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Synthesis and Characterization of Lactose-Amines with Respect to Oil-in-Water Emulsion Stability

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SYNTHESIS AND CHARACTERIZATION OF LACTOSE-AMINES WITH RESPECT TO OIL-IN-WATER EMULSION STABILITY

by

Nidhi Garg

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ABSTRACT

Synthesis and Characterization of Lactose-amines with Respect to Oil-in-Water Emulsion Stability

by

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Utah State University, 2008

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Fatty amines (hexadecyl-amine) can be esterified to lactose via Schiff-base formation at temperatures of 60° C. Extending the time of the reaction results in a darker colored product due to the Maillard reaction. Due to the amphiphilic properties of the lactose-amines, the emulsion stabilization characteristics were investigated.

In this study, synthesis of lactose-amines was done at four different heating and cooling cycles from 4 to 24 hours. Lactose-amines processed for 24 hours and 12 hours of constant heating and cooling cycles are named as 24H and 12H, respectively. Lactose-amines 4H and 8H were processed for 4 and 8 hours of constant heating at 60°C. The 24H and 12H samples were white in color as they were exposed to heat for short time (due to the cooling cycle) i.e. 2-2.5 and 1.5 hours, respectively, as compare to 4H and 8H (i.e. 4 hours and 8 hours, respectively). It was assumed that white colored compounds are early intermediates of Maillard browning reactions known as Amadori. The light brown color of the 4 hours heat-treated product might contain intermediate products of the

Maillard browning reaction. The dark brown colored after 8 hours of constant heating might have advanced Maillard products and polymers.

Each lactose-amine sample was used as emulsifiers in oil-in-water (20:80 ratio of oil: water) emulsion at four different concentrations (0.01%, 0.05%, 0.1%, and 1%). Negative controls consisted of hexadecyl-amine and lactose at the same concentrations as stated above, as well as an oil-in-water control. The positive control was an emulsion containing 2% whey protein (WP). Emulsions were formed with a microfluidizer 110S at a pressure of 6,900 psi. Emulsion stability was monitored by measuring the oil droplet sizes of each emulsion on day 0 and destabilization kinetics on day 1 and 5.

The oil droplet size distribution and destabilization kinetics of the emulsions prepared with lactose-amines (4H, 8H, 12H, and 24H) at 0.01% of concentration were closer towards the negative controls (lactose, fatty-amine, and o/w). At 1% concentration, emulsions prepared with all types of lactose-amines had smaller droplet size similar to WPC 80. Destabilization kinetic profiles of the emulsions show that 1% lactose-amines produced more stabilized emulsions as compared to WPC 80 with respect to time. Emulsions of 4H and 24H were following the similar trend of droplet size distribution and destabilization rate as of WPC 80. Lactose-amines 8H and 12H emulsions were showing more destabilization and bigger oil droplet size as compared to 4H, 24H, and WPC 80. Droplet size distribution at day 0 and destabilization kinetics from day 0 to day 5 showed that the types of lactose-amines and their increasing concentrations have great influence on the stability of emulsions. This research has shown that lactose-amines produced at treatments of 24 and 4 hours are effective at stabilizing emulsions at 1% concentration. (98 pages)

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Nidhi Garg

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LIST of SYMBOLS, NOTATIONS and DEFINITIONS

Abbreviation Key

µm	micrometer
12H	12 hours
24H	24 hours
4H	4 hours
8H	8 hours
ANOVA	analysis of variance
C	(degrees) Celcius
conc	concentration
$D_{(3,2)}$	surface-volume mean particle diameter
df	degree of freedom
FA	fatty amine
g	gram
GRAS	Generally Recognized as Safe
hrs	hours
L	lactose
HCA	hexadecyl amine
LA	lactose-amines
M	molar (moles per litre)
min	minute
ml	milliliter

mM	milimolar
mm	millimeter
n	number of observations
O/W	oil-in-water
obs	observations
pH	potential of hydrogen
rpm	revolutions per minute
SD	standard deviation
WP	whey protein
WPC	whey protein concentrate
WPI	whey protein isolates
Δ BS	change in backscattering

INTRODUCTION

Emulsions are a mixture of two immiscible liquids, which are unstable systems due to the dispersed phase, which divides into small droplets increasing the contact area between both liquid phases (Hui 2007). Some food and food products consist of complex emulsions (Bee et al. 1989; Larson and Friberg 1990). Some of the most commonly known examples of o/w (oil-in-water) emulsions are salad dressing, ice-cream, and mayonnaise.

Instabilities in Emulsions

Emulsion is the mixture of two unblendable or immiscible liquids like oil and water, by applying shear pressure causing changes in the interfacial layers of both the liquids. With the progression of time emulsions destabilize. Instabilities in emulsions are creaming, coalescence and flocculation.

Creaming is when the dispersed phase has a lower density than the continuous phase and can be coupled with coalescence or flocculation, which leads to a phase separation. An example of creaming is the rising of the layer of fat in raw milk. Coalescence and flocculation phenomena are physico-chemically very different, but they both lead to an increase in the size of the oil droplets. Coalescence is irreversible and leads to the fusion of the interfaces, hence the creation of one single oil drop, while flocculation is an aggregation of the oil droplets. These instabilities occur in emulsions due to insufficient emulsifiers which cover the entire oil-water interface protecting oil droplets from interacting with each other, leading to the inhibition of the flocculation and coalescence phenomenon.

Mechanism of Emulsifiers

Emulsifiers or surface-active agents can be used to make emulsions stable for a reasonable period of time (Hui 2007). The mechanisms of emulsifiers are based on their amphiphilic property. Amphiphilic nature means that they contain both hydrophobic groups (water-fearing group as their "tails") and hydrophilic groups (water-loving group as their "heads"); therefore, they are soluble in both organic solvents and water. An example is phospholipids. Emulsifiers aggregate in a liquid colloid, forming a micelle. A micelle is a structure where hydrophobic tails associate in the center shielded from the aqueous solution while the hydrophilic charged associate with the aqueous solution (Fig 1 (A) and (B)).

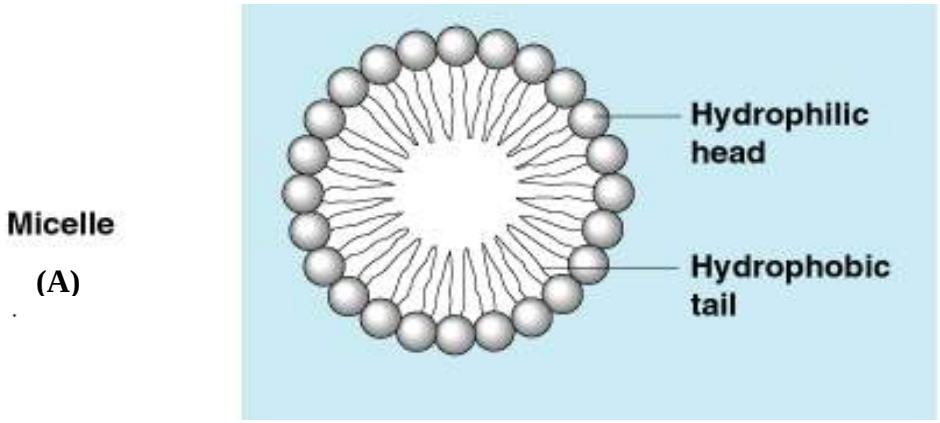


Fig 1 (A). Scheme of a micelle formed by phospholipids in an aqueous solution (Source: General, organic and biological chemistry, Platinum edition, 2004).

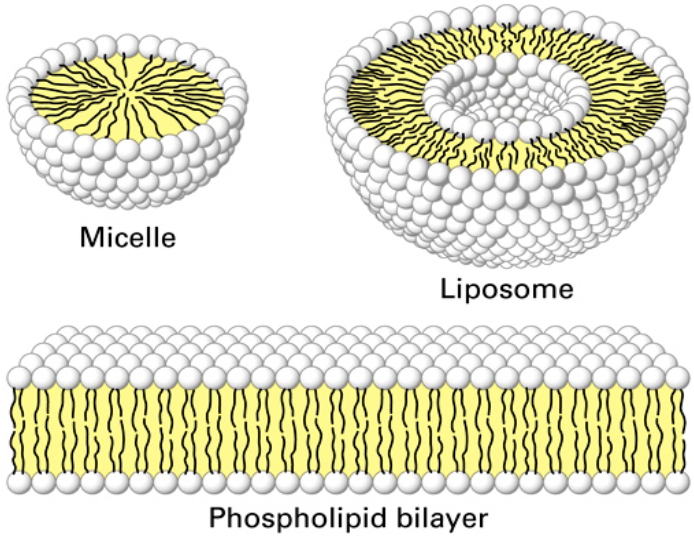


Fig 1 (B). Cross section view of the structures that can be formed by phospholipids in aqueous solutions (Source: General, organic and biological chemistry, Platinum edition, 2004).

There are two categories of emulsifiers that are widely used in the food industry. They are low molecular weight emulsifiers which includes phospholipids such as lecithin (found in egg yolk) and surfactants which includes sugar esters (sucrose esters), and high molecular weight emulsifiers consisting of polysaccharides (maltodextrin, gum Arabic) and proteins (caseins, whey proteins, gelatins) (Garti 1999). Both types of emulsifiers possess amphiphilic properties, which increases the stability of the emulsions for a prolonged period.

In the categories of emulsifiers, proteins stand as efficient emulsifying agents and stabilizers of food o/w emulsions (Dickinson and Stainby 1982). Because the free energy of protein is lower at the interface than it is in the bulk aqueous phase, spontaneous migration of protein occurs forming a highly visco-elastic film at the o/w interface.

Surface active properties of proteins are related to differences in protein conformation, which include adaptive nature within the environment, stability and flexibility of the polypeptide chain, and the distribution pattern of hydrophilic and hydrophobic groups on the protein surface (Damodaran 1996). Whey proteins purified from bovine milk are frequently used in various emulsion based food products such as ice cream, salad dressing, frozen desserts, and infant formulas (McClements 2004; Swaisgood 1996; Surh et al., 2006). Several studies have reported the ability of whey proteins to stabilize o/w emulsions. Martin-Diana et al. (2005) reported that whey proteins have significantly higher emulsifying activity index as compare to casein macropeptides. Other researchers have also found that whey protein-maltrodextrin conjugates act as an emulsifying agent and can be a good alternative to gum Arabic (Akhtar and Dickinson 2005).

Lactose

Lactose is a disaccharide and reducing sugar found in milk and milk products. It consists of β-D-galactose and α-D-glucose monosaccharides bonded through a β1-4 glycosidic linkage. This linkage is β1-4 glycosidic because galactose forms an acetal with a hydroxyl group of glucose at carbon 4 (Fig 2). Lactose possess a property of mutarotation, hence is a reducing sugar, due to the presence of the aldehyde group of glucose, which form α and β- lactose (Anonymous 2004).

Previous Studies on Synthesis of Lactose-amines

Based on the reducing and mutarotation properties of lactose, researchers found ease in modifying the lactose chemically (Dhruv et al. 2005). Presence of multi-hydroxyl

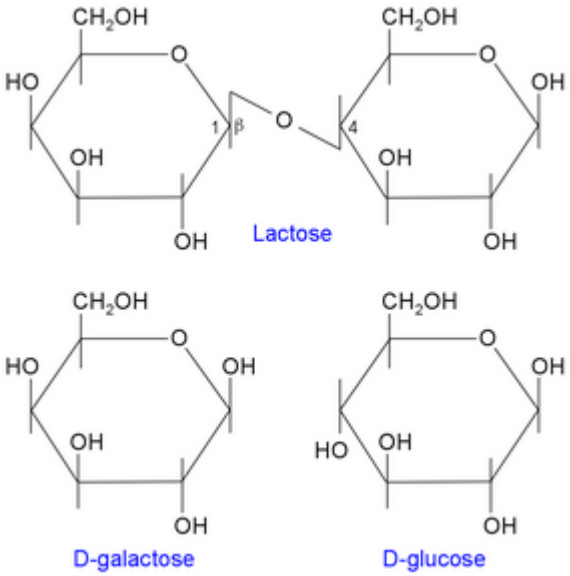


Fig. 2. Structure of beta-lactose and the products of hydrolysis (Source: General, organic and biological chemistry, Platinum edition, 2004).

groups in lactose can produce several synthesized products like hydrogels, and glycopolymers (Dhruv et al. 2005). Previous studies have shown that lactose cross-linked to fatty amides or fatty amines, becomes novel lactose based surfactants (Bhattacharya and Acharya 1999; Dhruv et al. 2005). These synthesized polymers due to their amphiphilic nature come into the class of "surfactants," which means surface active agents. Studies have shown that nonionic surfactants can stabilize o/w emulsions (Ponginebbi et al. 1999). Lactose was selected by the researchers in the past as lactose is a low cost product or, in other words, a waste from the cheese industry. Lactose is used in pharmaceuticals, infant formulas, and confectionary markets. It has been stated that a lactose-rich clean waste of the dairy industry can be considered a source of surfactants in the food industry (Lukondeh et al. 2003). Drummond and Wells (1998) found that

nonionic lactose and lacitol-based surfactants possess very similar physio-chemical properties and both exhibit good surface and interfacial activity suggesting their roles as effective emulsifiers.

On the basis of previous studies of Bhattacharya and Acharya (1999) and Dhruv et al. (2005), several points regarding lactose as a surfactant came into focus, leading to the concept of using this surfactant in the food industry. Bhattacharya and Acharya (1999) and Dhruv et al. (2005) synthesized lactose (disaccharide) with hexadecyl amine (C16 fatty amine) by going through maillard reactions. These Maillard reacted lactose-amines have the ability to form hydrogels (gels that can hold water in them for a prolonged period). Several researchers studied that hydrogels are the results of cross-linked polymerization and copolymerization of surfactants (Dhruv et al. 2005).

My study focused on a) the synthesis of lactose-amine with constant and cyclic heat treatments, which resulted in polymerized (brown colored Maillard reacted) and non-polymerized (non brown Maillard reacted) lactose-amines, and b) their influence with different concentrations on o/w emulsions as an emulsifier, in comparison to whey protein concentrate (protein emulsifier).

Hypothesis

- Lactose-amines synthesized via constant and cyclic heat treatments act as a surfactant at different concentrations by stabilizing o/w emulsions.

Objectives

- Synthesis of lactose-amines at four different heating times from 4 to 24 hours, to polymerized (brown colored maillard reacted) and non-polymerized (non brown maillard reacted) lactose-amines.
- Estimation of particle size of o/w emulsion with different concentrations of polymerized and non-polymerized lactose-amines comparable to whey protein concentrate (protein emulsifier) as positive control.
- Determine the optimum concentration of polymerized and non-polymerized lactose-amine synthesized in objective 1 that will stabilize o/w emulsions comparable to whey protein concentrate (protein emulsifier) as positive control, lactose, o/w and fatty-amine as negative controls.

REVIEW OF LITERATURE

Emulsion

Emulsions are a mixture of two immiscible liquids, which are thermodynamically unstable systems due to the dispersed phase, which divides into small droplets increasing the contact area between both liquid phases (Hui 2007). Emulsions are of two types "direct emulsion" and "inverse emulsion." Direct emulsions are the emulsions in which oil droplets are dispersed in water and inverse emulsion are those emulsions in which water is dispersed in oil (Mason et al. 2006). The dispersed phase droplet size generally ranges from 0.1 - 10 μ m. Examples of food oil-in-water (o/w) emulsions include milk, cream, ice cream, salad dressings and cake batters, while butter and margarine are water-in-oil (w/o) emulsions (Bee et al. 1989; Larson and Friberg 1990).

In emulsions, the thermodynamically lowest energy state is a layer of liquid (oil) having lower density on top of a liquid layer of higher density (water). To create an emulsion, energy (shear and pressure) is applied to rupture oil into small droplets which are dispersed in water phase (Mason et al. 2006).

To prepare these emulsions, high pressure homogenizers are considered to be the best choice, and widely used in the food industry (Manea et al. 2008). Several studies have shown that sheer is required to prepare o/w emulsions (Pearce and Kinsella 1978; Cameron et al. 1991; Yaghmur et al. 1999). These emulsions are homogenized in a microfluidizer (bench scale high pressure homogenizer) as it ruptures large oil droplets into smaller droplets (Garti et al.1998; Mason et al. 2006).

Homogenization is a mechanical treatment of the fat globules under high pressure, which results in a decrease in the average diameter and an increase in the number and surface area of the fat globules. Three factors that enhanced the stability of homogenized emulsion are; decrease in the mean diameter of the fat globules, decrease in the size distribution of the fat globules, and an increase in density of the globules (Dalgleish et al. 1996). This disruption of fat globules is done by a combination of factors such as turbulence and cavitation. Homogenization reduces fat globule size in milk from 3.5 µm to less than 0.1 µm, and increases the fat interfacial layer by four to six folds (Dalgleish et al. 1996).

Homogenizers works on two theories; first is the theory of globule disintegration or disruption by turbulent eddies (micro whirls), which work on the fact that an unlimited number of small eddies are created in liquid moving with high velocity. Higher velocity yields smaller eddies and if an eddy causes droplets to collide, the droplets will break up. Secondly the theory of cavitation suggested that when the liquid leaves the narrow gap in the homogenizer of 0.1 mm (where the fat globules are separated what does this mean?) due to back pressure, homogenization takes place. Homogenization can be done without considering the cavitation theory but it will reduce the efficiency of the homogenization process (Dalgleish et al. 1996).

Instability of emulsions results when there is high concentration of oil droplets in the creaming phase leading to aggregation (particles will adhere to each other and become larger particles), or coalescence i.e., fusing of particles (Dalgleish 1997). To avoid these destabilization effects, emulsifiers play an important role.

Creaming is a phenomenon of instability for emulsions, when the dispersed phase has a lower density than the continuous phase and can be coupled with coalescence or flocculation which leads to a phase separation. Sedimentation is a phenomenon encountered when the density of the dispersed phase is greater than the density of the continuous phase. Coalescence and flocculation phenomena are physico-chemically very different but they both lead to an increase in the size of the particles. Coalescence is irreversible and leads to the fusion of the interfaces, hence the creation of one single drop while flocculation is an aggregation of the particles.

Emulsifiers

Emulsions are thermodynamically unstable, certain emulsifiers or surface-active agents can be used to make emulsions kinetically (the rate at which molecules collide in order to react together) stable by allowing them to remain in a high state of energy (Dalgleish 1997; Hui 2007). These emulsifiers reduce the interfacial tension between the two immiscible phases, reduce the amount of work in dispersing these two phases, and provide stabilization of the dispersed droplets by inhibiting flocculation and coalescence (Garti 1999). Emulsifiers are absorbed into the newly formed surface of the oil droplet during the process of homogenization. Emulsifiers thus lower the interfacial tension and form a protective layer around the droplets, which results in decreasing droplet coalescence and resistance to rupture by generating repulsive interactions between droplets (Pallandre et al. 2007).

