (----*----)
                               ------+---------+---------+---------+--
-
                                  -35         0        35        70
```

Table A.11 Analysis of Variance to Compare the Effect Storage Time on Ultrasonicated Samples, using Adjusted SS for Tests.

One-way ANOVA: S75_AB_2min_4°C versus days

```
Source  DF     SS    MS     F      P
days     3    8,9   3,0  0,05  0,986
Error    8  516,1  64,5
Total   11  525,0

S = 8,032   R-Sq = 1,69%   R-Sq(adj) = 0,00%


                          Individual 95% CIs For Mean Based on Pooled
StDev
Level  N    Mean  StDev    --+---------+---------+---------+-------
 1     3  124,84  10,61      (-----------------*-----------------)
 7     3  124,16   5,67     (-----------------*-----------------)
14     3  125,55   6,30       (-----------------*-----------------)
30     3  123,22   8,58    (----------------*-----------------)
                           --+---------+---------+---------+-------
                          114,0     120,0     126,0     132,0

Pooled StDev = 8,03


Grouping Information Using Tukey Method

days  N     Mean  Grouping
14    3  125,554  A
 1    3  124,842  A
 7    3  124,164  A
30    3  123,220  A

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


days =  1 subtracted from:

days    Lower  Center   Upper  ---------+---------+---------+---------+
 7    -21,685  -0,678  20,328   (----------------*-----------------)
14    -20,295   0,712  21,718    (-----------------*----------------)
30    -22,628  -1,622  19,385  (-----------------*----------------)
                               ---------+---------+---------+---------+
                                      -12         0        12        24


days =  7 subtracted from:

days    Lower  Center   Upper  ---------+---------+---------+---------+
14    -19,616   1,390  22,396     (----------------*-----------------)
```

```
30    -21,950  -0,943  20,063   (----------------*----------------)
                                ---------+---------+---------+---------+
                                       -12         0        12        24


days = 14 subtracted from:

days    Lower  Center   Upper  ---------+---------+---------+---------+
30    -23,340  -2,333  18,673  (----------------*----------------)
                                ---------+---------+---------+---------+
                                       -12         0        12        24
```

One-way ANOVA: S75_AB_5min_4°C versus days

```
Source  DF     SS    MS     F      P
days     3   78,4  26,1  1,06  0,417
Error    8  196,8  24,6
Total   11  275,3

S = 4,960   R-Sq = 28,49%   R-Sq(adj) = 1,67%


                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean  StDev  ---+---------+---------+---------+------
 1     3  86,717  4,107     (------------*-------------)
 7     3  85,001  7,468  (------------*------------)
14     3  85,931  3,810    (------------*------------)
30     3  91,618  3,358                (------------*------------)
                        ---+---------+---------+---------+------
                        80,0      85,0      90,0      95,0

Pooled StDev = 4,960


Grouping Information Using Tukey Method

days  N    Mean  Grouping
30    3  91,618  A
 1    3  86,717  A
14    3  85,931  A
 7    3  85,001  A

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


days =  1 subtracted from:

days    Lower  Center   Upper    +---------+---------+---------+--------
-
 7    -14,689  -1,715  11,258         (------------*------------)
14    -13,760  -0,786  12,187          (------------*------------)
30     -8,073   4,901  17,875                (------------*------------)
```

```
                                +---------+---------+---------+--------
-
                              -20       -10         0        10


days =  7 subtracted from:

days    Lower  Center   Upper    +---------+---------+---------+--------
-
14    -12,044   0,929  13,903            (------------*------------)
30     -6,357   6,616  19,590                  (------------*-----------
-)
                                +---------+---------+---------+--------
-
                              -20       -10         0        10


days = 14 subtracted from:

days   Lower  Center   Upper    +---------+---------+---------+---------
30    -7,286   5,687  18,661                (------------*------------)
                               +---------+---------+---------+---------
                             -20       -10         0        10
```

One-way ANOVA: S75_AB_8min_4°C versus days

```
Source  DF     SS    MS     F      P
days     3   13,1   4,4  0,19  0,900
Error    8  183,9  23,0
Total   11  197,0

S = 4,794   R-Sq = 6,67%   R-Sq(adj) = 0,00%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev  --------+---------+---------+---------+-
 1     3  85,402  4,413        (---------------*---------------)
 7     3  83,170  7,897  (---------------*---------------)
14     3  85,691  1,117        (---------------*---------------)
30     3  85,627  2,975        (---------------*---------------)
                          --------+---------+---------+---------+-
                               80,0      84,0      88,0      92,0

Pooled StDev = 4,794


Grouping Information Using Tukey Method

days  N    Mean  Grouping
14    3  85,691  A
30    3  85,627  A
 1    3  85,402  A
 7    3  83,170  A

Means that do not share a letter are significantly different.


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

```
Individual confidence level = 98,74%


days =  1 subtracted from:

days    Lower  Center   Upper  ---------+---------+---------+---------+
 7    -14,771  -2,232  10,307   (--------------*---------------)
14    -12,249   0,290  12,829      (--------------*---------------)
30    -12,313   0,226  12,764      (--------------*---------------)
                                ---------+---------+---------+---------+
                                      -8,0       0,0       8,0      16,0


days =  7 subtracted from:

days    Lower  Center   Upper  ---------+---------+---------+---------+
14    -10,017   2,522  15,060        (---------------*---------------)
30    -10,082   2,457  14,996        (---------------*---------------)
                                ---------+---------+---------+---------+
                                      -8,0       0,0       8,0      16,0


days = 14 subtracted from:

days    Lower  Center   Upper  ---------+---------+---------+---------+
30    -12,603  -0,064  12,475     (---------------*---------------)
                                ---------+---------+---------+---------+
                                      -8,0       0,0       8,0      16,0
```

One-way ANOVA: S75_AB_2min_25°C versus days

```
Source  DF     SS    MS     F      P
days     3   50,8  16,9  0,43  0,739
Error    8  316,8  39,6
Total   11  367,6

S = 6,293   R-Sq = 13,82%   R-Sq(adj) = 0,00%


                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean  StDev  --------+---------+---------+---------+-
 1     3  128,38   3,80          (-------------*-------------)
 7     3  123,61   5,89  (-------------*-------------)
14     3  125,23   9,17     (-------------*-------------)
30     3  128,37   5,02          (-------------*-------------)
                        --------+---------+---------+---------+-
                            120,0     126,0     132,0     138,0

Pooled StDev = 6,29


Grouping Information Using Tukey Method

days  N     Mean  Grouping
 1    3  128,383  A
30    3  128,367  A
14    3  125,230  A
 7    3  123,612  A
```

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


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

Individual confidence level = 98,74%


days =  1 subtracted from:

days    Lower  Center   Upper  --------+---------+---------+---------+-
 7    -21,230  -4,771  11,688  (-------------*-------------)
14    -19,612  -3,153  13,306    (------------*-------------)
30    -16,476  -0,017  16,442      (-------------*-------------)
                                --------+---------+---------+---------+-
                                      -12         0        12        24


days =  7 subtracted from:

days    Lower  Center   Upper  --------+---------+---------+---------+-
14    -14,841   1,618  18,077        (-------------*-------------)
30    -11,705   4,754  21,213          (-------------*-------------)
                                --------+---------+---------+---------+-
                                      -12         0        12        24


days = 14 subtracted from:

days    Lower  Center   Upper  --------+---------+---------+---------+-
30    -13,323   3,136  19,595         (-------------*------------)
                                --------+---------+---------+---------+-
                                      -12         0        12        24
```

One-way ANOVA: S75_AB_5min_25°C versus days

```
Source  DF     SS    MS     F      P
days     3  140,0  46,7  1,81  0,224
Error    8  206,5  25,8
Total   11  346,4

S = 5,080   R-Sq = 40,40%   R-Sq(adj) = 18,05%


                        Individual 95% CIs For Mean Based on Pooled
StDev
Level  N   Mean  StDev     +---------+---------+---------+---------
 1     3  85,05   2,32     (-----------*----------)
 7     3  92,00   6,93                (----------*-----------)
14     3  88,46   5,98           (----------*-----------)
30     3  93,98   3,75                     (-----------*----------)
                           +---------+---------+---------+---------
                          78,0      84,0      90,0      96,0

Pooled StDev = 5,08


Grouping Information Using Tukey Method
```

```
days  N    Mean  Grouping
30    3  93,978  A
 7    3  92,001  A
14    3  88,457  A
 1    3  85,050  A

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


days =  1 subtracted from:

days   Lower  Center   Upper  ------+---------+---------+---------+---
 7    -6,335   6,951  20,238            (----------*----------)
14    -9,880   3,407  16,693         (----------*----------)
30    -4,358   8,928  22,215             (----------*----------)
                             ------+---------+---------+---------+---
                                 -12         0        12        24


days =  7 subtracted from:

days    Lower  Center   Upper  ------+---------+---------+---------+---
14    -16,832  -3,545   9,742    (----------*----------)
30    -11,310   1,977  15,264         (----------*----------)
                              ------+---------+---------+---------+---
                                  -12         0        12        24


days = 14 subtracted from:

days   Lower  Center   Upper  ------+---------+---------+---------+---
30    -7,765   5,522  18,809           (----------*----------)
                             ------+---------+---------+---------+---
                                 -12         0        12        24
```

One-way ANOVA: S75_AB_8min_25° versus days

```
Source  DF     SS    MS     F      P
days     3   76,5  25,5  0,70  0,577
Error    8  290,4  36,3
Total   11  367,0

S = 6,025   R-Sq = 20,86%   R-Sq(adj) = 0,00%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev   --+---------+---------+---------+-------
 1     3  84,735  8,412   (------------*-------------)
 7     3  90,170  3,907            (------------*-------------)
14     3  89,306  3,906          (-------------*------------)
30     3  91,428  6,628              (------------*-------------)
                          --+---------+---------+---------+-------
```

```
                          78,0      84,0      90,0      96,0

Pooled StDev = 6,025


Grouping Information Using Tukey Method

days  N    Mean  Grouping
30    3  91,428  A
 7    3  90,170  A
14    3  89,306  A
 1    3  84,735  A

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


days =  1 subtracted from:

days    Lower  Center   Upper  -----+---------+---------+---------+----
 7    -10,324   5,435  21,194        (-------------*------------)
14    -11,188   4,571  20,329        (-------------*------------)
30     -9,066   6,693  22,451         (-------------*------------)
                                -----+---------+---------+---------+----
                                   -12         0        12        24


days =  7 subtracted from:

days    Lower  Center   Upper  -----+---------+---------+---------+----
14    -16,623  -0,864  14,894   (------------*------------)
30    -14,501   1,258  17,017     (------------*------------)
                                -----+---------+---------+---------+----
                                   -12         0        12        24


days = 14 subtracted from:

days    Lower  Center   Upper  -----+---------+---------+---------+----
30    -13,636   2,122  17,881      (------------*------------)
                                -----+---------+---------+---------+----
                                   -12         0        12        24
```

One-way ANOVA: S75_DW_2min_4°C versus days

```
Source  DF     SS    MS     F      P
days     3  126,9  42,3  1,57  0,271
Error    8  215,9  27,0
Total   11  342,9

S = 5,195   R-Sq = 37,03%   R-Sq(adj) = 13,41%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
```

```
Level  N    Mean  StDev  -----+---------+---------+---------+----
 1     3  83,587  8,489              (----------*----------)
 7     3  76,027  2,386  (-----------*----------)
14     3  80,829  5,393          (-----------*----------)
30     3  75,978  1,057  (-----------*----------)
                         -----+---------+---------+---------+----
                           72,0      78,0      84,0      90,0

Pooled StDev = 5,195


Grouping Information Using Tukey Method

days  N    Mean  Grouping
 1    3  83,587  A
14    3  80,829  A
 7    3  76,027  A
30    3  75,978  A

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


days =  1 subtracted from:

days    Lower  Center   Upper   -+---------+---------+---------+--------
 7    -21,147  -7,560   6,027   (------------*-------------)
14    -16,346  -2,759  10,829        (------------*-------------)
30    -21,196  -7,609   5,978   (------------*-------------)
                                -+---------+---------+---------+--------
                               -20       -10         0        10


days =  7 subtracted from:

days    Lower  Center   Upper   -+---------+---------+---------+--------
14     -8,785   4,802  18,389              (-------------*------------)
30    -13,636  -0,049  13,538         (-------------*-------------)
                                -+---------+---------+---------+--------
                               -20       -10         0        10


days = 14 subtracted from:

days    Lower  Center  Upper   -+---------+---------+---------+--------
30    -18,438  -4,851  8,736      (------------*-------------)
                                -+---------+---------+---------+--------
                              -20       -10         0        10
```

One-way ANOVA: S75_DW_5min_4°C versus days

```
Source  DF     SS    MS     F      P
days     3  237,6  79,2  3,77  0,059
Error    8  168,0  21,0
Total   11  405,6
```

```
S = 4,582   R-Sq = 58,59%   R-Sq(adj) = 43,06%


                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean  StDev  ----+---------+---------+---------+-----
 1     3  45,480  3,517  (---------*---------)
 7     3  56,608  5,068                    (---------*----------)
14     3  55,105  6,428                  (---------*---------)
30     3  55,296  2,144                  (---------*---------)
                        ----+---------+---------+---------+-----
                         42,0      48,0      54,0      60,0

Pooled StDev = 4,582


Grouping Information Using Tukey Method

days  N    Mean  Grouping
 7    3  56,608  A
30    3  55,296  A
14    3  55,105  A
 1    3  45,480  A

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


days =  1 subtracted from:

days   Lower  Center   Upper  ---+---------+---------+---------+------
 7    -0,856  11,128  23,112              (-----------*-----------)
14    -2,359   9,625  21,608            (-----------*-----------)
30    -2,168   9,816  21,799            (-----------*-----------)
                           ---+---------+---------+---------+------
                            -10         0        10        20


days =  7 subtracted from:

days    Lower  Center   Upper  ---+---------+---------+---------+------
14    -13,487  -1,503  10,480  (----------*-----------)
30    -13,296  -1,312  10,671  (-----------*-----------)
                              ---+---------+---------+---------+------
                               -10         0        10        20


days = 14 subtracted from:

days    Lower  Center   Upper  ---+---------+---------+---------+------
30    -11,793   0,191  12,175   (-----------*-----------)
                              ---+---------+---------+---------+------
                               -10         0        10        20
```

One-way ANOVA: S75_DW_8min_4°C versus days

```
Source  DF     SS    MS     F      P
days     3   38,7  12,9  0,42  0,746
Error    8  248,4  31,1
Total   11  287,1

S = 5,572   R-Sq = 13,49%   R-Sq(adj) = 0,00%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev  ------+---------+---------+---------+---
 1     3  59,334  7,188           (--------------*--------------)
 7     3  54,648  4,161  (--------------*--------------)
14     3  57,066  6,440       (--------------*--------------)
30     3  55,412  3,708    (--------------*--------------)
                          ------+---------+---------+---------+---
                             50,0      55,0      60,0      65,0

Pooled StDev = 5,572


Grouping Information Using Tukey Method

days  N    Mean  Grouping
 1    3  59,334  A
14    3  57,066  A
30    3  55,412  A
 7    3  54,648  A

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


days =  1 subtracted from:

days    Lower  Center   Upper  ---------+---------+---------+---------+
 7    -19,260  -4,686   9,888  (--------------*--------------)
14    -16,842  -2,268  12,305    (--------------*-------------)
30    -18,496  -3,922  10,652   (--------------*--------------)
                               ---------+---------+---------+---------+
                                      -10         0        10        20


days =  7 subtracted from:

days    Lower  Center   Upper  ---------+---------+---------+---------+
14    -12,157   2,417  16,991         (--------------*--------------)
30    -13,810   0,764  15,338       (--------------*-------------)
                               ---------+---------+---------+---------+
                                      -10         0        10        20


days = 14 subtracted from:
```

```
days    Lower  Center   Upper  ---------+---------+---------+---------+
30    -16,227  -1,653  12,921     (-------------*--------------)
                                ---------+---------+---------+---------+
                                       -10         0        10        20
```

One-way ANOVA: S75_DW_2min_25°C versus days

```
Source  DF     SS    MS     F      P
days     3  129,7  43,2  1,86  0,215
Error    8  186,2  23,3
Total   11  315,8

S = 4,824   R-Sq = 41,06%   R-Sq(adj) = 18,96%


                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean  StDev  ------+---------+---------+---------+---
 1     3  83,587  3,495                (---------*----------)
 7     3  81,496  6,328             (----------*----------)
14     3  79,800  4,659          (----------*----------)
30     3  74,696  4,372  (---------*----------)
                        ------+---------+---------+---------+---
                           72,0      78,0      84,0      90,0

Pooled StDev = 4,824


Grouping Information Using Tukey Method

days  N    Mean  Grouping
 1    3  83,587  A
 7    3  81,496  A
14    3  79,800  A
30    3  74,696  A

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


days =  1 subtracted from:

days    Lower  Center   Upper  --------+---------+---------+---------+-
 7    -14,708  -2,092  10,524        (---------*----------)
14    -16,403  -3,787   8,829      (----------*---------)
30    -21,507  -8,891   3,725  (----------*---------)
                                --------+---------+---------+---------+-
                                      -12         0        12        24


days =  7 subtracted from:

days    Lower  Center   Upper  --------+---------+---------+---------+-
14    -14,312  -1,695  10,921        (----------*---------)
30    -19,416  -6,799   5,817    (---------*----------)
```

```
                                 --------+---------+---------+---------+-
                                       -12         0        12        24


days = 14 subtracted from:

days    Lower  Center  Upper  --------+---------+---------+---------+-
30    -17,720  -5,104  7,512     (----------*---------)
                                --------+---------+---------+---------+-
                                      -12         0        12        24
```

One-way ANOVA: S75_DW_5min_25°C versus days

```
Source  DF     SS     MS     F      P
days     3  325,7  108,6  3,97  0,053
Error    8  218,6   27,3
Total   11  544,3

S = 5,227   R-Sq = 59,84%   R-Sq(adj) = 44,78%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev  ----+---------+---------+---------+-----
 1     3  46,147  3,328  (---------*---------)
 7     3  57,967  6,638                (---------*---------)
14     3  59,586  5,965                  (---------*---------)
30     3  55,678  4,310             (---------*--------)
                          ----+---------+---------+---------+-----
                           42,0      49,0      56,0      63,0

Pooled StDev = 5,227


Grouping Information Using Tukey Method

days  N    Mean  Grouping
14    3  59,586  A
 7    3  57,967  A
30    3  55,678  A
 1    3  46,147  A

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


days =  1 subtracted from:

days   Lower  Center   Upper  -----+---------+---------+---------+----
 7    -1,851  11,820  25,491            (-----------*----------)
14    -0,232  13,439  27,110              (----------*-----------)
30    -4,140   9,531  23,202            (----------*----------)
                           -----+---------+---------+---------+----
                              -12         0        12        24
```

```
days =  7 subtracted from:

days    Lower  Center   Upper  -----+---------+---------+---------+----
14    -12,052   1,619  15,290       (-----------*-----------)
30    -15,960  -2,289  11,382    (-----------*----------)
                               -----+---------+---------+---------+----
                                  -12         0        12        24


days = 14 subtracted from:

days    Lower  Center  Upper  -----+---------+---------+---------+----
30    -17,579  -3,908  9,763  (-----------*----------)
                              -----+---------+---------+---------+----
                                 -12         0        12        24
```

One-way ANOVA: S75_DW_8min_25°C versus days

```
Source  DF     SS    MS     F      P
days     3   91,6  30,5  0,80  0,527
Error    8  304,6  38,1
Total   11  396,2

S = 6,170   R-Sq = 23,13%   R-Sq(adj) = 0,00%


                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level  N    Mean  StDev  ---------+---------+---------+---------+
 1     3  59,334  8,193      (-------------*-------------)
 7     3  64,472  8,237                (-------------*-------------)
14     3  61,814  3,904           (-------------*-------------)
30     3  57,065  1,434  (-------------*-------------)
                         ---------+---------+---------+---------+
                               54,0      60,0      66,0      72,0

Pooled StDev = 6,170


Grouping Information Using Tukey Method

days  N    Mean  Grouping
 7    3  64,472  A
14    3  61,814  A
 1    3  59,334  A
30    3  57,065  A

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


days =  1 subtracted from:
```

```
days    Lower  Center   Upper    +---------+---------+---------+--------
-
 7    -10,999   5,138  21,275              (------------*-------------)
14    -13,658   2,480  18,617            (------------*-------------)
30    -18,406  -2,269  13,868         (------------*-------------)
                                   +---------+---------+---------+--------
-
                                -24       -12         0        12


days =  7 subtracted from:

days    Lower  Center   Upper    +---------+---------+---------+--------
-
14    -18,796  -2,658  13,479        (-------------*------------)
30    -23,544  -7,407   8,730    (-------------*------------)
                                   +---------+---------+---------+--------
-
                                -24       -12         0        12


days = 14 subtracted from:

days    Lower  Center   Upper    +---------+---------+---------+--------
-
30    -20,886  -4,749  11,389       (------------*------------)
                                   +---------+---------+---------+--------
-
                                -24       -12         0        12
```

One-way ANOVA: S75_PB_2min_4°C versus days

```
Source  DF     SS    MS     F      P
days     3   7876  2625  4,67  0,036
Error    8   4498   562
Total   11  12374

S = 23,71   R-Sq = 63,65%   R-Sq(adj) = 50,02%


                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean  StDev  --+---------+---------+---------+-------
 1     3  234,41  26,38  (--------*--------)
 7     3  292,92  27,04                   (--------*--------)
14     3  291,63  28,04                  (--------*--------)
30     3  295,85   5,97                    (--------*--------)
                        --+---------+---------+---------+-------
                        210       245       280       315

Pooled StDev = 23,71


Grouping Information Using Tukey Method

days  N    Mean  Grouping
30    3  295,85  A
 7    3  292,92  A
14    3  291,63  A
 1    3  234,41  A
```

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


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

Individual confidence level = 98,74%


days =  1 subtracted from:

days  Lower  Center   Upper  ---+---------+---------+---------+------
 7    -3,50   58,51  120,53              (------------*-----------)
14    -4,80   57,22  119,24              (-----------*------------)
30    -0,57   61,45  123,46               (-----------*------------)
                           ---+---------+---------+---------+------
                            -50         0        50       100


days =  7 subtracted from:

days   Lower  Center  Upper  ---+---------+---------+---------+------
14    -63,31   -1,29  60,72  (------------*-----------)
30    -59,08    2,93  64,95   (------------*-----------)
                           ---+---------+---------+---------+------
                            -50         0        50       100


days = 14 subtracted from:

days   Lower  Center  Upper  ---+---------+---------+---------+------
30    -57,79    4,23  66,24   (------------*-----------)
                           ---+---------+---------+---------+------
                            -50         0        50       100
```

One-way ANOVA: S75_PB_5min_4°C versus days

```
Source  DF     SS    MS     F      P
days     3  225,1  75,0  1,04  0,427
Error    8  579,1  72,4
Total   11  804,2

S = 8,508   R-Sq = 27,99%   R-Sq(adj) = 0,99%


                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean  StDev  -------+---------+---------+---------+--
 1     3  163,95   9,46  (----------*----------)
 7     3  173,18   9,48           (----------*----------)
14     3  173,00  10,10           (----------*----------)
30     3  175,12   2,86             (----------*----------)
                        -------+---------+---------+---------+--
                             160       170       180       190

Pooled StDev = 8,51


Grouping Information Using Tukey Method
```

```
days  N     Mean  Grouping
30    3  175,119  A
 7    3  173,185  A
14    3  173,004  A
 1    3  163,952  A

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


days =  1 subtracted from:

days    Lower  Center   Upper  ------+---------+---------+---------+---
 7    -13,019   9,233  31,484         (--------------*--------------)
14    -13,200   9,052  31,304         (--------------*--------------)
30    -11,085  11,167  33,418           (-------------*--------------)
                                ------+---------+---------+---------+---
                                    -15         0        15        30


days =  7 subtracted from:

days    Lower  Center   Upper  ------+---------+---------+---------+---
14    -22,433  -0,181  22,071   (--------------*--------------)
30    -20,318   1,934  24,186    (--------------*--------------)
                                ------+---------+---------+---------+---
                                    -15         0        15        30


days = 14 subtracted from:

days    Lower  Center   Upper  ------+---------+---------+---------+---
30    -20,137   2,115  24,367     (-------------*--------------)
                                ------+---------+---------+---------+---
                                    -15         0        15        30
```

One-way ANOVA: S75_PB_8min_4°C versus days

```
Source  DF     SS    MS     F      P
days     3  264,4  88,1  1,04  0,425
Error    8  677,5  84,7
Total   11  941,9

S = 9,203   R-Sq = 28,07%   R-Sq(adj) = 1,10%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev  ----+---------+---------+---------+-----
 1     3  164,40  13,53        (-----------*-----------)
 7     3  158,56   9,53  (-----------*-----------)
14     3  170,64   3,95              (-----------*-----------)
30     3  169,02   7,01             (-----------*-----------)
                          ----+---------+---------+---------+-----
```

```
                          150       160       170       180

Pooled StDev = 9,20


Grouping Information Using Tukey Method

days  N     Mean  Grouping
14    3  170,642  A
30    3  169,023  A
 1    3  164,402  A
 7    3  158,559  A

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


days =  1 subtracted from:

days    Lower  Center   Upper  --------+---------+---------+---------+-
 7    -29,912  -5,843  18,225     (-----------*-----------)
14    -17,829   6,240  30,309           (-----------*-----------)
30    -19,449   4,620  28,689         (-----------*-----------)
                                --------+---------+---------+---------+-
                                      -20         0        20        40


days =  7 subtracted from:

days    Lower  Center   Upper  --------+---------+---------+---------+-
14    -11,986  12,083  36,152              (-----------*-----------)
30    -13,605  10,464  34,533            (-----------*-----------)
                                --------+---------+---------+---------+-
                                      -20         0        20        40


days = 14 subtracted from:

days    Lower  Center   Upper  --------+---------+---------+---------+-
30    -25,688  -1,620  22,449       (-----------*-----------)
                                --------+---------+---------+---------+-
                                      -20         0        20        40
```

One-way ANOVA: S75_PB_2min_25°C versus days

```
Source  DF     SS    MS      F      P
days     3  10903  3634  11,25  0,003
Error    8   2584   323
Total   11  13488

S = 17,97   R-Sq = 80,84%   R-Sq(adj) = 73,66%


                          Individual 95% CIs For Mean Based on Pooled
StDev
```

```
Level  N    Mean  StDev    +---------+---------+---------+---------
 1     3  233,74  30,50    (------*------)
 7     3  299,82  13,53                      (------*------)
14     3  303,40   8,69                       (------*------)
30     3  306,24  10,17                        (-----*------)
                           +---------+---------+---------+---------
                         210       245       280       315

Pooled StDev = 17,97


Grouping Information Using Tukey Method

days  N    Mean  Grouping
30    3  306,24  A
14    3  303,40  A
 7    3  299,82  A
 1    3  233,74    B

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


days =  1 subtracted from:

days  Lower  Center   Upper   -+---------+---------+---------+--------
 7    19,08   66,08  113,09                 (--------*---------)
14    22,65   69,66  116,66                  (--------*--------)
30    25,50   72,50  119,51                  (---------*--------)
                            -+---------+---------+---------+--------
                           -50         0        50       100


days =  7 subtracted from:

days   Lower  Center  Upper   -+---------+---------+---------+--------
14    -43,43    3,57  50,58     (---------*--------)
30    -40,59    6,42  53,42      (--------*---------)
                            -+---------+---------+---------+--------
                           -50         0        50       100


days = 14 subtracted from:

days   Lower  Center  Upper   -+---------+---------+---------+--------
30    -44,16    2,84  49,85     (---------*--------)
                            -+---------+---------+---------+--------
                           -50         0        50       100
```

One-way ANOVA: S75_PB_5min_25°C versus days

```
Source  DF    SS   MS     F      P
days     3   666  222  1,18  0,377
Error    8  1509  189
Total   11  2176
```

```
S = 13,74   R-Sq = 30,63%   R-Sq(adj) = 4,62%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev   -+---------+---------+---------+--------
 1     3  166,62  18,31   (-----------*-----------)
 7     3  179,22  12,25           (-----------*-----------)
14     3  183,64  13,10              (-----------*-----------)
30     3  185,88   9,89                (-----------*-----------)
                          -+---------+---------+---------+--------
                          150       165       180       195

Pooled StDev = 13,74


Grouping Information Using Tukey Method

days  N    Mean  Grouping
30    3  185,88  A
14    3  183,64  A
 7    3  179,22  A
 1    3  166,62  A

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


days =  1 subtracted from:

days   Lower  Center  Upper  -------+---------+---------+---------+--
 7    -23,32   12,60  48,53          (-------------*-------------)
14    -18,90   17,02  52,95           (--------------*-------------)
30    -16,66   19,26  55,19            (--------------*-------------)
                           -------+---------+---------+---------+--
                                -25         0        25        50


days =  7 subtracted from:

days   Lower  Center  Upper  -------+---------+---------+---------+--
14    -31,50    4,42  40,35      (--------------*-------------)
30    -29,26    6,66  42,58       (--------------*-------------)
                           -------+---------+---------+---------+--
                                -25         0        25        50


days = 14 subtracted from:

days   Lower  Center  Upper  -------+---------+---------+---------+--
30    -33,69    2,24  38,16      (-------------*-------------)
                           -------+---------+---------+---------+--
                                -25         0        25        50
```

One-way ANOVA: S75_PB_8min_25°C versus days

```
Source  DF    SS   MS     F      P
days     3   769  256  0,78  0,536
Error    8  2618  327
Total   11  3386

S = 18,09   R-Sq = 22,70%   R-Sq(adj) = 0,00%


                          Individual 95% CIs For Mean Based on Pooled
StDev
Level  N    Mean  StDev    +---------+---------+---------+---------
 1     3  164,40  31,99    (-----------*-----------)
 7     3  166,32   9,91     (-----------*-----------)
14     3  172,50   7,32        (-----------*-----------)
30     3  184,88  11,55              (-----------*-----------)
                             +---------+---------+---------+---------
                           140       160       180       200

Pooled StDev = 18,09


Grouping Information Using Tukey Method


days  N    Mean  Grouping
30    3  184,88  A
14    3  172,50  A
 7    3  166,32  A
 1    3  164,40  A

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


days =  1 subtracted from:

days   Lower  Center  Upper  ---------+---------+---------+---------+
 7    -45,39    1,92  49,22         (-------------*------------)
14    -39,21    8,10  55,41           (-------------*-------------)
30    -26,83   20,48  67,79              (-------------*------------)
                          ---------+---------+---------+---------+
                                  -35         0        35        70


days =  7 subtracted from:

days   Lower  Center  Upper  ---------+---------+---------+---------+
14    -41,13    6,18  53,49         (-------------*------------)
30    -28,75   18,56  65,87             (------------*-------------)
                          ---------+---------+---------+---------+
                                  -35         0        35        70


days = 14 subtracted from:
```

```
days   Lower  Center  Upper  ---------+---------+---------+---------+
30    -34,93   12,38  59,69           (-------------*------------)
                               ---------+---------+---------+---------+
                                      -35         0        35        70
```

Table A.12 Analysis of Variance to Compare the Effect Storage Time on Microfluidized Samples, using Adjusted SS for Tests.