Garti (1999) quoted the definition of emulsifiers and stabilizers, defined by Dickinson et al. (1988) as “a single chemical component, or mixture of components

having the capacity for promoting formulation and stabilization by interfacial action, and a stabilizer as chemical component, or a mixture of components, which can confer long term stability to an emulsion, possibly by a mechanism involving adsorption."

A good stabilizer keeps droplets apart in the emulsion once it has been formed during long-term storage. An emulsifier has the capacity to adsorb rapidly at the nascent o/w interface created during emulsification and protecting the newly formed droplets against re-coalescence. Polysaccharides (hydrocolloids) are used as stabilizers as they can form macromolecular barriers in the aqueous medium between dispersed droplets with their hydrophilicity and high molecular weight. Proteins are also commonly used emulsifiers due to their molecular flexibility which allows rapid adsorption and rearrangement at the interface to give a coherent molecular protective layer (Dickinson 1988).

There are two categories of emulsifiers; low molecular weight and high molecular weight. Low molecular weight includes monoglycerides, diglycerides, phospholipids and surfactants which include sugar esters such as sucrose esters. High molecular weight emulsifiers include polysaccharides and proteins (casein and whey proteins). Phospholipids, polysaccharides, proteins and sugar esters are all widely used in the food industry (Garti 1999).

Food Grade Emulsifiers

Food grade emulsifiers are those emulsifiers that are recognized and approved to be in the GRAS (Generally Recognized as Safe) category. These emulsifiers have a significant place in cosmetic, food and pharmaceutical industries. Several studies have

been done on the influence of food grade emulsifiers on the stabilization of o/w emulsions (Garti 1999).

High molecular weight emulsifiers

High molecular weight amphiphiles have been the topic of discussion in the field of emulsions and emulsifiers for years. Several studies have been done to understand the behavior of macromolecules at liquid or solid interfaces in foods and related industries (Finney 1982; Fox and Condon 1982; Tornberg and Ediriweera 1986; Barsh and Horbett 1987; Dickinson et al. 1988).

Maltodextrin is a polysaccharide that is used as a food additive. It is produced from starch and is usually found as a creamy-white hygroscopic powder. Maltodextrin is used in various emulsions, which give desirable viscosity, texture, and mouth feel to the emulsions (Dokic-Baucal et al. 2004). Dokic-Baucal et al. (2004) have also stated that emulsions with high maltodextrin concentration (25%) were stable compared to the low maltodextrin concentration (5%). Emulsion stability with high concentrations of maltodextrin is due to the branched molecules of maltodextrin which form tightly packed segments or are arranged like “fringes” (Chronakis 1997), forming a network structure which keep the droplets in place preventing coalescence (Dickinson et al. 1995).

The other most studied polysaccharide is gum Arabic, which is a mixture of saccharide and glycoprotein, used to stabilize emulsified flavored oils (McClements 2004; Tan 2004) at concentrations of 2% or less (Djordjevic et al. 2007). Gum Arabic adheres to the surface of the oil droplets during homogenization, where an interfacial layer is formed, which is thick and negatively charged, stabilizing the oil droplets

(Chanamai 2002). Addition of sodium alginate has been reported to improve the stability of o/w emulsions containing caseinate (Pallandre et al. 2007).

Protein emulsifiers includes 1% sodium caseinate (Kanafusa et al. 2007), whey proteins and gelatins which are widely studied and discussed for their role in influencing the interfacial activity of the o/w emulsions (Garti 1999). Of the total milk protein, 80% is casein (Wong et al. 1996). Sodium caseinate (NaCN) is a spray dried high quality milk protein or in other words, contains a soluble mixture of surface active caseins, which can act as an emulsifier and stabilizer at o/w interfaces (Dickinson et al. 1998; Shrinivasan et al. 2000; Ye and Singh 2001). Due to its iron chelating properties and ability to produce thick interfacial layers around the droplets, sodium caseinate protects emulsified oils from oxidation (Hu et al. 1995; Kanafusa et al. 2007).

Other proteins and polysaccharides used as emulsifiers include gelatin (Vaziri and Warburton 1994), xanthan (Evison et al. 1995), conjugates of casein-maltodextrin (Shepherd, et al. 2000), and, above all, whey proteins (Cornec et al. 1998; Onsaard et al. 2005; Akhtar and Dickinson 2007). Protein-polysaccharide conjugates are referred to as natural and non-toxic emulsifiers. Shepherd et al. (2000) reported that casein-glycoconjugates have significant potential as effective food emulsifiers or soluble protein additives for acidic sports drinks or nutritional supplements. Casein-glycoconjugates at a 2% concentration act as emulsifiers even in acidic solutions (Shepherd et al. 1995; Fencher et al. 2006). Use of casein-dextran conjugate as an emulsifier makes the oil droplets smaller and narrowly distributed in o/w emulsions (Fencher et al. 2006).

Whey Proteins

Whey protein (WP) is the name for a collection of globular proteins that can be isolated from whey, a by-product of cheese manufactured from cow's milk. The protein fraction in whey (approximately 10% of the total dry solids in liquid whey) is typically a mixture of beta-lactoglobulin (~55%), alpha-lactalbumin (~25%), serum albumin (~5%) and immunoglobulins (~15%) (Swaisgood 1996).

Whey proteins are an important ingredient in the commercial food industry due to their high nutritional value and versatile functional properties such as solubility, viscosity, water holding capacity, gelation, adhesion, emulsification (de Wit 1998; Huffman 1996; Boye et al. 1997; Corradini 1998; Kinekawa et al. 1998; Herceg et al. 2005). Two major forms of whey proteins are discussed in this chapter: isolate and concentrate. Whey protein isolates (WPI) are processed to remove fat and lactose and contain >90% protein. Whey protein concentrates (WPC) contain a low level of fat and lactose and the protein content may vary from 25% to 80% (Morr and Ha 1993; Kinsella and Whitehead 1998).

WPI, at acidic pH, stabilizes the interfacial layer around the oil droplets which is relatively thin (~2nm) and positively charged (+29mV at 100 mM NaCl at pH 3), this has been proven to increase the oxidative stability of emulsified polyunsaturated lipids and decrease iron-lipid interactions. Above all, WPI stabilized emulsions are stabilized to thermal processing operations such as pasteurization (Hu et al. 2004; McClements and Decker 2000; Djordjevic 2004). WPI created emulsions have been proven to be more stable compared to protein source fractions such as coconut skim milk proteins (Onsaard et al. 2005). The covalent complexes of WPI and maltodextrin have demonstrated

effectiveness in stabilizing emulsions at low pH stored for several weeks without any visible precipitation or phase separation (Akhtar and Dickinson 2006).

WPCs are readily available in the U.S. and have the surface active properties required to make an emulsion stable (Hogan et al. 2001; Herceg et al. 2005; Surh et al. 2005). WPC 60 (60% protein) and WPC 80 (80% protein) were used in various studies to compare emulsion properties (Arai and Watanbe 1988; Kato et al. 1994; Herceg et al. 2005). The ability of WPC to maintain the stability of oil droplets during spray-drying and also fulfilling the role of protective agent for the oil droplets makes it an effective emulsifying agent (Hogan, et al. 2001). Studies have proven the wide application of WPC as a natural emulsifier in food products (Surh et al. 2005).

WPI concentration ranging from 0.09% to 0.9% in 5 mM phosphate buffer, (Onsaard et al. 2005; Surh et al. 2005) are effective emulsifiers with 20:80 o/w emulsions. WPC 80 at 2% concentration has 1.6% protein (Herceg et al. 2005) and WPC 75 at 5% concentration has 3.75% protein, both of which work as effective emulsifiers for 20:80 o/w emulsion (Hogan et al. 2001).

Low molecular weight emulsifiers

Fats and oils are considered to be the best source of emulsifiers (Bee et al.1989; Larson and Friberg 1990; Hamilton 1995; Karleskind 1996; Garti 1999). Purifying emulsifiers from fats and oils produces more than 92% of pure monoglyceride esters, which are considered to be GRAS emulsifiers (Garti 1999). Fats and oils from every source contain small quantities of phospholipids and triglycerides. Phospholipids hold an

important position in the areas of emulsions (Karleskind 1996; Garti 1999) especially in food, agriculture, pharmaceutical, and cosmetics industries.

Lecithin is a synonym for pure phosphatidylcholine, a phospholipid. Lecithin is isolated either from egg yolk or soy beans. Due to its low solubility in water, in aqueous solution the phospholipid can form liposomes, bilayer sheets, micelles, or lamellar structures, depending on hydration and temperature. These properties results in a type of surfactant that is usually classified as amphoteric i.e., the molecule consist of both water and oil soluble portions (Jimenez et al. 1990; Iwata et al. 1993). Lecithin, as a primary emulsifier has been studied in o/w emulsion (Akhtar and Dickinson 2001). O/w emulsions have been reported consistently in research studies related to emulsions. Researchers (Johansson et al. 1995; Nieuwenhuyzen 2002) have reported that lecithin, when heated, improves emulsifying properties of o/w emulsions (Weete et al. 1994). O/w emulsions prepared with 2.5% lecithin are stable over a significant period of time (Knoth et al. 2005; Scherze et al. 2006). Egg lecithin used at 5% also formed stabilized emulsions (Thakur et al. 2007).

The high surface activity of phospholipids influences the interfacial properties of emulsions, and foams (Bos et al. 1997; Patino et al. 2007), and due to their strong tendency to absorb at fluid interfaces. These qualities make phospholipids a useful component in the manufacturing of stable food dispersions (Patino et al. 2007).

Surfactants

Surfactants are a surface-active, structurally diverse group of molecules synthesized by microorganisms or chemically and enzymatically synthesized (Nitschke

and Costa 2007). Due to their influence on interfacial activities and the surface tension of water, surfactants are also used as emulsifying and dispersing agents. Studies done on emulsion capabilities of surfactants, considered them an emulsifier (Garti 1999). Surfactants exhibit some special properties: low toxicity; a biodegradable nature; effectiveness at extreme temperatures, pH, and salinity; and, above all, ease of synthesis (Desai and Desai 1993). Rosenberg and Ron (1999) had suggested two categories of surfactants on the basis of molecular mass that are low-molecular-mass molecules with low surface and interfacial tensions (glycolipids, lipopeptides, and phospholipids), and high-molecular-mass polymers which act as an emulsion stabilizing agent, i.e., polymeric and particulate surfactants (Nitschke and Costa 2007).

The term surfactant is a blend of "surface acting agent". Surfactants are usually compounds that posses an amphiphilic nature, meaning they contain both hydrophobic groups (their "tails") and hydrophilic groups (their "heads"). Therefore, they are soluble in both organic solvents and water (Desai and Banat 1997). Surfactin is acyclic lipopeptides-amino acid lipid surfactants, which is capable of lowering the surface tension of water, and also of being stable at wide pH ranges (Arima et al. 1968; Garti 1999) and shares the category of low molecular weight polymers. Among the high molecular weight polymers, emulsan has proved to be the most efficient as it holds good surface properties and excellent emulsification capabilities due to the presence of fatty acids linked to an amino sugar backbone of the anionic polysaccharides (Gutnick 1987; Garti 1999).

Sugar-based surfactant products are based on the useable renewable resources (Hill and Rhode 1999). Studies have been done to modifying their amphiphilic structure

by attaching a carbohydrate group to a lipid as the hydrophilic group (Schulz 1992). Sugar-based surfactants include sorbitan esters, sucrose esters, alkyl polyglycosides, and fatty acids glucamides. Some of the sugar based surfactants and their uses are currently limited due to the economics involved in their processing (Hill and Rhode 1999). Most successful sugar based surfactants are alkyl polyglycosides and fatty acid glucamides, as they are multi-functional, competitively priced, and exhibit high product safety in addition to being made from renewable resources (Hill and Rhode 1999). Akoh (1992) suggested that emulsifier blends of potential fat substitutes with sugar ester emulsifiers, which are commercially approved by FDA, may act as an o/w emulsifiers at concentration of 0.5%- 1.0% at 10%-20% oil concentration (Akoh 1992; Piao and Adachi 2006). Studies have shown that surfactants can stabilize oil and water emulsions (Ponginebbi et al. 1999). The use of synthetic low molecular weight or polymeric surfactants has been documented in several research studies (Clark 1995; Bos et al. 1997; Knoth et al. 2005). On the basis of the above review of literature, the present study was conducted with different concentrations of lactose based surfactants and WPC 80, which is a well-known and established emulsifier in the food industry.

Other uses of surfactants

Surfactants have various applications in different industrial sectors other than the food industry such as organic chemicals, cosmetics and pharmaceuticals, petrochemicals and petroleum, mining and metallurgy, agrochemicals and fertilizers, and many others (Kosaric 1992). Surfactants are not only used as emulsifiers but also as wetting agents, spreading agents, foaming agents and as functional detergents (Kosaric 1992). They also

play an important role in emulsification of simple emulsions like kerosene /water in petroleum industries (Kosaric et al. 1987).

Lactose and Maillard Reaction

Lactose is a reducing sugar found in milk and milk products. It is made up of two monosaccharides, galactose and glucose. Lactose disaccharide exists as α and β anomers which can undergo mutarotation via the open chain formation in the solution. Lactose disaccharide forms hemiacetal when an aldehyde group reacts with one alcohol molecule and forms the open chain. In the process of mutarotation each isomer converts from the closed ring to the open chain and vice versa. On the closing and opening of the chain, carbon 1 and 2 bonds rotate, which leads to the shift of the hydroxyl group (-OH) between α- and β- positions (Anonymous 2004). The bond in lactose is a β-1-4 glycosidic bond (the glycosidic bond forms when an alcohol reacts with a cyclic hemiacetal to give an acetal). In the lactose, β- anomer of galactose forms acetal with the hydroxyl group of glucose. Due to the presence of hemiacetal carbon in glucose, lactose undergoes mutarotation to give α- and β- lactose (Fig 3) (Anonymous 2004).

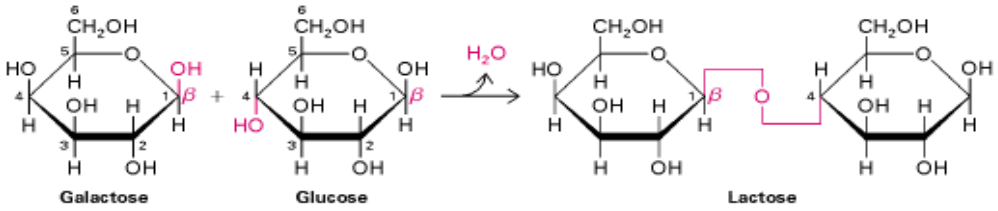


Fig. 3. Formation of beta-lactose (Source: General, organic and biological chemistry, Platinum edition, 2004)

Lactose undergoes mutarotation (classified as a reducing sugar) therefore it can participate in the Maillard reaction to form synthesized products like hydrogels, and glycopolymers (Dhruv et al. 2005). The Maillard reaction is a chemical reaction between primary amino group and a reducing sugar. This reaction can be the result when there is an increased heat to the system. It is a form of non-enzymatic browning (oxidative browning is a chemical process that produces a brown color in foods without enzymes). The two types of non-enzymatic browning are caramelization and the Maillard reaction. The reactive carbonyl group of the sugar reacts with the amino group and forms a variety of molecules responsible for a range of odors and flavors. This process generally takes place in an alkaline environment as the amino groups are deprotonated. The reducing sugar reacts with the amine group to form Schiff base (an imine, RHC=NHR'), which may cyclize to form a glycosylamine or N-glycoside. The Schiff base undergoes a reaction called the Amadori rearrangement (Fig. 4 A). The progression of the maillard reaction leads by condensation and polymerization reactions which further produce furfural and hydroxymethylfurfural (HMF) compounds (Boekel 2006; Liu et al. 2008). These compounds are brown, polymerized compounds of the Maillard reaction known as melanoidins (Boekel 2006; Liu et al. 2008). A furfural compound forms when there is a reaction with a pentose sugar and HMF is the result of a reaction with a hexose (glucose, saccharose) (Fig. 4 B) (Boekel 2006; Liu et al. 2008).

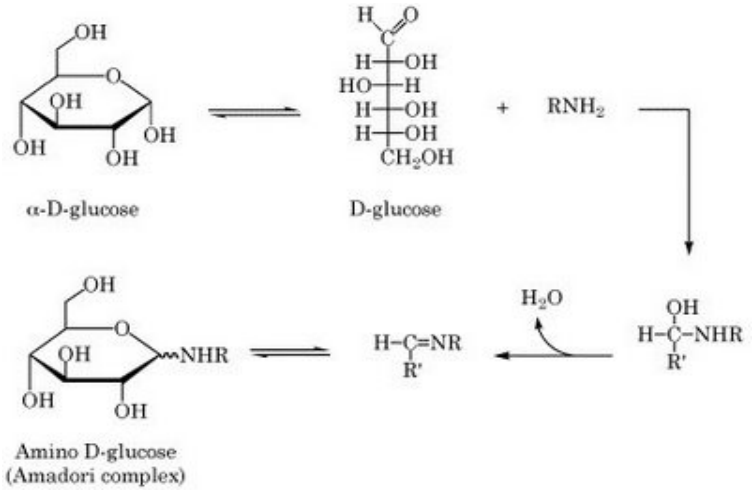


Fig. 4 (A) Formation of Amadori complex with Schiff base formation (Source: Dhruv et al. 2005).

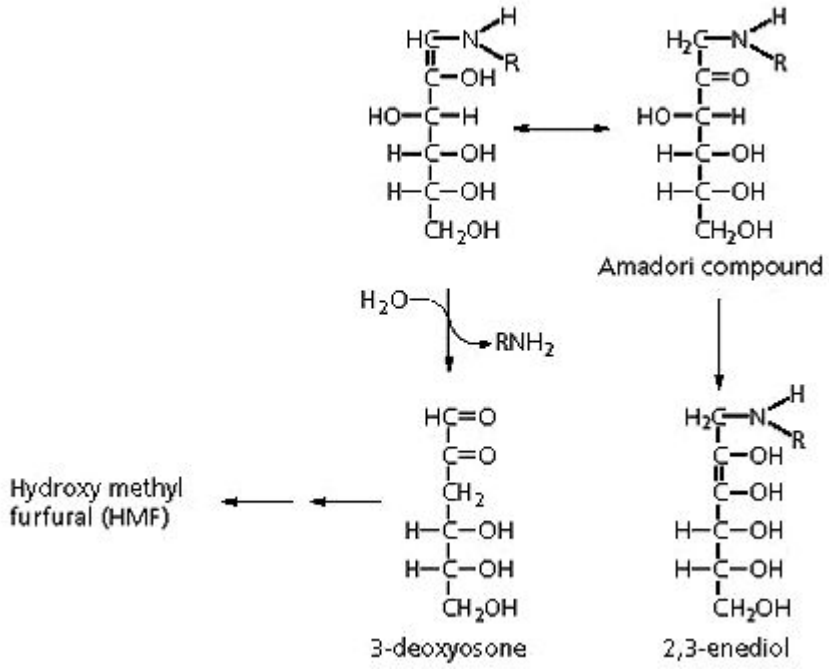


Fig. 4 (B) Formation of Amadori-rearrangement and Hydroxymethylfurfural compounds (Source: Dhruv et al. 2005).

Synthesis of Lactose-amines

Bhattacharya and Acharya 1999 have extensively studied the amphiphilic behavior of lactose and maltose coupled to fatty-amines and fatty acids. Their synthesized lactose-amines were gels that possessed thermoreversible properties as they are early intermediates (Amadori compounds) in the browning reactions (Martin et al. 2005; Boekel 2006; Liu et al. 2008). Lactose-amine gels turned into clear fluid on applying heat and returned back to a gel state by cooling the fluid (Bhattacharya and Acharya 1999; Dhruv et al. 2005). The present study was designed on the basis of the above studies done on the lactose-amine hydrogels, which possesses surfactant properties. The present study includes cyclic heat treated lactose-amine, and constant heat treated maillard reacted lactose-amine polymers. Reversible reactions were observed in cyclic heat treated lactose-amines when stored for a long period (Bhattacharya and Acharya 1999; Latge et al. 1992). Constant heat treatment was the continuation of the maillard reaction after the amadori rearrangements. Two potential products were formed with constant heat treatment, osones and hydroxymethylfurfural compounds (Martin et al. 2005; Boekel 2006; Liu et al. 2008).

Analytical Techniques for Measurement of Emulsions

Droplet size measurement

Emulsion droplet size measurements can be done using a light scattering instrument (LS Beckman Coulter LS230, Coulter Corporation, Miami, Florida, USA). This instrument is patented with an advanced technology of polarization intensity differential scattering (PIDS), as droplets below a few microns in diameter have very

similar light scattering patterns that are alike in both shape and intensity. The major benefit of acquiring PIDS data is that by simple interpretation of the raw data, presence of small droplets can be confirmed (Beckman Coulter Manual, BeckLS13320.pdf, Coulter Corporation, Miami, Florida, USA). The basis of the method is as follows, a laser light source is used to illuminate particulates, usually contained within a suitable sample cell. The light scattered by the droplets is then detected by silicon photo-detectors. The intensity of light on each detector measured as a function of angle is then subjected to mathematical analysis using a complex inversion matrix algorithm. The result is a droplet size distribution displayed as volume % in discrete size classes.

Droplet size measurements can be reported as mean $D_{3,2}$ values. As $D_{3,2}$ is the diameter of a sphere that has the same volume in ratio with surface area (McClements 2004). The $D_{3,2}$ is more accurate with smaller droplets measurements as compare to $d_{4,3}$ which is a weight-average mean droplet diameter and also sensitive to large droplet size (Herceg et al. 2005; Onsaard et al. 2005; Surh et al. 2005; Akhtar and Dickinson 2007). Studies have shown that mean $D_{3,2}$ value of a whey protein emulsion is 0.3-0.4µm (Hogan et al. 2001; Herceg et al. 2005; Onsaard et al. 2005; Akhtar and Dickinson 2007). Droplet size measurement is an important tool to measure the stability of the emulsions, the smaller the $D_{3,2}$ value the higher the stability of the emulsion (Hogan et al. 2001; Herceg et al. 2005; Onsaard et al. 2005; Akhtar and Dickinson 2007; Dalgeish 2006).

Emulsion stability

Turbiscan is an instrument that can be used to measure emulsion stability. It consists of a reading head moving along a flat-bottomed, cylindrical cell, which scans the

entire sample height and the reading head. The reading head consists of a pulsed, near-infrared light source used to read backscattering data. The backscattering detector (BS) receives the light backscattered by the sample at an angle of 135 °. The reading head acquires backscattering data every 40 µm on a maximum height of 80 mm. The obtained profile measures sample homogeneity and particle concentration of homogenized sample (HS) and is represented on the software screen as a curve showing the percentage of backscattered light in form of sample height (in mm). The acquisition along the product is then repeated with a set frequency to obtain the superimposition of sample fingerprints characterizing the stability or destability of the sample (Fig. 5).

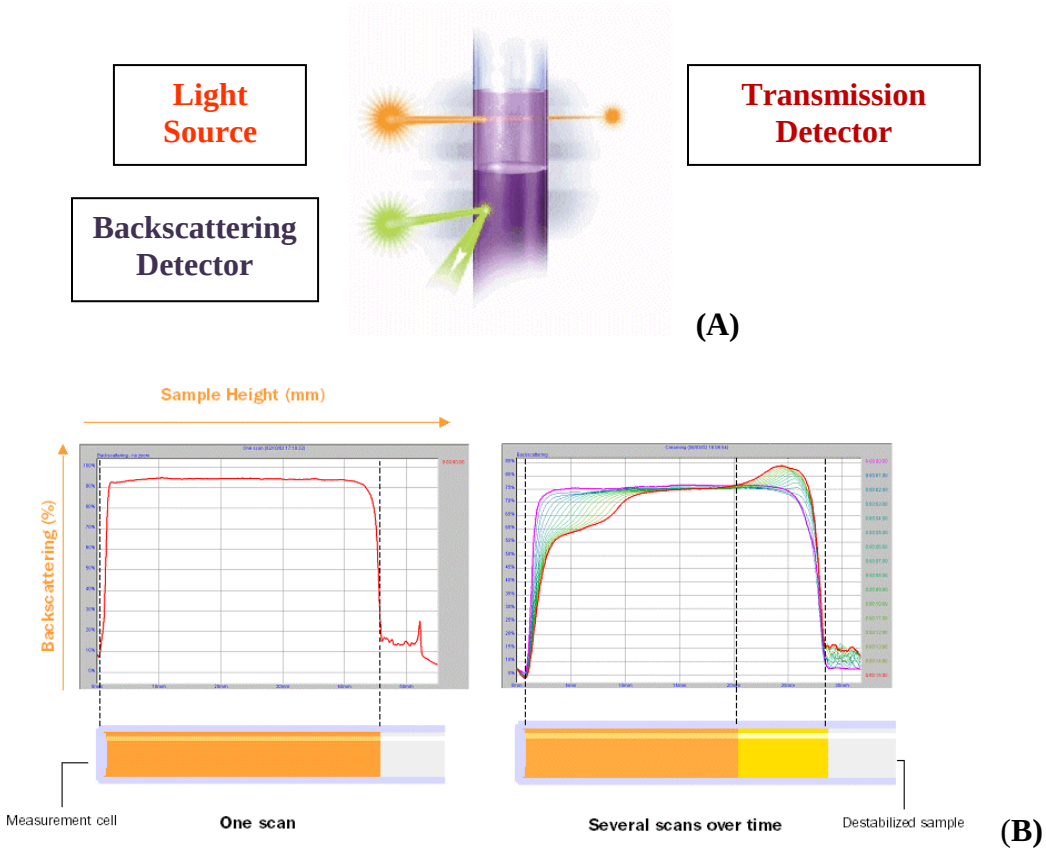


Fig. 5. (A) Measurement principle and (B) Backscattering profiles of turbiscan (Source: Turbiscan Manual, TurbiScan MA 2000, Formulaction, Toulouse, France).

Backscattering is defined as when a light beam is scattered, the rate at which scattered light beam reflects back after passing through the emulsion, and this rate of reflection of light is called % backscattering. Backscattering can be used to measure the stability of emulsions. The backscattering % increases with the decrease in droplet mean diameter and it decreases with an increase of the mean diameter of droplets in emulsion (Pearce and Kinsella 1978; Herceg et al. 2005;).

Interpretation of turbiscan results

There are few ways to interpret whether there is sedimentation or clarification at the bottom or any creaming present at the top layer of emulsions. Creaming is coupled with coalescence or flocculation and finally leads to a phase separation (Fig. 6). These phenomenon can be easily detected using the turbiscan as it records a variation of the concentration between the top and the bottom of the cell (Fig. 7, 8).

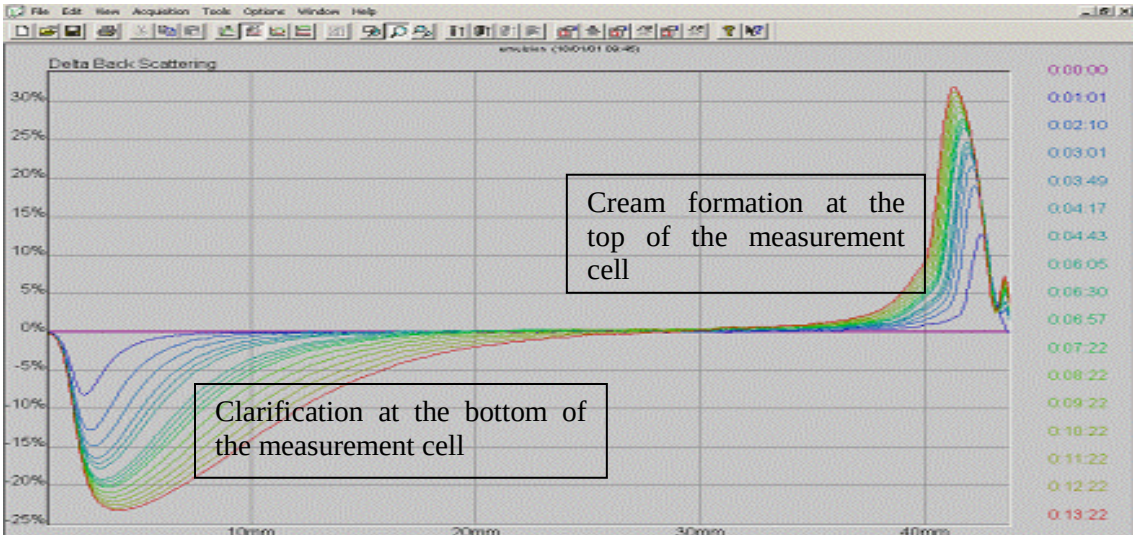


Fig. 6. Profile of creaming emulsions (Source: Turbiscan Manual, TurbiScan MA 2000, Formulaction, Toulouse, France)

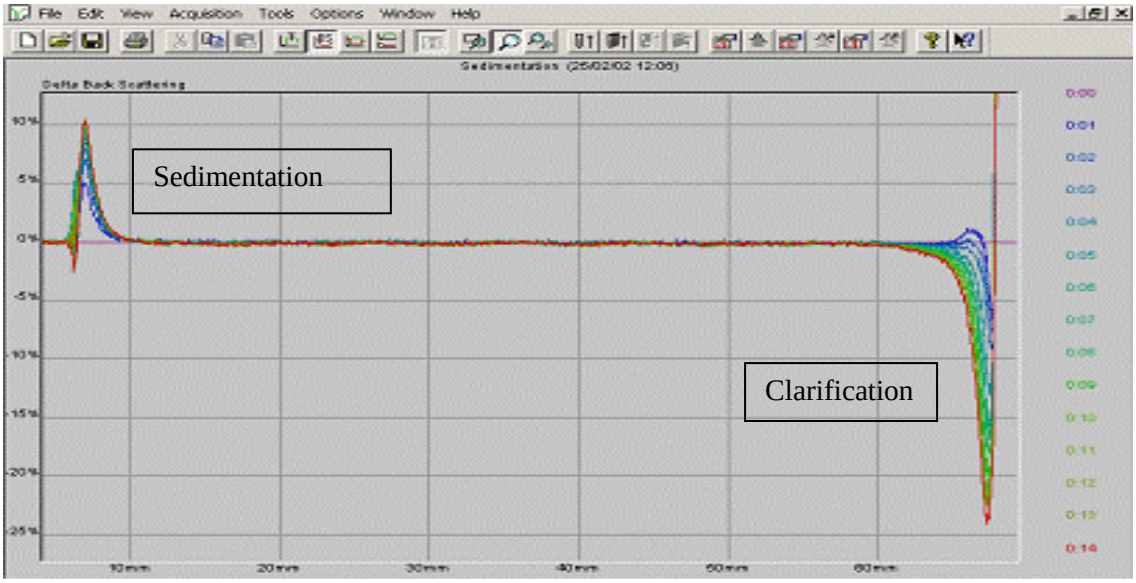


Fig. 7. Profile of sedimentation emulsions (Source: Turbiscan Manual, TurbiScan MA 2000, Formulaction, Toulouse, France)

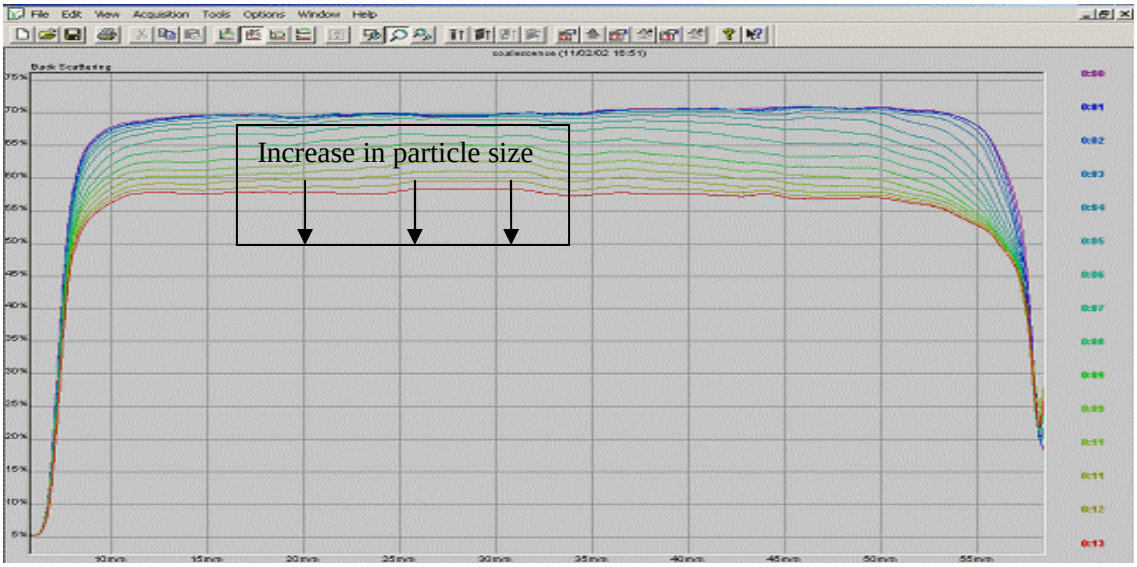


Fig. 8. Profile of flocculation and coalescence emulsions (Source: Turbiscan Manual, TurbiScan MA 2000, Formulaction, Toulouse, France)

MATERIAL AND METHODS

Materials

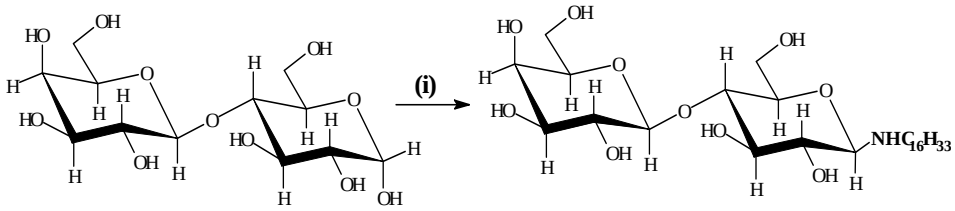
Lactose was donated by Proliant Inc., iso-propanol (90%) and hexadecyl-amines (HCA) (95%) (C_{16} fatty amine) were purchased from Sigma Aldrich**.** Whey protein concentrate (WPC) (80% protein, 5% lactose, 6% fat, 3% water, and 6% ash) was obtained from Saputo (St.-Hyacinthe, Quebec). The technical analysis were done using LS Beckman Coulter (LS230, Coulter Corporation, Miami, Florida, USA) for mean droplet size and $D_{(3,2)}$ and Turbiscan (TurbiScan MA 2000, Formulaction, Toulouse, France) for emulsion stability measurements.

Study Design

The experiment consisted of emulsions prepared with 8 groups including oil and water alone and oil and water with lactose and hexadecyl-amines (HCA) as negative controls. The treatment group included four different lactose-amine samples (4 hour, 8 hour, 12 hour, and 24 hour) treatments at four different concentrations (0.01%, 0.05%, 0.1%, and 1.0%) with 4 replicates at each concentration. WPC (2% protein in 50 mM phosphate, pH 7) was used as the positive control.

Synthesis of Lactose- amines

For the synthesis of lactose-amines, 250 milimolar solutions of HCA in 10 ml iso-propanol were added with 250 milimolar solutions of lactose in 10 ml distilled water (Fig. 9).



$C_{16}H_{33}NH_2$ +

(i) *n*-hexadecyl amine + 2-propanol and lactose + d H_2O, stir, 24 hr, with intermittent heating at ~60 °C to produce n-hexadecyl D-lactosylamine.

Fig. 9. Synthesis scheme of lactose-amines from lactose and hexadecyl-amine (Source: Bhattacharya and Acharya 1999; Dhruv et al. 2005)

.

The treatment groups were 4 hour (4H) and 8 hour (8H) lactose-amines which were processed for 4 and 8 hours of constant heating at 60°C, while 12 hour (12H) and 24 hour (24H) lactose-amines were processed for 12 and 24 hours of cyclic heating at 60°C followed by cooling cycles at room temperature. For the heating cycle, the solutions were kept in a hot water bath at 60°C with continuous monitoring of the temperature of the sample solution and hot water bath. During the heating cycle when the solutions turned transparent, the samples were removed from the hot water bath and were moved to a room temperature water bath for the cooling cycle until they become opaque again (Fig. 10).

After the synthesis of lactose-amines, the products were in the form of gels which were frozen to -80°C. After freezing, the product samples were freeze dried (Dura-Top microprocessor control freeze-dryer, FTS systems, NJ, USA) for 8 days. Dried and grounded (grinding was done with mortar and pestle) samples, in the powder state were

kept frozen at -4°C. Each lactose-amine samples (4H, 8H, 12H and 24H) was synthesized 4 times and the dried samples were pooled.