One-way ANOVA: S75_AB_1300_4°C versus DAYS

```
Source  DF      SS     MS      F      P
DAYS     3  1121,8  373,9  10,15  0,004
Error    8   294,8   36,9
Total   11  1416,6

S = 6,070   R-Sq = 79,19%   R-Sq(adj) = 71,39%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean   StDev  -----+---------+---------+---------+----
 1     3  38,590   3,855  (------*------)
 7     3  48,863   0,391           (------*-----)
14     3  46,903   3,848         (------*------)
30     3  65,261  10,844                       (-----*------)
                          -----+---------+---------+---------+----
                              36        48        60        72

Pooled StDev = 6,070


Grouping Information Using Tukey Method

DAYS  N    Mean  Grouping
30    3  65,261  A
 7    3  48,863    B
14    3  46,903    B
 1    3  38,590    B

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


DAYS =  1 subtracted from:

DAYS   Lower  Center   Upper  -------+---------+---------+---------+--
 7    -5,604  10,273  26,149              (-------*-------)
14    -7,564   8,313  24,190             (-------*-------)
30    10,794  26,671  42,548                      (-------*-------)
                          -------+---------+---------+---------+--
                               -20         0        20        40


DAYS =  7 subtracted from:
```

```
DAYS    Lower  Center   Upper  -------+---------+---------+---------+--
14    -17,836  -1,959  13,917          (-------*-------)
30      0,522  16,398  32,275                   (-------*-------)
                                -------+---------+---------+---------+--
                                     -20         0        20        40


DAYS = 14 subtracted from:

DAYS  Lower  Center   Upper  -------+---------+---------+---------+--
30    2,481  18,358  34,235                   (-------*-------)
                             -------+---------+---------+---------+--
                                  -20         0        20        40
```

One-way ANOVA: S75_AB_900_4°C versus DAYS

```
Source  DF     SS     MS      F      P
DAYS     3  782,4  260,8  14,81  0,001
Error    8  140,9   17,6
Total   11  923,3

S = 4,197   R-Sq = 84,74%   R-Sq(adj) = 79,01%


                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean  StDev  -----+---------+---------+---------+----
 1     3  49,822  5,738  (------*------)
 7     3  70,639  2,594                           (------*------)
14     3  53,156  4,466      (------*------)
30     3  54,085  3,295        (------*------)
                        -----+---------+---------+---------+----
                          48,0      56,0      64,0      72,0

Pooled StDev = 4,197


Grouping Information Using Tukey Method

DAYS  N    Mean  Grouping
 7    3  70,639  A
30    3  54,085    B
14    3  53,156    B
 1    3  49,822    B

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


DAYS =  1 subtracted from:

DAYS   Lower  Center   Upper  --------+---------+---------+---------+-
 7     9,840  20,817  31,793                           (------*------)
```

```
14    -7,643   3,334  14,310               (------*------)
30    -6,714   4,263  15,239                (------*------)
                                 --------+---------+---------+---------+-
                                       -16         0        16        32
```

```
DAYS =  7 subtracted from:

DAYS    Lower   Center   Upper  --------+---------+---------+---------+-
14    -28,460  -17,483  -6,506  (------*------)
30    -27,531  -16,554  -5,577   (------*------)
                                 --------+---------+---------+---------+-
                                       -16         0        16        32
```

```
DAYS = 14 subtracted from:

DAYS    Lower  Center   Upper  --------+---------+---------+---------+-
30    -10,048   0,929  11,906             (------*-----)
                                 --------+---------+---------+---------+-
                                       -16         0        16        32
```

One-way ANOVA: S75_AB_400_4°C versus DAYS

```
Source  DF     SS    MS     F      P
DAYS     3   97,4  32,5  1,16  0,385
Error    8  224,8  28,1
Total   11  322,2

S = 5,301   R-Sq = 30,22%   R-Sq(adj) = 4,06%
```

```
                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean  StDev  ---------+---------+---------+---------+
 1     3  79,708  7,266  (-----------*-----------)
 7     3  86,673  2,069             (-----------*-----------)
14     3  84,509  5,779          (-----------*-----------)
30     3  86,696  4,682             (-----------*-----------)
                        ---------+---------+---------+---------+
                              78,0      84,0      90,0      96,0

Pooled StDev = 5,301
```

```
Grouping Information Using Tukey Method

DAYS  N    Mean  Grouping
30    3  86,696  A
 7    3  86,673  A
14    3  84,509  A
 1    3  79,708  A

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

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

```
Individual confidence level = 98,74%


DAYS =  1 subtracted from:

DAYS   Lower  Center   Upper  ------+---------+---------+---------+---
 7    -6,900   6,964  20,828           (-------------*-------------)
14    -9,064   4,800  18,664         (-------------*-------------)
30    -6,877   6,987  20,851           (-------------*-------------)
                               ------+---------+---------+---------+---
                                   -10         0        10        20


DAYS =  7 subtracted from:

DAYS    Lower  Center   Upper  ------+---------+---------+---------+---
14    -16,028  -2,164  11,700  (-------------*-------------)
30    -13,841   0,023  13,887    (-------------*-------------)
                               ------+---------+---------+---------+---
                                   -10         0        10        20


DAYS = 14 subtracted from:

DAYS    Lower  Center   Upper  ------+---------+---------+---------+---
30    -11,677   2,187  16,051      (-------------*-------------)
                               ------+---------+---------+---------+---
                                   -10         0        10        20
```

One-way ANOVA: S75_AB_1300_25°C versus DAYS

```
Source  DF     SS    MS     F      P
DAYS     3  169,7  56,6  4,26  0,045
Error    8  106,2  13,3
Total   11  275,9

S = 3,643   R-Sq = 61,52%   R-Sq(adj) = 47,08%


                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean  StDev  ---+---------+---------+---------+------
 1     3  38,590  3,855  (---------*---------)
 7     3  47,960  2,281                    (---------*---------)
14     3  46,497  2,359                  (---------*---------)
30     3  47,120  5,240                    (--------*---------)
                        ---+---------+---------+---------+------
                        35,0      40,0      45,0      50,0

Pooled StDev = 3,643


Grouping Information Using Tukey Method

DAYS  N    Mean  Grouping
 7    3  47,960  A
30    3  47,120  A
14    3  46,497  A
 1    3  38,590  A
```

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


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

Individual confidence level = 98,74%


DAYS =  1 subtracted from:

DAYS   Lower  Center   Upper  ----+---------+---------+---------+-----
 7    -0,158   9,370  18,898               (-----------*-----------)
14    -1,621   7,907  17,435            (-----------*-----------)
30    -0,999   8,530  18,058             (-----------*-----------)
                           ----+---------+---------+---------+-----
                            -8,0       0,0       8,0      16,0


DAYS =  7 subtracted from:

DAYS    Lower  Center  Upper  ----+---------+---------+---------+-----
14    -10,991  -1,463  8,065  (-----------*-----------)
30    -10,369  -0,840  8,688   (-----------*-----------)
                           ----+---------+---------+---------+-----
                            -8,0       0,0       8,0      16,0


DAYS = 14 subtracted from:

DAYS   Lower  Center   Upper  ----+---------+---------+---------+-----
30    -8,906   0,623  10,151     (-----------*-----------)
                           ----+---------+---------+---------+-----
                            -8,0       0,0       8,0      16,0
```

One-way ANOVA: S75_AB_900_25°C versus DAYS

```
Source  DF     SS     MS      F      P
DAYS     3  59223  19741  64,69  0,000
Error    8   2441    305
Total   11  61664

S = 17,47   R-Sq = 96,04%   R-Sq(adj) = 94,56%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev  ------+---------+---------+---------+---
 1     3   49,82   5,74  (---*---)
 7     3   51,36   1,23   (---*--)
14     3   50,18   0,65  (---*---)
30     3  212,69  34,43                             (--*---)
                          ------+---------+---------+---------+---
                               60       120       180       240

Pooled StDev = 17,47


Grouping Information Using Tukey Method
```

```
DAYS  N    Mean  Grouping
30    3  212,69  A
 7    3   51,36    B
14    3   50,18    B
 1    3   49,82    B

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


DAYS =  1 subtracted from:

DAYS   Lower  Center   Upper  -------+---------+---------+---------+--
 7    -44,15    1,53   47,22              (---*---)
14    -45,33    0,36   46,05              (---*---)
30    117,18  162,86  208,55                          (---*--)
                            -------+---------+---------+---------+--
                                -120         0       120       240


DAYS =  7 subtracted from:

DAYS   Lower  Center   Upper  -------+---------+---------+---------+--
14    -46,86   -1,17   44,51              (---*---)
30    115,65  161,33  207,02                          (--*---)
                            -------+---------+---------+---------+--
                                -120         0       120       240


DAYS = 14 subtracted from:

DAYS   Lower  Center   Upper  -------+---------+---------+---------+--
30    116,82  162,50  208,19                          (---*--)
                            -------+---------+---------+---------+--
                                -120         0       120       240
```

One-way ANOVA: S75_AB_400_25°C versus DAYS

```
Source  DF     SS    MS     F      P
DAYS     3  171,5  57,2  2,64  0,121
Error    8  173,3  21,7
Total   11  344,8

S = 4,654   R-Sq = 49,75%   R-Sq(adj) = 30,90%


                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean  StDev  -------+---------+---------+---------+--
 1     3  79,708  7,266  (---------*---------)
 7     3  88,262  2,268             (---------*---------)
14     3  81,190  4,725    (---------*----------)
30     3  87,583  2,526             (---------*---------)
                        -------+---------+---------+---------+--
```

```
                              78,0      84,0      90,0      96,0

Pooled StDev = 4,654


Grouping Information Using Tukey Method

DAYS  N    Mean  Grouping
 7    3  88,262  A
30    3  87,583  A
14    3  81,190  A
 1    3  79,708  A

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


DAYS =  1 subtracted from:

DAYS    Lower  Center   Upper  ---------+---------+---------+---------+
 7     -3,619   8,554  20,727                (------------*-----------)
14    -10,691   1,482  13,655          (-----------*------------)
30     -4,298   7,875  20,048                (-----------*-----------)
                                 ---------+---------+---------+---------+
                                        -10         0        10        20


DAYS =  7 subtracted from:

DAYS    Lower  Center   Upper  ---------+---------+---------+---------+
14    -19,245  -7,072   5,101  (------------*-----------)
30    -12,852  -0,679  11,494        (-----------*-----------)
                                 ---------+---------+---------+---------+
                                       -10         0        10        20


DAYS = 14 subtracted from:

DAYS   Lower  Center   Upper  ---------+---------+---------+---------+
30    -5,780   6,393  18,566               (-----------*------------)
                                 ---------+---------+---------+---------+
                                       -10         0        10        20
```

One-way ANOVA: S75_PB_1300_4°C versus DAYS

```
Source  DF     SS    MS     F      P
DAYS     3  103,1  34,4  1,08  0,411
Error    8  255,0  31,9
Total   11  358,2

S = 5,646   R-Sq = 28,80%   R-Sq(adj) = 2,10%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
```

```
Level  N    Mean  StDev  -----+---------+---------+---------+----
 1     3  76,441  6,917  (-----------*------------)
 7     3  84,087  4,170               (-----------*------------)
14     3  82,999  4,060             (-----------*------------)
30     3  80,711  6,769         (------------*-----------)
                           -----+---------+---------+---------+----
                             72,0      78,0      84,0      90,0

Pooled StDev = 5,646

Grouping Information Using Tukey Method

DAYS  N    Mean  Grouping
 7    3  84,087  A
14    3  82,999  A
30    3  80,711  A
 1    3  76,441  A

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


DAYS =  1 subtracted from:

DAYS    Lower  Center   Upper  -----+---------+---------+---------+----
 7     -7,121   7,647  22,414           (-----------*------------)
14     -8,209   6,558  21,325          (-----------*------------)
30    -10,497   4,271  19,038        (------------*-----------)
                           -----+---------+---------+---------+----
                              -12         0        12        24


DAYS =  7 subtracted from:

DAYS    Lower  Center   Upper  -----+---------+---------+---------+----
14    -15,856  -1,089  13,679    (-----------*-----------)
30    -18,143  -3,376  11,391  (-----------*-----------)
                           -----+---------+---------+---------+----
                              -12         0        12        24


DAYS = 14 subtracted from:

DAYS    Lower  Center   Upper  -----+---------+---------+---------+----
30    -17,055  -2,287  12,480   (-----------*-----------)
                           -----+---------+---------+---------+----
                              -12         0        12        24
```

One-way ANOVA: S75_PB_900_4°C versus DAYS

```
Source  DF     SS    MS     F      P
DAYS     3  106,6  35,5  1,87  0,213
Error    8  152,2  19,0
Total   11  258,8
```

```
S = 4,362   R-Sq = 41,20%   R-Sq(adj) = 19,14%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev  --------+---------+---------+---------+-
 1     3  106,83   5,05  (-----------*----------)
 7     3  111,02   3,88          (-----------*-----------)
14     3  113,19   3,17               (----------*-----------)
30     3  114,76   5,05                  (-----------*----------)
                          --------+---------+---------+---------+-
                              105,0     110,0     115,0     120,0

Pooled StDev = 4,36


Grouping Information Using Tukey Method

DAYS  N     Mean  Grouping
30    3  114,761  A
14    3  113,190  A
 7    3  111,016  A
 1    3  106,828  A

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


DAYS =  1 subtracted from:

DAYS   Lower  Center   Upper  -----+---------+---------+---------+----
 7    -7,219   4,189  15,597          (----------*----------)
14    -5,046   6,362  17,770            (----------*----------)
30    -3,475   7,933  19,341              (----------*----------)
                           -----+---------+---------+---------+----
                              -10         0        10        20


DAYS =  7 subtracted from:

DAYS   Lower  Center   Upper  -----+---------+---------+---------+----
14    -9,235   2,173  13,582       (----------*----------)
30    -7,664   3,745  15,153         (----------*----------)
                           -----+---------+---------+---------+----
                              -10         0        10        20


DAYS = 14 subtracted from:

DAYS   Lower  Center   Upper  -----+---------+---------+---------+----
30    -9,837   1,571  12,979       (----------*----------)
                           -----+---------+---------+---------+----
                              -10         0        10        20
```

One-way ANOVA: S75_AB_400_25°C versus DAYS

```
Source  DF     SS    MS     F      P
DAYS     3  171,5  57,2  2,64  0,121
Error    8  173,3  21,7
Total   11  344,8

S = 4,654   R-Sq = 49,75%   R-Sq(adj) = 30,90%


                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level  N    Mean  StDev  -------+---------+---------+---------+--
 1     3  79,708  7,266  (---------*---------)
 7     3  88,262  2,268               (---------*---------)
14     3  81,190  4,725    (---------*----------)
30     3  87,583  2,526              (---------*---------)
                         -------+---------+---------+---------+--
                             78,0      84,0      90,0      96,0

Pooled StDev = 4,654


Grouping Information Using Tukey Method

DAYS  N    Mean  Grouping
 7    3  88,262  A
30    3  87,583  A
14    3  81,190  A
 1    3  79,708  A

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


DAYS =  1 subtracted from:

DAYS    Lower  Center   Upper  ---------+---------+---------+---------+
 7     -3,619   8,554  20,727                (------------*-----------)
14    -10,691   1,482  13,655          (-----------*------------)
30     -4,298   7,875  20,048                (-----------*-----------)
                                ---------+---------+---------+---------+
                                       -10         0        10        20


DAYS =  7 subtracted from:

DAYS    Lower  Center   Upper  ---------+---------+---------+---------+
14    -19,245  -7,072   5,101  (-----------*-----------)
30    -12,852  -0,679  11,494        (-----------*-----------)
                                ---------+---------+---------+---------+
                                       -10         0        10        20


DAYS = 14 subtracted from:
```

```
DAYS   Lower  Center   Upper  ---------+---------+---------+---------+
30    -5,780   6,393  18,566              (-----------*-----------)
                            ---------+---------+---------+---------+
                                   -10         0        10        20
```

One-way ANOVA: S75_PB_1300_4°C versus DAYS

```
Source  DF     SS    MS     F      P
DAYS     3  103,1  34,4  1,08  0,411
Error    8  255,0  31,9
Total   11  358,2

S = 5,646   R-Sq = 28,80%   R-Sq(adj) = 2,10%
```

```
                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev  -----+---------+---------+---------+----
 1     3  76,441  6,917  (-----------*-----------)
 7     3  84,087  4,170              (-----------*-----------)
14     3  82,999  4,060            (-----------*-----------)
30     3  80,711  6,769         (-----------*-----------)
                          -----+---------+---------+---------+----
                            72,0      78,0      84,0      90,0
```

```
Pooled StDev = 5,646
```

```
Grouping Information Using Tukey Method

DAYS  N    Mean  Grouping
 7    3  84,087  A
14    3  82,999  A
30    3  80,711  A
 1    3  76,441  A

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

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

Individual confidence level = 98,74%
```

```
DAYS =  1 subtracted from:

DAYS    Lower  Center   Upper  -----+---------+---------+---------+----
 7     -7,121   7,647  22,414           (-----------*-----------)
14     -8,209   6,558  21,325         (-----------*-----------)
30    -10,497   4,271  19,038       (-----------*-----------)
                          -----+---------+---------+---------+----
                             -12         0        12        24
```

```
DAYS =  7 subtracted from:

DAYS    Lower  Center   Upper  -----+---------+---------+---------+----
```

```
14    -15,856  -1,089  13,679    (-----------*-----------)
30    -18,143  -3,376  11,391  (-----------*-----------)
                               -----+---------+---------+---------+----
                                  -12         0        12        24


DAYS = 14 subtracted from:

DAYS    Lower  Center   Upper  -----+---------+---------+---------+----
30    -17,055  -2,287  12,480   (-----------*-----------)
                               -----+---------+---------+---------+----
                                  -12         0        12        24
```

One-way ANOVA: S75_PB_900_4°C versus DAYS

```
Source  DF     SS    MS     F      P
DAYS     3  106,6  35,5  1,87  0,213
Error    8  152,2  19,0
Total   11  258,8

S = 4,362   R-Sq = 41,20%   R-Sq(adj) = 19,14%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev  --------+---------+---------+---------+-
 1     3  106,83   5,05  (-----------*----------)
 7     3  111,02   3,88          (-----------*-----------)
14     3  113,19   3,17               (----------*-----------)
30     3  114,76   5,05                  (-----------*----------)
                          --------+---------+---------+---------+-
                              105,0     110,0     115,0     120,0

Pooled StDev = 4,36


Grouping Information Using Tukey Method

DAYS  N     Mean  Grouping
30    3  114,761  A
14    3  113,190  A
 7    3  111,016  A
 1    3  106,828  A

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


DAYS =  1 subtracted from:

DAYS   Lower  Center   Upper  -----+---------+---------+---------+----
 7    -7,219   4,189  15,597          (----------*----------)
14    -5,046   6,362  17,770            (----------*----------)
30    -3,475   7,933  19,341              (----------*----------)
                          -----+---------+---------+---------+----
```

```
                                -10         0        10        20


DAYS =  7 subtracted from:

DAYS   Lower  Center   Upper  -----+---------+---------+---------+----
14    -9,235   2,173  13,582        (----------*----------)
30    -7,664   3,745  15,153         (-----------*----------)
                               -----+---------+---------+---------+----
                                  -10         0        10        20


DAYS = 14 subtracted from:

DAYS   Lower  Center   Upper  -----+---------+---------+---------+----
30    -9,837   1,571  12,979       (-----------*----------)
                               -----+---------+---------+---------+----
                                  -10         0        10        20
```

One-way ANOVA: S75_PB_400_4°C versus DAYS

```
Source  DF      SS     MS     F      P
DAYS     3   504,0  168,0  2,43  0,141
Error    8   553,9   69,2
Total   11  1057,8

S = 8,321   R-Sq = 47,64%   R-Sq(adj) = 28,01%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev   -+---------+---------+---------+--------
 1     3  169,17   9,99            (----------*----------)
 7     3  177,35   4,57                    (----------*----------)
14     3  174,08   8,07                 (----------*----------)
30     3  160,16   9,55   (----------*----------)
                          -+---------+---------+---------+--------
                         150       160       170       180

Pooled StDev = 8,32


Grouping Information Using Tukey Method

DAYS  N     Mean  Grouping
 7    3  177,355  A
14    3  174,076  A
 1    3  169,168  A
30    3  160,164  A

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


DAYS =  1 subtracted from:

DAYS    Lower  Center   Upper  ---------+---------+---------+---------+
```

```
 7    -13,575   8,187  29,949              (----------*----------)
14    -16,854   4,908  26,669            (----------*----------)
30    -30,765  -9,004  12,758       (----------*----------)
                                 ---------+---------+---------+---------+
                                        -20         0        20        40
```

```
DAYS =  7 subtracted from:

DAYS    Lower   Center   Upper  ---------+---------+---------+---------+
14    -25,041   -3,279  18,483        (----------*----------)
30    -38,952  -17,190   4,571  (----------*----------)
                                 ---------+---------+---------+---------+
                                        -20         0        20        40
```

```
DAYS = 14 subtracted from:

DAYS    Lower   Center  Upper  ---------+---------+---------+---------+
30    -35,673  -13,911  7,850   (----------*----------)
                                 ---------+---------+---------+---------+
                                        -20         0        20        40
```

One-way ANOVA: S75_PB_1300_25°C versus DAYS

```
Source  DF      SS      MS      F      P
DAYS     3  4157,2  1385,7  15,62  0,001
Error    8   709,8    88,7
Total   11  4867,0

S = 9,419   R-Sq = 85,42%   R-Sq(adj) = 79,95%
```

```
                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean  StDev  --------+---------+---------+---------+-
 1     3   76,44   6,92  (-----*-----)
 7     3  120,32  13,00                      (-----*-----)
14     3  118,51   7,13                     (-----*------)
30     3  119,37   9,33                     (------*-----)
                        --------+---------+---------+---------+-
                               80       100       120       140

Pooled StDev = 9,42
```

```
Grouping Information Using Tukey Method

DAYS  N     Mean  Grouping
 7    3  120,317  A
30    3  119,375  A
14    3  118,507  A
 1    3   76,441    B

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

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

```
Individual confidence level = 98,74%


DAYS =  1 subtracted from:

DAYS   Lower  Center   Upper   -+---------+---------+---------+--------
 7    19,241  43,876  68,511                    (---------*--------)
14    17,432  42,067  66,701                   (---------*---------)
30    18,299  42,934  67,569                   (---------*---------)
                               -+---------+---------+---------+--------
                              -25         0        25        50


DAYS =  7 subtracted from:

DAYS    Lower  Center   Upper   -+---------+---------+---------+--------
14    -26,444  -1,809  22,826   (---------*---------)
30    -25,577  -0,942  23,693    (---------*--------)
                               -+---------+---------+---------+--------
                              -25         0        25        50


DAYS = 14 subtracted from:

DAYS    Lower  Center   Upper   -+---------+---------+---------+--------
30    -23,767   0,868  25,502    (---------*---------)
                               -+---------+---------+---------+--------
                              -25         0        25        50
```

One-way ANOVA: S75_PB_900_25°C versus DAYS

```
Source  DF      SS     MS      F      P
DAYS     3  2119,6  706,5  34,09  0,000
Error    8   165,8   20,7
Total   11  2285,4

S = 4,552   R-Sq = 92,75%   R-Sq(adj) = 90,03%


                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean  StDev  ------+---------+---------+---------+---
 1     3  106,83   5,05  (----*----)
 7     3  136,54   5,77                        (----*----)
14     3  138,04   3,48                         (----*----)
30     3  137,88   3,46                         (----*----)
                        ------+---------+---------+---------+---
                            108       120       132       144

Pooled StDev = 4,55


Grouping Information Using Tukey Method

DAYS  N     Mean  Grouping
14    3  138,045  A
30    3  137,885  A
 7    3  136,542  A
 1    3  106,828    B
```

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


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

Individual confidence level = 98,74%


DAYS =  1 subtracted from:

DAYS   Lower  Center   Upper  ---------+---------+---------+---------+
 7    17,809  29,715  41,621                      (-------*-------)
14    19,311  31,217  43,123                       (-------*-------)
30    19,151  31,057  42,963                       (-------*-------)
                           ---------+---------+---------+---------+
                                    0        15        30        45


DAYS =  7 subtracted from:

DAYS    Lower  Center   Upper  ---------+---------+---------+---------+
14    -10,403   1,503  13,409    (-------*-------)
30    -10,563   1,343  13,249    (-------*-------)
                           ---------+---------+---------+---------+
                                    0        15        30        45


DAYS = 14 subtracted from:

DAYS    Lower  Center   Upper  ---------+---------+---------+---------+
30    -12,066  -0,160  11,746   (-------*-------)
                           ---------+---------+---------+---------+
                                    0        15        30        45
```

One-way ANOVA: S75_PB_400_25°C versus DAYS

```
Source  DF     SS    MS     F      P
DAYS     3   63,8  21,3  0,28  0,839
Error    8  610,6  76,3
Total   11  674,4

S = 8,737   R-Sq = 9,46%   R-Sq(adj) = 0,00%


                        Individual 95% CIs For Mean Based on Pooled
StDev
Level  N    Mean  StDev     -+---------+---------+---------+--------
 1     3  169,17   9,99              (-------------*-------------)
 7     3  162,93   9,72     (-------------*-------------)
14     3  165,56   6,99        (-------------*-------------)
30     3  164,40   7,89       (-------------*-------------)
                           -+---------+---------+---------+--------
                         152,0     160,0     168,0     176,0

Pooled StDev = 8,74


Grouping Information Using Tukey Method
```

```
DAYS  N     Mean  Grouping
 1    3  169,168  A
14    3  165,558  A
30    3  164,399  A
 7    3  162,933  A

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


DAYS =  1 subtracted from:

DAYS    Lower  Center   Upper  ---------+---------+---------+---------+
 7    -29,085  -6,235  16,615  (--------------*-------------)
14    -26,460  -3,610  19,241   (---------------*--------------)
30    -27,619  -4,769  18,081   (---------------*--------------)
                               ---------+---------+---------+---------+
                                      -15         0        15        30


DAYS =  7 subtracted from:

DAYS    Lower  Center   Upper  ---------+---------+---------+---------+
14    -20,224   2,626  25,476        (--------------*--------------)
30    -21,384   1,466  24,317       (--------------*--------------)
                               ---------+---------+---------+---------+
                                      -15         0        15        30


DAYS = 14 subtracted from:

DAYS    Lower  Center   Upper  ---------+---------+---------+---------+
30    -24,009  -1,159  21,691     (--------------*--------------)
                               ---------+---------+---------+---------+
                                      -15         0        15        30
```

One-way ANOVA: E80_AB_1300_4°C versus DAYS

```
Source  DF      SS     MS     F      P
DAYS     3   94,52  31,51  5,87  0,020
Error    8   42,92   5,36
Total   11  137,43

S = 2,316   R-Sq = 68,77%   R-Sq(adj) = 57,06%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev  -------+---------+---------+---------+--
 1     3  60,057  1,657  (--------*-------)
 7     3  64,832  3,049              (--------*--------)
14     3  67,524  2,778                       (--------*--------)
30     3  66,107  1,304                    (--------*--------)
                         -------+---------+---------+---------+--
                             59,5      63,0      66,5      70,0
```

```
Pooled StDev = 2,316


Grouping Information Using Tukey Method

DAYS  N    Mean  Grouping
14    3  67,524  A
30    3  66,107  A B
 7    3  64,832  A B
 1    3  60,057    B

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


DAYS =  1 subtracted from:

DAYS   Lower  Center   Upper  -----+---------+---------+---------+----
 7    -1,283   4,775  10,832               (---------*---------)
14     1,408   7,466  13,524                   (---------*----------)
30    -0,008   6,050  12,108                 (---------*---------)
                            -----+---------+---------+---------+----
                              -6,0       0,0       6,0      12,0


DAYS =  7 subtracted from:

DAYS   Lower  Center  Upper  -----+---------+---------+---------+----
14    -3,366   2,692  8,750           (----------*----------)
30    -4,782   1,275  7,333         (----------*---------)
                           -----+---------+---------+---------+----
                             -6,0       0,0       6,0      12,0


DAYS = 14 subtracted from:

DAYS   Lower  Center  Upper  -----+---------+---------+---------+----
30    -7,474  -1,416  4,642     (---------*---------)
                           -----+---------+---------+---------+----
                             -6,0       0,0       6,0      12,0
```

One-way ANOVA: E80_AB_900_4°C versus DAYS

```
Source  DF     SS    MS     F      P
DAYS     3  119,6  39,9  1,16  0,383
Error    8  274,8  34,4
Total   11  394,4

S = 5,861   R-Sq = 30,32%   R-Sq(adj) = 4,19%


                        Individual 95% CIs For Mean Based on Pooled
StDev
Level  N   Mean  StDev    -+---------+---------+---------+--------
```

```
 1     3  93,65   1,57        (----------*----------)
 7     3  99,94   9,98                  (----------*----------)
14     3  91,38   2,37    (-----------*----------)
30     3  94,12   5,45        (----------*-----------)
                          -+---------+---------+---------+--------
                        84,0      91,0      98,0     105,0

Pooled StDev = 5,86


Grouping Information Using Tukey Method

DAYS  N    Mean  Grouping
 7    3  99,939  A
30    3  94,115  A
 1    3  93,654  A
14    3  91,384  A

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


DAYS =  1 subtracted from:

DAYS    Lower  Center   Upper    +---------+---------+---------+--------
-
 7     -9,044   6,285  21,614               (------------*------------)
14    -17,598  -2,269  13,059         (------------*------------)
30    -14,867   0,462  15,791            (-----------*------------)
                                  +---------+---------+---------+--------
-
                                -24       -12         0        12


DAYS =  7 subtracted from:

DAYS    Lower  Center  Upper    +---------+---------+---------+---------
14    -23,883  -8,555  6,774    (------------*------------)
30    -21,152  -5,823  9,506      (------------*------------)
                                 +---------+---------+---------+---------
                               -24       -12         0        12


DAYS = 14 subtracted from:

DAYS    Lower  Center   Upper    +---------+---------+---------+--------
-
30    -12,598   2,731  18,060              (-----------*------------)
                                  +---------+---------+---------+--------
-
                                -24       -12         0        12
```