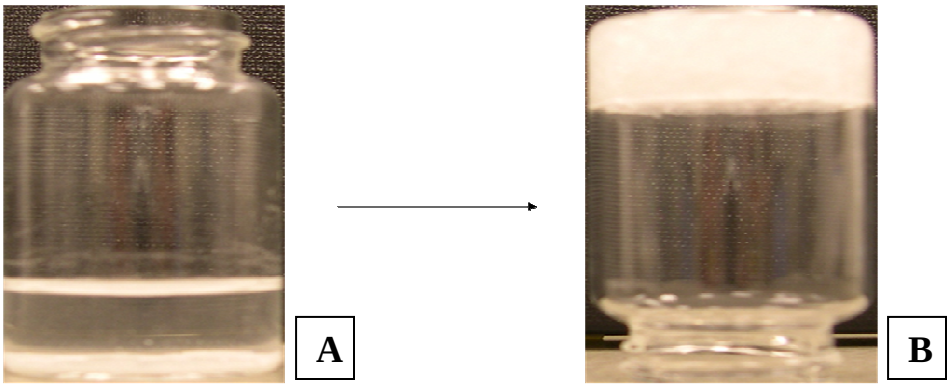


Fig.10. (A) Heating and (B) cooling cycle during synthesis of lactose-amines.

Droplet Size Determination

Preparation of o/w emulsions

Emulsions of negative controls and treatment groups were prepared with 80 ml of water and 20 ml of oil. Samples were mixed with a high speed blender (polytron) (Ultra-Turrax T25, Janke and Kunkel, Staufen, Germany) at 24000 rpm for 3 minutes with four different concentrations of lactose, hexadecyl-amines and lactose-amines (0.01%, 0.05%, 0.1% and 1.0%). For the positive control, 80 ml of solution of WPC and 20 ml of oil were mixed with polytron as described above. Each solution was homogenized in a microfluidizer (Microfludics Corporation, Newton, Massachusetts, USA) for 3-5 minutes at 6900 psi at room temperature.

Determination of droplet size distribution

The droplet size of the fat globules present in the emulsions was measured by using a LS Beckman Coulter droplet size analyzer. All the measurements were made on two freshly prepared emulsions from each treatment group (4H, 8H, 12H, and 24H) at each concentration (0.01%, 0.05%, 0.1%, and 1.0%) and the WPC control, except negative controls (lactose, HCA, and o/w) as they were too unstable to measure. Emulsion samples were added drop wise to the droplet size analyzer until PIDS obscuration reached 40%. Before measuring the droplet size of each sample, the instrument was rinsed, the background measured and the instrument calibrated. The results for each sample were given in volume (%) of droplet size distribution and droplet size (μm).

The oil droplet measurements were taken at angular dependence of the intensity of laser light (λ= 632.8nm) scattered by emulsions, and then mean oil droplet size was generated as the surface-volume mean particle diameter, using the following equation:

$$D_{3,2}(=\Sigma n_i d_i^3 / \Sigma n_i d_i^2),$$

where d is the diameter and n is the number of particles. The results were reported as means and standard deviation of $D_{(3,2)}$.

Emulsification Activity

The physicochemical stability of the o/w emulsions with lactose-amines, and both negative and positive controls, was done using Turbiscan, a vertical scan macroscopic analyzer. About 6 ml of each emulsion was put in the tubes for measuring the change in

backscattering (Δ BS %). Δ BS % were recorded every 15 minutes over 3 hours and then once a day for 5 days.

Statistical Analysis

Repeated measures of ANOVA were used to analyze the destabilization rate of o/w emulsions. Analysis of the data set was not satisfying the assumption of normality as a plot of normal quantile was long-tailed, a box plot was showing outliers, the approximate test of normality was showing significant values, and plots against predicted values and residuals were showing a triangular pattern which means there was a sign of heteroscedasticity. To remove these abnormalities, the data was transformed with the highest level of transformation (according to ladder of power of transformation), but still there were outliers in the analysis. Outliers were discarded and the analysis was done on day 1 and day 5 data using a two-way factorial analysis. Means and standard deviation were used to relate the droplet size estimation with the destabilization rate.

RESULTS AND DISCUSSION

Synthesis of Non- polymerized and Polymerized Lactose-amines

After synthesis, non-polymerized and polymerized lactose-amines are in a gel form which forms a dried product after freeze drying (Fig. 11). Grounded dried products were stored frozen at -4°C (Dhruv et al. 2005) to prevent the reverse reactions of Amadori compounds into lactose and fatty amine (Boekel 2006; Liu et al. 2008). Figure 12, shows lactose-amine products, every product posses a different color due to their heat exposure. Four hour (4H) and 8 hour (8H) samples were heat-treated for longer times as compare to 24 hour (24H) and 12 hour (12H) which results in different colored Maillard-reacted product. The color of 8H was brown as it was prepared with continuous and constant heating at 60°C for 8 hours and 4H was light brown as its exposure to heat was for 4 hours. The resultant brown color of the products may be the result of dehydration, cyclization, condensation and polymerization reactions (Boekel 2006; Liu et al. 2008). The 24H and 12H samples were white in color as they were exposed to heat for short time (due to the cooling cycle) i.e. 2-2.5 and 1.5 hours, respectively, as compare to 4H and 8H (i.e. 4 hours and 8 hours, respectively).

Based on the previous studies and facts of the Maillard reaction, it can be assumed that white colored compounds are early intermediates of Maillard browning reactions. These intermediates may share the designation of Amadori compounds and falls in the category of low molecular weight surfactants (LMW) (Dhruv et al. 2005). Studies have shown that there is a series of reversible reactions between reducing sugar and amine to form Schiff base and Amadori compounds (Boekel 2006; Liu et al. 2008). Amadori compounds further undergo irreversible reactions of dehydration, condensation

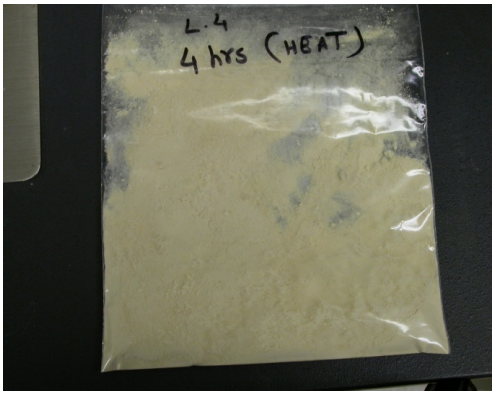
and polymerization (Martins et al. 2005; Boekel 2006; Liu et al. 2008) with continued heat. The light brown color of the 4 hours heat-treated product might contain intermediate products of the Mailard browning sequence. Dark brown color of the product can be considered as melanoidins, nitrogenous polymers and copolymers (Boekel 2006; Liu et al. 2008). After 8 hours of constant heating, it can be assumed that resultant product may contain advanced Maillard products which include polymers.

Droplet Size Measurement of O/w Emulsions

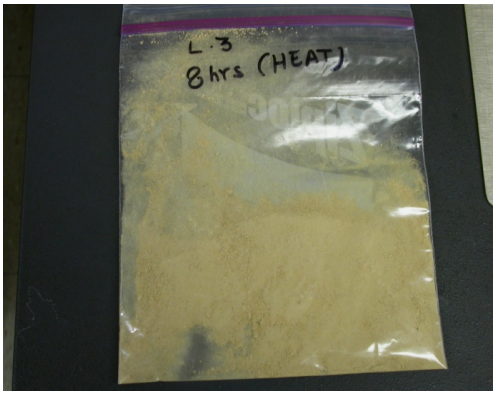
Figure 13 shows the $D_{(3,2)}$ profiles of emulsions formulated with lactose-amines (4H, 8H, 12H, and 24H) at various concentration (0.01%, 0.05%, 0.1%, and 1%) in comparison with WPC 80 at day 0 (no negative controls results were used as they were to destabilized to analyze). It can be clearly seen in Fig. 13, that there is a descending trend of $D_{(3,2)}$ observed from concentration 0.01% to 1%.

(Before- Gel form) **(After- Dried form)**

Fig. 11. Processed lactose-amines sample before and after freeze drying.

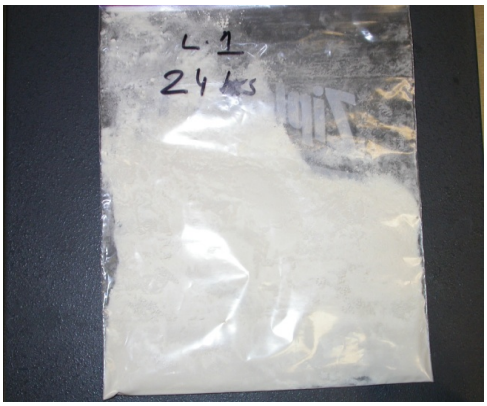


(A) 4H (Maillard product)



(B) 8H (Maillard product)

(C) 12H (LMW surfactant)



(D) 24H (LMW surfactant)

Fig. 12. Lactose-amines products in their powder state A) 4 hours of constant heat exposure at 60°C, B) 8 hours of constant heat exposure at 60°C, both the treatments produced Maillard reacted polymers. C) 1.5 hours of cyclic heat exposure at 60°C and cooling at room temperature D) 2.5 hours of cyclic heat exposure at 60°C and cooling at room temperature, resultant product of both the treatments were low molecular weight surfactants (LMW).

Although 12H is shows a slightly different trend, with the $D_{(3,2)}$ value at 0.1% higher as compare to other groups (Fig. 13). $D_{(3,2)}$ values of all the groups at 1% concentration are less than or equal to WPC 80 (Fig. 13). The reported values of $D_{(3,2)}$ of WPC 80 emulsions prepared with the 20% oil and 80% water, ranges between 0.3-0.4µm (Hogan et al. 2001; Herceg et al. 2005; Onsaard et al. 2005; Akhtar and Dickinson 2007) which is similar to the $D_{(3,2)}$ of present study i.e. 0.4±0.038 µm. The $D_{(3,2)}$ value of 24H and 12H at 1.0% concentration were found to be less than WPC 80, i.e. 0.32±0.002 and 0.37±0.028, respectively (Table A1 in the Appendix). As mentioned earlier $D_{(3,2)}$ is a tool to measure the stability of an emulsion, the smaller the value of $D_{(3,2),}$ the higher the stability of an emulsion (Hogan et al. 2001; Herceg et al. 2005; Onsaard et al. 2005; Dalgeish, 2006; Akhtar and Dickinson 2007).

Statistical analysis for droplet size at day 0 has shown that there is significant difference between droplet size of lactose-amines and WPC 80 emulsions. No results were presented for droplet size of emulsions of negative controls as they were highly unstable to analyze. LS mean comparison shown that 24H at day 0 has smaller droplet size as compare to other lactose-amines.

Figure 14 shows the droplet size distribution (the distribution of oil droplets of certain sizes in percent volume) of emulsions formulated with 24H with various concentrations of lactose-amines in comparison with WPC 80 at day 0. Droplet distribution profiles show the oil droplet distribution in relation to volume % with respect to droplet diameter (µm). WPC 80 has 14% of the volume oil droplets in the range of 0.1 µm to 1 µm, approximately 4.5% of the volume droplets were between 1 and 10 µm,

while the remaining oil droplets are distributed in very small fractions of the total volume of emulsion (Fig. 14).

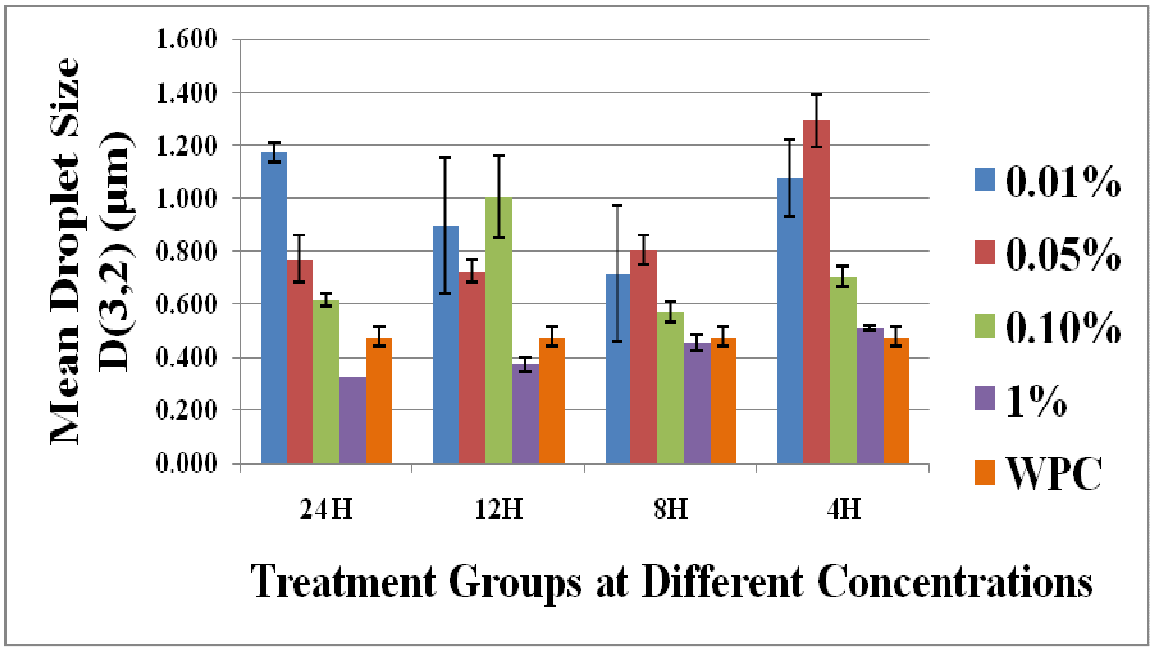


Fig. 13. Mean droplet size $D_{(3, 2)}$ of emulsions formulated with different lactose-amines and concentrations in comparison with WPC 80 (2% protein) at day 0. (n=2).

Emulsions prepared with 1% of 24H sample follow a similar droplet distribution as WPC 80 while a concentration of 0.01% of 24H has an oil droplet size of approximately 10 µm. Concentrations of 0.05% and 0.1% of 24H have very small percentage of oil droplets of less than 1 µm (Fig. 14). With increase in concentration of lactose-amines (12H, 8H and 4H), their higher volume% of droplet size is falling within the range of 0.1 µm to 1 µm (Fig. 15, 16, and 17).

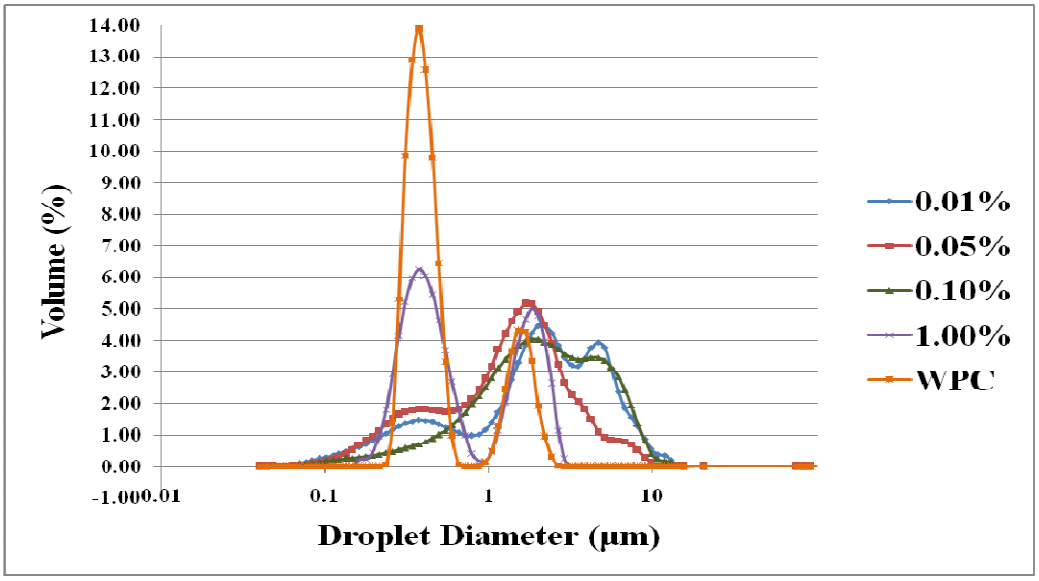


Fig. 14. Droplet distribution of emulsions formulated with lactose-amines (prepared under 24H condition) at different concentrations in comparison with WPC 80 at day 0.

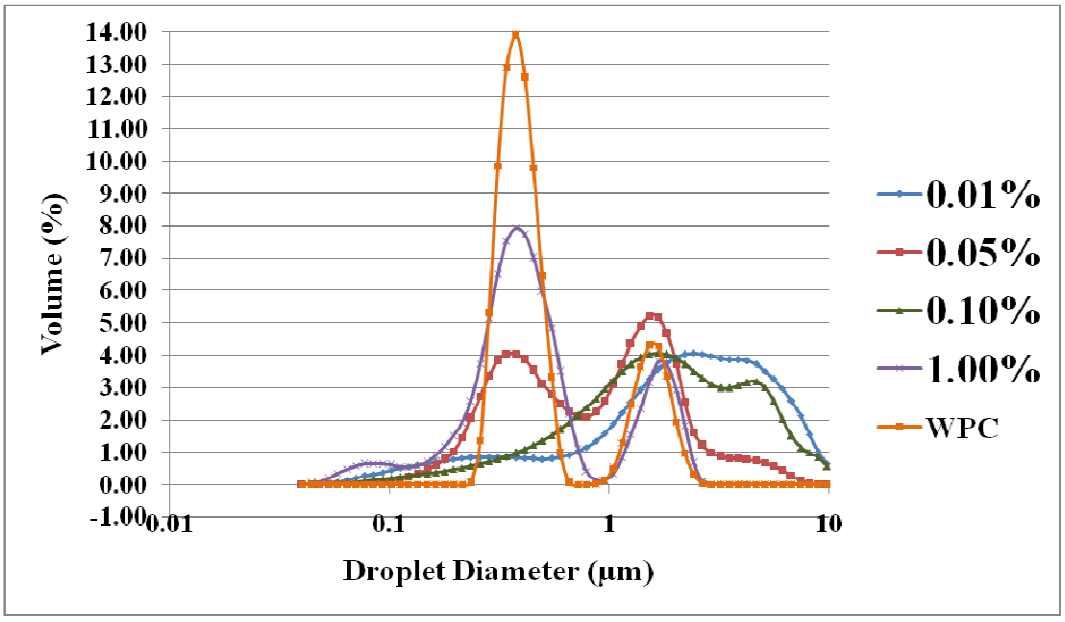


Fig. 15. Droplet distribution of emulsions formulated with 12H (12 hour) lactose-amines at different and concentrations in comparison with WPC 80 at day 0.

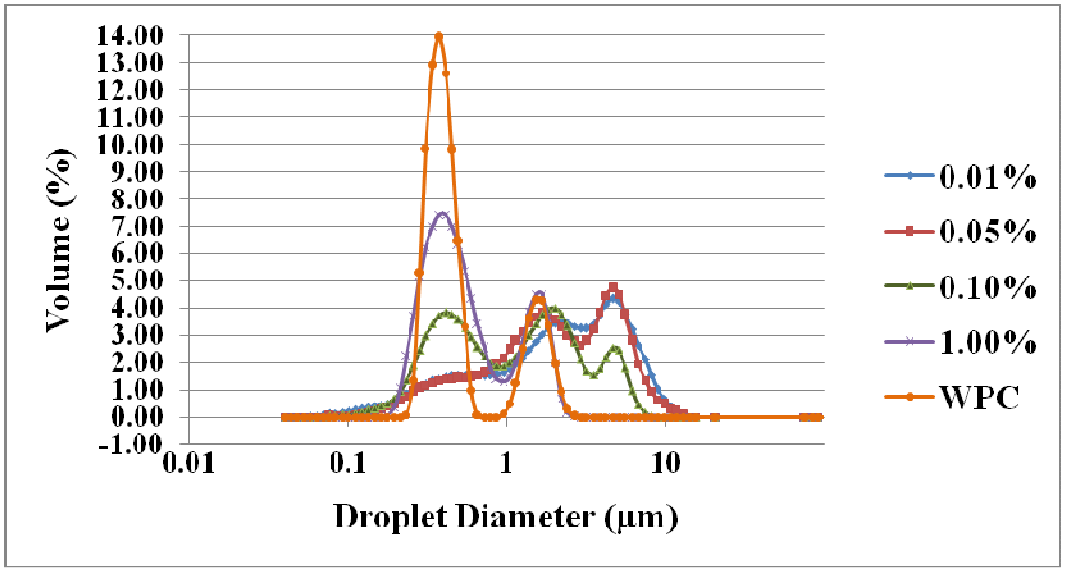


Fig. 16. Droplet distribution of emulsions formulated with 4H (4 hour) lactose-amines at different and concentrations in comparison with WPC 80 at day 0.

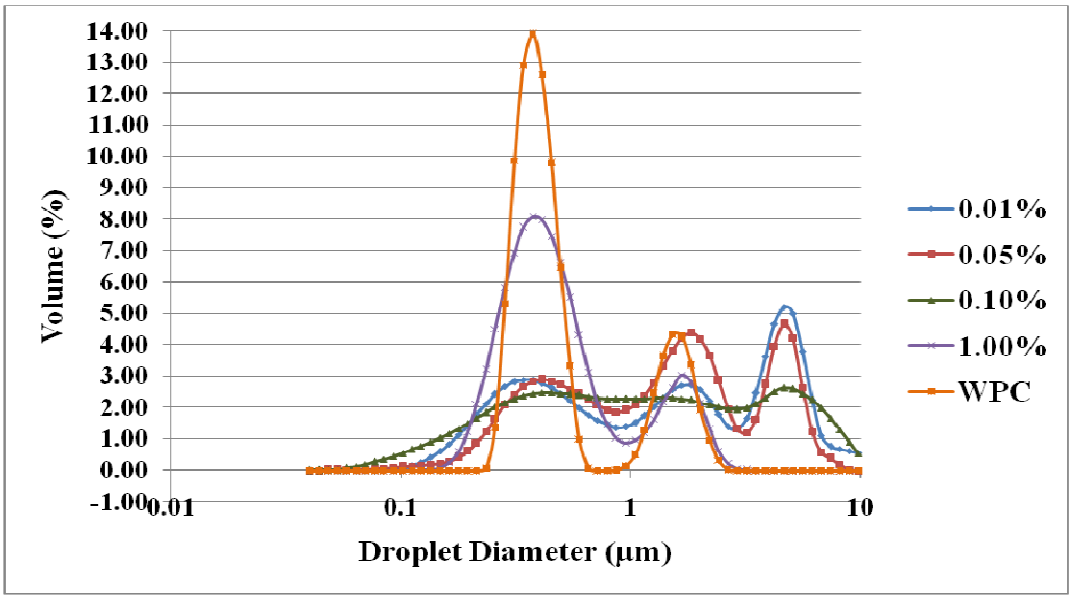


Fig. 17. Droplet distribution of emulsions formulated with 8H (8 hour) lactose-amines at different and concentrations in comparison with WPC 80 at day 0.

Destabilization Kinetics of O/w Emulsions

Descending trends in droplet-size measurements with an increase in concentration of lactose-amines were confirmed by measuring destabilization kinetics. Selective turbiscan data of changes in backscattering over the length of tubes are given in Fig. 14 A-E. Changes in backscattering is defined as the percent difference between the backscattering with respect to time (Δ BS %). In Fig. 18 C and D, Δ BS % profiles of emulsions formulated with 24H lactose-amines at 0.01% concentration show clarification at the bottom of the tube and an increase in droplet size over the tube length with creaming at the top of the tube. At 1.0% concentration there is less clarification at the bottom with constant droplet size till day 5 (144 hours) and creaming at the top of the tube. However, negative control lactose is showing clarification at the bottom of the tube and increase in particle size over the length of the tube at both 0.01% and 1.0% concentrations (Fig. 18 A and B). The WPC 80 at 2% protein is showing slight clarification at the bottom of the tube over time while no increase in droplet size was observed over the length of the tube (Fig. 18 E) (profiles with other groups or treatments are in Table B1 in Appendix B). Similar trends in the Δ BS% profiles have been followed for determining the destabilization kinetics of the o/w emulsions (Scuriatti et al, 2003; Palazolo et al, 2004). Presence of clarification at the bottom of the tube from 0-10 mm is evidence of emulsion destabilization. As mentioned earlier in the literature review, an increase in Δ BS % is directly related to destabilization of emulsions. On focusing on the bottom part of the tube (0-10 mm) in the backscattering profile, the absolute thickness of the clarification layer can be calculated. Figure 19 shows that at a concentration of 0.01%, emulsions prepared from lactose-amines exhibit a thick

clarification layer at the bottom similar to negative controls while at concentration 1.0% in Figure 20 emulsions prepared from lactose-amines were showing less clarification at the bottom of the tube similar to WPC 80 (Appendix C has additional absolute thickness in Figures C1 and C2).

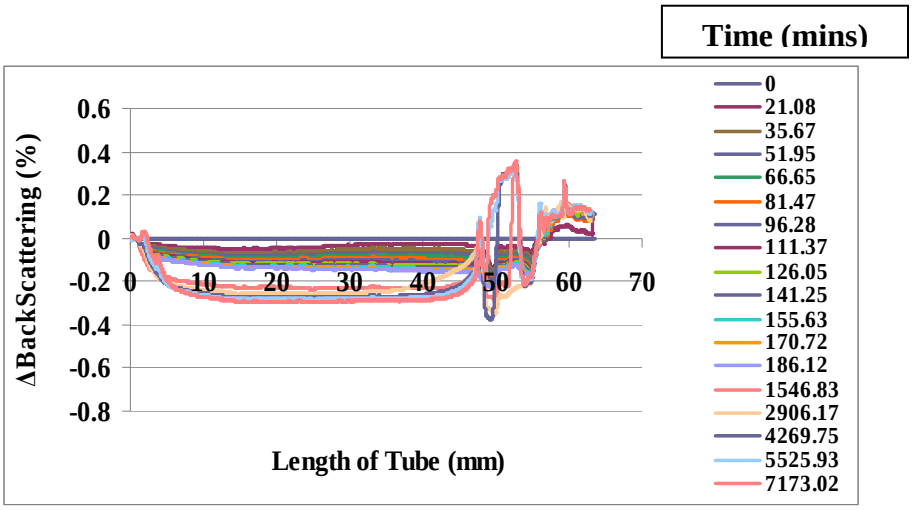


Fig. 18 A. Turbiscan view of o/w emulsions formulated with lactose at 0.01% concentration.

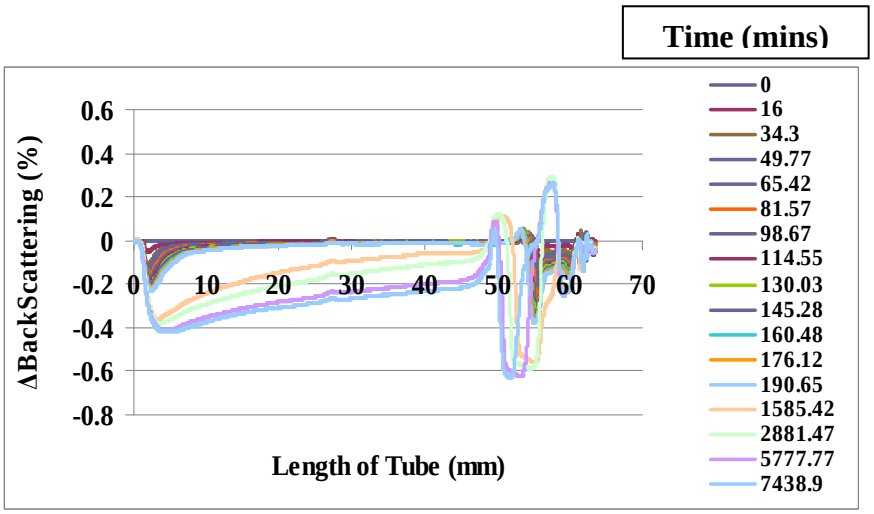


Fig. 18 B. Turbiscan view of o/w emulsions formulated with lactose at 1.0% concentration.

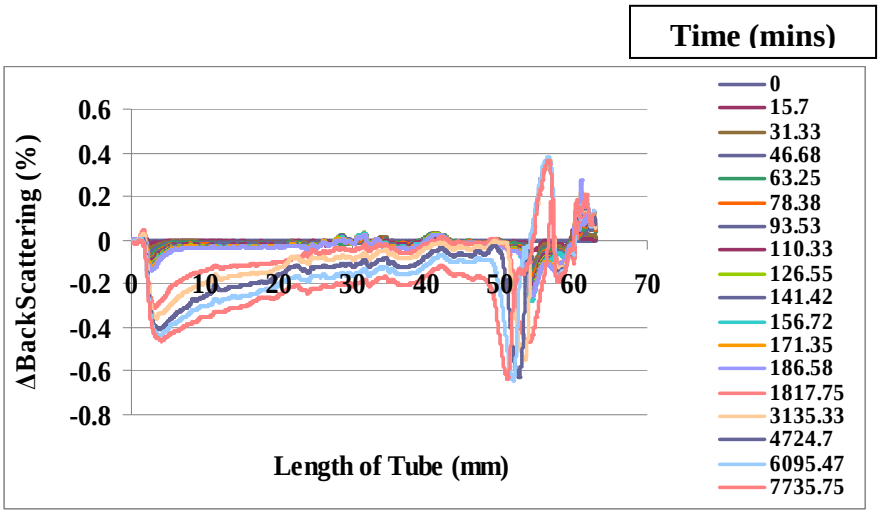


Fig. 18 C. Turbiscan view of o/w emulsions formulated with 24H lactose-amine at 0.01% concentration.

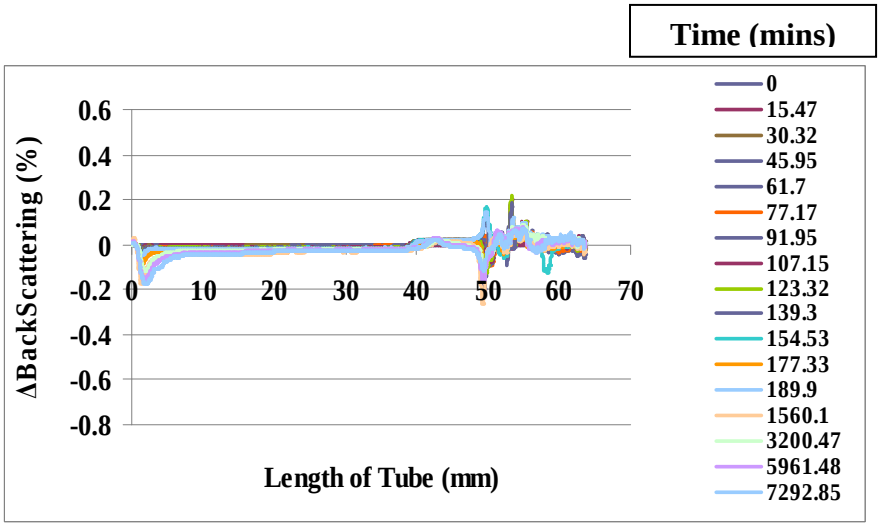


Fig. 18 D. Turbiscan view of o/w emulsions formulated with 24H lactose-amine at 1.0% concentration.

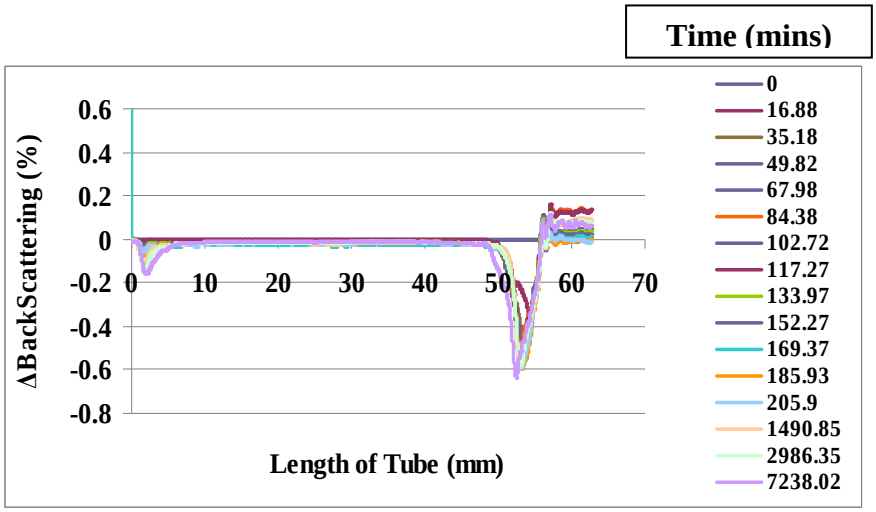


Fig. 18 E. Turbiscan view of o/w emulsions formulated with WPC 80 (2% protein).

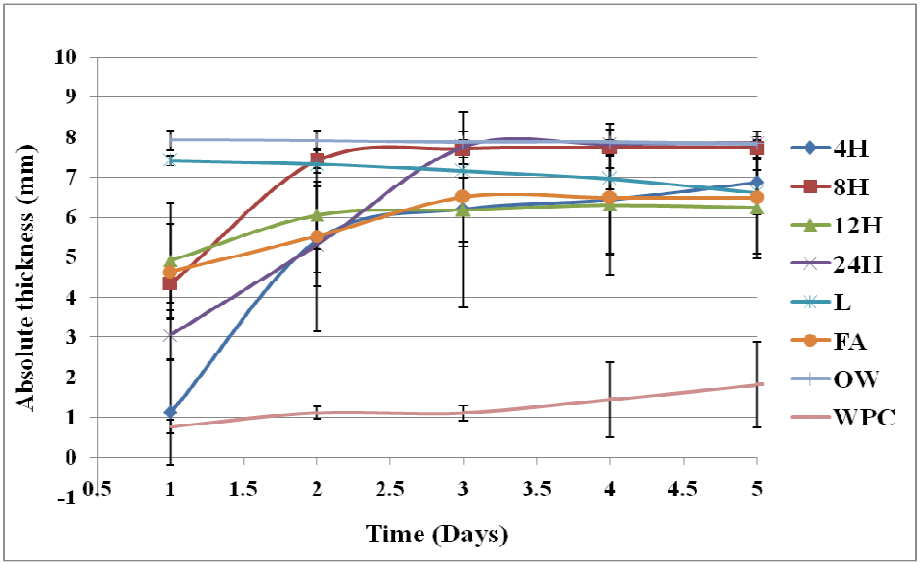


Fig.19. Absolute thickness (at the bottom of the tube from 0-10mm) of the clarification layer of emulsions formulated with different lactose-amines (4H, 8H, 12H and 24H), negative controls (L, FA and OW) at 0.01% concentration and WPC 80 (2% protein).

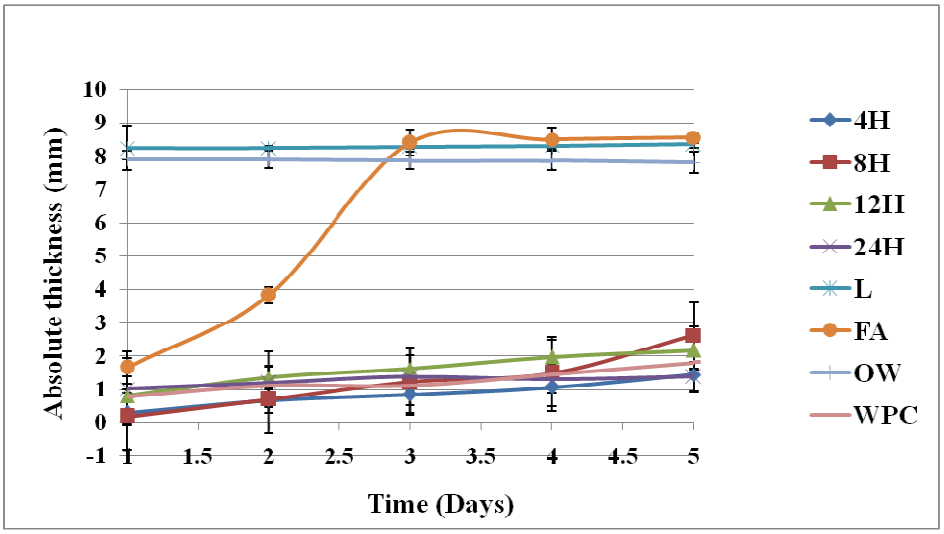


Fig. 20. Absolute thickness of clarification emulsions formulated with different (4H, 8H, 12H and 24H), negative controls (L, FA and OW) at 0.01% concentration and WPC 80 (2% protein).