One-way ANOVA: E80_AB_400_4°C versus DAYS

```
Source  DF     SS    MS     F      P
DAYS     3  201,4  67,1  0,97  0,455
Error    8  556,4  69,5
Total   11  757,8

S = 8,339   R-Sq = 26,58%   R-Sq(adj) = 0,00%


                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level  N    Mean  StDev  ---------+---------+---------+---------+
 1     3  154,75   3,14     (----------*----------)
 7     3  162,82  15,22              (----------*----------)
14     3  159,25   1,60          (----------*----------)
30     3  152,16   5,83  (----------*----------)
                         ---------+---------+---------+---------+
                                150       160       170       180

Pooled StDev = 8,34


Grouping Information Using Tukey Method

DAYS  N     Mean  Grouping
 7    3  162,819  A
14    3  159,246  A
 1    3  154,745  A
30    3  152,165  A

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


DAYS =  1 subtracted from:

DAYS    Lower  Center   Upper  ------+---------+---------+---------+---
 7    -13,736   8,074  29,885           (----------*----------)
14    -17,310   4,501  26,312         (----------*----------)
30    -24,391  -2,580  19,231      (----------*----------)
                                ------+---------+---------+---------+---
                                   -20         0        20        40


DAYS =  7 subtracted from:

DAYS    Lower   Center   Upper  ------+---------+---------+---------+---
14    -25,384   -3,573  18,237     (----------*----------)
30    -32,465  -10,654  11,156  (----------*----------)
                                ------+---------+---------+---------+---
                                    -20         0        20        40


DAYS = 14 subtracted from:
```

```
DAYS    Lower  Center   Upper  ------+---------+---------+---------+---
30    -28,892  -7,081  14,730    (---------*----------)
                               ------+---------+---------+---------+---
                                   -20         0        20        40
```

One-way ANOVA: E80_AB_1300_25°C versus DAYS

```
Source  DF      SS     MS     F      P
DAYS     3  112,16  37,39  5,08  0,029
Error    8   58,87   7,36
Total   11  171,03

S = 2,713   R-Sq = 65,58%   R-Sq(adj) = 52,67%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev  ---------+---------+---------+---------+
 1     3  60,057  1,657            (--------*--------)
 7     3  64,182  4,547                     (--------*--------)
14     3  61,811  2,013                (---------*--------)
30     3  55,818  1,400  (--------*--------)
                          ---------+---------+---------+---------+
                                56,0      60,0      64,0      68,0

Pooled StDev = 2,713


Grouping Information Using Tukey Method

DAYS  N    Mean  Grouping
 7    3  64,182  A
14    3  61,811  A B
 1    3  60,057  A B
30    3  55,818    B

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


DAYS =  1 subtracted from:

DAYS    Lower  Center   Upper  ---------+---------+---------+---------+
 7     -2,970   4,124  11,219                (--------*--------)
14     -5,341   1,754   8,848             (--------*--------)
30    -11,334  -4,239   2,855       (--------*--------)
                          ---------+---------+---------+---------+
                               -8,0       0,0       8,0      16,0


DAYS =  7 subtracted from:

DAYS    Lower  Center   Upper  ---------+---------+---------+---------+
14     -9,465  -2,370   4,724         (--------*--------)
30    -15,458  -8,364  -1,269  (--------*-------)
```

```
                                  ---------+---------+---------+---------+
                                        -8,0       0,0       8,0      16,0


DAYS = 14 subtracted from:

DAYS    Lower  Center  Upper  ---------+---------+---------+---------+
30    -13,088  -5,993  1,101     (--------*-------)
                                ---------+---------+---------+---------+
                                      -8,0       0,0       8,0      16,0
```

One-way ANOVA: E80_AB_900_25°C versus DAYS

```
Source  DF     SS     MS     F      P
DAYS     3  402,1  134,0  2,23  0,162
Error    8  481,0   60,1
Total   11  883,1

S = 7,754   R-Sq = 45,53%   R-Sq(adj) = 25,11%


                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level  N    Mean  StDev  -+---------+---------+---------+--------
 1     3   93,65   1,57      (----------*---------)
 7     3  103,70  12,71                (----------*---------)
14     3   89,84   8,53   (---------*---------)
30     3   89,25   1,91  (---------*----------)
                         -+---------+---------+---------+--------
                         80        90       100       110

Pooled StDev = 7,75


Grouping Information Using Tukey Method

DAYS  N     Mean  Grouping
 7    3  103,703  A
 1    3   93,654  A
14    3   89,843  A
30    3   89,252  A

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


DAYS =  1 subtracted from:

DAYS    Lower  Center   Upper  -------+---------+---------+---------+--
 7    -10,230  10,049  30,329              (---------*---------)
14    -24,090  -3,810  16,469       (---------*---------)
30    -24,681  -4,401  15,878       (---------*---------)
                                -------+---------+---------+---------+--
                                     -20         0        20        40
```

```
DAYS =  7 subtracted from:

DAYS    Lower   Center  Upper  -------+---------+---------+---------+--
14    -34,139  -13,860  6,420  (---------*---------)
30    -34,730  -14,451  5,829  (---------*---------)
                                -------+---------+---------+---------+--
                                     -20         0        20        40


DAYS = 14 subtracted from:

DAYS    Lower  Center   Upper  -------+---------+---------+---------+--
30    -20,870  -0,591  19,689         (---------*---------)
                                -------+---------+---------+---------+--
                                     -20         0        20        40
```

One-way ANOVA: E80_AB_400_25°C versus DAYS

```
Source  DF      SS     MS      F      P
DAYS     3  1115,2  371,7  10,22  0,004
Error    8   290,9   36,4
Total   11  1406,1

S = 6,030   R-Sq = 79,31%   R-Sq(adj) = 71,56%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev  -------+---------+---------+---------+--
 1     3  154,75   3,14                          (-------*-------)
 7     3  154,62   7,30                          (-------*-------)
14     3  143,92   3,14                (-------*-------)
30     3  131,28   8,51  (-------*-------)
                          -------+---------+---------+---------+--
                               130       140       150       160

Pooled StDev = 6,03


Grouping Information Using Tukey Method

DAYS  N     Mean  Grouping
 1    3  154,745  A
 7    3  154,621  A
14    3  143,919  A B
30    3  131,279    B

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


DAYS =  1 subtracted from:
```

```
DAYS    Lower   Center   Upper    +---------+---------+---------+-------
--
 7    -15,895   -0,124  15,646               (-------*-------)
14    -26,597  -10,826   4,944           (-------*------)
30    -39,236  -23,466  -7,695    (-------*-------)
                                   +---------+---------+---------+-------
--
                                -40       -20         0        20


DAYS =  7 subtracted from:

DAYS    Lower   Center   Upper    +---------+---------+---------+-------
--
14    -26,473  -10,702   5,069           (-------*-------)
30    -39,112  -23,341  -7,571    (-------*-------)
                                   +---------+---------+---------+-------
--
                                -40       -20         0        20


DAYS = 14 subtracted from:

DAYS    Lower   Center  Upper    +---------+---------+---------+--------
-
30    -28,410  -12,639  3,131          (-------*-------)
                                  +---------+---------+---------+--------
-
                               -40       -20         0        20
```

One-way ANOVA: E80_PB_1300_4°C versus DAYS

```
Source  DF     SS    MS     F      P
DAYS     3  136,5  45,5  1,05  0,421
Error    8  346,1  43,3
Total   11  482,5

S = 6,577   R-Sq = 28,28%   R-Sq(adj) = 1,38%


                        Individual 95% CIs For Mean Based on Pooled
StDev
Level  N    Mean  StDev     +---------+---------+---------+---------
 1     3  81,750  2,629         (------------*-----------)
 7     3  87,068  8,016                   (-----------*------------)
14     3  78,701  2,842     (-----------*------------)
30     3  86,088  9,685               (------------*-----------)
                           +---------+---------+---------+---------
                        70,0      77,0      84,0      91,0

Pooled StDev = 6,577


Grouping Information Using Tukey Method

DAYS  N    Mean  Grouping
 7    3  87,068  A
30    3  86,088  A
 1    3  81,750  A
14    3  78,701  A
```

Means that do not share a letter are significantly different.

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

Individual confidence level = 98,74%

DAYS = 1 subtracted from:

DAYS	Lower	Center	Upper	----+---------+---------+---------+--
7	-11,884	5,318	22,520	(-----------*----------)
14	-20,251	-3,049	14,153	(-----------*----------)
30	-12,864	4,338	21,540	(-----------*----------)
				----+---------+---------+---------+--
				-15 0 15 30

DAYS = 7 subtracted from:

DAYS	Lower	Center	Upper	----+---------+---------+---------+--
14	-25,570	-8,367	8,835	(----------*----------)
30	-18,182	-0,980	16,222	(----------*----------)
				----+---------+---------+---------+--
				-15 0 15 30

DAYS = 14 subtracted from:

DAYS	Lower	Center	Upper	----+---------+---------+---------+--
30	-9,815	7,387	24,590	(-----------*----------)
				----+---------+---------+---------+--
				-15 0 15 30

One-way ANOVA: E80_PB_900_4°C versus DAYS

Source	DF	SS	MS	F	P
DAYS	3	68,6	22,9	0,28	0,837
Error	8	649,5	81,2		
Total	11	718,1			

S = 9,010 R-Sq = 9,56% R-Sq(adj) = 0,00%

Level	N	Mean	StDev	Individual 95% CIs For Mean Based on Pooled StDev
1	3	112,46	4,61	(--------------*--------------)
7	3	118,75	6,57	(--------------*--------------)
14	3	115,22	15,66	(--------------*--------------)
30	3	113,48	3,86	(--------------*--------------)
				----+---------+---------+---------+-----
				104,0 112,0 120,0 128,0

Pooled StDev = 9,01

Grouping Information Using Tukey Method

```
DAYS  N     Mean  Grouping
 7    3  118,752  A
14    3  115,220  A
30    3  113,476  A
 1    3  112,465  A

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


DAYS =  1 subtracted from:

DAYS    Lower  Center   Upper  ---------+---------+---------+---------+
 7    -17,277   6,288  29,853         (---------------*---------------)
14    -20,810   2,755  26,320       (---------------*---------------)
30    -22,554   1,011  24,576     (---------------*--------------)
                                ---------+---------+---------+---------+
                                       -15         0        15        30


DAYS =  7 subtracted from:

DAYS    Lower  Center   Upper  ---------+---------+---------+---------+
14    -27,098  -3,533  20,033   (---------------*--------------)
30    -28,842  -5,277  18,289  (--------------*---------------)
                                ---------+---------+---------+---------+
                                       -15         0        15        30


DAYS = 14 subtracted from:

DAYS    Lower  Center   Upper  ---------+---------+---------+---------+
30    -25,309  -1,744  21,821    (---------------*---------------)
                                ---------+---------+---------+---------+
                                       -15         0        15        30
```

One-way ANOVA: E80_PB_400_4°C versus DAYS

```
Source  DF    SS   MS     F      P
DAYS     3    99   33  0,26  0,852
Error    8  1007  126
Total   11  1106

S = 11,22   R-Sq = 8,92%   R-Sq(adj) = 0,00%


                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean  StDev  ------+---------+---------+---------+---
 1     3  185,04  11,07        (--------------*--------------)
 7     3  179,60  10,51   (--------------*--------------)
14     3  184,87  12,67        (--------------*--------------)
30     3  178,89  10,49  (--------------*--------------)
                        ------+---------+---------+---------+---
```

```
                            170       180       190       200

Pooled StDev = 11,22


Grouping Information Using Tukey Method

DAYS  N    Mean  Grouping
 1    3  185,04  A
14    3  184,87  A
 7    3  179,60  A
30    3  178,89  A

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


DAYS =  1 subtracted from:

DAYS   Lower  Center  Upper  --------+---------+---------+---------+-
 7    -34,79   -5,44  23,90   (-------------*--------------)
14    -29,51   -0,17  29,17     (--------------*--------------)
30    -35,49   -6,15  23,20  (--------------*--------------)
                            --------+---------+---------+---------+-
                                  -20         0        20        40


DAYS =  7 subtracted from:

DAYS   Lower  Center  Upper  --------+---------+---------+---------+-
14    -24,07    5,27  34,62        (--------------*-------------)
30    -30,05   -0,71  28,64     (--------------*-------------)
                            --------+---------+---------+---------+-
                                  -20         0        20        40


DAYS = 14 subtracted from:

DAYS   Lower  Center  Upper  --------+---------+---------+---------+-
30    -35,32   -5,98  23,37  (--------------*--------------)
                            --------+---------+---------+---------+-
                                  -20         0        20        40
```

One-way ANOVA: E80_PB_1300_25°C versus DAYS

```
Source  DF     SS    MS     F      P
DAYS     3  260,4  86,8  1,62  0,261
Error    8  429,6  53,7
Total   11  689,9

S = 7,328   R-Sq = 37,74%   R-Sq(adj) = 14,39%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
```

```
Level  N    Mean   StDev  -------+---------+---------+---------+--
 1     3  81,750   2,629         (-----------*-----------)
 7     3  76,545   6,404  (------------*-----------)
14     3  79,038  11,370      (-----------*-----------)
30     3  88,993   6,130                  (-----------*-----------)
                          -------+---------+---------+---------+--
                              72,0      80,0      88,0      96,0

Pooled StDev = 7,328


Grouping Information Using Tukey Method

DAYS  N    Mean  Grouping
30    3  88,993  A
 1    3  81,750  A
14    3  79,038  A
 7    3  76,545  A

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


DAYS =  1 subtracted from:

DAYS    Lower  Center   Upper    +---------+---------+---------+--------
-
 7    -24,370  -5,206  13,959         (-----------*-----------)
14    -21,877  -2,713  16,452          (-----------*-----------)
30    -11,922   7,242  26,407                (-----------*-----------)
                          +---------+---------+---------+--------
-
                        -32       -16         0        16


DAYS =  7 subtracted from:

DAYS    Lower  Center   Upper    +---------+---------+---------+--------
-
14    -16,672   2,493  21,658             (-----------*-----------)
30     -6,717  12,448  31,613                   (-----------*----------
-)
                          +---------+---------+---------+--------
-
                        -32       -16         0        16


DAYS = 14 subtracted from:

DAYS   Lower  Center   Upper    +---------+---------+---------+---------
30    -9,210   9,955  29,120                (-----------*-----------)
                        +---------+---------+---------+---------
                      -32       -16         0        16
```

One-way ANOVA: E80_PB_900_25°C versus DAYS

```
Source  DF     SS    MS     F      P
DAYS     3   14,6   4,9  0,07  0,976
Error    8  581,5  72,7
Total   11  596,1

S = 8,525   R-Sq = 2,45%   R-Sq(adj) = 0,00%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev  ---------+---------+---------+---------+
 1     3  112,46   4,61     (----------------*---------------)
 7     3  112,89  12,96      (---------------*---------------)
14     3  110,78   4,68   (----------------*---------------)
30     3  110,26   8,92  (----------------*---------------)
                          ---------+---------+---------+---------+
                               105,0     112,0     119,0     126,0

Pooled StDev = 8,53


Grouping Information Using Tukey Method

DAYS  N     Mean  Grouping
 7    3  112,886  A
 1    3  112,465  A
14    3  110,780  A
30    3  110,260  A

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


DAYS =  1 subtracted from:

DAYS    Lower  Center   Upper  -------+---------+---------+---------+--
 7    -21,876   0,421  22,719    (--------------*--------------)
14    -23,982  -1,685  20,613   (--------------*--------------)
30    -24,502  -2,205  20,093   (--------------*-------------)
                               -------+---------+---------+---------+--
                                    -15         0        15        30


DAYS =  7 subtracted from:

DAYS    Lower  Center   Upper  -------+---------+---------+---------+--
14    -24,404  -2,106  20,191   (--------------*-------------)
30    -24,923  -2,626  19,672  (--------------*--------------)
                               -------+---------+---------+---------+--
                                    -15         0        15        30


DAYS = 14 subtracted from:
```

```
DAYS    Lower  Center   Upper  -------+---------+---------+---------+--
30    -22,817  -0,520  21,778    (--------------*--------------)
                                -------+---------+---------+---------+--
                                     -15         0        15        30
```

One-way ANOVA: E80_PB_400_25°C versus DAYS

```
Source  DF    SS   MS     F      P
DAYS     3    47   16  0,13  0,941
Error    8   989  124
Total   11  1036

S = 11,12   R-Sq = 4,56%   R-Sq(adj) = 0,00%


                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean  StDev  ---+---------+---------+---------+------
 1     3  185,04  11,07     (--------------*--------------)
 7     3  185,04  11,07     (--------------*--------------)
14     3  186,92  11,44       (--------------*--------------)
30     3  181,44  10,87  (--------------*--------------)
                        ---+---------+---------+---------+------
                         170       180       190       200

Pooled StDev = 11,12


Grouping Information Using Tukey Method

DAYS  N    Mean  Grouping
14    3  186,92  A
 7    3  185,04  A
 1    3  185,04  A
30    3  181,44  A

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


DAYS =  1 subtracted from:

DAYS   Lower  Center  Upper  -------+---------+---------+---------+--
 7    -29,08    0,00  29,08    (--------------*--------------)
14    -27,20    1,88  30,95     (--------------*--------------)
30    -32,68   -3,60  25,48   (--------------*--------------)
                           -------+---------+---------+---------+--
                                -20         0        20        40


DAYS =  7 subtracted from:

DAYS   Lower  Center  Upper  -------+---------+---------+---------+--
14    -27,20    1,88  30,95     (--------------*--------------)
30    -32,68   -3,60  25,48   (--------------*--------------)
```

```
                              -------+---------+---------+---------+--
                                   -20         0        20        40


DAYS = 14 subtracted from:

DAYS   Lower  Center  Upper  -------+---------+---------+---------+--
30    -34,55   -5,48  23,60  (-------------*--------------)
                              -------+---------+---------+---------+--
                                   -20         0        20        40
```

One-way ANOVA: S75_DW_1300_4°C versus DAYS

```
Source  DF     SS    MS     F      P
DAYS     3  18,43  6,14  1,47  0,294
Error    8  33,41  4,18
Total   11  51,84

S = 2,044   R-Sq = 35,55%   R-Sq(adj) = 11,38%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev  -----+---------+---------+---------+----
 1     3  31,444  0,823  (----------*----------)
 7     3  31,798  0,979   (----------*----------)
14     3  34,473  3,358              (----------*----------)
30     3  31,636  1,948   (----------*---------)
                          -----+---------+---------+---------+----
                            30,0      32,5      35,0      37,5

Pooled StDev = 2,044


Grouping Information Using Tukey Method

DAYS  N    Mean  Grouping
14    3  34,473  A
 7    3  31,798  A
30    3  31,636  A
 1    3  31,444  A

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


DAYS =  1 subtracted from:

DAYS   Lower  Center  Upper  ------+---------+---------+---------+---
 7    -4,991   0,354  5,699        (----------*---------)
14    -2,316   3,029  8,374             (----------*----------)
30    -5,153   0,192  5,537        (---------*----------)
                              ------+---------+---------+---------+---
                                 -5,0       0,0       5,0      10,0
```

```
DAYS =  7 subtracted from:

DAYS   Lower  Center  Upper  ------+---------+---------+---------+---
14    -2,670   2,675  8,020             (---------*----------)
30    -5,508  -0,163  5,183       (----------*---------)
                             ------+---------+---------+---------+---
                                -5,0       0,0       5,0      10,0


DAYS = 14 subtracted from:

DAYS   Lower  Center  Upper  ------+---------+---------+---------+---
30    -8,183  -2,838  2,507  (---------*----------)
                             ------+---------+---------+---------+---
                                -5,0       0,0       5,0      10,0
```

One-way ANOVA: S75_DW_400_4°C versus DAYS

```
Source  DF      SS     MS     F      P
DAYS     3   695,8  231,9  4,45  0,041
Error    8   416,8   52,1
Total   11  1112,6

S = 7,218   R-Sq = 62,54%   R-Sq(adj) = 48,49%


                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level  N    Mean  StDev  ---------+---------+---------+---------+
 1     3  60,962  7,642  (---------*---------)
 7     3  76,549  8,035                (---------*--------)
14     3  81,549  6,429                     (---------*--------)
30     3  74,355  6,642               (--------*---------)
                         ---------+---------+---------+---------+
                                  60        70        80        90

Pooled StDev = 7,218


Grouping Information Using Tukey Method

DAYS  N    Mean  Grouping
14    3  81,549  A
 7    3  76,549  A B
30    3  74,355  A B
 1    3  60,962    B

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


DAYS =  1 subtracted from:

DAYS   Lower  Center   Upper  ---+---------+---------+---------+------
```

```
 7    -3,291  15,587  34,465             (---------*--------)
14     1,709  20,587  39,465                (--------*---------)
30    -5,485  13,394  32,272           (---------*--------)
                           ---+---------+---------+---------+------
                            -20         0        20        40
```

```
DAYS =  7 subtracted from:

DAYS    Lower  Center   Upper  ---+---------+---------+---------+------
14    -13,878   5,000  23,878        (---------*--------)
30    -21,071  -2,193  16,685    (---------*--------)
                           ---+---------+---------+---------+------
                            -20         0        20        40
```

```
DAYS = 14 subtracted from:

DAYS    Lower  Center   Upper  ---+---------+---------+---------+------
30    -26,071  -7,193  11,685  (--------*---------)
                           ---+---------+---------+---------+------
                            -20         0        20        40
```

One-way ANOVA: S75_DW_UTO_4°C versus DAYS

```
Source  DF     SS    MS     F      P
DAYS     3   8194  2731  7,61  0,010
Error    8   2870   359
Total   11  11064

S = 18,94   R-Sq = 74,06%   R-Sq(adj) = 64,34%
```

```
                        Individual 95% CIs For Mean Based on Pooled
StDev
Level  N    Mean  StDev    +---------+---------+---------+---------
 1     3  355,19  23,51                       (-------*--------)
 7     3  340,12  13,73                   (-------*--------)
14     3  297,66  11,99     (-------*--------)
30     3  295,37  23,45    (-------*--------)
                           +---------+---------+---------+---------
                        270       300       330       360

Pooled StDev = 18,94
```

```
Grouping Information Using Tukey Method

DAYS  N    Mean  Grouping
 1    3  355,19  A
 7    3  340,12  A B
14    3  297,66    B
30    3  295,37    B

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

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

```
Individual confidence level = 98,74%


DAYS =  1 subtracted from:

DAYS    Lower  Center   Upper  --------+---------+---------+---------+-
 7     -64,61  -15,07   34,46         (-------*--------)
14    -107,07  -57,53   -8,00  (-------*--------)
30    -109,36  -59,82  -10,28  (-------*-------)
                              --------+---------+---------+---------+-
                                    -60         0        60       120


DAYS =  7 subtracted from:

DAYS   Lower  Center  Upper  --------+---------+---------+---------+-
14    -92,00  -42,46   7,08     (-------*-------)
30    -94,28  -44,75   4,79    (--------*-------)
                             --------+---------+---------+---------+-
                                   -60         0        60       120


DAYS = 14 subtracted from:

DAYS   Lower  Center  Upper  --------+---------+---------+---------+-
30    -51,82   -2,29  47,25           (--------*-------)
                             --------+---------+---------+---------+-
                                   -60         0        60       120
```

One-way ANOVA: S75_DW_1300_25°C versus DAYS

```
Source  DF     SS     MS      F      P
DAYS     3  458,1  152,7  13,52  0,002
Error    8   90,4   11,3
Total   11  548,5

S = 3,361   R-Sq = 83,53%   R-Sq(adj) = 77,35%


                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean  StDev  -----+---------+---------+---------+----
 1     3  31,444  0,823  (------*-------)
 7     3  44,973  3,401                       (-------*------)
14     3  45,925  1,302                         (-------*------)
30     3  46,133  5,589                         (-------*------)
                        -----+---------+---------+---------+----
                          30,0      36,0      42,0      48,0

Pooled StDev = 3,361


Grouping Information Using Tukey Method

DAYS  N    Mean  Grouping
30    3  46,133  A
14    3  45,925  A
 7    3  44,973  A
 1    3  31,444    B
```

Means that do not share a letter are significantly different.

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

Individual confidence level = 98,74%

DAYS = 1 subtracted from:

DAYS	Lower	Center	Upper	+---------+---------+---------+---------
7	4,740	13,529	22,319	(--------*-------)
14	5,691	14,481	23,270	(-------*--------)
30	5,899	14,689	23,479	(--------*-------)
				+---------+---------+---------+---------
				-10 0 10 20

DAYS = 7 subtracted from:

DAYS	Lower	Center	Upper	+---------+---------+---------+---------
14	-7,838	0,952	9,741	(--------*--------)
30	-7,630	1,160	9,949	(--------*--------)
				+---------+---------+---------+---------
				-10 0 10 20

DAYS = 14 subtracted from:

DAYS	Lower	Center	Upper	+---------+---------+---------+---------
30	-8,581	0,208	8,998	(--------*--------)
				+---------+---------+---------+---------
				-10 0 10 20

One-way ANOVA: S75_DW_400_25°C versus DAYS

Source	DF	SS	MS	F	P
DAYS	3	4121,9	1374,0	25,90	0,000
Error	8	424,5	53,1		
Total	11	4546,4			

S = 7,284 R-Sq = 90,66% R-Sq(adj) = 87,16%

Level	N	Mean	StDev	Individual 95% CIs For Mean Based on Pooled StDev
1	3	60,96	7,64	(-----*-----)
7	3	105,31	4,45	(-----*-----)
14	3	102,73	4,91	(-----*-----)
30	3	103,07	10,48	(-----*-----)
				--------+---------+---------+---------+-
				64 80 96 112

Pooled StDev = 7,28

Grouping Information Using Tukey Method

```
DAYS  N     Mean  Grouping
 7    3  105,306  A
30    3  103,070  A
14    3  102,730  A
 1    3   60,962    B

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


DAYS =  1 subtracted from:

DAYS   Lower  Center   Upper  ---------+---------+---------+---------+
 7    25,293  44,344  63,395                    (-------*------)
14    22,718  41,769  60,820                   (-------*------)
30    23,057  42,108  61,159                   (-------*------)
                            ---------+---------+---------+---------+
                                     0        25        50        75


DAYS =  7 subtracted from:

DAYS    Lower  Center   Upper  ---------+---------+---------+---------+
14    -21,626  -2,575  16,475  (-------*-------)
30    -21,287  -2,236  16,815  (-------*-------)
                               ---------+---------+---------+---------+
                                        0        25        50        75


DAYS = 14 subtracted from:

DAYS    Lower  Center   Upper  ---------+---------+---------+---------+
30    -18,711   0,339  19,390    (------*-------)
                               ---------+---------+---------+---------+
                                        0        25        50        75
```

One-way ANOVA: S75_DW_UTO_25°C versus DAYS

```
Source  DF      SS      MS       F      P
DAYS     3  347143  115714  196,47  0,000
Error    8    4712     589
Total   11  351855

S = 24,27   R-Sq = 98,66%   R-Sq(adj) = 98,16%


                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean  StDev  ----+---------+---------+---------+-----
 1     3  355,19  23,51   (--*-)
 7     3  343,97  13,50  (--*-)
14     3  757,56  11,35                                (--*--)
30     3  564,63  38,63                    (--*--)
                        ----+---------+---------+---------+-----
                          360       480       600       720
```

```
Pooled StDev = 24,27


Grouping Information Using Tukey Method

DAYS  N    Mean  Grouping
14    3  757,56  A
30    3  564,63    B
 1    3  355,19      C
 7    3  343,97      C

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


DAYS =  1 subtracted from:

DAYS   Lower  Center   Upper  ---------+---------+---------+---------+
 7    -74,70  -11,22   52,25                (--*-)
14    338,89  402,37  465,84                              (-*--)
30    145,96  209,44  272,91                        (-*--)
                           ---------+---------+---------+---------+
                                 -250         0       250       500


DAYS =  7 subtracted from:

DAYS   Lower  Center   Upper  ---------+---------+---------+---------+
14    350,12  413,59  477,06                                (--*-)
30    157,19  220,66  284,13                        (--*-)
                           ---------+---------+---------+---------+
                                 -250         0       250       500


DAYS = 14 subtracted from:

DAYS    Lower   Center    Upper  ---------+---------+---------+---------
+
30    -256,40  -192,93  -129,46           (-*--)
                                ---------+---------+---------+---------
+
                                     -250         0       250
500
```

One-way ANOVA: E80_DW_1300_4°C versus DAYS

```
Source  DF     SS    MS     F      P
DAYS     3  172,7  57,6  3,48  0,071
Error    8  132,5  16,6
Total   11  305,1

S = 4,069   R-Sq = 56,59%   R-Sq(adj) = 40,31%
```

```
                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev  --------+---------+---------+---------+-
 1     3  45,969  5,230                (--------*--------)
 7     3  45,690  1,925               (--------*--------)
14     3  36,545  3,837  (--------*--------)
30     3  42,988  4,523            (--------*--------)
                          --------+---------+---------+---------+-
                               36,0      42,0      48,0      54,0

Pooled StDev = 4,069


Grouping Information Using Tukey Method

DAYS  N    Mean  Grouping
 1    3  45,969  A
 7    3  45,690  A
30    3  42,988  A
14    3  36,545  A

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


DAYS =  1 subtracted from:

DAYS    Lower  Center   Upper    +---------+---------+---------+--------
-
 7    -10,921  -0,278  10,365             (----------*---------)
14    -20,066  -9,424   1,219    (----------*---------)
30    -13,623  -2,981   7,662          (----------*----------)
                                +---------+---------+---------+--------
-
                              -20       -10         0        10


DAYS =  7 subtracted from:

DAYS    Lower  Center  Upper    +---------+---------+---------+---------
14    -19,788  -9,145  1,497    (----------*---------)
30    -13,345  -2,702  7,940           (---------*----------)
                                +---------+---------+---------+---------
                              -20       -10         0        10


DAYS = 14 subtracted from:

DAYS   Lower  Center   Upper    +---------+---------+---------+---------
30    -4,200   6,443  17,086                   (---------*----------)
                                +---------+---------+---------+---------
                              -20       -10         0        10
```

One-way ANOVA: E80_DW_400_4°C versus DAYS

```
Source  DF      SS     MS     F      P
DAYS     3   826,6  275,5  6,30  0,017
Error    8   349,9   43,7
Total   11  1176,6

S = 6,614   R-Sq = 70,26%   R-Sq(adj) = 59,10%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev  -------+---------+---------+---------+--
 1     3  103,63   9,50                       (--------*-------)
 7     3   85,87   5,08      (--------*--------)
14     3   86,90   3,49       (--------*--------)
30     3   82,02   6,83  (--------*--------)
                          -------+---------+---------+---------+--
                                80        90       100       110

Pooled StDev = 6,61


Grouping Information Using Tukey Method

DAYS  N     Mean  Grouping
 1    3  103,632  A
14    3   86,902  A B
 7    3   85,873    B
30    3   82,019    B

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


DAYS =  1 subtracted from:

DAYS    Lower   Center   Upper  ----+---------+---------+---------+-----
 7    -35,057  -17,759  -0,461    (----------*----------)
14    -34,028  -16,730   0,568     (----------*---------)
30    -38,911  -21,613  -4,315  (---------*----------)
                               ----+---------+---------+---------+-----
                                 -32       -16         0        16


DAYS =  7 subtracted from:

DAYS    Lower  Center   Upper  ----+---------+---------+---------+-----
14    -16,269   1,029  18,327               (----------*---------)
30    -21,152  -3,854  13,444            (----------*---------)
                               ----+---------+---------+---------+-----
                                 -32       -16         0        16


DAYS = 14 subtracted from:
```

```
DAYS    Lower  Center   Upper  ----+---------+---------+---------+-----
30    -22,181  -4,883  12,415            (----------*----------)
                                ----+---------+---------+---------+-----
                                  -32       -16         0        16
```

One-way ANOVA: E80_DW_UTO_4°C versus DAYS

```
Source  DF      SS     MS       F      P
DAYS     3  268770  89590  237,22  0,000
Error    8    3021    378
Total   11  271791

S = 19,43   R-Sq = 98,89%   R-Sq(adj) = 98,47%


                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level  N    Mean  StDev  -----+---------+---------+---------+----
 1     3  677,94  26,77                              (-*--)
 7     3  335,41  11,77   (-*-)
14     3  333,55  10,28   (-*-)
30     3  328,18  23,45  (-*--)
                         -----+---------+---------+---------+----
                            360       480       600       720

Pooled StDev = 19,43


Grouping Information Using Tukey Method

DAYS  N    Mean  Grouping
 1    3  677,94  A
 7    3  335,41    B
14    3  333,55    B
30    3  328,18    B

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


DAYS =  1 subtracted from:

DAYS    Lower   Center    Upper  ---+---------+---------+---------+-----
-
 7    -393,36  -342,53  -291,71  (---*----)
14    -395,22  -344,40  -293,57  (---*----)
30    -400,59  -349,77  -298,94  (---*---)
                                ---+---------+---------+---------+-----
-
                                -360      -240      -120         0


DAYS =  7 subtracted from:

DAYS   Lower  Center  Upper  ---+---------+---------+---------+------
```

```
14    -52,69   -1,86  48,96                              (---*---)
30    -58,06   -7,23  43,59                            (---*----)
                           ---+---------+---------+---------+------
                           -360      -240      -120         0
```

```
DAYS = 14 subtracted from:

DAYS   Lower  Center  Upper  ---+---------+---------+---------+------
30    -56,20   -5,37  45,46                            (----*---)
                           ---+---------+---------+---------+------
                           -360      -240      -120         0
```

One-way ANOVA: E80_DW_1300_25°C versus DAYS

```
Source  DF     SS    MS     F      P
DAYS     3   16,8   5,6  0,24  0,865
Error    8  185,2  23,2
Total   11  202,0

S = 4,811   R-Sq = 8,33%   R-Sq(adj) = 0,00%


                          Individual 95% CIs For Mean Based on Pooled
StDev
Level  N    Mean  StDev    -+---------+---------+---------+--------
 1     3  45,969  5,230    (---------------*---------------)
 7     3  48,237  4,884          (---------------*---------------)
14     3  48,724  1,607           (---------------*---------------)
30     3  48,952  6,230           (---------------*---------------)
                           -+---------+---------+---------+--------
                          40,0      44,0      48,0      52,0

Pooled StDev = 4,811


Grouping Information Using Tukey Method

DAYS  N    Mean  Grouping
30    3  48,952  A
14    3  48,724  A
 7    3  48,237  A
 1    3  45,969  A

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


DAYS =  1 subtracted from:

DAYS    Lower  Center   Upper  -------+---------+---------+---------+--
 7    -10,316   2,268  14,852      (---------------*---------------)
14     -9,829   2,755  15,339       (---------------*---------------)
30     -9,601   2,983  15,567       (---------------*---------------)
                          -------+---------+---------+---------+--
```

```
                                  -8,0       0,0       8,0      16,0


DAYS =  7 subtracted from:

DAYS    Lower  Center   Upper  -------+---------+---------+---------+--
14    -12,097   0,487  13,071    (---------------*--------------)
30    -11,869   0,715  13,299    (---------------*---------------)
                                -------+---------+---------+---------+--
                                  -8,0       0,0       8,0      16,0


DAYS = 14 subtracted from:

DAYS    Lower  Center   Upper  -------+---------+---------+---------+--
30    -12,356   0,228  12,812    (--------------*--------------)
                                -------+---------+---------+---------+--
                                  -8,0       0,0       8,0      16,0
```

One-way ANOVA: E80_DW_400_4°C versus DAYS

```
Source  DF      SS     MS     F      P
DAYS     3   826,6  275,5  6,30  0,017
Error    8   349,9   43,7
Total   11  1176,6

S = 6,614   R-Sq = 70,26%   R-Sq(adj) = 59,10%


                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level  N    Mean  StDev  -------+---------+---------+---------+--
 1     3  103,63   9,50                        (--------*-------)
 7     3   85,87   5,08      (--------*--------)
14     3   86,90   3,49       (--------*--------)
30     3   82,02   6,83  (--------*--------)
                         -------+---------+---------+---------+--
                               80        90       100       110

Pooled StDev = 6,61


Grouping Information Using Tukey Method

DAYS  N     Mean  Grouping
 1    3  103,632  A
14    3   86,902  A B
 7    3   85,873    B
30    3   82,019    B

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


DAYS =  1 subtracted from:
```

```
DAYS    Lower   Center   Upper  ----+---------+---------+---------+-----
 7    -35,057  -17,759  -0,461    (----------*----------)
14    -34,028  -16,730   0,568     (----------*---------)
30    -38,911  -21,613  -4,315  (---------*----------)
                                 ----+---------+---------+---------+-----
                                   -32       -16         0        16


DAYS =  7 subtracted from:

DAYS    Lower  Center   Upper  ----+---------+---------+---------+-----
14    -16,269   1,029  18,327               (----------*---------)
30    -21,152  -3,854  13,444            (----------*---------)
                                 ----+---------+---------+---------+-----
                                   -32       -16         0        16


DAYS = 14 subtracted from:

DAYS    Lower  Center   Upper  ----+---------+---------+---------+-----
30    -22,181  -4,883  12,415           (----------*----------)
                                 ----+---------+---------+---------+-----
                                   -32       -16         0        16
```

One-way ANOVA: E80_DW_UTO_4°C versus DAYS

```
Source  DF      SS     MS       F      P
DAYS     3  268770  89590  237,22  0,000
Error    8    3021    378
Total   11  271791

S = 19,43   R-Sq = 98,89%   R-Sq(adj) = 98,47%


                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level  N    Mean  StDev  -----+---------+---------+---------+----
 1     3  677,94  26,77                              (-*--)
 7     3  335,41  11,77   (-*-)
14     3  333,55  10,28   (-*-)
30     3  328,18  23,45  (-*--)
                         -----+---------+---------+---------+----
                            360       480       600       720

Pooled StDev = 19,43


Grouping Information Using Tukey Method

DAYS  N    Mean  Grouping
 1    3  677,94  A
 7    3  335,41     B
14    3  333,55     B
30    3  328,18     B

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
```

```
All Pairwise Comparisons among Levels of DAYS

Individual confidence level = 98,74%


DAYS =  1 subtracted from:

DAYS    Lower   Center    Upper  ---+---------+---------+---------+-----
-
 7    -393,36  -342,53  -291,71  (---*----)
14    -395,22  -344,40  -293,57  (---*----)
30    -400,59  -349,77  -298,94  (---*---)
                                 ---+---------+---------+---------+-----
-
                                 -360      -240      -120         0


DAYS =  7 subtracted from:

DAYS   Lower  Center  Upper  ---+---------+---------+---------+------
14    -52,69   -1,86  48,96                          (---*---)
30    -58,06   -7,23  43,59                        (---*----)
                           ---+---------+---------+---------+------
                           -360      -240      -120         0


DAYS = 14 subtracted from:

DAYS   Lower  Center  Upper  ---+---------+---------+---------+------
30    -56,20   -5,37  45,46                        (----*---)
                           ---+---------+---------+---------+------
                           -360      -240      -120         0
```

One-way ANOVA: E80_DW_1300_25°C versus DAYS

```
Source  DF     SS    MS     F      P
DAYS     3   16,8   5,6  0,24  0,865
Error    8  185,2  23,2
Total   11  202,0

S = 4,811   R-Sq = 8,33%   R-Sq(adj) = 0,00%


                        Individual 95% CIs For Mean Based on Pooled
StDev
Level  N    Mean  StDev    -+---------+---------+---------+--------
 1     3  45,969  5,230    (---------------*---------------)
 7     3  48,237  4,884          (---------------*---------------)
14     3  48,724  1,607           (---------------*---------------)
30     3  48,952  6,230           (---------------*---------------)
                              -+---------+---------+---------+--------
                            40,0      44,0      48,0      52,0

Pooled StDev = 4,811


Grouping Information Using Tukey Method

DAYS  N    Mean  Grouping
30    3  48,952  A
```

```
14    3  48,724  A
 7    3  48,237  A
 1    3  45,969  A

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


DAYS =  1 subtracted from:

DAYS    Lower  Center   Upper  -------+---------+---------+---------+--
 7    -10,316   2,268  14,852      (---------------*---------------)
14     -9,829   2,755  15,339       (---------------*---------------)
30     -9,601   2,983  15,567       (---------------*---------------)
                                -------+---------+---------+---------+--
                                    -8,0       0,0       8,0      16,0


DAYS =  7 subtracted from:

DAYS    Lower  Center   Upper  -------+---------+---------+---------+--
14    -12,097   0,487  13,071    (---------------*--------------)
30    -11,869   0,715  13,299    (---------------*---------------)
                                -------+---------+---------+---------+--
                                    -8,0       0,0       8,0      16,0


DAYS = 14 subtracted from:

DAYS    Lower  Center   Upper  -------+---------+---------+---------+--
30    -12,356   0,228  12,812    (--------------*---------------)
                                -------+---------+---------+---------+--
                                    -8,0       0,0       8,0      16,0
```

One-way ANOVA: E80_DW_400_25°C versus DAYS

```
Source  DF     SS    MS     F      P
DAYS     3  118,3  39,4  0,84  0,511
Error    8  377,2  47,2
Total   11  495,5

S = 6,867   R-Sq = 23,87%   R-Sq(adj) = 0,00%


                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean  StDev  ------+---------+---------+---------+---
 1     3  103,63   9,50   (------------*------------)
 7     3  103,08   5,50  (------------*------------)
14     3  109,96   7,48            (------------*------------)
30     3  109,25   3,48           (------------*------------)
                        ------+---------+---------+---------+---
                           98,0     105,0     112,0     119,0

Pooled StDev = 6,87
```

```
Grouping Information Using Tukey Method

DAYS  N     Mean  Grouping
14    3  109,961  A
30    3  109,250  A
 1    3  103,632  A
 7    3  103,084  A

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


DAYS =  1 subtracted from:

DAYS    Lower  Center   Upper  -------+---------+---------+---------+--
 7    -18,508  -0,548  17,411       (-----------*-----------)
14    -11,631   6,329  24,288           (-----------*-----------)
30    -12,342   5,618  23,577           (-----------*-----------)
                                -------+---------+---------+---------+--
                                     -15         0        15        30


DAYS =  7 subtracted from:

DAYS    Lower  Center   Upper  -------+---------+---------+---------+--
14    -11,083   6,877  24,837            (-----------*-----------)
30    -11,794   6,166  24,126           (-----------*-----------)
                                -------+---------+---------+---------+--
                                     -15         0        15        30


DAYS = 14 subtracted from:

DAYS    Lower  Center   Upper  -------+---------+---------+---------+--
30    -18,671  -0,711  17,249       (-----------*----------)
                                -------+---------+---------+---------+--
                                     -15         0        15        30
```

One-way ANOVA: E80_DW_UTO_25°C versus DAYS

```
Source  DF      SS     MS       F      P
DAYS     3  160196  53399  179,84  0,000
Error    8    2375    297
Total   11  162572

S = 17,23   R-Sq = 98,54%   R-Sq(adj) = 97,99%


                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean  StDev  ----+---------+---------+---------+-----
 1     3  677,94  26,77                                (-*-)
 7     3  642,88   4,94                             (-*--)
14     3  556,61   8,97                       (--*-)
30     3  379,23  19,13  (-*-)
```

```
                        ----+---------+---------+---------+-----
                          400       500       600       700

Pooled StDev = 17,23


Grouping Information Using Tukey Method

DAYS  N    Mean  Grouping
 1    3  677,94  A
 7    3  642,88  A
14    3  556,61    B
30    3  379,23      C

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,74%


DAYS =  1 subtracted from:

DAYS    Lower   Center    Upper  -------+---------+---------+---------+-
-
 7     -80,13   -35,06    10,00               (-*--)
14    -166,40  -121,34   -76,27           (-*-)
30    -343,78  -298,71  -253,64  (-*-)
                                -------+---------+---------+---------+-
-
                                    -200         0       200       400


DAYS =  7 subtracted from:

DAYS    Lower   Center    Upper  -------+---------+---------+---------+-
-
14    -131,34   -86,27   -41,21            (--*-)
30    -308,72  -263,65  -218,58    (-*-)
                                -------+---------+---------+---------+-
-
                                    -200         0       200       400


DAYS = 14 subtracted from:

DAYS    Lower   Center    Upper  -------+---------+---------+---------+-
-
30    -222,44  -177,38  -132,31        (-*-)
                                -------+---------+---------+---------+-
-
                                    -200         0       200       400
```

Table A.13 Analysis of Variance and Correlation Analysis to Compare the Effect of Microfluidization Pressure and Ultrasonication Time on Zeta Potential, using Adjusted SS for Tests.

```
Source    DF     SS   MS     F      P
Pressure   2    840  420  0,86  0,435
Error     24  11684  487
Total     26  12524

S = 22,06   R-Sq = 6,71%   R-Sq(adj) = 0,00%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev  ------+---------+---------+---------+---
   0   9  -27,50  30,49  (------------*------------)
 400   9  -18,19  18,26           (------------*-----------)
1300   9  -14,18  14,05               (-----------*------------)
                          ------+---------+---------+---------+---
                              -36       -24       -12         0

Pooled StDev = 22,06


Grouping Information Using Tukey Method

Pressure  N    Mean  Grouping
1300      9  -14,18  A
 400      9  -18,19  A
   0      9  -27,50  A

Means that do not share a letter are significantly different.


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

Individual confidence level = 98,02%


Pressure =    0 subtracted from:

Pressure   Lower  Center  Upper  -----+---------+---------+---------+---
-
 400      -16,66    9,31  35,27          (------------*------------)
1300      -12,65   13,32  39,28            (------------*------------)
                                -----+---------+---------+---------+---
-
                                   -20         0        20        40


Pressure =  400 subtracted from:

Pressure   Lower  Center  Upper  -----+---------+---------+---------+---
-
1300      -21,95    4,01  29,97       (------------*------------)
```

```
                                  -----+---------+---------+---------+---
-
                                     -20         0        20        40
```

General Linear Model: ZETA versus Solvent; Pressure

```
Factor    Type   Levels  Values
Solvent   fixed       3  AB; DW; PB
Pressure  fixed       3  0; 400; 1300


Analysis of Variance for ZETA_DAY1, using Adjusted SS for Tests

Source            DF   Seq SS  Adj SS  Adj MS      F      P
Solvent            2   8966,4  8966,4  4483,2  82,35  0,000
Pressure           2    840,0   840,0   420,0   7,71  0,004
Solvent*Pressure   4   1738,2  1738,2   434,5   7,98  0,001
Error             18    979,9   979,9    54,4
Total             26  12524,4


S = 7,37825   R-Sq = 92,18%   R-Sq(adj) = 88,70%


Grouping Information Using Tukey Method and 95,0% Confidence

Solvent  N   Mean  Grouping
PB       9    0,8  A
AB       9  -17,1    B
DW       9  -43,6      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure  N   Mean  Grouping
1300      9  -14,2  A
 400      9  -18,2  A
   0      9  -27,5    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Solvent  Pressure  N   Mean  Grouping
PB          0      3    1,9  A
PB        400      3    1,2  A
PB       1300      3   -0,7  A
AB        400      3  -15,5  A B
AB       1300      3  -17,1  A B
AB          0      3  -18,7  A B
DW       1300      3  -24,8    B C
DW        400      3  -40,2      C
DW          0      3  -65,7        D
```

Means that do not share a letter are significantly different.

General Linear Model: US ZETA_DAY1 versus US Time; Solvent

```
Factor   Type   Levels  Values
US Time  fixed       3  2; 5; 8
Solvent  fixed       3  AB; DW; PB


Analysis of Variance for US ZETA_DAY1, using Adjusted SS for Tests

Source           DF   Seq SS   Adj SS   Adj MS       F      P
US Time           2    16,25    16,25     8,12    0,58  0,570
Solvent           2  6113,94  6113,94  3056,97  218,22  0,000
US Time*Solvent   4   137,98   137,98    34,50    2,46  0,082
Error            18   252,16   252,16    14,01
Total            26  6520,33


S = 3,74283   R-Sq = 96,13%   R-Sq(adj) = 94,41%



Grouping Information Using Tukey Method and 95,0% Confidence

US
Time  N   Mean  Grouping
2     9  -16,3  A
5     9  -17,0  A
8     9  -18,2  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Solvent  N   Mean  Grouping
PB       9   -0,5  A
AB       9  -14,0    B
DW       9  -36,9      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

US
Time  Solvent  N   Mean  Grouping
5     PB       3    0,1  A
2     PB       3   -0,6  A
8     PB       3   -0,9  A
5     AB       3  -12,1    B
8     AB       3  -13,5    B
2     AB       3  -16,5    B
2     DW       3  -31,8      C
5     DW       3  -39,0      C
8     DW       3  -40,1      C
```

Means that do not share a letter are significantly different.

Correlations: US Zeta_Day1; US_ParticleSize_Day1

Pearson correlation of US ZETA_DAY1 and US_ParticleSize_Day1 = 0,812
P-Value = 0,000

Correlations: MF_Zeta_Day1; MF_ParticleSize_Day1

Pearson correlation of MF_Zeta_Day1 and MF_Particle Size_Day1 = 0,156
P-Value = 0,436

Table A.14 Analysis of Variance and Particle Size Correlation for DAY1 T_2, using Adjusted SS for Tests.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pressure	2	43089.0	43089.0	21544.5	588.27	0.000
LType	1	6985.1	6985.1	6985.1	190.73	0.000
Solvent	1	16270.9	16270.9	16270.9	444.28	0.000
Pressure*LType	2	1586.3	1586.3	793.2	21.66	0.000
Pressure*Solvent	2	1455.2	1455.2	727.6	19.87	0.000
LType*Solvent	1	3221.4	3221.4	3221.4	87.96	0.000
Pressure*LType*Solvent	2	693.7	693.7	346.9	9.47	0.001
Error	24	879.0	879.0	36.6		
Total	35	74180.7				

Grouping Information Using Tukey Method and 95.0% Confidence for DAY1 T2

Pressure	N	Mean	Grouping
1300	12	1610.0	A
900	12	1495.7	B
400	12	1167.8	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for DAY1 T2

LType	N	Mean	Grouping
E80	18	1496.3	A
S75	18	1352.7	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for DAY1 T2

Solvent	N	Mean	Grouping
AB	18	1436.1	A
PB	18	1412.9	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for DAY1 T2

Pressure	LType	N	Mean	Grouping
1300	E80	6	1632.9	A
1300	S75	6	1587.2	B
900	E80	6	1583.1	B
900	S75	6	1408.2	C
400	E80	6	1272.9	D
400	S75	6	1062.8	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for DAY1 T2

Pressure	Solvent	N	Mean	Grouping
1300	AB	6	1718.1	A
1300	PB	6	1501.9	B
900	AB	6	1496.5	B
900	PB	6	1494.9	B
400	PB	6	1241.9	C
400	AB	6	1093.7	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for DAY1 T2

LType	Solvent	N	Mean	Grouping
E80	AB	9	1520.7	A
E80	PB	9	1471.9	B
S75	PB	9	1354.0	C
S75	AB	9	1351.5	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for DAY1 T2

Pressure	LType	Solvent	N	Mean	Grouping
1300	E80	AB	3	1741.8	A
1300	S75	AB	3	1694.4	A
900	E80	AB	3	1593.3	B
900	E80	PB	3	1572.9	B
1300	E80	PB	3	1523.9	B C
1300	S75	PB	3	1479.9	C D
900	S75	PB	3	1416.8	D E
900	S75	AB	3	1399.7	E
400	E80	PB	3	1318.7	F
400	E80	AB	3	1227.0	G
400	S75	PB	3	1165.2	G
400	S75	AB	3	960.4	H

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for DAY1 PZL

Pressure	N	Mean	Grouping
400	12	147.2	A
900	12	90.7	B
1300	12	64.2	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for DAY1 PZL

LType	N	Mean	Grouping
E80	18	114.6	A
S75	18	86.8	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for DAY1
PZL

Solvent	N	Mean	Grouping
PB	18	121.9	A
AB	18	79.4	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for DAY1
PZL

Pressure	LType	N	Mean	Grouping
400	E80	6	169.9	A
400	S75	6	124.4	B
900	E80	6	103.1	C
900	S75	6	78.3	D
1300	E80	6	70.9	D
1300	S75	6	57.5	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for DAY1
PZL

Pressure	Solvent	N	Mean	Grouping
400	PB	6	177.1	A
400	AB	6	117.2	B
900	PB	6	109.6	B
1300	PB	6	79.1	C
900	AB	6	71.7	C
1300	AB	6	49.3	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for DAY1
PZL

LType	Solvent	N	Mean	Grouping
E80	PB	9	126.4	A
S75	PB	9	117.5	B
E80	AB	9	102.8	C
S75	AB	9	56.0	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence for DAY1
PZL

Pressure	LType	Solvent	N	Mean	Grouping
400	E80	PB	3	185.0	A
400	S75	PB	3	169.2	A B
400	E80	AB	3	154.7	B
900	E80	PB	3	112.5	C
900	S75	PB	3	106.8	C D
900	E80	AB	3	93.7	D E

1300	E80	PB	3	81.8	E
400	S75	AB	3	79.7	E
1300	S75	PB	3	76.4	E F
1300	E80	AB	3	60.1	F G
900	S75	AB	3	49.8	G H
1300	S75	AB	3	38.6	H

Means that do not share a letter are significantly different.

Correlations: DAY1 T2; DAY1 Particle Size for S75_AB

Pearson correlation of DAY1 T2 and DAY1 PZL = -0.958
P-Value = 0.000

Correlations: DAY1 T2; DAY1 Particle Size for S75_PB

Pearson correlation of DAY1 T2 and DAY1 PZL = -0.955
P-Value = 0.000

Correlations: DAY1 T2; DAY1 Particle Size for E80_AB

Pearson correlation of DAY1 T2 and DAY1 PZL = -0.985
P-Value = 0.000

Correlations: DAY1 T2; DAY1 Particle Size for E80_PB

Pearson correlation of DAY1 T2 and DAY1 PZL = -0.861
P-Value = 0.003

Table A.15 Analysis of Variance for T_2 Times with respect to Temperature for 400 Bar Microfluidized Samples, using Adjusted SS for Tests.

One-way ANOVA: S75_AB_4°C versus Time

```
Source  DF     SS     MS      F      P
Time     7  91438  13063  90,94  0,000
Error   16   2298    144
Total   23  93736

S = 11,99   R-Sq = 97,55%   R-Sq(adj) = 96,48%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev  -----+---------+---------+---------+----
 1     3   960,4   11,6  (-*-)
 4     3  1019,8   16,0           (-*-)
 7     3  1046,3   15,6              (-*--)
10     3  1038,2    9,1             (-*-)
14     3  1089,9   13,9                      (-*-)
21     3  1111,2    7,7                         (-*-)
28     3  1109,6    2,7                        (--*-)
35     3  1173,6   13,1                                 (-*-)
                          -----+---------+---------+---------+----
                             980      1050      1120      1190

Pooled StDev = 12,0


Grouping Information Using Tukey Method

Time  N     Mean  Grouping
35    3  1173,56  A
21    3  1111,20     B
28    3  1109,55     B
14    3  1089,93     B
 7    3  1046,27       C
10    3  1038,23       C
 4    3  1019,80       C
 1    3   960,37         D

Means that do not share a letter are significantly different.


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

Individual confidence level = 99,68%


Time =  1 subtracted from:

Time   Lower  Center   Upper  ------+---------+---------+---------+---
 4     25,52   59,42   93,33                    (--*--)
 7     51,99   85,90  119,81                      (--*--)
10     43,96   77,86  111,77                      (-*--)
14     95,66  129,56  163,47                          (--*--)
```

```
21    116,92  150,83  184,73                          (--*-)
28    115,27  149,18  183,09                          (-*--)
35    179,28  213,19  247,09                               (--*--)
                              ------+---------+---------+---------+---
                                 -120         0       120       240
```

Time = 4 subtracted from:

```
Time   Lower  Center   Upper  ------+---------+---------+---------+---
 7     -7,43   26,48   60,38               (--*--)
10    -15,47   18,44   52,35               (--*-)
14     36,23   70,14  104,04                   (--*--)
21     57,50   91,40  125,31                     (--*-)
28     55,85   89,76  123,66                     (-*--)
35    119,86  153,76  187,67                          (--*--)
                              ------+---------+---------+---------+---
                                 -120         0       120       240
```

Time = 7 subtracted from:

```
Time   Lower  Center   Upper  ------+---------+---------+---------+---
10    -41,94   -8,04   25,87            (-*--)
14      9,76   43,66   77,57                (--*-)
21     31,02   64,93   98,83                  (-*--)
28     29,37   63,28   97,19                 (--*--)
35     93,38  127,29  161,19                       (--*-)
                              ------+---------+---------+---------+---
                                 -120         0       120       240
```

Time = 10 subtracted from:

```
Time   Lower  Center   Upper  ------+---------+---------+---------+---
14     17,79   51,70   85,60                (--*--)
21     39,06   72,96  106,87                  (--*--)
28     37,41   71,32  105,22                  (--*--)
35    101,42  135,33  169,23                       (--*--)
                              ------+---------+---------+---------+---
                                 -120         0       120       240
```

Time = 14 subtracted from:

```
Time   Lower  Center   Upper  ------+---------+---------+---------+---
21    -12,64   21,27   55,17               (--*--)
28    -14,29   19,62   53,52               (--*-)
35     49,72   83,63  117,53                    (--*--)
                              ------+---------+---------+---------+---
                                 -120         0       120       240
```

Time = 21 subtracted from:

```
Time   Lower  Center  Upper  ------+---------+---------+---------+---
28    -35,55   -1,65  32,26            (--*--)
35     28,45   62,36  96,27                 (--*--)
                              ------+---------+---------+---------+---
                                 -120         0       120       240
```

```
Time = 28 subtracted from:

Time  Lower  Center  Upper  ------+---------+---------+---------+---
35    30,10   64,01  97,91                    (-*--)
                            ------+---------+---------+---------+---
                               -120         0       120       240
```

One-way ANOVA: S75_PB_4°C versus Time

```
Source  DF      SS     MS      F      P
Time     7  610912  87273  78,30  0,000
Error   16   17835   1115
Total   23  628747

S = 33,39   R-Sq = 97,16%   R-Sq(adj) = 95,92%


                         Individual 95% CIs For Mean Based on Pooled
StDev
Level  N    Mean  StDev     +---------+---------+---------+---------
 1     3  1165,2   17,9     (--*-)
 4     3  1210,1   17,7        (--*-)
 7     3  1292,1    8,5             (--*-)
10     3  1338,3   47,3                (--*-)
14     3  1715,1   64,6                                      (-*--)
21     3  1468,6   11,7                        (--*-)
28     3  1384,7   13,2                    (--*-)
35     3  1359,4   38,5                 (--*--)
                      +---------+---------+---------+---------
                     1120      1280      1440      1600

Pooled StDev = 33,4


Grouping Information Using Tukey Method

Time  N     Mean  Grouping
14    3  1715,06  A
21    3  1468,65     B
28    3  1384,74     B C
35    3  1359,38       C
10    3  1338,28       C
 7    3  1292,12       C D
 4    3  1210,10         D E
 1    3  1165,17           E

Means that do not share a letter are significantly different.