Statistical analysis (two-way factorial design) was done for day1 and day 5 data, on the absolute thickness of Δ BS % at the 1-10 mm portion of the tube as clarification was started at the bottom of the tube (Table 2), for day 1 data and day 5 data you just stated this in the same sentence. From day 0 to day 1, the data recorded were absolute zero figure or in other words the emulsions were to stable to record any other value then zero. Statistical analysis of day 0 to day 1 data showed outliers in the results (Anova tables in appendix D). Therefore, day 0 to day 1 data was discarded from the statistical analysis. Statistical analysis for day 1 shows that there was a significant interaction between treatments and concentration ($p < 0.0001$) while there was no significant interaction between treatments and replicates. There are significant differences between treatments ($p < 0.0001$) and between concentrations ($p< 0.001$). Results show that there

are significant differences in emulsion between 4H and 8H, 12H, 24H, lactose, hexadecyl-amine, and o/w. For the concentration analysis, a significant difference was recorded within all the groups between concentrations 0.01% and concentrations 0.05%, 0.1%, 1.0% on day 1.

Statistical analysis (two-way factorial design) for day 5 shows that there are significant interactions between treatments and concentration ($p < 0.0001$). There are significant differences in treatments ($p < 0.0001$) and in concentrations ($p < 0.001$). Results show that there is a significant difference between treatments 12H and 24H while no significant difference was found between treatments 4H, 8H and 24H. There is also no significant difference between treatments 4H and 12H ($p < 0.001$). There are significant differences among negative controls of lactose, hexadecyl-amine, and o/w and treatment groups. These significant differences were also seen between lactose-amines groups and WPC with comparison of LS means. The results show that the destabilization rate of 24H is close to that of WPC 80 at 1.0% concentration. For the concentration analysis, there was a significant difference between the concentration 0.01% and concentrations of 0.05%, 0.1%, and 1.0% but there are no significant differences between concentrations of 0.1% and 1.0% on Day 5. Maillard reacted lactose-amine 4H follows the similar trend as 24H and WPC 80 in destabilization rate of emulsion at 1.0% concentration. In table 2, it can clearly be seen that absolute thickness of clarification layer of emulsions prepared with Maillard reacted 4H lactose-amines at 1.0% concentration shows no significant difference with WPC 80 and 24H of low molecular weight lactose-amine.

Table 1. Mean thickness of clarification layer (0-10mm at bottom) of emulsions formulated with different treatments at different concentrations on day 1 and day 5

Concentration ➡ / Treatments ⬇	0.01g		0.05g		0.10g		1.0g	
	Day1	Day 5	Day1	Day 5	Day1	Day 5	Day1	Day 5
4 hour	1.13 ± 1.30^{Ad}	6.87 ± 0.75^{Abc}	1.1 ± 0.78^{Bd}	3.45 ± 0.08^{Bbc}	0.77 ± 0.51^{Bd}	2.18 ± 0.26^{Cbc}	0.29 ± 0.32^{Bd}	1.45 ± 0.54^{Cbc}
8 hour	4.33 ± 0.47^{Ac}	7.73 ± 0.28^{Abc}	0.88 ± 0.59^{Bc}	3.32 ± 0.16^{Bbc}	0.72 ± 0.23^{Bc}	1.88 ± 0.25^{Cbc}	0.18 ± 0.23^{Bc}	2.61 ± 1.32^{Cbc}
12 hour	4.92 ± 1.43^{Ac}	6.24 ± 1.27^{Ac}	1.15 ± 0.25^{Bc}	2.82 ± 0.36^{Bc}	0.86 ± 0.02^{Bc}	2.26 ± 0.25^{Cc}	0.8 ± 0.21^{Bc}	2.17 ± 0.70^{Cc}
24hour	3.06 ± 0.61^{Ac}	7.85 ± 0.09^{Ab}	1.21 ± 0.34^{Bc}	3.92 ± 0.61^{Bb}	1.01 ± 0.1^{Bc}	3.22 ± 0.33^{Cb}	1.02 ± 1.11^{Bc}	1.38 ± 0.44^{Cb}
lactose	7.41 ± 0.13^{Aa}	6.62 ± 0.54^{Aa}	7.47 ± 0.14^{Ba}	7.24 ± 0.13^{Ba}	7.7 ± 0.34^{Ba}	8.03 ± 0.33^{Ca}	8.24 ± 0.65^{Ba}	8.35 ± 0.10^{Ca}
fatty amides	4.64 ± 1.18^{Ab}	6.49 ± 1.40^{Aa}	2.13 ± 0.63^{Bb}	8.4 ± 0.20^{Ba}	2.48 ± 0.40^{Bb}	8.45 ± 0.24^{Ca}	1.66 ± 0.27^{Bb}	8.55 ± 0.15^{Ca}
oil- water	7.92 ± 0.25^{Aa}	7.82 ± 0.32^{Aa}	7.92 ± 0.25^{Ba}	7.82 ± 0.32^{Ba}	7.92 ± 0.25^{Ba}	7.82 ± 0.32^{Ca}	7.92 ± 0.25^{Ba}	7.82 ± 0.32^{Ca}
WPC (2.5g/100ml)	0.77 ± 0.18^{Ad}							1.81 ± 0.19^{Cd}

^{abcd} mean with same letter are not significantly different in each column

^{ABC} capitalized letter represents significant differences in concentrations across the rows

Concentration 0.05% is significantly different from concentrations 0.01%, 0.1%, and 1.0%. On day 1, interactions between treatment and concentration show varying significant differences among themselves and with controls also. Fatty amines with all the 4 concentrations show no significant difference with 4H at 0.01%, 0.05% and 0.1%. No significant difference was observed between lactose-amines at 1.0% and WPC 80. On day 5, lactose-amines at 0.1% and 1.0% were non-significantly different from WPC 80 and also among themselves, while lactose-amines at 0.01% and 0.05% were significantly different from WPC 80 but not significantly different from lactose and fatty amines at 4 concentrations (Anova tables in appendix D).

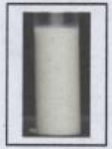
Figure 19 supports the results by showing the difference between WPC and other lactose-amines (4H, 8H, 12H, and 24H), including negative controls lactose, fatty amines and o/w. Whey protein at 2.5g/100ml (2% protein) has less thickness of clarification layer as compared to other groups at 0.01% of concentration (Fig. 19). Lactose-amines 4H, 8H, 12H, and 24H are closer towards the negative controls at 0.01% of concentration (Fig.19). On day 1 and day 5, a difference in the absolute thickness of the clarification layer in the emulsions was observed in Table 2. Figure 20, shows the thickness of clarification layer from day 1 to day 5 at 1.0% concentrations of all the groups and their comparison with WPC 80 (2% protein). At 1.0% concentration all the lactose-amines were showing similar thickness of clarification layer as of WPC 80, and on day 5, 4H and 24H were following the similar trend of clarification as of WPC 80 while other lactose-amines i.e 8H and 12H were showing more clarification on day 5 as compare to 4H, 24H and WPC 80.

All the analysis and results are supported by Table. 2. A macroscopic view of the destabilization of o/w emulsions of different treatments with different concentrations. This table contains pictures of day 1 and day 5 samples. In these pictures separation of both phases are evident for some samples.

Based on the above results, two groups of lactose-amines were produced. These two groups are surfactant lactose-amines, including 24H and 12H, and advanced Maillard reacted polymers, including 4H and 8H. The above results have shown that 24H and 4H can stabilize o/w emulsions for 5 days comparable to WPC 80. Previous studies have proven that lactose-amines prepared with cyclic heating possess low molecular weight surfactant properties, but can also be reversed back into lactose and fatty amines on prolonged storage (Bhattacharya and Acharya 1999). As mentioned earlier, studies have shown that Maillard browning is the result of an reversible and irreversible series of condensation and polymerization reactions with prolonged heating (Boekel 2006; Liu et al. 2008). Fatty amine groups may have interacted covalently with the Maillard intermediate products forming polymers. The new polymers with both hydrophobic and hydrophilic characteristics might have formed after 4 hours of constant heating. Due to the presence of both hydrophobic and hydrophilic compound in 4H lactose-amine (light brown color), emulsification activity was recorded as compared to the 8H lactose-amine. The dark brown colored 8H lactose-amine was showing less emulsification activity, which could be due to decomposition of the polymers with, prolong heating. I assumed that both types of non- polymerized and polymerized lactose-amines have hydrophilic and hydrophobic ends which may have stabilized the emulsions.

Table 2. Macroscopic view of o/w emulsions formulated with hexadecyl- amine, lactose, lactose- amines at different concentrations and WPC 80.

Concentration → / Treatments ↓	0.01%		0.05%		0.10%		1.0%	
	Day1	Day 5	Day1	Day 5	Day1	Day 5	Day1	Day 5
4 hour								
8 hour								
12 hour								

Concentration → / Treatments ↓	0.01%		0.05%		0.10%		1.0%	
	Day1	Day 5	Day1	Day 5	Day1	Day 5	Day1	Day 5
24hour								
Lactose								
Fatty amides								
O/W			WPC (2.5g/100ml)					

It can be concluded, on the basis of the droplet size distribution at day 0 and destabilization kinetics from day 0 to day 5, that concentration has a great influence on the activity of lactose-amines as an emulsifier. At concentration 1% all the types of lactose-amines have smaller droplet size similar to WPC 80 and also Δ BS% profiles show that 1% lactose-amines are more stable as compare to WPC 80 with respect to time.

Statistical analysis on oil droplet size and destabilization rate of o/w emulsions shows that 24H lactose-amines have greater stability as compared to 12H lactose-amines at 1% concentration. While brown colored polymerized lactose-amines group, 4H at 1.0% concentration have greater efficiency to stabilize o/w emulsions as compare to 8H. Therefore, 24H and 4H lactose-amines at a 1% concentration can be recommended as emulsifiers.

SUMMARY AND FUTURE RESEARCH

In this study, synthesis of lactose-amines was done at four different heating and cooling cycles from 4 to 24 hours. Lactose-amines processed for 24 hours and 12 hours of constant heating and cooling cycles are named as 24H and 12H, respectively. Lactose-amines 4H and 8H were processed for 4 and 8 hours of constant heating at 60°C. The 24H and 12H samples were white in color as they were exposed to heat for short time (due to the cooling cycle) i.e. 2-2.5 and 1.5 hours, respectively, as compare to 4H and 8H (i.e. 4 hours and 8 hours, respectively). It was assumed that white colored compounds are early intermediates of Maillard browning reactions known as Amadori. It can be assumed that white colored compounds are early intermediates of Maillard browning reactions known as Amadori compounds. The light brown color of the 4H product might contain intermediate products of the Maillard browning sequence. After 8 hours of constant heating, it can be assumed that resultant product may contain advanced Maillard products which include polymers.

Lactose-amines, lactose and hexadecyl-amine were each used in o/w emulsions at 4 different concentrations (0.01%, 0.05%, 0.1%, and 1.0%) and each concentration had 4 replicates. Observations based on the experiments were, that stability of o/w emulsions is dependent on the concentration of lactose-amines. O/w emulsions produce with lactose-amines are stable for days, comparable to WPC 80. This research has determined the influence of treatments and concentration of lactose-amines on the stability of o/w emulsions.

Emulsions prepared by lactose-amines at different concentration showed different oil droplet sizes, droplet size distributions and emulsion destabilization kinetics. Observations showed that 24H lactose-amine at 1% concentration produced stable emulsion comparable to WPC 80. Oil droplet diameter at day 0 showed a decreasing trend as the concentrations increased from 0.01% to 1.0% for all lactose-amines. At day 0, 24H at 1.0% concentration and WPC 80, both had small oil droplet sizes as compared to other lactose-amines. Destabilization kinetics to day 5 showed that at 0.01% concentration, lactose-amines had similar destabilization kinetics as the negative controls of lactose and hexadecyl-amine. Emulsion stability was significantly higher than the negative controls at lactose-amines concentrations greater than 0.05%. There was a decrease in stabilization for each treatment and concentration of lactose-amines, as well as negative controls, over time. This research has shown that lactose-amines produced at treatments of 24H and 4H are effective at stabilizing emulsions at concentrations of 0.05% to 1%. Lactose-amines 24H at 1.0% concentration showed a small separation of o/w phases as compare to WPC 80.

Further research is needed to complete the study on the influence of lactose-amines on o/w emulsions. Stabilization of o/w emulsions with respect to time with higher concentration of lactose-amines, after day 5 up to one month, is required to know the stabilization rate of the emulsions for prolong period. Work must be done to know the rate of hydrolysis of lactose-amines and its influence on the stability of the o/w emulsions. Due to the different heating treatments used to prepare lactose-amines, their molecular weights are presumed different but undefined. Studies are also required to test the assumptions of polymerized and non- polymerized lactose-amines definitions.

Determining the beneficial usage in food and pharmaceuticals industries by comparing the functionality of lactose-amines with present emulsifiers such as sugar esters will increase its future prospects. Further research is required to find out whether lactose-amines fit to the GRAS (Generally Recognized as Safe) category.

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APPENDICES

Appendix A

Droplet size measurement of o/w emulsions

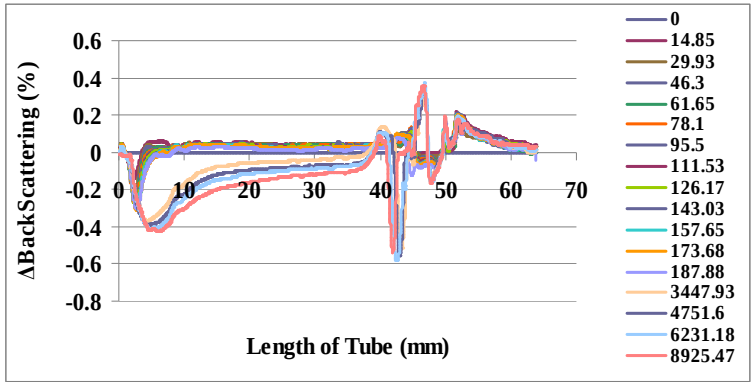
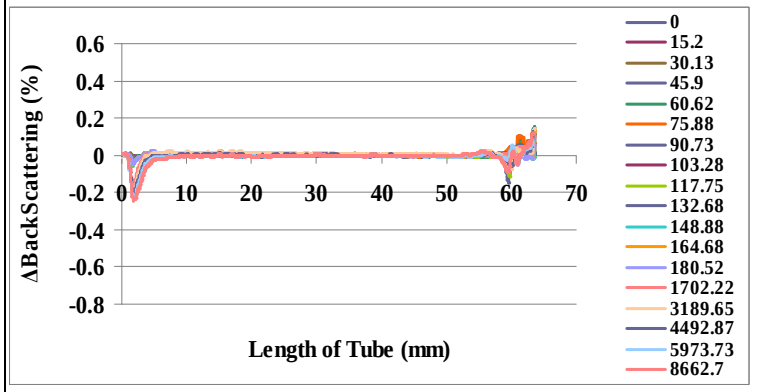
Table A1. Droplet mean diameter ($d_{3,2}$) of emulsions formulated with different lactose-amines and concentrations in comparison with WPC 80.

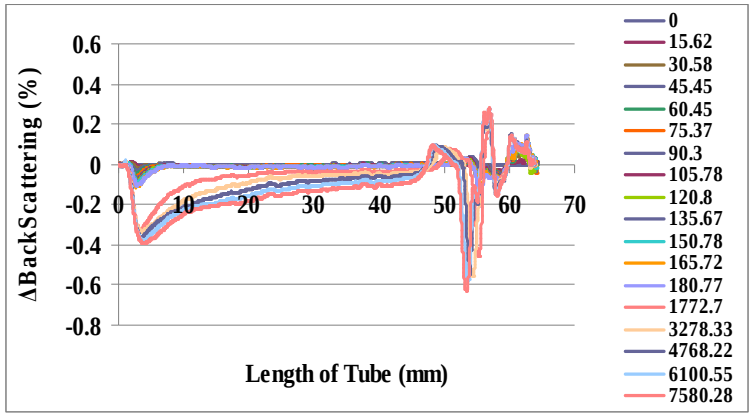
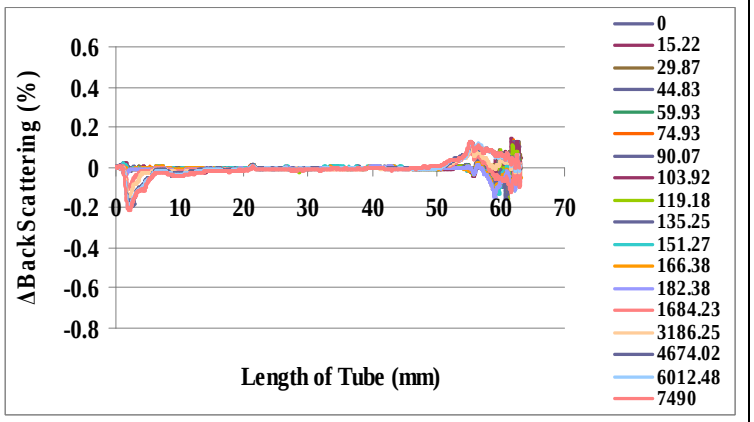
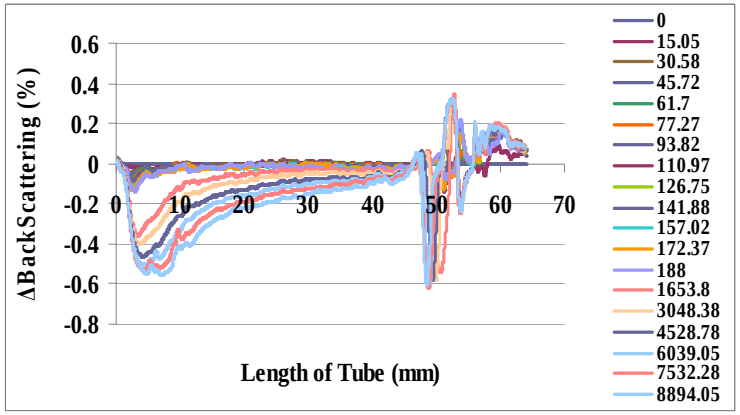
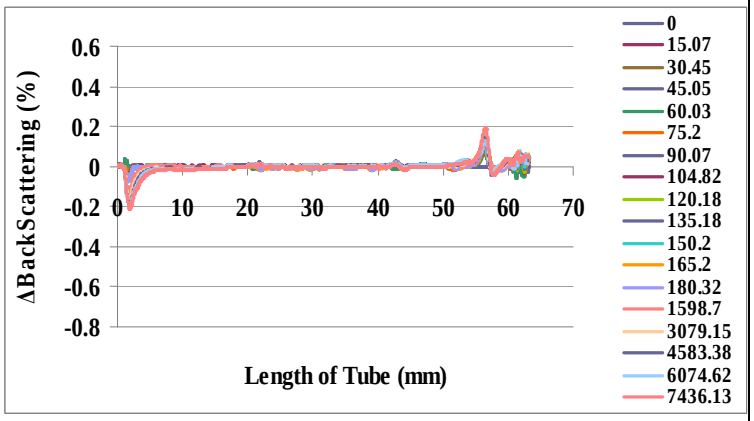
Concentrations	**0.01%**	**0.05%**	**0.1%**	**1.0%**
Treatments	**Average D(3,2)**			
WPC	**0.479 ±0.038**			
4H	**1.294±0.098**	**1.077±0.074**	**0.705±0.038**	**0.512±0.008**
8H	**0.808±0.057**	**0.721±0.148**	**0.570±0.037**	**0.456±0.030**
12H	**1.007±0.153**	**0.900±0.257**	**0.727±0.041**	**0.374±0.028**
24H	**1.175±0.255**	**0.772±0.090**	**0.620±0.025**	**0.329±0.002**

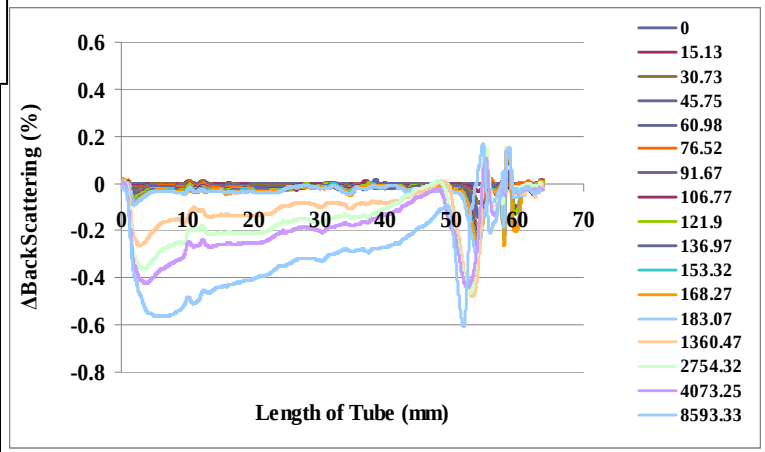
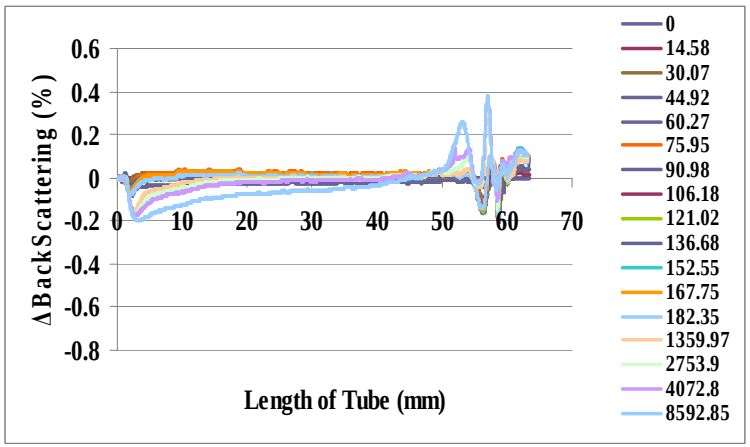
Appendix B

Destabilization profiles of o/w emulsions

Table. B1. Turbiscan view of o/w emulsions formulated with lactose-amines, negative groups at different concentrations.

Concentration → / Treatments ↓	**0.01%**	**1.0%**
	Time (mins)	**Time (mins)**
4H		

Concentration → / Treatments ↓	0.01%	1.0%
8H		
12H		

Concentration → / Treatments ↓	0.01% Time (mins)	1.0% Time (mins)
FA		

Destabilization profiles of o/w emulsions

Fig. C1. Absolute thickness (at the bottom of the tube from 0-10mm) of the clarification layer of the emulsions formulated with different lactose-amines, negative controls at 0.05% concentration and WPC 80 (2% protein).

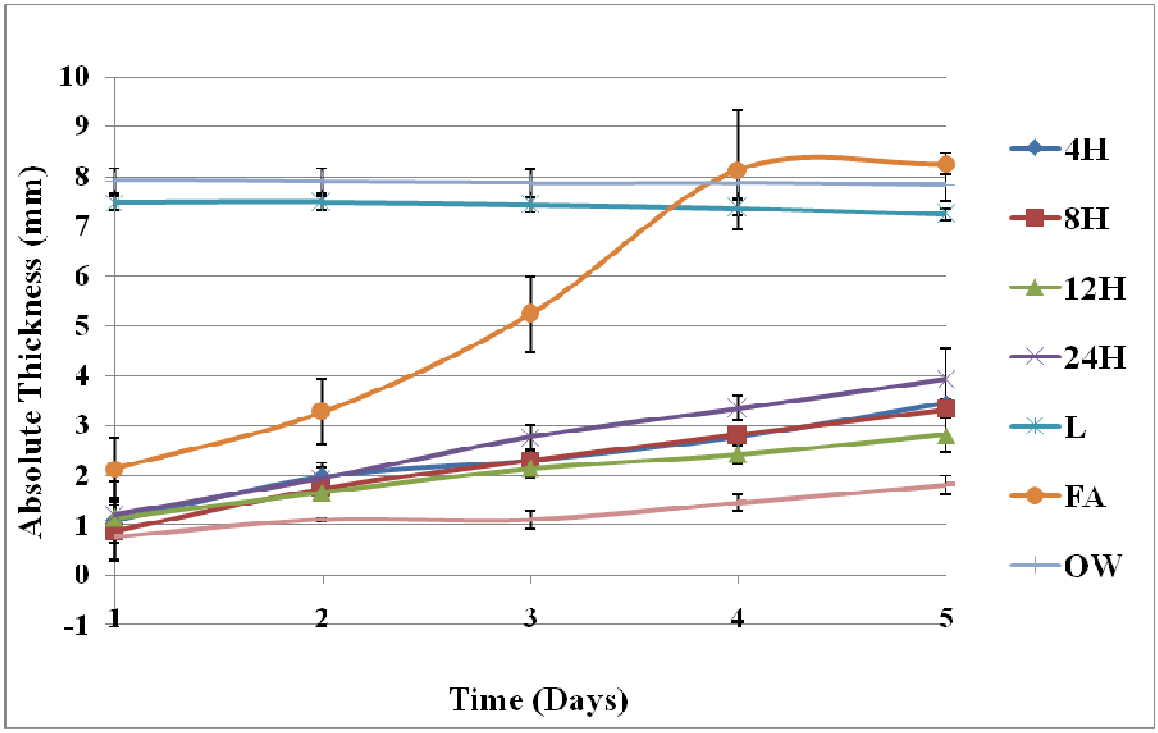
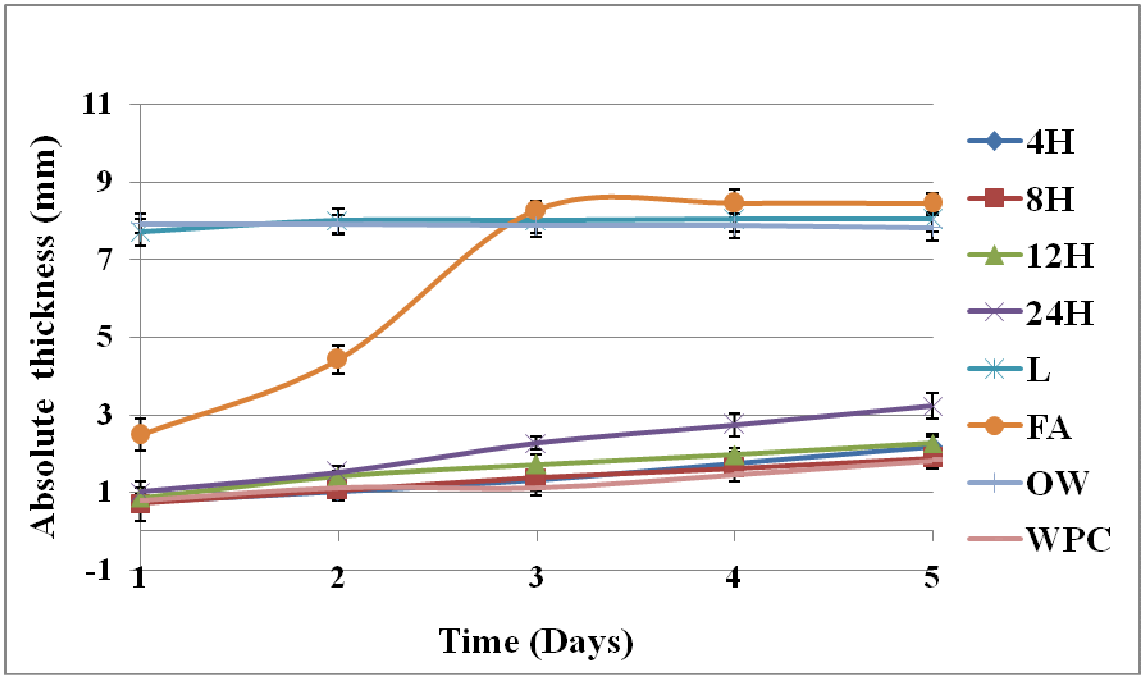


Fig. C2. Absolute thickness (at the bottom of the tube from 0-10mm) of the clarification layer of the emulsions formulated with different lactose-amines, negative controls at 0.1% concentration and WPC 80 (2% protein).



Appendix D

Destabilization of o/w emulsions(day1.sas)
A two way factorial design

The GLM Procedure

Class Level Information		
Class	**Levels**	**Values**
treat	8	A B C D E F G H
c	4	1 2 3 4

Number of observations	128

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	31	1166.688550	37.635115	132.83	<.0001
Error	96	27.200800	0.283342		
Corrected Total	127	1193.889350			

R-Square	Coeff Var	Root MSE	Dstabrate Mean
0.977217	21.45824	0.532298	2.480625

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treat	7	931.312750 00	133.0446786	469.56	<.0001
c	3	103.757525 0	34.5858417	122.06	<.0001
treat*c	21	131.618275 0	6.2675369	22.12	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treat	7	931.3127500	133.0446786	469.56	<.0001
c	3	103.7575250	34.5858417	122.06	<.0001
treat*c	21	131.6182750	6.2675369	22.12	<.0001

NOTE: This test controls the Type I experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	96
Error Mean Square	0.283342

Number of Means	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Critical Range	0.477745	0.518559 6	0.540802 1	0.555843 3	0.5670 4	0.5670 4	0.583187 4

Means with the same letter are not significantly different.				
REGWQ Grouping		**Mean**	**N**	**treat**
	A	7.9200	16	G
	B	6.0550	16	E
	C	1.9325	16	C
	C			
D	C	1.5125	16	B
D				
D	E	1.0675	16	D

Means with the same letter are not significantly different.				
REGWQ Grouping		**Mean**	**N**	**treat**
	E			
F	E	0.7875	16	A
F				
F	G	0.5100	16	F
	G			
	G	0.0600	16	H

NOTE This test controls the Type I experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	96
Error Mean Square	0.283342

Number of Means	**2**	**3**	**4**
Critical Range	0.3023517	0.316801	0.3479382

Means with the same letter are not significantly different.			
REGWQ Grouping	**Mean**	**N**	**c**
A	3.8581	32	1
B	2.4738	32	2
B			
B	2.2456	32	3
C	1.3450	32	4

treat	c	Dstabrate LSMEAN	LSMEAN Number
A	**1**	1.12500000	1
A	**2**	1.10000000	2
A	**3**	0.76500000	3
A	**4**	0.16000000	4
B	**1**	4.33000000	5
B	**2**	0.88000000	6
B	**3**	0.66000000	7
B	**4**	0.18000000	8
C	**1**	4.92000000	9
C	**2**	1.15000000	10
C	**3**	0.86000000	11
C	**4**	0.80000000	12
D	**1**	3.06000000	13
D	**2**	1.21000000	14
D	**3**	-0.00000000	15

Means with the same letter are not significantly different.				
REGWQ Grouping			**Mean**	**N**	**c**
D	**4**	-0.00000000	16	
E	**1**	7.41000000	17	
E	**2**	7.47000000	18	
E	**3**	7.70000000	19	
E	**4**	1.64000000	20	
F	**1**	2.04000000	21	
F	**2**	-0.00000000	22	
F	**3**	-0.00000000	23	
F	**4**	-0.00000000	24	
G	**1**	7.92000000	25	
G	**2**	7.92000000	26	
G	**3**	7.92000000	27	
G	**4**	7.92000000	28	
H	**1**	0.06000000	29	
H	**2**	0.06000000	30	
H	**3**	0.06000000	31	
H	**4**	0.06000000	32	

Least Squares Means for effect treat*c
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: Dstabrate

i/j	1	2	3	4	5	6	7	8	9	10	11	12	13
1		1.0000	1.0000	0.7549	<.0001	1.0000	1.0000	0.7894	<.0001	1.0000	1.0000	1.0000	0.0006
2	1.0000		1.0000	0.7977	<.0001	1.0000	1.0000	0.8291	<.0001	1.0000	1.0000	1.0000	0.0005
3	1.0000	1.0000		0.9992	<.0001	1.0000	1.0000	0.9996	<.0001	1.0000	1.0000	1.0000	<.0001
4	0.7549	0.7977	0.9992		<.0001	0.9882	1.0000	1.0000	<.0001	0.7088	0.9920	0.9980	<.0001
5	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	0.9995	<.0001	<.0001	<.0001	0.2032
6	1.0000	1.0000	1.0000	0.9882	<.0001		1.0000	0.9920	<.0001	1.0000	1.0000	1.0000	<.0001
7	1.0000	1.0000	1.0000	1.0000	<.0001	1.0000		1.0000	<.0001	1.0000	1.0000	1.0000	<.0001
8	0.7894	0.8291	0.9996	1.0000	<.0001	0.9920	1.0000		<.0001	0.7459	0.9948	0.9988	<.0001
9	<.0001	<.0001	<.0001	<.0001	0.9995	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	0.0013
10	1.0000	1.0000	1.0000	0.7088	<.0001	1.0000	1.0000	0.7459	<.0001		1.0000	1.0000	0.0008
11	1.0000	1.0000	1.0000	0.9920	<.0001	1.0000	1.0000	0.9948	<.0001	1.0000		1.0000	<.0001
12	1.0000	1.0000	1.0000	0.9980	<.0001	1.0000	1.0000	0.9988	<.0001	1.0000	1.0000		<.0001
13	0.0006	0.0005	<.0001	<.0001	0.2032	<.0001	<.0001	<.0001	0.0013	0.0008	<.0001	<.0001	
14	1.0000	1.0000	1.0000	0.5896	<.0001	1.0000	0.9999	0.6302	<.0001	1.0000	1.0000	1.0000	0.0015
15	0.4384	0.4878	0.9740	1.0000	<.0001	0.8833	0.9967	1.0000	<.0001	0.3909	0.9059	0.9559	<.0001

Least Squares Means for effect treat*c
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: Dstabrate

i/j	1	2	3	4	5	6	7	8	9	10	11	12	13
16	0.4384	0.4878	0.9740	1.0000	<.0001	0.8833	0.9967	1.0000	<.0001	0.3909	0.9059	0.9559	<.0001
17	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
18	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
19	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
20	1.0000	0.9999	0.8893	0.0454	<.0001	0.9760	0.7276	0.0532	<.0001	1.0000	0.9671	0.9255	0.0725
21	0.8365	0.7977	0.1971	0.0011	<.0001	0.3726	0.0974	0.0013	<.0001	0.8709	0.3372	0.2431	0.6503
22	0.4384	0.4878	0.9740	1.0000	<.0001	0.8833	0.9967	1.0000	<.0001	0.3909	0.9059	0.9559	<.0001
23	0.4384	0.4878	0.9740	1.0000	<.0001	0.8833	0.9967	1.0000	<.0001	0.3909	0.9059	0.9559	<.0001
24	0.4384	0.4878	0.9740	1.0000	<.0001	0.8833	0.9967	1.0000	<.0001	0.3909	0.9059	0.9559	<.0001
25	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
26	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
27	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
28	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
29	0.5589	0.6099	0.9912	1.0000	<.0001	0.9421	0.9993	1.0000	<.0001	0.5080	0.9559	0.9829	<.0001
30	0.5589	0.6099	0.9912	1.0000	<.0001	0.9421	0.9993	1.0000	<.0001	0.5080	0.9559	0.9829	<.0001

Least Squares Means for effect treat*c
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: Dstabrate

i/j	1	2	3	4	5	6	7	8	9	10	11	12	13
31	0.5589	0.6099	0.9912	1.0000	<.0001	0.9421	0.9993	1.0000	<.0001	0.5080	0.9559	0.9829	<.0001
32	0.5589	0.6099	0.9912	1.0000	<.0001	0.9421	0.9993	1.0000	<.0001	0.5080	0.9559	0.9829	<.0001

Least Squares Means for effect treat*c
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: Dstabrate

i/j	14	15	16	17	18	19	20	21	22	23	24	25	26
1	1.0000	0.4384	0.4384	<.0001	<.0001	<.0001	1.0000	0.8365	0.4384	0.4384	0.4384	<.0001	<.0001
2	1.0000	0.4878	0.4878	<.0001	<.0001	<.0001	0.9999	0.7977	0.4878	0.4878	0.4878	<.0001	<.0001
3	1.0000	0.9740	0.9740	<.0001	<.0001	<.0001	0.8893	0.1971	0.9740	0.9740	0.9740	<.0001	<.0001
4	0.5896	1.0000	1.0000	<.0001	<.0001	<.0001	0.0454	0.0011	1.0000	1.0000	1.0000	<.0001	<.0001
5	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
6	1.0000	0.8833	0.8833	<.0001	<.0001	<.0001	0.9760	0.3726	0.8833	0.8833	0.8833	<.0001	<.0001
7	0.9999	0.9967	0.9967	<.0001	<.0001	<.0001	0.7276	0.0974	0.9967	0.9967	0.9967	<.0001	<.0001
8	0.6302	1.0000	1.0000	<.0001	<.0001	<.0001	0.0532	0.0013	1.0000	1.0000	1.0000	<.0001	<.0001
9	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
10	1.0000	0.3909	0.3909	<.0001	<.0001	<.0001	1.0000	0.8709	0.3909	0.3909	0.3909	<.0001	<.0001