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

Individual confidence level = 99,68%


Time =  1 subtracted from:

Time   Lower  Center   Upper  -------+---------+---------+---------+--
 4    -49,52   44,93  139,38                   (-*--)
```

```
 7     32,50  126,95  221,40                    (--*-)
10     78,66  173,11  267,56                     (--*--)
14    455,44  549,89  644,34                               (--*-)
21    209,03  303,48  397,93                          (--*-)
28    125,12  219,57  314,03                        (-*--)
35     99,76  194,22  288,67                       (--*-)
                              -------+---------+---------+---------+--
                                  -350         0       350       700
```

Time = 4 subtracted from:

```
Time   Lower  Center   Upper  -------+---------+---------+---------+--
 7    -12,43   82,02  176,47                   (-*--)
10     33,73  128,18  222,63                    (--*-)
14    410,51  504,96  599,41                              (-*--)
21    164,10  258,55  353,01                         (-*--)
28     80,20  174,65  269,10                      (--*--)
35     54,84  149,29  243,74                      (-*--)
                              -------+---------+---------+---------+--
                                  -350         0       350       700
```

Time = 7 subtracted from:

```
Time   Lower  Center   Upper  -------+---------+---------+---------+--
10    -48,29   46,16  140,61                  (-*--)
14    328,49  422,94  517,39                            (--*--)
21     82,08  176,53  270,98                     (--*--)
28     -1,83   92,63  187,08                   (--*-)
35    -27,18   67,27  161,72                  (--*--)
                              -------+---------+---------+---------+--
                                  -350         0       350       700
```

Time = 10 subtracted from:

```
Time   Lower  Center   Upper  -------+---------+---------+---------+--
14    282,33  376,78  471,23                           (--*-)
21     35,92  130,37  224,82                    (--*-)
28    -47,99   46,46  140,92                  (-*--)
35    -73,35   21,11  115,56                 (--*-)
                              -------+---------+---------+---------+--
                                  -350         0       350       700
```

Time = 14 subtracted from:

```
Time    Lower   Center    Upper  -------+---------+---------+---------+-
-
21    -340,86  -246,41  -151,96         (--*--)
28    -424,77  -330,32  -235,87       (--*-)
35    -450,13  -355,68  -261,22      (--*--)
                                 -------+---------+---------+---------+-
-
                                     -350         0       350       700
```

Time = 21 subtracted from:

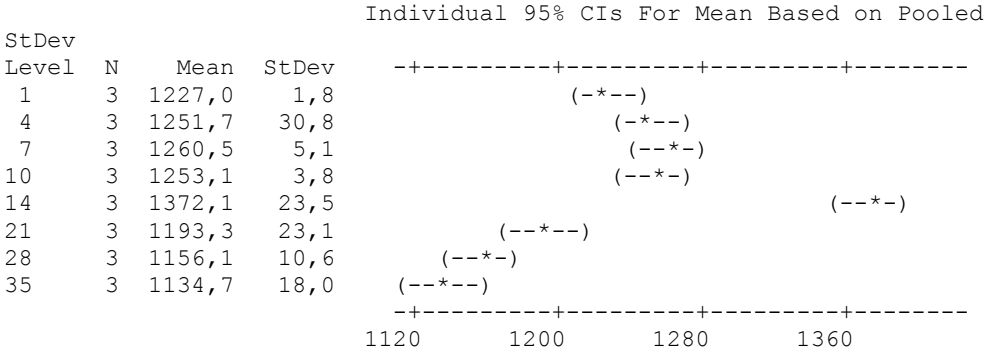
```
Time    Lower   Center   Upper  -------+---------+---------+---------+--
```

```
28    -178,36   -83,91   10,54              (--*-)
35    -203,72  -109,27  -14,81             (--*--)
                                 -------+---------+---------+---------+--
                                     -350         0       350       700
```

```
Time = 28 subtracted from:

Time    Lower  Center  Upper  -------+---------+---------+---------+--
35    -119,81  -25,36  69,09               (-*--)
                               -------+---------+---------+---------+--
                                   -350         0       350       700
```

One-way ANOVA: E80_AB_4°C versus Time

```
Source  DF      SS     MS      F      P
Time     7  114092  16299  51,83  0,000
Error   16    5031    314
Total   23  119124

S = 17,73   R-Sq = 95,78%   R-Sq(adj) = 93,93%
```




```
Pooled StDev = 17,7
```

```
Grouping Information Using Tukey Method

Time  N     Mean  Grouping
14    3  1372,13  A
 7    3  1260,50    B
10    3  1253,14    B
 4    3  1251,65    B
 1    3  1227,05    B C
21    3  1193,31      C D
28    3  1156,11        D E
35    3  1134,67          E

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

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

Individual confidence level = 99,68%
```

Time = 1 subtracted from:

Time	Lower	Center	Upper	---------+---------+---------+---------+
4	-25,56	24,61	74,77	(---*--)
7	-16,71	33,45	83,62	(--*---)
10	-24,07	26,10	76,26	(---*--)
14	94,92	145,09	195,25	(---*--)
21	-83,90	-33,74	16,43	(---*--)
28	-121,10	-70,94	-20,77	(--*---)
35	-142,55	-92,38	-42,21	(---*--)
				---------+---------+---------+---------+
				-150 0 150 300

Time = 4 subtracted from:

Time	Lower	Center	Upper	---------+---------+---------+---------+
7	-41,32	8,85	59,01	(---*--)
10	-48,68	1,49	51,65	(--*--)
14	70,31	120,48	170,65	(--*--)
21	-108,51	-58,35	-8,18	(--*--)
28	-145,71	-95,54	-45,38	(---*--)
35	-167,15	-116,99	-66,82	(--*---)
				---------+---------+---------+---------+
				-150 0 150 300

Time = 7 subtracted from:

Time	Lower	Center	Upper	---------+---------+---------+---------+
10	-57,53	-7,36	42,81	(---*--)
14	61,47	111,63	161,80	(--*---)
21	-117,36	-67,19	-17,03	(---*--)
28	-154,56	-104,39	-54,22	(--*--)
35	-176,00	-125,84	-75,67	(---*--)
				---------+---------+---------+---------+
				-150 0 150 300

Time = 10 subtracted from:

Time	Lower	Center	Upper	---------+---------+---------+---------+
14	68,83	118,99	169,16	(--*--)
21	-110,00	-59,83	-9,67	(--*--)
28	-147,20	-97,03	-46,87	(---*--)
35	-168,64	-118,48	-68,31	(--*--)
				---------+---------+---------+---------+
				-150 0 150 300

Time = 14 subtracted from:

Time	Lower	Center	Upper	---------+---------+---------+---------+
21	-228,99	-178,83	-128,66	(--*--)
28	-266,19	-216,02	-165,86	(---*--)
35	-287,63	-237,47	-187,30	(--*---)
				---------+---------+---------+---------

```
                                    -150         0       150
300


Time = 21 subtracted from:

Time    Lower  Center  Upper  ---------+---------+---------+---------+
28     -87,36  -37,20  12,97               (---*--)
35    -108,81  -58,64  -8,48             (--*--)
                            ---------+---------+---------+---------+
                                  -150         0       150       300


Time = 28 subtracted from:

Time   Lower  Center  Upper  ---------+---------+---------+---------+
35    -71,61  -21,44  28,72               (---*--)
                            ---------+---------+---------+---------+
                                  -150         0       150       300
```

One-way ANOVA: E80_PB_4°C versus Time

```
Source  DF      SS     MS     F      P
Time     7  238328  34047  9,11  0,000
Error   16   59818   3739
Total   23  298147

S = 61,14   R-Sq = 79,94%   R-Sq(adj) = 71,16%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev  -------+---------+---------+---------+--
 1     3  1318,7   17,0  (----*----)
 4     3  1365,7   35,8     (----*----)
 7     3  1499,3   15,1              (----*----)
10     3  1471,7   27,8            (----*----)
14     3  1662,6  157,2                        (----*----)
21     3  1541,5   45,5                 (----*----)
28     3  1436,8   23,5          (----*----)
35     3  1429,4    3,8         (----*----)
                          -------+---------+---------+---------+--
                              1350      1500      1650      1800

Pooled StDev = 61,1


Grouping Information Using Tukey Method

Time  N     Mean  Grouping
14    3  1662,59  A
21    3  1541,52  A B
 7    3  1499,27  A B C
10    3  1471,72    B C D
28    3  1436,81    B C D
35    3  1429,42    B C D
 4    3  1365,72      C D
 1    3  1318,67        D

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

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

Individual confidence level = 99,68%

Time = 1 subtracted from:

Time	Lower	Center	Upper	---------+---------+---------+---------+
4	-125,93	47,05	220,02	(------*------)
7	7,62	180,60	353,58	(------*------)
10	-19,93	153,05	326,03	(------*------)
14	170,94	343,92	516,90	(------*------)
21	49,87	222,85	395,83	(------*------)
28	-54,84	118,14	291,11	(------*------)
35	-62,23	110,75	283,73	(-----*------)
				---------+---------+---------+---------+
				-250 0 250 500

Time = 4 subtracted from:

Time	Lower	Center	Upper	---------+---------+---------+---------+
7	-39,43	133,55	306,53	(------*------)
10	-66,97	106,00	278,98	(------*------)
14	123,89	296,87	469,85	(------*------)
21	2,82	175,80	348,78	(------*------)
28	-101,89	71,09	244,07	(------*------)
35	-109,28	63,70	236,68	(------*-----)
				---------+---------+---------+---------+
				-250 0 250 500

Time = 7 subtracted from:

Time	Lower	Center	Upper	---------+---------+---------+---------+
10	-200,53	-27,55	145,43	(------*------)
14	-9,66	163,32	336,30	(------*-----)
21	-130,73	42,25	215,23	(------*------)
28	-235,44	-62,46	110,52	(------*-----)
35	-242,83	-69,85	103,13	(------*------)
				---------+---------+---------+---------+
				-250 0 250 500

Time = 10 subtracted from:

Time	Lower	Center	Upper	---------+---------+---------+---------+
14	17,89	190,87	363,85	(------*------)
21	-103,18	69,80	242,78	(------*------)
28	-207,89	-34,91	138,07	(------*------)
35	-215,28	-42,30	130,68	(------*------)
				---------+---------+---------+---------+
				-250 0 250 500

Time = 14 subtracted from:

Time	Lower	Center	Upper	---------+---------+---------+---------+

```
21    -294,05  -121,07   51,91          (------*------)
28    -398,76  -225,78  -52,80      (------*------)
35    -406,15  -233,17  -60,19      (------*------)
                                 ---------+---------+---------+---------+
                                       -250         0       250       500


Time = 21 subtracted from:

Time    Lower   Center  Upper  ---------+---------+---------+---------+
28    -277,69  -104,71  68,27          (------*------)
35    -285,08  -112,10  60,88          (------*-----)
                                 ---------+---------+---------+---------+
                                       -250         0       250       500


Time = 28 subtracted from:

Time    Lower  Center   Upper  ---------+---------+---------+---------+
35    -180,37   -7,39  165,59              (------*------)
                                 ---------+---------+---------+---------+
                                       -250         0       250       500
```

One-way ANOVA: S75_AB_25°C versus Time

```
Source  DF     SS    MS      F      P
Time     7  49152  7022  42,47  0,000
Error   16   2645   165
Total   23  51797

S = 12,86   R-Sq = 94,89%   R-Sq(adj) = 92,66%


                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean  StDev  ------+---------+---------+---------+---
 1     3  1133,4    6,8  (--*--)
 4     3  1234,9    7,3                     (--*--)
 7     3  1244,0    6,7                       (--*--)
10     3  1260,6    7,9                          (--*--)
14     3  1269,7    9,7                            (--*--)
21     3  1220,6    3,9                  (--*--)
28     3  1221,5   15,4                  (--*--)
35     3  1156,7   27,8      (--*--)
                        ------+---------+---------+---------+---
                           1150      1200      1250      1300

Pooled StDev = 12,9


Grouping Information Using Tukey Method

Time  N     Mean  Grouping
14    3  1269,67  A
10    3  1260,62  A
 7    3  1244,02  A B
 4    3  1234,93  A B
28    3  1221,49    B
21    3  1220,56    B
35    3  1156,71      C
```

```
 1    3  1133,39      C

Means that do not share a letter are significantly different.


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

Individual confidence level = 99,68%


Time =  1 subtracted from:

Time   Lower  Center   Upper  -----+---------+---------+---------+----
 4     65,17  101,54  137,92                       (--*---)
 7     74,26  110,63  147,01                       (---*---)
10     90,86  127,23  163,61                         (---*--)
14     99,90  136,28  172,66                          (---*--)
21     50,79   87,17  123,55                      (---*--)
28     51,72   88,10  124,48                      (---*--)
35    -13,06   23,32   59,69              (--*---)
                           -----+---------+---------+---------+----
                             -100         0       100       200


Time =  4 subtracted from:

Time    Lower  Center   Upper  -----+---------+---------+---------+----
 7     -27,28    9,09   45,47             (---*---)
10     -10,68   25,69   62,07               (---*--)
14      -1,64   34,74   71,11                (--*---)
21     -50,75  -14,37   22,00           (---*--)
28     -49,82  -13,44   22,93           (---*--)
35    -114,60  -78,22  -41,85      (--*---)
                           -----+---------+---------+---------+----
                             -100         0       100       200


Time =  7 subtracted from:

Time    Lower  Center   Upper  -----+---------+---------+---------+----
10     -19,77   16,60   52,98              (---*--)
14     -10,73   25,65   62,02               (---*--)
21     -59,84  -23,46   12,91          (---*--)
28     -58,91  -22,53   13,84          (---*--)
35    -123,69  -87,31  -50,94     (--*---)
                           -----+---------+---------+---------+----
                             -100         0       100       200


Time = 10 subtracted from:

Time    Lower   Center   Upper  -----+---------+---------+---------+----
14     -27,33     9,05   45,42             (---*---)
21     -76,44   -40,06   -3,69         (---*---)
28     -75,51   -39,13   -2,76         (---*---)
35    -140,29  -103,92  -67,54   (---*--)
                           -----+---------+---------+---------+----
                             -100         0       100       200
```

```
Time = 14 subtracted from:

Time    Lower   Center   Upper  -----+---------+---------+---------+----
21     -85,49   -49,11  -12,73        (---*---)
28     -84,56   -48,18  -11,80         (--*---)
35    -149,34  -112,96  -76,59  (---*--)
                                -----+---------+---------+---------+----
                                  -100         0       100       200


Time = 21 subtracted from:

Time    Lower  Center   Upper  -----+---------+---------+---------+----
28     -35,45    0,93   37,31             (---*---)
35    -100,23  -63,85  -27,48       (---*--)
                               -----+---------+---------+---------+----
                                 -100         0       100       200


Time = 28 subtracted from:

Time    Lower  Center   Upper  -----+---------+---------+---------+----
35    -101,16  -64,78  -28,41       (---*--)
                               -----+---------+---------+---------+----
                                 -100         0       100       200
```

One-way ANOVA: S75_PB_25°C versus Time

```
Source  DF      SS     MS      F      P
Time     7  195479  27926  23,89  0,000
Error   16   18699   1169
Total   23  214179

S = 34,19   R-Sq = 91,27%   R-Sq(adj) = 87,45%


                        Individual 95% CIs For Mean Based on Pooled
StDev
Level  N    Mean  StDev    -+---------+---------+---------+--------
 1     3  1234,4   15,4    (---*----)
 4     3  1443,4   22,9                       (---*----)
 7     3  1535,6   69,7                                 (----*---)
10     3  1426,2   26,2                     (----*---)
14     3  1520,1   26,1                               (---*---)
21     3  1377,9   32,1                 (---*---)
28     3  1379,0   25,7                 (---*---)
35     3  1478,8   26,0                           (---*---)
                        -+---------+---------+---------+--------
                       1200      1300      1400      1500

Pooled StDev = 34,2


Grouping Information Using Tukey Method

Time  N     Mean  Grouping
 7    3  1535,55  A
14    3  1520,08  A B
35    3  1478,83  A B
 4    3  1443,41  A B C
10    3  1426,25    B C
28    3  1379,01      C
```

```
21    3  1377,92      C
 1    3  1234,39        D

Means that do not share a letter are significantly different.


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

Individual confidence level = 99,68%


Time =  1 subtracted from:

Time   Lower  Center   Upper  ---+---------+---------+---------+------
 4    112,31  209,02  305,73                    (---*----)
 7    204,44  301,16  397,87                        (----*----)
10     95,14  191,86  288,57                   (----*---)
14    188,98  285,69  382,40                       (----*----)
21     46,82  143,53  240,24                (----*----)
28     47,90  144,62  241,33                (----*----)
35    147,72  244,43  341,15                     (----*----)
                           ---+---------+---------+---------+------
                           -200         0       200       400


Time =  4 subtracted from:

Time    Lower  Center   Upper  ---+---------+---------+---------+------
 7      -4,57   92,14  188,85              (----*---)
10    -113,88  -17,16   79,55         (----*----)
14     -20,04   76,67  173,38             (----*----)
21    -162,20  -65,49   31,22       (----*----)
28    -161,11  -64,40   32,31       (----*----)
35     -61,30   35,42  132,13            (----*----)
                           ---+---------+---------+---------+------
                           -200         0       200       400


Time =  7 subtracted from:

Time    Lower   Center   Upper  ---+---------+---------+---------+------
10    -206,02  -109,30  -12,59     (----*---)
14    -112,18   -15,47   81,24         (----*----)
21    -254,34  -157,63  -60,92  (----*----)
28    -253,25  -156,54  -59,83  (----*----)
35    -153,44   -56,72   39,99       (----*----)
                           ---+---------+---------+---------+------
                           -200         0       200       400


Time = 10 subtracted from:

Time    Lower  Center   Upper  ---+---------+---------+---------+------
14      -2,88   93,83  190,55              (----*----)
21    -145,04  -48,33   48,39        (----*---)
28    -143,95  -47,24   49,47        (----*---)
35     -44,13   52,58  149,29             (----*---)
                           ---+---------+---------+---------+------
                           -200         0       200       400
```

```
Time = 14 subtracted from:

Time    Lower   Center   Upper  ---+---------+---------+---------+------
21    -238,87  -142,16  -45,45   (----*----)
28    -237,78  -141,07  -44,36   (----*----)
35    -137,97   -41,25   55,46        (----*----)
                                ---+---------+---------+---------+------
                                -200         0       200       400


Time = 21 subtracted from:

Time   Lower  Center   Upper  ---+---------+---------+---------+------
28    -95,62    1,09   97,80          (----*----)
35      4,19  100,91  197,62               (----*----)
                              ---+---------+---------+---------+------
                              -200         0       200       400


Time = 28 subtracted from:

Time  Lower  Center   Upper  ---+---------+---------+---------+------
35     3,10   99,82  196,53               (----*----)
                            ---+---------+---------+---------+------
                            -200         0       200       400
```

One-way ANOVA: E80_AB_25°C versus Time

```
Source  DF      SS     MS       F      P
Time     7  128860  18409  100,84  0,000
Error   16    2921    183
Total   23  131781

S = 13,51   R-Sq = 97,78%   R-Sq(adj) = 96,81%


                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean  StDev  ----+---------+---------+---------+-----
 1     3  930,15   3,80                           (--*--)
 4     3  953,85  14,77                               (--*--)
 7     3  950,52  28,24                               (-*--)
10     3  878,57  11,41                     (-*--)
14     3  876,91   4,86                    (--*--)
21     3  775,83   6,95   (-*--)
28     3  771,39   3,89  (--*-)
35     3  784,62  14,61    (--*--)
                        ----+---------+---------+---------+-----
                          780       840       900       960

Pooled StDev = 13,51


Grouping Information Using Tukey Method

Time  N    Mean  Grouping
 4    3  953,85  A
 7    3  950,52  A
 1    3  930,15  A
```

```
10    3  878,57    B
14    3  876,91    B
35    3  784,62      C
21    3  775,83      C
28    3  771,39      C

Means that do not share a letter are significantly different.


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

Individual confidence level = 99,68%


Time =  1 subtracted from:

Time    Lower   Center    Upper  --------+---------+---------+---------
+-
 4     -14,52    23,71    61,93                   (--*--)
 7     -17,85    20,37    58,60                   (--*--)
10     -89,80   -51,58   -13,35             (--*--)
14     -91,45   -53,23   -15,01            (---*--)
21    -192,54  -154,32  -116,09    (--*--)
28    -196,98  -158,76  -120,54    (--*--)
35    -183,75  -145,53  -107,30     (--*--)
                                  --------+---------+---------+---------
+-
                                       -120         0       120       240


Time =  4 subtracted from:

Time    Lower   Center    Upper  --------+---------+---------+---------
+-
 7     -41,56    -3,33    34,89                 (--*--)
10    -113,51   -75,28   -37,06           (--*--)
14    -115,16   -76,94   -38,71          (---*--)
21    -216,25  -178,02  -139,80  (--*--)
28    -220,69  -182,47  -144,24  (--*--)
35    -207,46  -169,23  -131,01   (--*--)
                                  --------+---------+---------+---------
+-
                                       -120         0       120       240


Time =  7 subtracted from:

Time    Lower   Center    Upper  --------+---------+---------+---------
+-
10    -110,17   -71,95   -33,73           (--*--)
14    -111,83   -73,60   -35,38           (--*--)
21    -212,91  -174,69  -136,46  (--*---)
28    -217,36  -179,13  -140,91  (--*--)
35    -204,12  -165,90  -127,67   (--*--)
                                  --------+---------+---------+---------
+-
                                       -120         0       120       240


Time = 10 subtracted from:
```

```
Time    Lower   Center   Upper  --------+---------+---------+---------+-
14     -39,88    -1,65   36,57                (--*--)
21    -140,96  -102,74  -64,51        (--*---)
28    -145,41  -107,18  -68,96        (--*--)
35    -132,17   -93,95  -55,72         (--*--)
                                --------+---------+---------+---------+-
                                     -120         0       120       240
```

```
Time = 14 subtracted from:

Time    Lower   Center   Upper  --------+---------+---------+---------+-
21    -139,31  -101,08  -62,86        (---*--)
28    -143,75  -105,53  -67,30        (--*--)
35    -130,52   -92,30  -54,07         (--*--)
                                --------+---------+---------+---------+-
                                     -120         0       120       240
```

```
Time = 21 subtracted from:

Time   Lower  Center  Upper  --------+---------+---------+---------+-
28    -42,67   -4,44  33,78               (---*--)
35    -29,43    8,79  47,01                 (--*--)
                           --------+---------+---------+---------+-
                                -120         0       120       240
```

```
Time = 28 subtracted from:

Time   Lower  Center  Upper  --------+---------+---------+---------+-
35    -24,99   13,23  51,46                 (--*--)
                           --------+---------+---------+---------+-
                                -120         0       120       240
```

One-way ANOVA: E80_PB_25°C versus Time

```
Source  DF      SS     MS      F      P
Time     7  624954  89279  10,17  0,000
Error   16  140408   8775
Total   23  765362

S = 93,68   R-Sq = 81,65%   R-Sq(adj) = 73,63%
```

```
                        Individual 95% CIs For Mean Based on
                        Pooled StdDev
Level  N    Mean  StdDev  ----+---------+---------+---------+-----
 1     3  1225,8   17,4  (----*-----)
 4     3  1446,9   28,2            (----*-----)
 7     3  1694,4   72,0                      (-----*----)
10     3  1610,2  161,7                   (-----*----)
14     3  1763,7  146,6                         (-----*-----)
21     3  1598,6  124,6                   (-----*-----)
28     3  1432,9   20,5             (-----*----)
35     3  1447,9   18,2              (----*-----)
                       ----+---------+---------+---------+-----
                        1200      1400      1600      1800
```

Pooled StDev = 93,7

Grouping Information Using Tukey Method

Time	N	Mean	Grouping
14	3	1763,66	A
7	3	1694,41	A B
10	3	1610,21	A B
21	3	1598,57	A B
35	3	1447,87	B C
4	3	1446,93	B C
28	3	1432,87	B C
1	3	1225,83	C

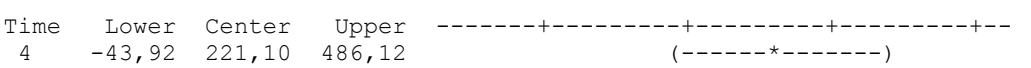
Means that do not share a letter are significantly different.

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

Individual confidence level = 99,68%

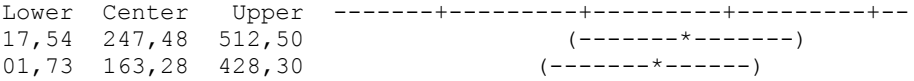
Time = 1 subtracted from:

Time	Lower	Center	Upper
4	-43,92	221,10	486,12
7	203,56	468,58	733,60
10	119,37	384,38	649,40
14	272,82	537,84	802,85
21	107,73	372,74	637,76
28	-57,97	207,04	472,06
35	-42,97	222,04	487,06



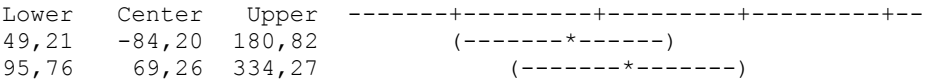
Time = 4 subtracted from:

Time	Lower	Center	Upper
7	-17,54	247,48	512,50
10	-101,73	163,28	428,30
14	51,72	316,74	581,75
21	-113,37	151,64	416,66
28	-279,07	-14,06	250,96
35	-264,07	0,94	265,96



Time = 7 subtracted from:

Time	Lower	Center	Upper
10	-349,21	-84,20	180,82
14	-195,76	69,26	334,27
21	-360,85	-95,84	169,18
28	-526,55	-261,54	3,48
35	-511,55	-246,54	18,48



```
Time = 10 subtracted from:

Time    Lower   Center   Upper  -------+---------+---------+---------+--
14    -111,56   153,45  418,47               (------*-------)
21    -276,66   -11,64  253,37          (-------*------)
28    -442,36  -177,34   87,67      (--------*-------)
35    -427,36  -162,34  102,68       (-------*-------)
                                -------+---------+---------+---------+--
                                    -350         0       350       700


Time = 14 subtracted from:

Time    Lower   Center   Upper  -------+---------+---------+---------+--
21    -430,11  -165,10   99,92       (-------*-------)
28    -595,81  -330,80  -65,78  (--------*------)
35    -580,81  -315,79  -50,78  (--------*-------)
                                -------+---------+---------+---------+--
                                    -350         0       350       700


Time = 21 subtracted from:

Time    Lower   Center   Upper  -------+---------+---------+---------+--
28    -430,72  -165,70   99,31       (-------*-------)
35    -415,71  -150,70  114,32       (--------*------)
                                -------+---------+---------+---------+--
                                    -350         0       350       700


Time = 28 subtracted from:

Time    Lower  Center   Upper  -------+---------+---------+---------+--
35    -250,01   15,00  280,02            (------*-------)
                               -------+---------+---------+---------+--
                                   -350         0       350       700
```

Table A.16 Analysis of Variance for T_2 Times with respect to Temperature for 900 Bar Microfluidized Samples, using Adjusted SS for Tests.

One-way ANOVA: S75_AB_4°C versus Time

```
Source  DF     SS    MS     F      P
Time     7   9753  1393  6,00  0,001
Error   16   3714   232
Total   23  13466

S = 15,23   R-Sq = 72,42%   R-Sq(adj) = 60,36%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev  --------+---------+---------+---------+-
 1     3  1399,7    3,8  (-------*------)
 4     3  1408,2   22,0      (------*-------)
 7     3  1422,5    7,0             (------*------)
10     3  1426,4    7,8              (-------*------)
14     3  1401,4   27,3   (-------*------)
21     3  1441,1    7,8                    (------*-------)
28     3  1460,2   18,5                            (------*-------)
35     3  1404,4   10,1    (-------*------)
                          --------+---------+---------+---------+-
                               1400      1425      1450      1475

Pooled StDev = 15,2


Grouping Information Using Tukey Method

Time  N     Mean  Grouping
28    3  1460,24  A
21    3  1441,05  A B
10    3  1426,41  A B
 7    3  1422,52  A B
 4    3  1408,15    B
35    3  1404,41    B
14    3  1401,39    B
 1    3  1399,67    B

Means that do not share a letter are significantly different.


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

Individual confidence level = 99,68%


Time =  1 subtracted from:

Time   Lower  Center   Upper  -------+---------+---------+---------+--
 4    -34,62    8,48   51,58             (------*-------)
 7    -20,25   22,85   65,95                (------*------)
10    -16,36   26,74   69,84                (------*-------)
```

```
14    -41,38    1,72   44,82            (------*------)
21     -1,72   41,38   84,48                   (------*------)
28     17,47   60,56  103,66                      (------*------)
35    -38,36    4,74   47,83             (------*------)
                             -------+---------+---------+---------+--
                                  -60         0        60       120
```

```
Time =  4 subtracted from:

Time   Lower  Center  Upper  -------+---------+---------+---------+--
 7    -28,73   14,37  57,47             (------*-------)
10    -24,84   18,26  61,36              (------*------)
14    -49,86   -6,76  36,34           (------*------)
21    -10,20   32,90  76,00                 (------*-------)
28      8,99   52,08  95,18                    (-------*------)
35    -46,84   -3,74  39,36           (------*-------)
                           -------+---------+---------+---------+--
                                -60         0        60       120
```

```
Time =  7 subtracted from:

Time   Lower  Center  Upper  -------+---------+---------+---------+--
10    -39,21    3,89  46,99           (-------*------)
14    -64,22  -21,13  21,97        (------*-------)
21    -24,56   18,54  61,64              (------*------)
28     -5,38   37,72  80,82                 (------*------)
35    -61,21  -18,11  24,99         (------*------)
                           -------+---------+---------+---------+--
                                -60         0        60       120
```

```
Time = 10 subtracted from:

Time   Lower  Center  Upper  -------+---------+---------+---------+--
14    -68,12  -25,02  18,08       (------*------)
21    -28,45   14,64  57,74             (------*-------)
28     -9,27   33,83  76,93                (-------*------)
35    -65,10  -22,00  21,10        (------*-------)
                           -------+---------+---------+---------+--
                                -60         0        60       120
```

```
Time = 14 subtracted from:

Time   Lower  Center   Upper  -------+---------+---------+---------+--
21     -3,44   39,66   82,76                (-------*------)
28     15,75   58,84  101,94                    (------*------)
35    -40,08    3,02   46,11           (-------*------)
                            -------+---------+---------+---------+--
                                 -60         0        60       120
```

```
Time = 21 subtracted from:

Time   Lower  Center  Upper  -------+---------+---------+---------+--
28    -23,92   19,18  62,28              (------*------)
35    -79,75  -36,65   6,45      (------*------)
                           -------+---------+---------+---------+--
                                -60         0        60       120
```

```
Time = 28 subtracted from:

Time   Lower  Center   Upper  -------+---------+---------+---------+--
35    -98,93  -55,83  -12,73   (------*------)
                              -------+---------+---------+---------+--
                                   -60         0        60       120
```

One-way ANOVA: S75_PB_4°C versus Time

```
Source  DF      SS     MS      F      P
Time     7  230099  32871  90,17  0,000
Error   16    5833    365
Total   23  235931

S = 19,09   R-Sq = 97,53%   R-Sq(adj) = 96,45%


                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level  N    Mean  StDev   --+---------+---------+---------+-------
 1     3  1416,8   19,5    (--*-)
 4     3  1405,3   12,6   (--*-)
 7     3  1454,6   14,2        (-*--)
10     3  1507,9    9,3             (--*-)
14     3  1523,6   33,7               (-*--)
21     3  1684,4   29,4                                (-*--)
28     3  1666,6    7,3                              (--*-)
35     3  1522,6    5,8               (-*--)
                       --+---------+---------+---------+-------
                      1400      1500      1600      1700

Pooled StDev = 19,1


Grouping Information Using Tukey Method

Time  N     Mean  Grouping
21    3  1684,41  A
28    3  1666,57  A
14    3  1523,57    B
35    3  1522,56    B
10    3  1507,88    B C
 7    3  1454,62      C D
 1    3  1416,79        D
 4    3  1405,33        D

Means that do not share a letter are significantly different.


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

Individual confidence level = 99,68%


Time =  1 subtracted from:

Time   Lower  Center   Upper  -------+---------+---------+---------+--
```

```
 4    -65,48  -11,46   42,55                (-*--)
 7    -16,19   37,83   91,84                  (--*--)
10     37,08   91,09  145,11                     (--*-)
14     52,77  106,78  160,80                      (-*--)
21    213,61  267,62  321,64                              (-*--)
28    195,77  249,78  303,80                            (-*--)
35     51,75  105,77  159,78                      (-*--)
                              -------+---------+---------+---------+--
                                  -200         0       200       400
```

Time = 4 subtracted from:

```
Time   Lower  Center   Upper  -------+---------+---------+---------+--
 7     -4,72   49,29  103,30                  (-*--)
10     48,54  102,55  156,57                    (--*--)
14     64,23  118,25  172,26                     (--*--)
21    225,07  279,09  333,10                             (--*--)
28    207,23  261,25  315,26                           (--*--)
35     63,22  117,23  171,24                     (--*--)
                              -------+---------+---------+---------+--
                                  -200         0       200       400
```

Time = 7 subtracted from:

```
Time   Lower  Center   Upper  -------+---------+---------+---------+--
10     -0,75   53,26  107,28                  (--*-)
14     14,94   68,96  122,97                   (-*--)
21    175,78  229,80  283,81                           (-*--)
28    157,94  211,96  265,97                         (--*-)
35     13,93   67,94  121,95                   (-*--)
                              -------+---------+---------+---------+--
                                  -200         0       200       400
```

Time = 10 subtracted from:

```
Time   Lower  Center   Upper  -------+---------+---------+---------+--
14    -38,32   15,69   69,71                (--*-)
21    122,52  176,53  230,55                       (--*--)
28    104,68  158,69  212,70                      (--*--)
35    -39,34   14,68   68,69                (--*-)
                              -------+---------+---------+---------+--
                                  -200         0       200       400
```

Time = 14 subtracted from:

```
Time   Lower  Center   Upper  -------+---------+---------+---------+--
21    106,83  160,84  214,85                       (--*--)
28     88,99  143,00  197,01                     (--*--)
35    -55,03   -1,02   53,00                (--*--)
                              -------+---------+---------+---------+--
                                  -200         0       200       400
```

Time = 21 subtracted from:

```
Time    Lower   Center    Upper  -------+---------+---------+---------+-
-
```

```
28     -71,85   -17,84    36,17                (--*--)
35    -215,87  -161,86  -107,84         (--*--)
                                  -------+---------+---------+---------+-
-
                                      -200         0       200       400


Time = 28 subtracted from:

Time    Lower   Center   Upper  -------+---------+---------+---------+--
35    -198,03  -144,02  -90,00         (--*-)
                                  -------+---------+---------+---------+--
                                      -200         0       200       400
```

One-way ANOVA: E80_AB_4°C versus Time

```
Source  DF     SS    MS     F      P
Time     7  63379  9054  5,35  0,003
Error   16  27070  1692
Total   23  90449

S = 41,13   R-Sq = 70,07%   R-Sq(adj) = 56,98%


                          Individual 95% CIs For Mean Based on Pooled
StDev
Level  N    Mean  StDev     +---------+---------+---------+---------
 1     3  1593,3   28,0     (-------*------)
 4     3  1743,2    8,1                          (------*------)
 7     3  1695,4   23,7                    (------*------)
10     3  1719,5   51,0                       (-------*------)
14     3  1678,7   30,1                  (------*------)
21     3  1747,9   73,2                            (------*------)
28     3  1758,1   35,0                             (------*------)
35     3  1663,4   45,2               (-------*------)
                          +---------+---------+---------+---------
                         1540      1610      1680      1750

Pooled StDev = 41,1


Grouping Information Using Tukey Method

Time  N     Mean  Grouping
28    3  1758,09  A
21    3  1747,93  A
 4    3  1743,21  A
10    3  1719,50  A
 7    3  1695,37  A B
14    3  1678,71  A B
35    3  1663,35  A B
 1    3  1593,28    B

Means that do not share a letter are significantly different.


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

Individual confidence level = 99,68%
```

```
Time =  1 subtracted from:

Time   Lower  Center   Upper  ----+---------+---------+---------+-----
 4     33,57  149,93  266,29                (-------*-------)
 7    -14,27  102,09  218,45              (-------*-------)
10      9,85  126,22  242,58                (------*-------)
14    -30,93   85,43  201,79              (-------*------)
21     38,29  154,65  271,02                   (------*-------)
28     48,45  164,81  281,18                   (-------*-------)
35    -46,29   70,08  186,44             (-------*------)
                           ----+---------+---------+---------+-----
                            -150         0       150       300


Time =  4 subtracted from:

Time    Lower  Center   Upper  ----+---------+---------+---------+-----
 7    -164,20  -47,84   68,52     (-------*-------)
10    -140,08  -23,71   92,65       (-------*-------)
14    -180,87  -64,50   51,86    (-------*------)
21    -111,64    4,72  121,09         (-------*-------)
28    -101,48   14,88  131,24         (-------*-------)
35    -196,22  -79,86   36,51   (-------*------)
                           ----+---------+---------+---------+-----
                            -150         0       150       300


Time =  7 subtracted from:

Time    Lower  Center   Upper  ----+---------+---------+---------+-----
10     -92,24   24,13  140,49          (-------*------)
14    -133,02  -16,66   99,70       (-------*-------)
21     -63,80   52,56  168,93            (-------*------)
28     -53,64   62,72  179,08            (-------*-------)
35    -148,38  -32,02   84,35      (-------*-------)
                           ----+---------+---------+---------+-----
                            -150         0       150       300


Time = 10 subtracted from:

Time    Lower  Center   Upper  ----+---------+---------+---------+-----
14    -157,15  -40,79   75,58      (------*-------)
21     -87,93   28,44  144,80          (-------*-------)
28     -77,77   38,59  154,96           (-------*------)
35    -172,51  -56,14   60,22    (-------*-------)
                           ----+---------+---------+---------+-----
                            -150         0       150       300


Time = 14 subtracted from:

Time    Lower  Center   Upper  ----+---------+---------+---------+-----
21     -47,14   69,22  185,59             (-------*------)
28     -36,98   79,38  195,75              (------*-------)
35    -131,72  -15,35  101,01       (-------*-------)
                           ----+---------+---------+---------+-----
                            -150         0       150       300
```

```
Time = 21 subtracted from:

Time    Lower  Center   Upper  ----+---------+---------+---------+-----
28    -106,20   10,16  126,52         (-------*------)
35    -200,94  -84,58   31,78   (------*-------)
                                ----+---------+---------+---------+-----
                                 -150         0       150       300


Time = 28 subtracted from:

Time    Lower  Center  Upper  ----+---------+---------+---------+-----
35    -211,10  -94,74  21,63  (-------*------)
                              ----+---------+---------+---------+-----
                               -150         0       150       300
```

One-way ANOVA: E80_PB_4°C versus Time

```
Source  DF      SS     MS      F      P
Time     7  222265  31752  23,24  0,000
Error   16   21856   1366
Total   23  244121

S = 36,96   R-Sq = 91,05%   R-Sq(adj) = 87,13%


                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean  StDev  --------+---------+---------+---------+-
 1     3  1572,9   39,1   (---*----)
 4     3  1560,4   11,0  (---*----)
 7     3  1616,6   14,5       (----*---)
10     3  1620,4   15,7        (---*----)
14     3  1713,6   59,4                 (---*----)
21     3  1773,7   47,9                       (---*----)
28     3  1852,6   53,9                               (---*----)
35     3  1632,8   10,1         (---*----)
                   --------+---------+---------+---------+-
                        1600      1700      1800      1900

Pooled StDev = 37,0


Grouping Information Using Tukey Method

Time  N     Mean  Grouping
28    3  1852,64  A
21    3  1773,68  A B
14    3  1713,58    B C
35    3  1632,84      C D
10    3  1620,44      C D
 7    3  1616,63      C D
 1    3  1572,94        D
 4    3  1560,41        D

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

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

Individual confidence level = 99,68%


Time =  1 subtracted from:

Time    Lower  Center   Upper     +---------+---------+---------+-------
--
 4    -117,09  -12,53   92,03                   (----*-----)
 7     -60,87   43,69  148,25                      (----*----)
10     -57,06   47,50  152,06                      (----*-----)
14      36,08  140,64  245,20                           (----*----)
21      96,18  200,74  305,30                              (----*----)
28     175,14  279,70  384,26                                   (----*---
-)
35     -44,66   59,90  164,46                        (----*----)
                                +---------+---------+---------+-------
--
                              -400      -200         0       200


Time =  4 subtracted from:

Time   Lower  Center   Upper     +---------+---------+---------+--------
-
 7    -48,34   56,22  160,78                        (----*----)
10    -44,53   60,03  164,59                        (----*----)
14     48,61  153,17  257,73                            (-----*----)
21    108,71  213,27  317,83                               (-----*----)
28    187,68  292,23  396,79                                   (-----*---
-)
35    -32,13   72,43  176,99                        (-----*----)
                               +---------+---------+---------+--------
-
                             -400      -200         0       200


Time =  7 subtracted from:

Time    Lower  Center   Upper     +---------+---------+---------+-------
--
10    -100,75    3,81  108,37                     (----*----)
14      -7,61   96,95  201,51                          (----*----)
21      52,49  157,05  261,61                             (----*----)
28     131,45  236,01  340,57                                 (----*----)
35     -88,35   16,21  120,77                      (----*----)
                                +---------+---------+---------+-------
--
                              -400      -200         0       200


Time = 10 subtracted from:

Time   Lower  Center   Upper     +---------+---------+---------+--------
-
14    -11,42   93,14  197,70                          (-----*----)
21     48,68  153,24  257,80                             (-----*----)
28    127,65  232,20  336,76                                 (-----*----)
35    -92,16   12,40  116,96                     (-----*----)
```

```
                                  +---------+---------+---------+--------
-
                                -400      -200         0       200


Time = 14 subtracted from:

Time    Lower  Center   Upper     +---------+---------+---------+-------
--
21     -44,46   60,10  164,66                       (----*----)
28      34,50  139,06  243,62                           (----*----)
35    -185,30  -80,74   23,82                 (----*----)
                                  +---------+---------+---------+-------
--
                                -400      -200         0       200


Time = 21 subtracted from:

Time    Lower   Center   Upper     +---------+---------+---------+------
---
28     -25,60    78,96  183,52                          (----*----)
35    -245,40  -140,84  -36,28               (----*----)
                                   +---------+---------+---------+------
---
                                 -400      -200         0       200


Time = 28 subtracted from:

Time    Lower   Center    Upper     +---------+---------+---------+-----
----
35    -324,36  -219,80  -115,25           (----*----)
                                    +---------+---------+---------+-----
----
                                  -400      -200         0       200
```

One-way ANOVA: S75_AB_25°C versus Time

```
Source  DF      SS     MS      F      P
Time     7  147664  21095  50,27  0,000
Error   16    6714    420
Total   23  154378

S = 20,48   R-Sq = 95,65%   R-Sq(adj) = 93,75%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev  -------+---------+---------+---------+--
 1     3  1412,7   10,2  (---*--)
 4     3  1519,1   31,2                (--*--)
 7     3  1573,6   18,8                       (--*--)
10     3  1622,3   25,8                             (--*--)
14     3  1607,9   10,0                          (--*--)
21     3  1630,0   19,4                             (--*--)
28     3  1671,4   10,5                                  (--*--)
35     3  1508,0   26,0             (--*---)
                          -------+---------+---------+---------+--
                              1440      1520      1600      1680
```

Pooled StDev = 20,5

Grouping Information Using Tukey Method

Time	N	Mean	Grouping
28	3	1671,39	A
21	3	1629,98	A B
10	3	1622,31	A B
14	3	1607,92	B
7	3	1573,56	B C
4	3	1519,09	C D
35	3	1507,98	D
1	3	1412,69	E

Means that do not share a letter are significantly different.

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

Individual confidence level = 99,68%

Time = 1 subtracted from:

Time	Lower	Center	Upper	-----+---------+---------+---------+----
4	48,45	106,40	164,35	(---*---)
7	102,92	160,87	218,83	(---*---)
10	151,67	209,62	267,57	(---*---)
14	137,29	195,24	253,19	(---*---)
21	159,34	217,29	275,24	(--*---)
28	200,75	258,70	316,65	(---*---)
35	37,34	95,29	153,24	(---*---)
				-----+---------+---------+---------+----
				-150 0 150 300

Time = 4 subtracted from:

Time	Lower	Center	Upper	-----+---------+---------+---------+----
7	-3,48	54,47	112,42	(---*--)
10	45,27	103,22	161,17	(---*---)
14	30,88	88,83	146,79	(---*---)
21	52,94	110,89	168,84	(--*---)
28	94,35	152,30	210,25	(---*---)
35	-69,06	-11,11	46,84	(---*---)
				-----+---------+---------+---------+----
				-150 0 150 300

Time = 7 subtracted from:

Time	Lower	Center	Upper	-----+---------+---------+---------+----
10	-9,20	48,75	106,70	(---*---)
14	-23,59	34,36	92,31	(---*---)
21	-1,53	56,42	114,37	(---*---)
28	39,87	97,83	155,78	(---*--)
35	-123,53	-65,58	-7,63	(---*--)
				-----+---------+---------+---------+----

```
                                  -150         0       150       300

Time = 10 subtracted from:

Time    Lower   Center   Upper  -----+---------+---------+---------+----
14     -72,34   -14,39   43,56            (---*---)
21     -50,28     7,67   65,62              (---*--)
28      -8,87    49,08  107,03                (---*---)
35    -172,28  -114,33  -56,38      (--*---)
                                 -----+---------+---------+---------+----
                                   -150         0       150       300

Time = 14 subtracted from:

Time    Lower  Center   Upper  -----+---------+---------+---------+----
21     -35,90   22,05   80,01               (--*---)
28       5,51   63,46  121,41                 (---*---)
35    -157,89  -99,94  -41,99      (---*---)
                               -----+---------+---------+---------+----
                                 -150         0       150       300

Time = 21 subtracted from:

Time    Lower   Center   Upper  -----+---------+---------+---------+----
28     -16,54    41,41   99,36               (---*---)
35    -179,95  -122,00  -64,05     (---*---)
                                 -----+---------+---------+---------+----
                                   -150         0       150       300

Time = 28 subtracted from:

Time    Lower   Center    Upper  -----+---------+---------+---------+---
-
35    -221,36  -163,41  -105,46  (---*---)
                                  -----+---------+---------+---------+---
-
                                    -150         0       150       300
```

One-way ANOVA: S75_PB_25°C versus Time

```
Source  DF      SS     MS     F      P
Time     7  119790  17113  6,26  0,001
Error   16   43707   2732
Total   23  163497

S = 52,27   R-Sq = 73,27%   R-Sq(adj) = 61,57%

                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev   --+---------+---------+---------+-------
 1     3  1544,0   26,2   (-----*------)
 4     3  1539,4   48,9   (-----*-----)
 7     3  1642,4   74,0             (-----*------)
10     3  1699,4   26,9                   (-----*-----)
14     3  1658,1   52,7              (------*-----)
```

```
21     3  1709,9   15,9                    (-----*-----)
28     3  1745,7   84,5                       (------*-----)
35     3  1611,7   49,0          (-----*------)
                          --+---------+---------+---------+-------
                         1500      1600      1700      1800
```

Pooled StDev = 52,3

Grouping Information Using Tukey Method

Time	N	Mean	Grouping
28	3	1745,69	A
21	3	1709,91	A
10	3	1699,39	A
14	3	1658,08	A B
7	3	1642,37	A B
35	3	1611,69	A B
1	3	1544,02	B
4	3	1539,42	B

Means that do not share a letter are significantly different.

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

Individual confidence level = 99,68%

Time = 1 subtracted from:

```
Time    Lower  Center   Upper  --------+---------+---------+---------+-
 4    -152,46   -4,60  143,26            (-------*------)
 7     -49,51   98,35  246,21                  (------*------)
10       7,51  155,37  303,23                    (-------*------)
14     -33,80  114,06  261,92                  (-------*------)
21      18,03  165,89  313,75                     (------*-------)
28      53,81  201,67  349,53                       (------*------)
35     -80,19   67,67  215,53               (------*-------)
                          --------+---------+---------+---------+-
                               -200         0       200       400
```

Time = 4 subtracted from:

```
Time   Lower  Center   Upper  --------+---------+---------+---------+-
 7    -44,91  102,95  250,81                (------*-------)
10     12,11  159,97  307,83                   (------*------)
14    -29,20  118,66  266,52                 (------*------)
21     22,63  170,49  318,34                   (-------*------)
28     58,41  206,27  354,13                     (------*-------)
35    -75,59   72,27  220,13               (-------*------)
                          --------+---------+---------+---------+-
                               -200         0       200       400
```

Time = 7 subtracted from:

```
Time    Lower  Center   Upper  --------+---------+---------+---------+-
10     -90,84   57,02  204,88               (-------*------)
```

```
14    -132,15   15,71  163,57             (-------*------)
21     -80,32   67,54  215,40                (------*-------)
28     -44,54  103,32  251,18                  (------*-------)
35    -178,54  -30,68  117,18           (------*-------)
                                --------+---------+---------+---------+-
                                     -200         0       200       400
```

```
Time = 10 subtracted from:

Time    Lower  Center   Upper  --------+---------+---------+---------+-
14    -189,16  -41,31  106,55          (------*------)
21    -137,34   10,52  158,38            (-------*------)
28    -101,55   46,30  194,16              (------*-------)
35    -235,56  -87,70   60,16       (-------*------)
                                --------+---------+---------+---------+-
                                     -200         0       200       400
```

```
Time = 14 subtracted from:

Time    Lower  Center   Upper  --------+---------+---------+---------+-
21     -96,03   51,82  199,68              (-------*------)
28     -60,25   87,61  235,47                (------*-------)
35    -194,25  -46,39  101,47         (-------*------)
                                --------+---------+---------+---------+-
                                     -200         0       200       400
```

```
Time = 21 subtracted from:

Time    Lower  Center   Upper  --------+---------+---------+---------+-
28    -112,07   35,79  183,64             (-------*------)
35    -246,08  -98,22   49,64       (------*------)
                                --------+---------+---------+---------+-
                                     -200         0       200       400
```

```
Time = 28 subtracted from:

Time    Lower   Center  Upper  --------+---------+---------+---------+-
35    -281,86  -134,00  13,86      (------*-------)
                                --------+---------+---------+---------+-
                                     -200         0       200       400
```

One-way ANOVA: E80_AB_25°C versus Time

```
Source  DF     SS     MS      F      P
Time     7  87665  12524  18,52  0,000
Error   16  10819    676
Total   23  98484

S = 26,00   R-Sq = 89,01%   R-Sq(adj) = 84,21%
```

```
                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean  StDev  ----+---------+---------+---------+-----
 1     3  1601,6   27,8          (----*---)
 4     3  1699,5   17,3                        (----*---)
```

```
 7     3  1712,1   11,0                          (----*---)
10     3  1740,4   54,8                              (----*---)
14     3  1676,1   20,1                       (---*----)
21     3  1656,1   20,6                    (----*---)
28     3  1637,2   16,0                 (----*---)
35     3  1540,4   11,4  (---*----)
                        ----+---------+---------+---------+-----
                         1540      1610      1680      1750
```

Pooled StDev = 26,0

Grouping Information Using Tukey Method

```
Time  N     Mean  Grouping
10    3  1740,36  A
 7    3  1712,11  A B
 4    3  1699,53  A B C
14    3  1676,05  A B C
21    3  1656,14    B C D
28    3  1637,22      C D
 1    3  1601,56        D E
35    3  1540,36          E
```

Means that do not share a letter are significantly different.

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

Individual confidence level = 99,68%

Time = 1 subtracted from:

```
Time    Lower  Center   Upper  --------+---------+---------+---------+-
 4      24,41   97,97  171,54                     (----*---)
 7      36,99  110,55  184,11                     (----*----)
10      65,23  138,79  212,36                       (----*----)
14       0,93   74,49  148,05                    (----*----)
21     -18,99   54,58  128,14                   (----*----)
28     -37,91   35,66  109,22                 (----*----)
35    -134,77  -61,20   12,36           (----*----)
                        --------+---------+---------+---------+-
                             -150         0       150       300
```

Time = 4 subtracted from:

```
Time    Lower   Center   Upper  --------+---------+---------+---------+-
 7     -60,99    12,58   86,14                (----*----)
10     -32,74    40,82  114,39                  (----*----)
14     -97,05   -23,48   50,08              (---*----)
21    -116,96   -43,40   30,17            (----*----)
28    -135,88   -62,31   11,25           (----*----)
35    -232,74  -159,18  -85,61    (----*----)
                        --------+---------+---------+---------+-
                             -150         0       150       300
```

Time = 7 subtracted from:

```
Time    Lower   Center   Upper  --------+---------+---------+---------+-
10     -45,32    28,24  101,81                (----*----)
14    -109,62   -36,06   37,50             (----*----)
21    -129,54   -55,98   17,59           (----*----)
28    -148,46   -74,89   -1,33         (----*----)
35    -245,32  -171,75  -98,19    (----*---)
                                --------+---------+---------+---------+-
                                     -150         0       150       300


Time = 10 subtracted from:

Time    Lower   Center    Upper  --------+---------+---------+---------
+-
14    -137,87   -64,30     9,26           (----*----)
21    -157,78   -84,22   -10,65         (----*----)
28    -176,70  -103,13   -29,57        (----*----)
35    -273,56  -200,00  -126,43  (----*----)
                                 --------+---------+---------+---------
+-
                                      -150         0       150       300


Time = 14 subtracted from:

Time    Lower   Center   Upper  --------+---------+---------+---------+-
21     -93,48   -19,91   53,65              (----*----)
28    -112,39   -38,83   34,73             (---*----)
35    -209,26  -135,69  -62,13      (----*----)
                                --------+---------+---------+---------+-
                                     -150         0       150       300


Time = 21 subtracted from:

Time    Lower   Center   Upper  --------+---------+---------+---------+-
28     -92,48   -18,92   54,65              (----*----)
35    -189,34  -115,78  -42,22       (----*----)
                                --------+---------+---------+---------+-
                                     -150         0       150       300


Time = 28 subtracted from:

Time    Lower  Center   Upper  --------+---------+---------+---------+-
35    -170,43  -96,86  -23,30         (----*---)
                               --------+---------+---------+---------+-
                                    -150         0       150       300
```

One-way ANOVA: E80_PB_25°C versus Time

```
Source  DF      SS     MS     F      P
Time     7  205542  29363  7,47  0,000
Error   16   62871   3929
Total   23  268413

S = 62,69   R-Sq = 76,58%   R-Sq(adj) = 66,33%
```

```
                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev  ------+---------+---------+---------+---
 1     3  1562,7   27,9  (-----*------)
 4     3  1824,7   57,0                       (-----*-----)
 7     3  1864,0   15,9                          (-----*------)
10     3  1833,0   26,6                       (------*-----)
14     3  1808,1   41,8                     (------*-----)
21     3  1877,5    7,3                            (-----*------)
28     3  1773,0  152,7                  (------*-----)
35     3  1781,7   36,6                    (-----*------)
                          ------+---------+---------+---------+---
                             1560      1680      1800      1920

Pooled StDev = 62,7


Grouping Information Using Tukey Method

Time  N     Mean  Grouping
21    3  1877,47  A
 7    3  1863,96  A
10    3  1832,95  A
 4    3  1824,68  A
14    3  1808,11  A
35    3  1781,71  A
28    3  1773,01  A
 1    3  1562,71     B

Means that do not share a letter are significantly different.


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

Individual confidence level = 99,68%


Time =  1 subtracted from:

Time   Lower  Center   Upper  ----+---------+---------+---------+-----
 4     84,64  261,98  439,31                   (--------*--------)
 7    123,92  301,26  478,59                     (--------*--------)
10     92,91  270,25  447,58                    (--------*-------)
14     68,07  245,40  422,74                   (--------*--------)
21    137,42  314,76  492,10                       (--------*--------)
28     32,97  210,31  387,64                  (--------*-------)
35     41,67  219,00  396,34                  (--------*--------)
                           ----+---------+---------+---------+-----
                            -200         0       200       400


Time =  4 subtracted from:

Time    Lower  Center   Upper  ----+---------+---------+---------+-----
 7    -138,06   39,28  216,62         (--------*--------)
10    -169,07    8,27  185,61        (--------*--------)
14    -193,91  -16,57  160,77      (--------*--------)
21    -124,55   52,79  230,12          (--------*--------)
28    -229,01  -51,67  125,67     (-------*--------)
35    -220,31  -42,97  134,37     (--------*--------)
```

```
                                  ----+---------+---------+---------+-----
                                   -200         0       200       400
```

```
Time =  7 subtracted from:

Time    Lower  Center   Upper  ----+---------+---------+---------+-----
10    -208,35  -31,01  146,33      (-------*--------)
14    -233,19  -55,85  121,49    (--------*--------)
21    -163,83   13,50  190,84        (--------*--------)
28    -268,29  -90,95   86,39   (-------*--------)
35    -259,59  -82,25   95,08   (--------*--------)
                                  ----+---------+---------+---------+-----
                                   -200         0       200       400
```

```
Time = 10 subtracted from:

Time    Lower  Center   Upper  ----+---------+---------+---------+-----
14    -202,18  -24,84  152,50      (--------*--------)
21    -132,82   44,51  221,85         (--------*--------)
28    -237,28  -59,94  117,40    (--------*--------)
35    -228,58  -51,24  126,09     (-------*--------)
                                  ----+---------+---------+---------+-----
                                   -200         0       200       400
```

```
Time = 14 subtracted from:

Time    Lower  Center   Upper  ----+---------+---------+---------+-----
21    -107,98   69,36  246,69           (-------*--------)
28    -212,44  -35,10  142,24     (--------*--------)
35    -203,74  -26,40  150,94      (--------*--------)
                                  ----+---------+---------+---------+-----
                                   -200         0       200       400
```

```
Time = 21 subtracted from:

Time    Lower   Center  Upper  ----+---------+---------+---------+-----
28    -281,79  -104,46  72,88  (--------*--------)
35    -273,09   -95,76  81,58  (--------*--------)
                                  ----+---------+---------+---------+-----
                                   -200         0       200       400
```

```
Time = 28 subtracted from:

Time    Lower  Center   Upper  ----+---------+---------+---------+-----
35    -168,64    8,70  186,04        (-------*--------)
                                  ----+---------+---------+---------+-----
                                   -200         0       200       400
```

One-way ANOVA: S75_AB_4°C versus Time

```
Source  DF     SS    MS     F      P
Time     7   9753  1393  6,00  0,001
Error   16   3714   232
Total   23  13466
```

```
S = 15,23   R-Sq = 72,42%   R-Sq(adj) = 60,36%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev  --------+---------+---------+---------+-
 1     3  1399,7    3,8  (-------*------)
 4     3  1408,2   22,0      (------*-------)
 7     3  1422,5    7,0            (------*------)
10     3  1426,4    7,8             (-------*------)
14     3  1401,4   27,3   (-------*------)
21     3  1441,1    7,8                   (------*-------)
28     3  1460,2   18,5                           (------*-------)
35     3  1404,4   10,1    (-------*------)
                          --------+---------+---------+---------+-
                               1400      1425      1450      1475

Pooled StDev = 15,2


Grouping Information Using Tukey Method

Time  N     Mean  Grouping
28    3  1460,24  A
21    3  1441,05  A B
10    3  1426,41  A B
 7    3  1422,52  A B
 4    3  1408,15    B
35    3  1404,41    B
14    3  1401,39    B
 1    3  1399,67    B

Means that do not share a letter are significantly different.


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

Individual confidence level = 99,68%


Time =  1 subtracted from:

Time   Lower  Center   Upper  -------+---------+---------+---------+--
 4    -34,62    8,48   51,58             (------*-------)
 7    -20,25   22,85   65,95                (------*------)
10    -16,36   26,74   69,84                (------*-------)
14    -41,38    1,72   44,82            (------*------)
21     -1,72   41,38   84,48                   (------*------)
28     17,47   60,56  103,66                      (------*------)
35    -38,36    4,74   47,83             (------*------)
                        -------+---------+---------+---------+--
                             -60         0        60       120


Time =  4 subtracted from:

Time   Lower  Center  Upper  -------+---------+---------+---------+--
 7    -28,73   14,37  57,47             (------*-------)
10    -24,84   18,26  61,36              (------*------)
```

```
14    -49,86   -6,76  36,34           (------*------)
21    -10,20   32,90  76,00                 (------*-------)
28      8,99   52,08  95,18                    (-------*------)
35    -46,84   -3,74  39,36           (------*-------)
                              -------+---------+---------+---------+--
                                   -60         0        60       120
```

```
Time =  7 subtracted from:

Time   Lower  Center  Upper  -------+---------+---------+---------+--
10    -39,21    3,89  46,99           (-------*------)
14    -64,22  -21,13  21,97       (------*-------)
21    -24,56   18,54  61,64              (------*------)
28     -5,38   37,72  80,82                 (------*------)
35    -61,21  -18,11  24,99        (------*------)
                              -------+---------+---------+---------+--
                                   -60         0        60       120
```

```
Time = 10 subtracted from:

Time   Lower  Center  Upper  -------+---------+---------+---------+--
14    -68,12  -25,02  18,08       (------*------)
21    -28,45   14,64  57,74             (------*-------)
28     -9,27   33,83  76,93                (-------*------)
35    -65,10  -22,00  21,10       (------*-------)
                              -------+---------+---------+---------+--
                                   -60         0        60       120
```

```
Time = 14 subtracted from:

Time   Lower  Center   Upper  -------+---------+---------+---------+--
21     -3,44   39,66   82,76                 (-------*------)
28     15,75   58,84  101,94                    (------*------)
35    -40,08    3,02   46,11            (-------*------)
                               -------+---------+---------+---------+--
                                    -60         0        60       120
```

```
Time = 21 subtracted from:

Time   Lower  Center  Upper  -------+---------+---------+---------+--
28    -23,92   19,18  62,28              (------*------)
35    -79,75  -36,65   6,45     (------*------)
                              -------+---------+---------+---------+--
                                   -60         0        60       120
```

```
Time = 28 subtracted from:

Time   Lower  Center   Upper  -------+---------+---------+---------+--
35    -98,93  -55,83  -12,73   (------*------)
                               -------+---------+---------+---------+--
                                    -60         0        60       120
```

One-way ANOVA: S75_PB_4°C versus Time

```
Source  DF      SS     MS      F      P
Time     7  230099  32871  90,17  0,000
Error   16    5833    365
Total   23  235931

S = 19,09   R-Sq = 97,53%   R-Sq(adj) = 96,45%


                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level  N    Mean  StDev   --+---------+---------+---------+-------
 1     3  1416,8   19,5     (--*-)
 4     3  1405,3   12,6   (--*-)
 7     3  1454,6   14,2        (-*--)
10     3  1507,9    9,3             (--*-)
14     3  1523,6   33,7               (-*--)
21     3  1684,4   29,4                                (-*--)
28     3  1666,6    7,3                              (--*-)
35     3  1522,6    5,8               (-*--)
                          --+---------+---------+---------+-------
                         1400      1500      1600      1700

Pooled StDev = 19,1


Grouping Information Using Tukey Method

Time  N     Mean  Grouping
21    3  1684,41  A
28    3  1666,57  A
14    3  1523,57    B
35    3  1522,56    B
10    3  1507,88    B C
 7    3  1454,62      C D
 1    3  1416,79        D
 4    3  1405,33        D

Means that do not share a letter are significantly different.


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

Individual confidence level = 99,68%


Time =  1 subtracted from:

Time   Lower  Center   Upper  -------+---------+---------+---------+--
 4    -65,48  -11,46   42,55               (-*--)
 7    -16,19   37,83   91,84                 (--*--)
10     37,08   91,09  145,11                    (--*-)
14     52,77  106,78  160,80                     (-*--)
21    213,61  267,62  321,64                              (-*--)
28    195,77  249,78  303,80                            (-*--)
35     51,75  105,77  159,78                     (-*--)
                              -------+---------+---------+---------+--
                                  -200         0       200       400
```

```
Time =  4 subtracted from:

Time   Lower  Center   Upper  -------+---------+---------+---------+--
 7     -4,72   49,29  103,30                  (-*--)
10     48,54  102,55  156,57                    (--*--)
14     64,23  118,25  172,26                     (--*--)
21    225,07  279,09  333,10                             (--*--)
28    207,23  261,25  315,26                           (--*--)
35     63,22  117,23  171,24                     (--*--)
                              -------+---------+---------+---------+--
                                  -200         0       200       400


Time =  7 subtracted from:

Time   Lower  Center   Upper  -------+---------+---------+---------+--
10     -0,75   53,26  107,28                  (--*-)
14     14,94   68,96  122,97                   (-*--)
21    175,78  229,80  283,81                           (-*--)
28    157,94  211,96  265,97                         (--*-)
35     13,93   67,94  121,95                   (-*--)
                              -------+---------+---------+---------+--
                                  -200         0       200       400


Time = 10 subtracted from:

Time   Lower  Center   Upper  -------+---------+---------+---------+--
14    -38,32   15,69   69,71                (--*-)
21    122,52  176,53  230,55                       (--*--)
28    104,68  158,69  212,70                     (--*--)
35    -39,34   14,68   68,69                (--*-)
                              -------+---------+---------+---------+--
                                  -200         0       200       400


Time = 14 subtracted from:

Time   Lower  Center   Upper  -------+---------+---------+---------+--
21    106,83  160,84  214,85                      (--*--)
28     88,99  143,00  197,01                    (--*--)
35    -55,03   -1,02   53,00              (--*--)
                              -------+---------+---------+---------+--
                                  -200         0       200       400


Time = 21 subtracted from:

Time    Lower   Center    Upper  -------+---------+---------+---------+-
-
28     -71,85   -17,84    36,17              (--*--)
35    -215,87  -161,86  -107,84       (--*--)
                             -------+---------+---------+---------+-
-
                                 -200         0       200       400


Time = 28 subtracted from:

Time    Lower   Center   Upper  -------+---------+---------+---------+--
```

```
35    -198,03  -144,02  -90,00         (--*-)
                                -------+---------+---------+---------+--
                                    -200         0       200       400
```

One-way ANOVA: E80_AB_4°C versus Time

```
Source  DF     SS    MS     F      P
Time     7  63379  9054  5,35  0,003
Error   16  27070  1692
Total   23  90449

S = 41,13   R-Sq = 70,07%   R-Sq(adj) = 56,98%


                          Individual 95% CIs For Mean Based on Pooled
StDev
Level  N    Mean  StDev     +---------+---------+---------+---------
 1     3  1593,3   28,0     (-------*------)
 4     3  1743,2    8,1                          (------*------)
 7     3  1695,4   23,7                    (------*------)
10     3  1719,5   51,0                       (-------*------)
14     3  1678,7   30,1                  (------*------)
21     3  1747,9   73,2                            (------*------)
28     3  1758,1   35,0                             (------*------)
35     3  1663,4   45,2               (-------*------)
                                   +---------+---------+---------+---------
                                  1540      1610      1680      1750

Pooled StDev = 41,1


Grouping Information Using Tukey Method

Time  N     Mean  Grouping
28    3  1758,09  A
21    3  1747,93  A
 4    3  1743,21  A
10    3  1719,50  A
 7    3  1695,37  A B
14    3  1678,71  A B
35    3  1663,35  A B
 1    3  1593,28    B

Means that do not share a letter are significantly different.


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

Individual confidence level = 99,68%


Time =  1 subtracted from:

Time   Lower  Center   Upper  ----+---------+---------+---------+-----
 4     33,57  149,93  266,29                  (-------*-------)
 7    -14,27  102,09  218,45               (-------*-------)
10      9,85  126,22  242,58                 (------*-------)
14    -30,93   85,43  201,79              (-------*------)
21     38,29  154,65  271,02                   (------*-------)
```

```
28     48,45  164,81  281,18                   (-------*-------)
35    -46,29   70,08  186,44             (-------*------)
                                ----+---------+---------+---------+-----
                                 -150         0       150       300


Time =  4 subtracted from:

Time    Lower  Center   Upper  ----+---------+---------+---------+-----
 7    -164,20  -47,84   68,52     (-------*-------)
10    -140,08  -23,71   92,65       (------*-------)
14    -180,87  -64,50   51,86    (-------*------)
21    -111,64    4,72  121,09         (------*-------)
28    -101,48   14,88  131,24         (-------*-------)
35    -196,22  -79,86   36,51   (-------*------)
                                ----+---------+---------+---------+-----
                                 -150         0       150       300


Time =  7 subtracted from:

Time    Lower  Center   Upper  ----+---------+---------+---------+-----
10     -92,24   24,13  140,49          (-------*------)
14    -133,02  -16,66   99,70       (-------*-------)
21     -63,80   52,56  168,93            (-------*------)
28     -53,64   62,72  179,08            (-------*-------)
35    -148,38  -32,02   84,35      (-------*-------)
                                ----+---------+---------+---------+-----
                                 -150         0       150       300


Time = 10 subtracted from:

Time    Lower  Center   Upper  ----+---------+---------+---------+-----
14    -157,15  -40,79   75,58      (------*-------)
21     -87,93   28,44  144,80          (-------*-------)
28     -77,77   38,59  154,96           (-------*------)
35    -172,51  -56,14   60,22    (-------*-------)
                                ----+---------+---------+---------+-----
                                 -150         0       150       300


Time = 14 subtracted from:

Time    Lower  Center   Upper  ----+---------+---------+---------+-----
21     -47,14   69,22  185,59             (-------*------)
28     -36,98   79,38  195,75              (------*-------)
35    -131,72  -15,35  101,01       (-------*-------)
                                ----+---------+---------+---------+-----
                                 -150         0       150       300


Time = 21 subtracted from:

Time    Lower  Center   Upper  ----+---------+---------+---------+-----
28    -106,20   10,16  126,52         (-------*------)
35    -200,94  -84,58   31,78   (------*-------)
                                ----+---------+---------+---------+-----
                                 -150         0       150       300
```

```
Time = 28 subtracted from:

Time    Lower  Center  Upper  ----+---------+---------+---------+-----
35    -211,10  -94,74  21,63  (-------*------)
                              ----+---------+---------+---------+-----
                               -150         0       150       300
```

One-way ANOVA: E80_PB_4°C versus Time

```
Source  DF      SS     MS      F      P
Time     7  222265  31752  23,24  0,000
Error   16   21856   1366
Total   23  244121

S = 36,96   R-Sq = 91,05%   R-Sq(adj) = 87,13%


                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level  N    Mean  StDev  --------+---------+---------+---------+-
 1     3  1572,9   39,1   (---*----)
 4     3  1560,4   11,0  (---*----)
 7     3  1616,6   14,5       (----*---)
10     3  1620,4   15,7        (---*----)
14     3  1713,6   59,4                 (---*----)
21     3  1773,7   47,9                       (---*----)
28     3  1852,6   53,9                               (---*----)
35     3  1632,8   10,1         (---*----)
                         --------+---------+---------+---------+-
                              1600      1700      1800      1900

Pooled StDev = 37,0


Grouping Information Using Tukey Method

Time  N     Mean  Grouping
28    3  1852,64  A
21    3  1773,68  A B
14    3  1713,58    B C
35    3  1632,84      C D
10    3  1620,44      C D
 7    3  1616,63      C D
 1    3  1572,94        D
 4    3  1560,41        D

Means that do not share a letter are significantly different.


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

Individual confidence level = 99,68%


Time =  1 subtracted from:

Time    Lower  Center   Upper     +---------+---------+---------+-------
--
 4    -117,09  -12,53   92,03                   (----*-----)
```

```
 7     -60,87   43,69  148,25                    (----*----)
10     -57,06   47,50  152,06                    (----*-----)
14      36,08  140,64  245,20                         (----*----)
21      96,18  200,74  305,30                            (----*----)
28     175,14  279,70  384,26                                (----*---
-)
35     -44,66   59,90  164,46                     (----*----)
                                  +---------+---------+---------+-------
--
                                -400      -200         0       200


Time =  4 subtracted from:

Time   Lower  Center   Upper     +---------+---------+---------+--------
-
 7    -48,34   56,22  160,78                    (----*----)
10    -44,53   60,03  164,59                    (----*----)
14     48,61  153,17  257,73                        (-----*----)
21    108,71  213,27  317,83                           (-----*----)
28    187,68  292,23  396,79                               (-----*---
-)
35    -32,13   72,43  176,99                    (-----*----)
                                +---------+---------+---------+--------
-
                              -400      -200         0       200


Time =  7 subtracted from:

Time    Lower  Center   Upper     +---------+---------+---------+-------
--
10    -100,75    3,81  108,37                  (----*----)
14      -7,61   96,95  201,51                       (----*----)
21      52,49  157,05  261,61                          (----*----)
28     131,45  236,01  340,57                              (----*----)
35     -88,35   16,21  120,77                   (----*----)
                                  +---------+---------+---------+-------
--
                               -400      -200         0       200


Time = 10 subtracted from:

Time   Lower  Center   Upper     +---------+---------+---------+--------
-
14    -11,42   93,14  197,70                       (-----*----)
21     48,68  153,24  257,80                          (-----*----)
28    127,65  232,20  336,76                              (-----*----)
35    -92,16   12,40  116,96                  (-----*----)
                                +---------+---------+---------+--------
-
                              -400      -200         0       200


Time = 14 subtracted from:

Time    Lower  Center   Upper     +---------+---------+---------+-------
--
21     -44,46   60,10  164,66                       (----*----)
28      34,50  139,06  243,62                           (----*----)
```

```
35    -185,30  -80,74   23,82                (----*----)
                                  +---------+---------+---------+-------
--
                                -400      -200         0       200


Time = 21 subtracted from:

Time    Lower   Center   Upper     +---------+---------+---------+------
---
28     -25,60    78,96  183,52                        (----*----)
35    -245,40  -140,84  -36,28             (----*----)
                                  +---------+---------+---------+------
---
                                -400      -200         0       200


Time = 28 subtracted from:

Time    Lower   Center    Upper     +---------+---------+---------+-----
----
35    -324,36  -219,80  -115,25         (----*----)
                                   +---------+---------+---------+-----
----
                                 -400      -200         0       200
```

One-way ANOVA: S75_AB_25°C versus Time

```
Source  DF      SS     MS      F      P
Time     7  147664  21095  50,27  0,000
Error   16    6714    420
Total   23  154378

S = 20,48   R-Sq = 95,65%   R-Sq(adj) = 93,75%


                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean  StDev  -------+---------+---------+---------+--
 1     3  1412,7   10,2  (---*--)
 4     3  1519,1   31,2               (--*--)
 7     3  1573,6   18,8                      (--*--)
10     3  1622,3   25,8                            (--*--)
14     3  1607,9   10,0                         (--*--)
21     3  1630,0   19,4                            (--*--)
28     3  1671,4   10,5                                 (--*--)
35     3  1508,0   26,0             (--*---)
                        -------+---------+---------+---------+--
                            1440      1520      1600      1680

Pooled StDev = 20,5


Grouping Information Using Tukey Method

Time  N     Mean  Grouping
28    3  1671,39  A
21    3  1629,98  A B
10    3  1622,31  A B
14    3  1607,92    B
```

```
 7    3  1573,56    B C
 4    3  1519,09      C D
35    3  1507,98        D
 1    3  1412,69          E

Means that do not share a letter are significantly different.


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

Individual confidence level = 99,68%


Time =  1 subtracted from:

Time   Lower  Center   Upper  -----+---------+---------+---------+----
 4     48,45  106,40  164,35                   (---*---)
 7    102,92  160,87  218,83                       (---*---)
10    151,67  209,62  267,57                          (---*---)
14    137,29  195,24  253,19                        (---*---)
21    159,34  217,29  275,24                          (--*---)
28    200,75  258,70  316,65                            (---*---)
35     37,34   95,29  153,24                 (---*---)
                            -----+---------+---------+---------+----
                              -150         0       150       300


Time =  4 subtracted from:

Time   Lower  Center   Upper  -----+---------+---------+---------+----
 7     -3,48   54,47  112,42               (---*--)
10     45,27  103,22  161,17                  (---*---)
14     30,88   88,83  146,79                (---*---)
21     52,94  110,89  168,84                  (--*---)
28     94,35  152,30  210,25                    (---*---)
35    -69,06  -11,11   46,84          (---*---)
                            -----+---------+---------+---------+----
                              -150         0       150       300


Time =  7 subtracted from:

Time    Lower  Center   Upper  -----+---------+---------+---------+----
10      -9,20   48,75  106,70               (---*---)
14     -23,59   34,36   92,31              (---*---)
21      -1,53   56,42  114,37                (---*---)
28      39,87   97,83  155,78                   (---*--)
35    -123,53  -65,58   -7,63        (---*--)
                             -----+---------+---------+---------+----
                               -150         0       150       300


Time = 10 subtracted from:

Time    Lower   Center   Upper  -----+---------+---------+---------+----
14     -72,34   -14,39   43,56           (---*---)
21     -50,28     7,67   65,62             (---*--)
28      -8,87    49,08  107,03               (---*---)
35    -172,28  -114,33  -56,38      (--*---)
                             -----+---------+---------+---------+----
```

```
                                  -150         0       150       300


Time = 14 subtracted from:

Time    Lower  Center   Upper  -----+---------+---------+---------+----
21     -35,90   22,05   80,01               (--*---)
28       5,51   63,46  121,41                 (---*---)
35    -157,89  -99,94  -41,99      (---*---)
                                -----+---------+---------+---------+----
                                  -150         0       150       300


Time = 21 subtracted from:

Time    Lower   Center   Upper  -----+---------+---------+---------+----
28     -16,54    41,41   99,36               (---*---)
35    -179,95  -122,00  -64,05     (---*---)
                                -----+---------+---------+---------+----
                                  -150         0       150       300


Time = 28 subtracted from:

Time    Lower   Center    Upper  -----+---------+---------+---------+---
-
35    -221,36  -163,41  -105,46  (---*---)
                                -----+---------+---------+---------+---
-
                                    -150         0       150       300
```

One-way ANOVA: S75_PB_25°C versus Time

```
Source  DF      SS     MS     F      P
Time     7  119790  17113  6,26  0,001
Error   16   43707   2732
Total   23  163497

S = 52,27   R-Sq = 73,27%   R-Sq(adj) = 61,57%


                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean  StDev   --+---------+---------+---------+-------
 1     3  1544,0   26,2   (-----*------)
 4     3  1539,4   48,9   (-----*-----)
 7     3  1642,4   74,0             (-----*------)
10     3  1699,4   26,9                   (-----*-----)
14     3  1658,1   52,7              (------*-----)
21     3  1709,9   15,9                    (-----*-----)
28     3  1745,7   84,5                       (------*-----)
35     3  1611,7   49,0           (-----*------)
                         --+---------+---------+---------+-------
                        1500      1600      1700      1800

Pooled StDev = 52,3


Grouping Information Using Tukey Method
```

```
Time  N     Mean  Grouping
28    3  1745,69  A
21    3  1709,91  A
10    3  1699,39  A
14    3  1658,08  A B
 7    3  1642,37  A B
35    3  1611,69  A B
 1    3  1544,02    B
 4    3  1539,42    B

Means that do not share a letter are significantly different.


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

Individual confidence level = 99,68%


Time =  1 subtracted from:

Time    Lower  Center   Upper  --------+---------+---------+---------+-
 4    -152,46   -4,60  143,26           (-------*------)
 7     -49,51   98,35  246,21                 (------*------)
10       7,51  155,37  303,23                   (-------*------)
14     -33,80  114,06  261,92                 (-------*------)
21      18,03  165,89  313,75                    (------*-------)
28      53,81  201,67  349,53                      (------*------)
35     -80,19   67,67  215,53               (------*-------)
                        --------+---------+---------+---------+-
                              -200         0       200       400


Time =  4 subtracted from:

Time   Lower  Center   Upper  --------+---------+---------+---------+-
 7    -44,91  102,95  250,81               (------*-------)
10     12,11  159,97  307,83                  (------*------)
14    -29,20  118,66  266,52                (------*------)
21     22,63  170,49  318,34                  (-------*------)
28     58,41  206,27  354,13                    (------*-------)
35    -75,59   72,27  220,13              (-------*------)
                       --------+---------+---------+---------+-
                             -200         0       200       400


Time =  7 subtracted from:

Time    Lower  Center   Upper  --------+---------+---------+---------+-
10     -90,84   57,02  204,88              (-------*------)
14    -132,15   15,71  163,57            (-------*------)
21     -80,32   67,54  215,40               (------*-------)
28     -44,54  103,32  251,18                 (------*-------)
35    -178,54  -30,68  117,18          (------*-------)
                        --------+---------+---------+---------+-
                              -200         0       200       400


Time = 10 subtracted from:

Time    Lower  Center   Upper  --------+---------+---------+---------+-
```

```
14    -189,16  -41,31  106,55           (------*------)
21    -137,34   10,52  158,38             (-------*------)
28    -101,55   46,30  194,16               (------*-------)
35    -235,56  -87,70   60,16         (--------*------)
                                -------+---------+---------+---------+-
                                     -200         0       200       400


Time = 14 subtracted from:

Time    Lower  Center   Upper  -------+---------+---------+---------+-
21     -96,03   51,82  199,68              (-------*------)
28     -60,25   87,61  235,47                (------*-------)
35    -194,25  -46,39  101,47          (-------*------)
                                -------+---------+---------+---------+-
                                     -200         0       200       400


Time = 21 subtracted from:

Time    Lower  Center   Upper  -------+---------+---------+---------+-
28    -112,07   35,79  183,64             (-------*------)
35    -246,08  -98,22   49,64        (-------*------)
                                -------+---------+---------+---------+-
                                     -200         0       200       400


Time = 28 subtracted from:

Time    Lower   Center  Upper  -------+---------+---------+---------+-
35    -281,86  -134,00  13,86      (------*-------)
                                -------+---------+---------+---------+-
                                     -200         0       200       400
```

One-way ANOVA: E80_AB_25°C versus Time

```
Source  DF     SS     MS      F      P
Time     7  87665  12524  18,52  0,000
Error   16  10819    676
Total   23  98484

S = 26,00   R-Sq = 89,01%   R-Sq(adj) = 84,21%


                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean  StDev  ----+---------+---------+---------+-----
 1     3  1601,6   27,8          (----*---)
 4     3  1699,5   17,3                       (----*---)
 7     3  1712,1   11,0                         (----*---)
10     3  1740,4   54,8                             (----*---)
14     3  1676,1   20,1                       (---*----)
21     3  1656,1   20,6                   (----*---)
28     3  1637,2   16,0                (----*---)
35     3  1540,4   11,4  (---*----)
                        ----+---------+---------+---------+-----
                         1540      1610      1680      1750

Pooled StDev = 26,0
```

Grouping Information Using Tukey Method

Time	N	Mean	Grouping
10	3	1740,36	A
7	3	1712,11	A B
4	3	1699,53	A B C
14	3	1676,05	A B C
21	3	1656,14	B C D
28	3	1637,22	C D
1	3	1601,56	D E
35	3	1540,36	E

Means that do not share a letter are significantly different.

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

Individual confidence level = 99,68%

Time = 1 subtracted from:

Time	Lower	Center	Upper	--------+---------+---------+---------+-
4	24,41	97,97	171,54	(----*---)
7	36,99	110,55	184,11	(----*----)
10	65,23	138,79	212,36	(----*----)
14	0,93	74,49	148,05	(----*----)
21	-18,99	54,58	128,14	(----*----)
28	-37,91	35,66	109,22	(----*----)
35	-134,77	-61,20	12,36	(----*----)
				--------+---------+---------+---------+-
				-150 0 150 300

Time = 4 subtracted from:

Time	Lower	Center	Upper	--------+---------+---------+---------+-
7	-60,99	12,58	86,14	(----*----)
10	-32,74	40,82	114,39	(----*----)
14	-97,05	-23,48	50,08	(---*----)
21	-116,96	-43,40	30,17	(----*----)
28	-135,88	-62,31	11,25	(----*----)
35	-232,74	-159,18	-85,61	(----*----)
				--------+---------+---------+---------+-
				-150 0 150 300

Time = 7 subtracted from:

Time	Lower	Center	Upper	--------+---------+---------+---------+-
10	-45,32	28,24	101,81	(----*----)
14	-109,62	-36,06	37,50	(----*----)
21	-129,54	-55,98	17,59	(----*----)
28	-148,46	-74,89	-1,33	(----*----)
35	-245,32	-171,75	-98,19	(----*---)
				--------+---------+---------+---------+-
				-150 0 150 300

```
Time = 10 subtracted from:

Time    Lower   Center    Upper  --------+---------+---------+---------
+-
14    -137,87   -64,30     9,26           (----*----)
21    -157,78   -84,22   -10,65         (----*----)
28    -176,70  -103,13   -29,57        (----*----)
35    -273,56  -200,00  -126,43  (----*----)
                                --------+---------+---------+---------
+-
                                     -150         0       150       300


Time = 14 subtracted from:

Time    Lower   Center   Upper  --------+---------+---------+---------+-
21     -93,48   -19,91   53,65             (----*----)
28    -112,39   -38,83   34,73            (---*----)
35    -209,26  -135,69  -62,13      (----*----)
                                --------+---------+---------+---------+-
                                     -150         0       150       300


Time = 21 subtracted from:

Time    Lower   Center   Upper  --------+---------+---------+---------+-
28     -92,48   -18,92   54,65             (----*----)
35    -189,34  -115,78  -42,22       (----*----)
                                --------+---------+---------+---------+-
                                     -150         0       150       300


Time = 28 subtracted from:

Time    Lower  Center   Upper  --------+---------+---------+---------+-
35    -170,43  -96,86  -23,30         (----*---)
                               --------+---------+---------+---------+-
                                    -150         0       150       300
```

One-way ANOVA: E80_PB_25°C versus Time

```
Source  DF      SS     MS     F      P
Time     7  205542  29363  7,47  0,000
Error   16   62871   3929
Total   23  268413

S = 62,69   R-Sq = 76,58%   R-Sq(adj) = 66,33%


                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean  StDev  ------+---------+---------+---------+---
 1     3  1562,7   27,9  (-----*------)
 4     3  1824,7   57,0                      (-----*-----)
 7     3  1864,0   15,9                         (-----*------)
10     3  1833,0   26,6                      (------*-----)
14     3  1808,1   41,8                    (------*-----)
21     3  1877,5    7,3                         (-----*------)
28     3  1773,0  152,7                  (------*-----)
35     3  1781,7   36,6                   (-----*------)
```

```
                        ------+---------+---------+---------+---
                           1560      1680      1800      1920
```

Pooled StDev = 62,7

Grouping Information Using Tukey Method

Time	N	Mean	Grouping
21	3	1877,47	A
7	3	1863,96	A
10	3	1832,95	A
4	3	1824,68	A
14	3	1808,11	A
35	3	1781,71	A
28	3	1773,01	A
1	3	1562,71	B

Means that do not share a letter are significantly different.

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

Individual confidence level = 99,68%

Time = 1 subtracted from:

Time	Lower	Center	Upper	----+---------+---------+---------+-----
4	84,64	261,98	439,31	(--------*--------)
7	123,92	301,26	478,59	(--------*--------)
10	92,91	270,25	447,58	(--------*-------)
14	68,07	245,40	422,74	(--------*--------)
21	137,42	314,76	492,10	(--------*--------)
28	32,97	210,31	387,64	(--------*-------)
35	41,67	219,00	396,34	(--------*--------)

```
                           ----+---------+---------+---------+-----
                            -200         0       200       400
```

Time = 4 subtracted from:

Time	Lower	Center	Upper	----+---------+---------+---------+-----
7	-138,06	39,28	216,62	(--------*--------)
10	-169,07	8,27	185,61	(-------*--------)
14	-193,91	-16,57	160,77	(--------*--------)
21	-124,55	52,79	230,12	(--------*--------)
28	-229,01	-51,67	125,67	(-------*--------)
35	-220,31	-42,97	134,37	(--------*--------)

```
                           ----+---------+---------+---------+-----
                            -200         0       200       400
```

Time = 7 subtracted from:

Time	Lower	Center	Upper	----+---------+---------+---------+-----
10	-208,35	-31,01	146,33	(-------*--------)
14	-233,19	-55,85	121,49	(--------*-------)
21	-163,83	13,50	190,84	(--------*--------)
28	-268,29	-90,95	86,39	(-------*--------)

```
35    -259,59  -82,25   95,08   (--------*--------)
                                ----+---------+---------+---------+-----
                                 -200         0       200       400


Time = 10 subtracted from:

Time    Lower  Center   Upper  ----+---------+---------+---------+-----
14    -202,18  -24,84  152,50      (--------*--------)
21    -132,82   44,51  221,85         (--------*--------)
28    -237,28  -59,94  117,40    (--------*--------)
35    -228,58  -51,24  126,09     (--------*--------)
                                ----+---------+---------+---------+-----
                                 -200         0       200       400


Time = 14 subtracted from:

Time    Lower  Center   Upper  ----+---------+---------+---------+-----
21    -107,98   69,36  246,69           (-------*--------)
28    -212,44  -35,10  142,24     (--------*--------)
35    -203,74  -26,40  150,94      (--------*--------)
                                ----+---------+---------+---------+-----
                                 -200         0       200       400


Time = 21 subtracted from:

Time    Lower   Center  Upper  ----+---------+---------+---------+-----
28    -281,79  -104,46  72,88  (--------*--------)
35    -273,09   -95,76  81,58  (--------*--------)
                                ----+---------+---------+---------+-----
                                 -200         0       200       400


Time = 28 subtracted from:

Time    Lower  Center   Upper  ----+---------+---------+---------+-----
35    -168,64    8,70  186,04        (-------*--------)
                                ----+---------+---------+---------+-----
                                 -200         0       200       400
```

Table A.17 Analysis of Variance for T_2 Times with respect to Temperature for 1300 Bar Microfluidized Samples, using Adjusted SS for Tests.

One-way ANOVA: S75_AB_4°C versus Time

```
Source  DF     SS    MS     F      P
Time     7  12326  1761  2,08  0,107
Error   16  13568   848
Total   23  25894

S = 29,12   R-Sq = 47,60%   R-Sq(adj) = 24,68%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev  ------+---------+---------+---------+---
 1     3  1694,4   19,9             (--------*--------)
 4     3  1720,6   48,2                   (--------*--------)
 7     3  1651,8   12,9  (--------*--------)
10     3  1684,4   38,2          (--------*--------)
14     3  1709,8   28,4                 (-------*--------)
21     3  1680,2   14,5          (--------*--------)
28     3  1711,4   36,1                 (--------*--------)
35     3  1723,4   10,9                    (--------*--------)
                          ------+---------+---------+---------+---
                             1640      1680      1720      1760

Pooled StDev = 29,1


Grouping Information Using Tukey Method

Time  N     Mean  Grouping
35    3  1723,37  A
 4    3  1720,55  A
28    3  1711,37  A
14    3  1709,81  A
 1    3  1694,40  A
10    3  1684,41  A
21    3  1680,20  A
 7    3  1651,83  A

Means that do not share a letter are significantly different.


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

Individual confidence level = 99,68%


Time =  1 subtracted from:

Time    Lower  Center   Upper  ---------+---------+---------+---------+
 4     -56,23   26,15  108,53              (---------*----------)
 7    -124,96  -42,58   39,80     (----------*---------)
10     -92,37   -9,99   72,39         (----------*---------)
```

```
14     -66,98   15,40   97,78             (---------*---------)
21     -96,59  -14,21   68,17          (---------*----------)
28     -65,41   16,97   99,35             (---------*---------)
35     -53,42   28,96  111,35              (----------*---------)
                                 ---------+---------+---------+---------+
                                        -80         0        80       160
```

Time = 4 subtracted from:

```
Time    Lower  Center  Upper  ---------+---------+---------+---------+
 7    -151,11  -68,72  13,66  (---------*----------)
10    -118,52  -36,14  46,24      (---------*----------)
14     -93,13  -10,74  71,64         (----------*---------)
21    -122,74  -40,36  42,03      (---------*---------)
28     -91,56   -9,18  73,20          (---------*---------)
35     -79,56    2,82  85,20           (---------*----------)
                         ---------+---------+---------+---------+
                                -80         0        80       160
```

Time = 7 subtracted from:

```
Time   Lower  Center   Upper  ---------+---------+---------+---------+
10    -49,79   32,59  114,97               (---------*---------)
14    -24,40   57,98  140,36                  (---------*----------)
21    -54,01   28,37  110,75               (----------*---------)
28    -22,83   59,55  141,93                  (---------*----------)
35    -10,84   71,54  153,92                    (---------*---------)
                         ---------+---------+---------+---------+
                                -80         0        80       160
```

Time = 10 subtracted from:

```
Time   Lower  Center   Upper  ---------+---------+---------+---------+
14    -56,99   25,39  107,77             (---------*---------)
21    -86,60   -4,22   78,16          (---------*----------)
28    -55,42   26,96  109,34             (---------*----------)
35    -43,43   38,95  121,33               (---------*---------)
                         ---------+---------+---------+---------+
                                -80         0        80       160
```

Time = 14 subtracted from:

```
Time    Lower  Center  Upper  ---------+---------+---------+---------+
21    -111,99  -29,61  52,77       (---------*----------)
28     -80,81    1,57  83,95          (---------*---------)
35     -68,82   13,56  95,94           (----------*---------)
                         ---------+---------+---------+---------+
                                -80         0        80       160
```

Time = 21 subtracted from:

```
Time   Lower  Center   Upper  ---------+---------+---------+---------+
28    -51,20   31,18  113,56               (---------*---------)
35    -39,21   43,17  125,55                (---------*----------)
                         ---------+---------+---------+---------+
                                -80         0        80       160
```

```
Time = 28 subtracted from:

Time   Lower  Center  Upper  ---------+---------+---------+---------+
35    -70,39   11,99  94,38            (---------*----------)
                               ---------+---------+---------+---------+
                                      -80         0        80       160
```

One-way ANOVA: S75_PB_4°C versus Time

```
Source  DF      SS     MS      F      P
Time     7  388012  55430  24,15  0,000
Error   16   36725   2295
Total   23  424737

S = 47,91   R-Sq = 91,35%   R-Sq(adj) = 87,57%


                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level  N    Mean  StDev   --+---------+---------+---------+-------
 1     3  1479,9   21,4   (----*----)
 4     3  1499,2   19,1     (----*----)
 7     3  1504,6   24,1     (----*----)
10     3  1563,4   17,1          (----*----)
14     3  1658,4   84,6                (----*----)
21     3  1808,6   70,5                             (----*----)
28     3  1823,8   41,5                              (----*----)
35     3  1626,9   53,2              (----*---)
                         --+---------+---------+---------+-------
                        1440      1560      1680      1800

Pooled StDev = 47,9


Grouping Information Using Tukey Method

Time  N     Mean  Grouping
28    3  1823,79  A
21    3  1808,58  A
14    3  1658,40    B
35    3  1626,93    B C
10    3  1563,37    B C D
 7    3  1504,61      C D
 4    3  1499,23      C D
 1    3  1479,94        D

Means that do not share a letter are significantly different.


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

Individual confidence level = 99,68%


Time =  1 subtracted from:

Time    Lower  Center   Upper  --------+---------+---------+---------+-
```

```
 4    -116,25   19,29  154,83               (-----*----)
 7    -110,86   24,67  160,21                (----*----)
10     -52,11   83,43  218,96                  (----*-----)
14      42,93  178,46  314,00                      (----*-----)
21     193,11  328,64  464,18                            (----*-----)
28     208,31  343,85  479,39                            (-----*----)
35      11,46  146,99  282,53                   (-----*----)
                                --------+---------+---------+---------+-
                                     -250         0       250       500
```

Time = 4 subtracted from:

```
Time    Lower  Center   Upper  --------+---------+---------+---------+-
 7    -130,15    5,38  140,92               (----*-----)
10     -71,40   64,14  199,67                 (-----*----)
14      23,64  159,17  294,71                     (----*-----)
21     173,82  309,35  444,89                           (----*-----)
28     189,02  324,56  460,10                            (----*----)
35      -7,83  127,70  263,24                   (----*-----)
                                --------+---------+---------+---------+-
                                     -250         0       250       500
```

Time = 7 subtracted from:

```
Time   Lower  Center   Upper  --------+---------+---------+---------+-
10    -76,78   58,75  194,29               (----*-----)
14     18,25  153,79  289,33                   (----*-----)
21    168,43  303,97  439,51                         (----*-----)
28    183,64  319,17  454,71                         (-----*----)
35    -13,22  122,32  257,86                  (-----*----)
                                --------+---------+---------+---------+-
                                     -250         0       250       500
```

Time = 10 subtracted from:

```
Time   Lower  Center   Upper  --------+---------+---------+---------+-
14    -40,50   95,04  230,57                (-----*----)
21    109,68  245,22  380,75                      (-----*----)
28    124,89  260,42  395,96                       (----*-----)
35    -71,97   63,57  199,10               (-----*----)
                                --------+---------+---------+---------+-
                                     -250         0       250       500
```

Time = 14 subtracted from:

```
Time    Lower  Center   Upper  --------+---------+---------+---------+-
21      14,64  150,18  285,72                   (----*----)
28      29,85  165,39  300,92                   (-----*----)
35    -167,01  -31,47  104,07            (-----*----)
                                --------+---------+---------+---------+-
                                     -250         0       250       500
```

Time = 21 subtracted from:

```
Time    Lower   Center   Upper  --------+---------+---------+---------+-
28    -120,33    15,20  150,74               (-----*----)
```

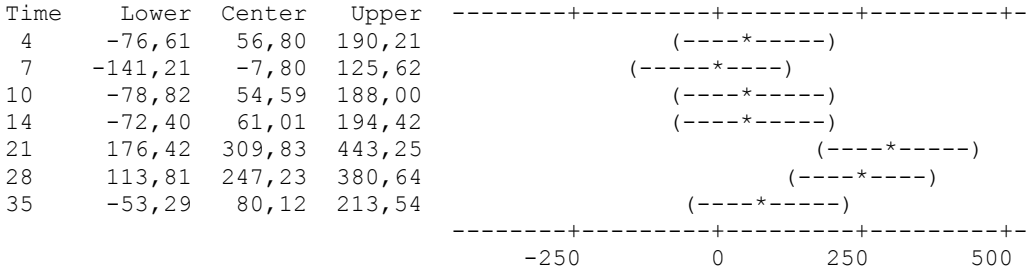
```
35    -317,19  -181,65  -46,11       (-----*----)
                                  ---------+---------+---------+---------+-
                                       -250         0       250       500


Time = 28 subtracted from:

Time    Lower   Center   Upper  ---------+---------+---------+---------+-
35    -332,39  -196,86  -61,32       (----*-----)
                                  ---------+---------+---------+---------+-
                                       -250         0       250       500
```

One-way ANOVA: E80_AB_4°C versus Time

```
Source  DF      SS     MS      F      P
Time     7  279508  39930  17,95  0,000
Error   16   35583   2224
Total   23  315091

S = 47,16   R-Sq = 88,71%   R-Sq(adj) = 83,77%


                          Individual 95% CIs For Mean Based on Pooled
StDev
Level  N    Mean  StDev     +---------+---------+---------+---------
 1     3  1741,8   56,7     (----*----)
 4     3  1798,6   27,1          (----*----)
 7     3  1734,0    9,3     (----*---)
10     3  1796,4   55,2          (----*----)
14     3  1802,8   12,3          (----*----)
21     3  2051,7   67,3                              (----*----)
28     3  1989,1   53,0                         (----*----)
35     3  1822,0   56,7            (----*----)
                               +---------+---------+---------+---------
                             1680      1800      1920      2040

Pooled StDev = 47,2


Grouping Information Using Tukey Method

Time  N     Mean  Grouping
21    3  2051,67  A
28    3  1989,06  A
35    3  1821,96    B
14    3  1802,84    B
 4    3  1798,63    B
10    3  1796,42    B
 1    3  1741,83    B
 7    3  1734,04    B

Means that do not share a letter are significantly different.


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

Individual confidence level = 99,68%
```

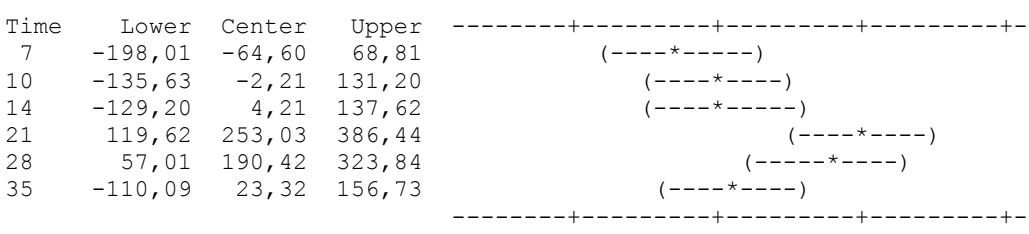
Time = 1 subtracted from:

Time	Lower	Center	Upper
4	-76,61	56,80	190,21
7	-141,21	-7,80	125,62
10	-78,82	54,59	188,00
14	-72,40	61,01	194,42
21	176,42	309,83	443,25
28	113,81	247,23	380,64
35	-53,29	80,12	213,54



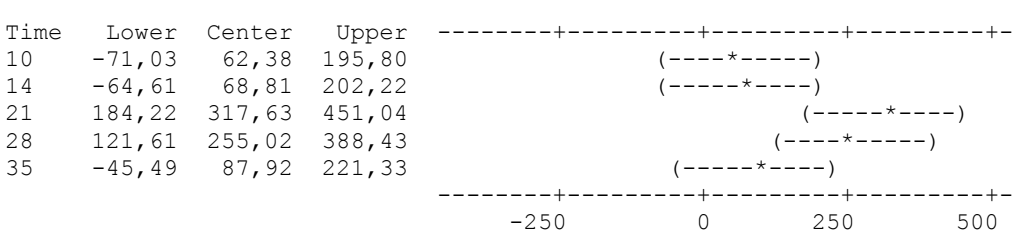
Time = 4 subtracted from:

Time	Lower	Center	Upper
7	-198,01	-64,60	68,81
10	-135,63	-2,21	131,20
14	-129,20	4,21	137,62
21	119,62	253,03	386,44
28	57,01	190,42	323,84
35	-110,09	23,32	156,73



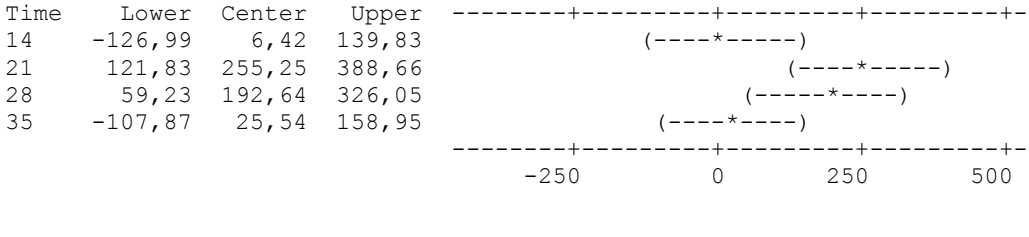
Time = 7 subtracted from:

Time	Lower	Center	Upper
10	-71,03	62,38	195,80
14	-64,61	68,81	202,22
21	184,22	317,63	451,04
28	121,61	255,02	388,43
35	-45,49	87,92	221,33



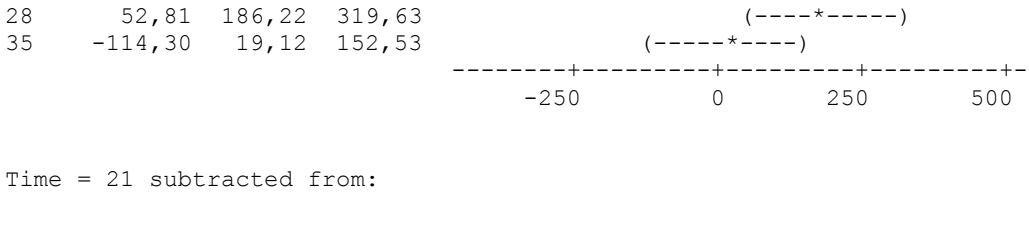
Time = 10 subtracted from:

Time	Lower	Center	Upper
14	-126,99	6,42	139,83
21	121,83	255,25	388,66
28	59,23	192,64	326,05
35	-107,87	25,54	158,95



Time = 14 subtracted from:

Time	Lower	Center	Upper
21	115,41	248,82	382,24
28	52,81	186,22	319,63
35	-114,30	19,12	152,53



Time = 21 subtracted from:

```
Time    Lower   Center   Upper  --------+---------+---------+---------+-
28    -196,02   -62,61   70,80            (----*-----)
35    -363,12  -229,71  -96,30     (-----*----)
                                 --------+---------+---------+---------+-
                                      -250         0       250       500


Time = 28 subtracted from:

Time    Lower   Center   Upper  --------+---------+---------+---------+-
35    -300,51  -167,10  -33,69        (----*-----)
                                 --------+---------+---------+---------+-
                                      -250         0       250       500
```

One-way ANOVA: E80_PB_4°C versus Time

```
Source  DF      SS     MS      F      P
Time     7  463041  66149  33,67  0,000
Error   16   31434   1965
Total   23  494475

S = 44,32   R-Sq = 93,64%   R-Sq(adj) = 90,86%


                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean  StDev  --------+---------+---------+---------+-
 1     3  1523,9   15,3  (----*----)
 4     3  1607,9   28,7         (----*----)
 7     3  1544,0   32,3    (----*---)
10     3  1652,0   20,1              (----*---)
14     3  1874,6   27,0                                (---*----)
21     3  1843,2   95,8                             (----*---)
28     3  1870,5   42,6                               (----*---)
35     3  1799,0   38,6                          (----*---)
                        --------+---------+---------+---------+-
                             1560      1680      1800      1920

Pooled StDev = 44,3


Grouping Information Using Tukey Method

Time  N     Mean  Grouping
14    3  1874,59  A
28    3  1870,53  A
21    3  1843,19  A
35    3  1799,01  A
10    3  1651,99    B
 4    3  1607,87    B C
 7    3  1543,96    B C
 1    3  1523,95      C

Means that do not share a letter are significantly different.


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

```
Individual confidence level = 99,68%


Time =  1 subtracted from:

Time    Lower  Center   Upper  --------+---------+---------+---------+-
 4     -41,47   83,92  209,32                  (----*----)
 7    -105,38   20,02  145,41                (----*----)
10       2,65  128,04  253,43                   (----*----)
14     225,25  350,64  476,03                            (----*----)
21     193,85  319,25  444,64                          (----*----)
28     221,18  346,58  471,97                           (----*----)
35     149,67  275,07  400,46                       (----*----)
                              --------+---------+---------+---------+-
                                   -250         0       250       500


Time =  4 subtracted from:

Time    Lower  Center   Upper  --------+---------+---------+---------+-
 7    -189,30  -63,91   61,49           (----*----)
10     -81,27   44,12  169,51                (----*----)
14     141,33  266,72  392,11                        (----*----)
21     109,93  235,32  360,72                      (----*----)
28     137,26  262,65  388,05                       (-----*----)
35      65,75  191,14  316,54                     (----*----)
                              --------+---------+---------+---------+-
                                   -250         0       250       500


Time =  7 subtracted from:

Time   Lower  Center   Upper  --------+---------+---------+---------+-
10    -17,37  108,03  233,42                  (----*----)
14    205,23  330,63  456,02                           (----*----)
21    173,84  299,23  424,62                         (----*----)
28    201,17  326,56  451,95                          (----*----)
35    129,66  255,05  380,44                      (----*----)
                            --------+---------+---------+---------+-
                                 -250         0       250       500


Time = 10 subtracted from:

Time  Lower  Center   Upper  --------+---------+---------+---------+-
14    97,21  222,60  347,99                       (----*----)
21    65,81  191,20  316,60                     (----*----)
28    93,14  218,54  343,93                       (----*----)
35    21,63  147,02  272,42                   (----*----)
                          --------+---------+---------+---------+-
                               -250         0       250       500


Time = 14 subtracted from:

Time    Lower  Center   Upper  --------+---------+---------+---------+-
21    -156,79  -31,40   94,00             (----*----)
28    -129,46   -4,06  121,33              (----*----)
35    -200,97  -75,58   49,82           (----*----)
                              --------+---------+---------+---------+-
                                   -250         0       250       500
```

```
Time = 21 subtracted from:

Time    Lower  Center   Upper  --------+---------+---------+---------+-
28     -98,06   27,33  152,72               (----*----)
35    -169,57  -44,18   81,21            (----*----)
                                --------+---------+---------+---------+-
                                     -250         0       250       500


Time = 28 subtracted from:

Time    Lower  Center  Upper  --------+---------+---------+---------+-
35    -196,90  -71,51  53,88           (----*----)
                               --------+---------+---------+---------+-
                                    -250         0       250       500
```

One-way ANOVA: S75_AB_25°C versus Time

```
Source  DF     SS    MS     F      P
Time     7  43996  6285  2,27  0,083
Error   16  44285  2768
Total   23  88281

S = 52,61   R-Sq = 49,84%   R-Sq(adj) = 27,89%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev  -------+---------+---------+---------+--
 1     3  1698,7    9,2  (---------*--------)
 4     3  1708,4  136,3    (--------*--------)
 7     3  1721,3   21,1      (--------*--------)
10     3  1769,5   21,4             (--------*--------)
14     3  1727,4   29,0       (--------*--------)
21     3  1830,4   20,8                    (--------*---------)
28     3  1761,6    1,1           (---------*--------)
35     3  1794,9   35,9                (--------*---------)
                          -------+---------+---------+---------+--
                              1680      1750      1820      1890

Pooled StDev = 52,6


Grouping Information Using Tukey Method

Time  N     Mean  Grouping
21    3  1830,36  A
35    3  1794,93  A
10    3  1769,48  A
28    3  1761,60  A
14    3  1727,44  A
 7    3  1721,34  A
 4    3  1708,42  A
 1    3  1698,67  A

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

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

Individual confidence level = 99,68%


Time =  1 subtracted from:

Time    Lower  Center   Upper  --------+---------+---------+---------+-
 4    -139,09    9,75  158,58           (---------*---------)
 7    -126,16   22,67  171,51            (---------*--------)
10     -78,03   70,81  219,64               (---------*---------)
14    -120,07   28,77  177,60            (---------*---------)
21     -17,15  131,69  280,52                    (---------*---------)
28     -85,91   62,93  211,76              (---------*---------)
35     -52,58   96,25  245,09                (---------*---------)
                       --------+---------+---------+---------+-
                            -150         0       150       300


Time =  4 subtracted from:

Time    Lower  Center   Upper  --------+---------+---------+---------+-
 7    -135,91   12,93  161,76           (---------*---------)
10     -87,77   61,06  209,90              (---------*---------)
14    -129,81   19,02  167,86           (---------*---------)
21     -26,89  121,94  270,78                  (---------*---------)
28     -95,66   53,18  202,01              (---------*--------)
35     -62,33   86,51  235,34                (---------*---------)
                       --------+---------+---------+---------+-
                            -150         0       150       300


Time =  7 subtracted from:

Time    Lower  Center   Upper  --------+---------+---------+---------+-
10    -100,70   48,13  196,97             (---------*---------)
14    -142,74    6,09  154,93          (---------*---------)
21     -39,82  109,02  257,85                (---------*---------)
28    -108,58   40,25  189,09             (---------*---------)
35     -75,25   73,58  222,42               (---------*---------)
                       --------+---------+---------+---------+-
                            -150         0       150       300


Time = 10 subtracted from:

Time    Lower  Center   Upper  --------+---------+---------+---------+-
14    -190,88  -42,04  106,79       (---------*---------)
21     -87,95   60,88  209,72              (---------*---------)
28    -156,72   -7,88  140,95          (---------*---------)
35    -123,39   25,45  174,28            (---------*---------)
                       --------+---------+---------+---------+-
                            -150         0       150       300


Time = 14 subtracted from:

Time    Lower  Center   Upper  --------+---------+---------+---------+-
21     -45,91  102,92  251,76                 (---------*---------)
28    -114,68   34,16  182,99             (---------*---------)
```

```
35     -81,35   67,49  216,32               (--------*---------)
                                  --------+---------+---------+---------+-
                                       -150         0       150       300


Time = 21 subtracted from:

Time    Lower  Center   Upper  --------+---------+---------+---------+-
28    -217,60  -68,76   80,07     (---------*---------)
35    -184,27  -35,43  113,40        (---------*---------)
                                  --------+---------+---------+---------+-
                                       -150         0       150       300


Time = 28 subtracted from:

Time    Lower  Center   Upper  --------+---------+---------+---------+-
35    -115,51   33,33  182,16           (---------*---------)
                                  --------+---------+---------+---------+-
                                       -150         0       150       300
```

One-way ANOVA: S75_PB_25°C versus Time

```
Source  DF      SS     MS      F      P
Time     7  170227  24318  15,04  0,000
Error   16   25875   1617
Total   23  196102

S = 40,21   R-Sq = 86,81%   R-Sq(adj) = 81,03%


                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean  StDev   --+---------+---------+---------+-------
 1     3  1466,5   17,5   (---*---)
 4     3  1685,6   45,0                    (---*----)
 7     3  1655,5   28,4                  (---*---)
10     3  1678,1   36,0                    (---*---)
14     3  1709,1   49,3                      (---*----)
21     3  1711,0   31,0                      (----*---)
28     3  1777,2   63,7                            (---*---)
35     3  1657,2   32,3                  (---*---)
                        --+---------+---------+---------+-------
                       1440      1560      1680      1800

Pooled StDev = 40,2


Grouping Information Using Tukey Method

Time  N     Mean  Grouping
28    3  1777,18  A
21    3  1711,03  A B
14    3  1709,06  A B
 4    3  1685,58  A B
10    3  1678,11  A B
35    3  1657,24    B
 7    3  1655,47    B
 1    3  1466,50      C
```

Means that do not share a letter are significantly different.

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

Individual confidence level = 99,68%

Time = 1 subtracted from:

Time	Lower	Center	Upper	--+---------+---------+---------+-------
4	105,31	219,08	332,85	(-----*-----)
7	75,20	188,97	302,74	(----*-----)
10	97,84	211,61	325,38	(-----*----)
14	128,80	242,56	356,33	(-----*-----)
21	130,76	244,53	358,30	(----*-----)
28	196,91	310,68	424,45	(-----*----)
35	76,97	190,74	304,51	(-----*----)
				--+---------+---------+---------+-------
				-200 0 200 400

Time = 4 subtracted from:

Time	Lower	Center	Upper	--+---------+---------+---------+-------
7	-143,88	-30,11	83,66	(----*-----)
10	-121,24	-7,47	106,30	(-----*----)
14	-90,28	23,48	137,25	(-----*-----)
21	-88,32	25,45	139,22	(----*-----)
28	-22,17	91,60	205,37	(-----*----)
35	-142,11	-28,34	85,43	(-----*----)
				--+---------+---------+---------+-------
				-200 0 200 400

Time = 7 subtracted from:

Time	Lower	Center	Upper	--+---------+---------+---------+-------
10	-91,13	22,64	136,40	(-----*-----)
14	-60,17	53,59	167,36	(-----*----)
21	-58,21	55,56	169,33	(-----*----)
28	7,94	121,71	235,47	(-----*-----)
35	-112,00	1,77	115,53	(-----*-----)
				--+---------+---------+---------+-------
				-200 0 200 400

Time = 10 subtracted from:

Time	Lower	Center	Upper	--+---------+---------+---------+-------
14	-82,81	30,96	144,72	(-----*----)
21	-80,84	32,92	146,69	(-----*----)
28	-14,70	99,07	212,84	(-----*-----)
35	-134,64	-20,87	92,90	(-----*-----)
				--+---------+---------+---------+-------
				-200 0 200 400

Time = 14 subtracted from:

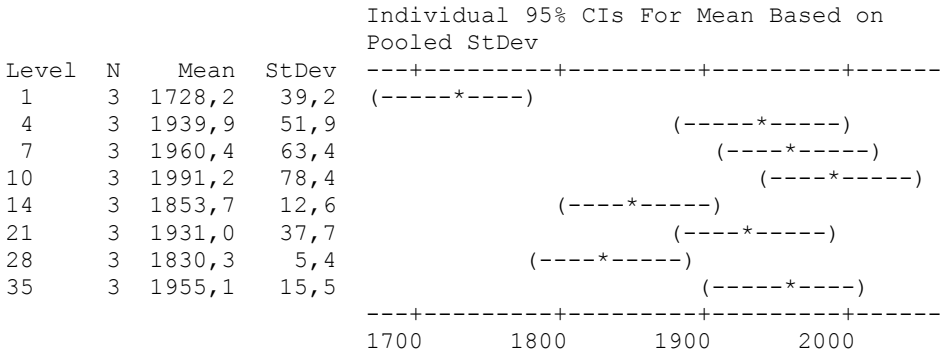
```
Time    Lower  Center   Upper   --+---------+---------+---------+-------
21    -111,80    1,97  115,73         (-----*-----)
28     -45,65   68,11  181,88             (----*-----)
35    -165,59  -51,83   61,94        (----*-----)
                                  --+---------+---------+---------+-------
                                 -200         0       200       400
```

```
Time = 21 subtracted from:

Time    Lower  Center   Upper   --+---------+---------+---------+-------
28     -47,62   66,15  179,91             (----*-----)
35    -167,56  -53,79   59,97        (----*-----)
                                  --+---------+---------+---------+-------
                                 -200         0       200       400
```

```
Time = 28 subtracted from:

Time    Lower   Center  Upper   --+---------+---------+---------+-------
35    -233,71  -119,94  -6,17   (-----*-----)
                                  --+---------+---------+---------+-------
                                 -200         0       200       400
```

One-way ANOVA: E80_AB_25°C versus Time

```
Source  DF      SS     MS      F      P
Time     7  162192  23170  11,41  0,000
Error   16   32497   2031
Total   23  194690

S = 45,07   R-Sq = 83,31%   R-Sq(adj) = 76,01%
```




```
Pooled StDev = 45,1
```

```
Grouping Information Using Tukey Method

Time  N     Mean  Grouping
10    3  1991,25  A
 7    3  1960,44  A B
35    3  1955,08  A B C
 4    3  1939,88  A B C
21    3  1931,03  A B C
14    3  1853,65    B C D
```

```
28    3  1830,27      C D
 1    3  1728,24        D

Means that do not share a letter are significantly different.


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

Individual confidence level = 99,68%


Time =  1 subtracted from:

Time   Lower  Center   Upper  ----+---------+---------+---------+-----
 4     84,14  211,63  339,13                   (------*-----)
 7    104,70  232,19  359,69                    (------*-----)
10    135,51  263,01  390,50                      (-----*------)
14     -2,09  125,41  252,90                (-----*------)
21     75,29  202,79  330,29                    (-----*------)
28    -25,47  102,03  229,52               (-----*-----)
35     99,34  226,84  354,33                     (-----*------)
                           ----+---------+---------+---------+-----
                            -200         0       200       400


Time =  4 subtracted from:

Time    Lower   Center   Upper  ----+---------+---------+---------+-----
 7   -106,93    20,56  148,06           (-----*-----)
10    -76,12    51,37  178,87            (------*-----)
14   -213,72   -86,22   41,27     (------*-----)
21   -136,34    -8,84  118,65         (------*-----)
28   -237,10  -109,61   17,89    (------*-----)
35   -112,29    15,21  142,70          (------*-----)
                           ----+---------+---------+---------+-----
                            -200         0       200       400


Time =  7 subtracted from:

Time    Lower   Center   Upper  ----+---------+---------+---------+-----
10    -96,69    30,81  158,31           (------*-----)
14   -234,28  -106,79   20,71    (------*-----)
21   -156,90   -29,40   98,09        (------*-----)
28   -257,67  -130,17   -2,67   (-----*------)
35   -132,85    -5,36  122,14         (------*-----)
                           ----+---------+---------+---------+-----
                            -200         0       200       400


Time = 10 subtracted from:

Time    Lower   Center   Upper  ----+---------+---------+---------+-----
14   -265,09  -137,60  -10,10   (-----*-----)
21   -187,71   -60,22   67,28       (-----*-----)
28   -288,48  -160,98  -33,48  (-----*-----)
35   -163,66   -36,17   91,33        (-----*------)
                           ----+---------+---------+---------+-----
                            -200         0       200       400
```

```
Time = 14 subtracted from:

Time    Lower  Center   Upper  ----+---------+---------+---------+-----
21     -50,11   77,38  204,88             (------*-----)
28    -150,88  -23,38  104,11        (------*-----)
35     -26,07  101,43  228,93               (-----*-----)
                                ----+---------+---------+---------+-----
                                -200         0       200       400


Time = 21 subtracted from:

Time    Lower   Center   Upper  ----+---------+---------+---------+-----
28    -228,26  -100,76   26,73     (-----*-----)
35    -103,45    24,05  151,54           (-----*------)
                                ----+---------+---------+---------+-----
                                 -200         0       200       400


Time = 28 subtracted from:

Time  Lower  Center   Upper  ----+---------+---------+---------+-----
35    -2,68  124,81  252,31               (-----*------)
                           ----+---------+---------+---------+-----
                            -200         0       200       400
```

One-way ANOVA: E80_PB_25°C versus Time

```
Source  DF      SS     MS      F      P
Time     7  306659  43808  12,32  0,000
Error   16   56907   3557
Total   23  363566

S = 59,64   R-Sq = 84,35%   R-Sq(adj) = 77,50%


                        Individual 95% CIs For Mean Based on
                        Pooled StDev
Level  N    Mean  StDev  ----+---------+---------+---------+-----
 1     3  1506,1   24,6  (---*----)
 4     3  1873,8   41,0                          (----*----)
 7     3  1812,2   38,5                       (----*----)
10     3  1864,6   62,2                          (----*----)
14     3  1797,9    9,1                       (----*----)
21     3  1834,2  124,3                         (----*----)
28     3  1870,9   40,9                          (----*----)
35     3  1806,5   60,0                       (---*----)
                        ----+---------+---------+---------+-----
                         1500      1650      1800      1950

Pooled StDev = 59,6


Grouping Information Using Tukey Method

Time  N     Mean  Grouping
 4    3  1873,78  A
28    3  1870,87  A
10    3  1864,61  A
```

```
21    3  1834,23  A
 7    3  1812,23  A
35    3  1806,45  A
14    3  1797,93  A
 1    3  1506,14    B
```

Means that do not share a letter are significantly different.

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

Individual confidence level = 99,68%

Time = 1 subtracted from:

```
Time   Lower  Center   Upper   --+---------+---------+---------+-------
 4    198,93  367,64  536,36                        (-------*--------)
 7    137,37  306,09  474,81                      (-------*--------)
10    189,76  358,47  527,19                        (--------*-------)
14    123,08  291,79  460,51                     (--------*-------)
21    159,37  328,09  496,81                       (-------*--------)
28    196,01  364,73  533,44                         (-------*--------)
35    131,60  300,31  469,03                      (-------*-------)
                           --+---------+---------+---------+-------
                          -200         0       200       400
```

Time = 4 subtracted from:

```
Time    Lower  Center   Upper   --+---------+---------+---------+-------
 7    -230,27  -61,55  107,16   (--------*-------)
10    -177,89   -9,17  159,55      (--------*-------)
14    -244,56  -75,85   92,87   (-------*--------)
21    -208,27  -39,55  129,17     (-------*-------)
28    -171,63   -2,91  165,80      (--------*-------)
35    -236,05  -67,33  101,39   (--------*-------)
                           --+---------+---------+---------+-------
                          -200         0       200       400
```

Time = 7 subtracted from:

```
Time    Lower  Center   Upper   --+---------+---------+---------+-------
10    -116,33   52,38  221,10         (--------*-------)
14    -183,01  -14,30  154,42      (-------*--------)
21    -146,71   22,00  190,72        (-------*--------)
28    -110,08   58,64  227,35         (--------*-------)
35    -174,49   -5,78  162,94      (--------*-------)
                           --+---------+---------+---------+-------
                          -200         0       200       400
```

Time = 10 subtracted from:

```
Time    Lower  Center   Upper   --+---------+---------+---------+-------
14    -235,39  -66,68  102,04   (--------*-------)
21    -199,10  -30,38  138,34     (-------*--------)
28    -162,46    6,26  174,97       (-------*--------)
35    -226,88  -58,16  110,56    (-------*--------)
```

```
                                 --+---------+---------+---------+-------
                                -200         0       200       400
```

Time = 14 subtracted from:

```
Time    Lower  Center   Upper   --+---------+---------+---------+-------
21    -132,42   36,30  205,01        (--------*-------)
28     -95,78   72,93  241,65          (--------*-------)
35    -160,20    8,52  177,24       (-------*--------)
                                 --+---------+---------+---------+-------
                                -200         0       200       400
```

Time = 21 subtracted from:

```
Time    Lower  Center   Upper   --+---------+---------+---------+-------
28    -132,08   36,64  205,35        (--------*-------)
35    -196,49  -27,78  140,94     (--------*-------)
                                 --+---------+---------+---------+-------
                                -200         0       200       400
```

Time = 28 subtracted from:

```
Time    Lower  Center   Upper   --+---------+---------+---------+-------
35    -233,13  -64,41  104,30   (--------*-------)
                                 --+---------+---------+---------+-------
                                -200         0       200       400
```