Least Squares Means for effect treat*c
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: Dstabrate

i/j	14	15	16	17	18	19	20	21	22	23	24	25	26
11	1.0000	0.9059	0.9059	<.0001	<.0001	<.0001	0.9671	0.3372	0.9059	0.9059	0.9059	<.0001	<.0001
12	1.0000	0.9559	0.9559	<.0001	<.0001	<.0001	0.9255	0.2431	0.9559	0.9559	0.9559	<.0001	<.0001
13	0.0015	<.0001	<.0001	<.0001	<.0001	<.0001	0.0725	0.6503	<.0001	<.0001	<.0001	<.0001	<.0001
14		0.2878	0.2878	<.0001	<.0001	<.0001	1.0000	0.9341	0.2878	0.2878	0.2878	<.0001	<.0001
15	0.2878		1.0000	<.0001	<.0001	<.0001	0.0114	0.0002	1.0000	1.0000	1.0000	<.0001	<.0001
16	0.2878	1.0000		<.0001	<.0001	<.0001	0.0114	0.0002	1.0000	1.0000	1.0000	<.0001	<.0001
17	<.0001	<.0001	<.0001		1.0000	1.0000	<.0001	<.0001	<.0001	<.0001	<.0001	1.0000	1.0000
18	<.0001	<.0001	<.0001	1.0000		1.0000	<.0001	<.0001	<.0001	<.0001	<.0001	1.0000	1.0000
19	<.0001	<.0001	<.0001	1.0000	1.0000		<.0001	<.0001	<.0001	<.0001	<.0001	1.0000	1.0000
20	1.0000	0.0114	0.0114	<.0001	<.0001	<.0001		1.0000	0.0114	0.0114	0.0114	<.0001	<.0001
21	0.9341	0.0002	0.0002	<.0001	<.0001	<.0001	1.0000		0.0002	0.0002	0.0002	<.0001	<.0001
22	0.2878	1.0000	1.0000	<.0001	<.0001	<.0001	0.0114	0.0002		1.0000	1.0000	<.0001	<.0001
23	0.2878	1.0000	1.0000	<.0001	<.0001	<.0001	0.0114	0.0002	1.0000		1.0000	<.0001	<.0001
24	0.2878	1.0000	1.0000	<.0001	<.0001	<.0001	0.0114	0.0002	1.0000	1.0000		<.0001	<.0001
25	<.0001	<.0001	<.0001	1.0000	1.0000	1.0000	<.0001	<.0001	<.0001	<.0001	<.0001		1.0000

Least Squares Means for effect treat*c
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: Dstabrate

i/j	14	15	16	17	18	19	20	21	22	23	24	25	26
26	<.0001	<.0001	<.0001	1.0000	1.0000	1.0000	<.0001	<.0001	<.0001	<.0001	<.0001	1.0000	
27	<.0001	<.0001	<.0001	1.0000	1.0000	1.0000	<.0001	<.0001	<.0001	<.0001	<.0001	1.0000	1.0000
28	<.0001	<.0001	<.0001	1.0000	1.0000	1.0000	<.0001	<.0001	<.0001	<.0001	<.0001	1.0000	1.0000
29	0.3909	1.0000	1.0000	<.0001	<.0001	<.0001	0.0195	0.0004	1.0000	1.0000	1.0000	<.0001	<.0001
30	0.3909	1.0000	1.0000	<.0001	<.0001	<.0001	0.0195	0.0004	1.0000	1.0000	1.0000	<.0001	<.0001
31	0.3909	1.0000	1.0000	<.0001	<.0001	<.0001	0.0195	0.0004	1.0000	1.0000	1.0000	<.0001	<.0001
32	0.3909	1.0000	1.0000	<.0001	<.0001	<.0001	0.0195	0.0004	1.0000	1.0000	1.0000	<.0001	<.0001

Least Squares Means for effect treat*c
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: Dstabrate

i/j	27	28	29	30	31	32
1	<.0001	<.0001	0.5589	0.5589	0.5589	0.5589
2	<.0001	<.0001	0.6099	0.6099	0.6099	0.6099
3	<.0001	<.0001	0.9912	0.9912	0.9912	0.9912
4	<.0001	<.0001	1.0000	1.0000	1.0000	1.0000
5	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Least Squares Means for effect treat*c
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: Dstabrate

i/j	27	28	29	30	31	32
6	<.0001	<.0001	0.9421	0.9421	0.9421	0.9421
7	<.0001	<.0001	0.9993	0.9993	0.9993	0.9993
8	<.0001	<.0001	1.0000	1.0000	1.0000	1.0000
9	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
10	<.0001	<.0001	0.5080	0.5080	0.5080	0.5080
11	<.0001	<.0001	0.9559	0.9559	0.9559	0.9559
12	<.0001	<.0001	0.9829	0.9829	0.9829	0.9829
13	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
14	<.0001	<.0001	0.3909	0.3909	0.3909	0.3909
15	<.0001	<.0001	1.0000	1.0000	1.0000	1.0000
16	<.0001	<.0001	1.0000	1.0000	1.0000	1.0000
17	1.0000	1.0000	<.0001	<.0001	<.0001	<.0001
18	1.0000	1.0000	<.0001	<.0001	<.0001	<.0001
19	1.0000	1.0000	<.0001	<.0001	<.0001	<.0001
20	<.0001	<.0001	0.0195	0.0195	0.0195	0.0195

Least Squares Means for effect treat*c Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: Dstabrate						
i/j	**27**	**28**	**29**	**30**	**31**	**32**
21	<.0001	<.0001	0.0004	0.0004	0.0004	0.0004
22	<.0001	<.0001	1.0000	1.0000	1.0000	1.0000
23	<.0001	<.0001	1.0000	1.0000	1.0000	1.0000
24	<.0001	<.0001	1.0000	1.0000	1.0000	1.0000
25	1.0000	1.0000	<.0001	<.0001	<.0001	<.0001
26	1.0000	1.0000	<.0001	<.0001	<.0001	<.0001
27		1.0000	<.0001	<.0001	<.0001	<.0001
28	1.0000		<.0001	<.0001	<.0001	<.0001
29	<.0001	<.0001		1.0000	1.0000	1.0000
30	<.0001	<.0001	1.0000		1.0000	1.0000
31	<.0001	<.0001	1.0000	1.0000		1.0000
32	<.0001	<.0001	1.0000	1.0000	1.0000	

Appendix D

Destabilization of o/w emulsions(day5.sas)
A two way factorial design

Class Level Information		
Class	**Levels**	**Values**
treat	8	A B C D E F G H
c	4	1 2 3 4

Number of observations	128

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	31	944.5081875	30.4680060	110.69	<.0001
Error	96	26.4236000	0.2752458		
Corrected Total	127	970.9317875			

R-Square	Coeff Var	Root MSE	Dstabrate Mean
0.972785	10.57140	0.524639	4.962813

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treat	7	637.5402875	91.0771839	330.89	<.0001
c	3	97.0404375	32.3468125	117.52	<.0001
treat*c	21	209.9274625	9.9965458	36.32	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treat	7	637.5402875	91.0771839	330.89	<.0001
c	3	97.0404375	32.3468125	117.52	<.0001
treat*c	21	209.9274625	9.9965458	36.32	<.0001

NOTE This test controls the Type I experimentwise error
: rate.

Alpha	0.05
Error Degrees of Freedom	96
Error Mean Square	0.275246

Number of Means	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Critical Range	0.4708703	0.5110976	0.5330201	0.5478448	0.5588803	0.5588803	0.5747954

Means with the same letter are not significantly different.				
REGWQ Grouping		**Mean**	**N**	**treat**
	A	7.8200	16	G
	A			
	A	7.8200	16	F
	A			
	A	7.5500	16	E
	B	3.9550	16	D
	B			
	B	3.8950	16	B

Means with the same letter are not significantly different.				
REGWQ Grouping		**Mean**	**N**	**treat**
	B			
C	B	3.4875	16	A
C				
C		3.3650	16	C
	D	1.8100	16	H

NOTE This test controls the Type I experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	96
Error Mean Square	0.275246

Number of Means	**2**	**3**	**4**
Critical Range	0.2980009	0.3122423	0.3429314

Means with the same letter are not significantly different.			
REGWQ Grouping	**Mean**	**N**	**c**
A	6.4250	32	1
B	4.8138	32	2
C	4.3775	32	3
C			
C	4.2350	32	4

treat	c	Dstabrate LSMEAN	LSMEAN Number
A	**1**	6.87000000	1
A	**2**	3.45000000	2
A	**3**	2.18000000	3
A	**4**	1.45000000	4
B	**1**	7.73000000	5
B	**2**	3.32000000	6
B	**3**	1.92000000	7
B	**4**	2.61000000	8

Means with the same letter are not significantly different.				
REGWQ Grouping		**Mean**	**N**	**c**
C	**1**	6.21000000	9	
C	**2**	2.82000000	10	
C	**3**	2.26000000	11	
C	**4**	2.17000000	12	
D	**1**	7.85000000	13	
D	**2**	3.92000000	14	
D	**3**	2.74000000	15	
D	**4**	1.31000000	16	
E	**1**	6.62000000	17	
E	**2**	7.24000000	18	
E	**3**	8.03000000	19	
E	**4**	8.31000000	20	
F	**1**	6.49000000	21	
F	**2**	8.13000000	22	
F	**3**	8.26000000	23	

Means with the same letter are not significantly different.				
REGWQ Grouping			Mean	N
F	4	8.40000000		24
G	1	7.82000000		25
G	2	7.82000000		26
G	3	7.82000000		27
G	4	7.82000000		28
H	1	1.81000000		29
H	2	1.81000000		30
H	3	1.81000000		31
H	4	1.81000000		32

Least Squares Means for effect treat*c
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: Dstabrate

i/j	1	2	3	4	5	6	7	8	9	10	11	12	13
1		<.0001	<.0001	<.0001	0.8921	<.0001	<.0001	<.0001	0.9959	<.0001	<.0001	<.0001	0.7006
2	<.0001		0.1811	0.0002	<.0001	1.0000	0.0248	0.9139	<.0001	0.9980	0.2919	0.1698	<.0001
3	<.0001	0.1811		0.9827	<.0001	0.3787	1.0000	1.0000	<.0001	0.9974	1.0000	1.0000	<.0001
4	<.0001	0.0002	0.9827		<.0001	0.0009	1.0000	0.3425	<.0001	0.0906	0.9407	0.9856	<.0001
5	0.8921	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	0.0271	<.0001	<.0001	<.0001	1.0000
6	<.0001	1.0000	0.3787	0.0009	<.0001		0.0723	0.9881	<.0001	1.0000	0.5375	0.3604	<.0001
7	<.0001	0.0248	1.0000	1.0000	<.0001	0.0723		0.9920	<.0001	0.8392	1.0000	1.0000	<.0001
8	<.0001	0.9139	1.0000	0.3425	<.0001	0.9881	0.9920		<.0001	1.0000	1.0000	1.0000	<.0001
9	0.9959	<.0001	<.0001	<.0001	0.0271	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	0.0091
10	<.0001	0.9980	0.9974	0.0906	<.0001	1.0000	0.8392	1.0000	<.0001		0.9997	0.9967	<.0001
11	<.0001	0.2919	1.0000	0.9407	<.0001	0.5375	1.0000	1.0000	<.0001	0.9997		1.0000	<.0001
12	<.0001	0.1698	1.0000	0.9856	<.0001	0.3604	1.0000	1.0000	<.0001	0.9967	1.0000		<.0001
13	0.7006	<.0001	<.0001	<.0001	1.0000	<.0001	<.0001	<.0001	0.0091	<.0001	<.0001	<.0001	
14	<.0001	1.0000	0.0035	<.0001	<.0001	0.9991	0.0002	0.1389	<.0001	0.4559	0.0076	0.0031	<.0001

Least Squares Means for effect treat*c
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: Dstabrate

i/j	1	2	3	4	5	6	7	8	9	10	11	12	13
15	<.0001	0.9881	0.9997	0.1590	<.0001	0.9995	0.9325	1.0000	<.0001	1.0000	1.0000	0.9996	<.0001
16	<.0001	<.0001	0.8801	1.0000	<.0001	0.0002	0.9988	0.1487	<.0001	0.0295	0.7569	0.8921	<.0001
17	1.0000	<.0001	<.0001	<.0001	0.4361	<.0001	<.0001	<.0001	1.0000	<.0001	<.0001	<.0001	0.2320
18	1.0000	<.0001	<.0001	<.0001	1.0000	<.0001	<.0001	<.0001	0.5997	<.0001	<.0001	<.0001	0.9988
19	0.3425	<.0001	<.0001	<.0001	1.0000	<.0001	<.0001	<.0001	0.0015	<.0001	<.0001	<.0001	1.0000
20	0.0528	<.0001	<.0001	<.0001	0.9995	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	1.0000
21	1.0000	<.0001	<.0001	<.0001	0.2184	<.0001	<.0001	<.0001	1.0000	<.0001	<.0001	<.0001	0.0975
22	0.1930	<.0001	<.0001	<.0001	1.0000	<.0001	<.0001	<.0001	0.0005	<.0001	<.0001	<.0001	1.0000
23	0.0780	<.0001	<.0001	<.0001	0.9999	<.0001	<.0001	<.0001	0.0001	<.0001	<.0001	<.0001	1.0000
24	0.0248	<.0001	<.0001	<.0001	0.9948	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.9998
25	0.7569	<.0001	<.0001	<.0001	1.0000	<.0001	<.0001	<.0001	0.0121	<.0001	<.0001	<.0001	1.0000
26	0.7569	<.0001	<.0001	<.0001	1.0000	<.0001	<.0001	<.0001	0.0121	<.0001	<.0001	<.0001	1.0000
27	0.7569	<.0001	<.0001	<.0001	1.0000	<.0001	<.0001	<.0001	0.0121	<.0001	<.0001	<.0001	1.0000
28	0.7569	<.0001	<.0001	<.0001	1.0000	<.0001	<.0001	<.0001	0.0121	<.0001	<.0001	<.0001	1.0000

Least Squares Means for effect treat*c
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: Dstabrate

i/j	1	2	3	4	5	6	7	8	9	10	11	12	13
29	<.0001	0.0091	1.0000	1.0000	<.0001	0.0295	1.0000	0.9481	<.0001	0.6408	1.0000	1.0000	<.0001
30	<.0001	0.0091	1.0000	1.0000	<.0001	0.0295	1.0000	0.9481	<.0001	0.6408	1.0000	1.0000	<.0001
31	<.0001	0.0091	1.0000	1.0000	<.0001	0.0295	1.0000	0.9481	<.0001	0.6408	1.0000	1.0000	<.0001
32	<.0001	0.0091	1.0000	1.0000	<.0001	0.0295	1.0000	0.9481	<.0001	0.6408	1.0000	1.0000	<.0001

Least Squares Means for effect treat*c
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: Dstabrate

i/j	14	15	16	17	18	19	20	21	22	23	24	25	26
1	<.0001	<.0001	<.0001	1.0000	1.0000	0.3425	0.0528	1.0000	0.1930	0.0780	0.0248	0.7569	0.7569
2	1.0000	0.9881	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
3	0.0035	0.9997	0.8801	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
4	<.0001	0.1590	1.0000	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
5	<.0001	<.0001	<.0001	0.4361	1.0000	1.0000	0.9995	0.2184	1.0000	0.9999	0.9948	1.0000	1.0000
6	0.9991	0.9995	0.0002	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
7	0.0002	0.9325	0.9988	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Least Squares Means for effect treat*c Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: Dstabrate													
i/j	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**
8	0.1389	1.0000	0.1487	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
9	<.0001	<.0001	<.0001	1.0000	0.5997	0.0015	<.0001	1.0000	0.0005	0.0001	<.0001	0.0121	0.0121
10	0.4559	1.0000	0.0295	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
11	0.0076	1.0000	0.7569	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
12	0.0031	0.9996	0.8921	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
13	<.0001	<.0001	<.0001	0.2320	0.9988	1.0000	1.0000	0.0975	1.0000	1.0000	0.9998	1.0000	1.0000
14		0.3083	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
15	0.3083		0.0572	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
16	<.0001	0.0572		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
17	<.0001	<.0001	<.0001		0.9985	0.0669	0.0057	1.0000	0.0295	0.0091	0.0023	0.2761	0.2761
18	<.0001	<.0001	<.0001	0.9985		0.9549	0.5169	0.9756	0.8536	0.6203	0.3425	0.9995	0.9995
19	<.0001	<.0001	<.0001	0.0669	0.9549		1.0000	0.0228	1.0000	1.0000	1.0000	1.0000	1.0000
20	<.0001	<.0001	<.0001	0.0057	0.5169	1.0000		0.0015	1.0000	1.0000	1.0000	1.0000	1.0000
21	<.0001	<.0001	<.0001	1.0000	0.9756	0.0228	0.0015		0.0091	0.0025	0.0006	0.1210	0.1210

Least Squares Means for effect treat*c
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: Dstabrate

i/j	14	15	16	17	18	19	20	21	22	23	24	25	26
22	<.0001	<.0001	<.0001	0.0295	0.8536	1.0000	1.0000	0.0091		1.0000	1.0000	1.0000	1.0000
23	<.0001	<.0001	<.0001	0.0091	0.6203	1.0000	1.0000	0.0025	1.0000		1.0000	1.0000	1.0000
24	<.0001	<.0001	<.0001	0.0023	0.3425	1.0000	1.0000	0.0006	1.0000	1.0000		0.9995	0.9995
25	<.0001	<.0001	<.0001	0.2761	0.9995	1.0000	1.0000	0.1210	1.0000	1.0000	0.9995		1.0000
26	<.0001	<.0001	<.0001	0.2761	0.9995	1.0000	1.0000	0.1210	1.0000	1.0000	0.9995	1.0000	
27	<.0001	<.0001	<.0001	0.2761	0.9995	1.0000	1.0000	0.1210	1.0000	1.0000	0.9995	1.0000	1.0000
28	<.0001	<.0001	<.0001	0.2761	0.9995	1.0000	1.0000	0.1210	1.0000	1.0000	0.9995	1.0000	1.0000
29	<.0001	0.7918	1.0000	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
30	<.0001	0.7918	1.0000	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
31	<.0001	0.7918	1.0000	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
32	<.0001	0.7918	1.0000	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Least Squares Means for effect treat*c Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: Dstabrate						
i/j	**27**	**28**	**29**	**30**	**31**	**32**
1	0.7569	0.7569	<.0001	<.0001	<.0001	<.0001
2	<.0001	<.0001	0.0091	0.0091	0.0091	0.0091
3	<.0001	<.0001	1.0000	1.0000	1.0000	1.0000
4	<.0001	<.0001	1.0000	1.0000	1.0000	1.0000
5	1.0000	1.0000	<.0001	<.0001	<.0001	<.0001
6	<.0001	<.0001	0.0295	0.0295	0.0295	0.0295
7	<.0001	<.0001	1.0000	1.0000	1.0000	1.0000
8	<.0001	<.0001	0.9481	0.9481	0.9481	0.9481
9	0.0121	0.0121	<.0001	<.0001	<.0001	<.0001
10	<.0001	<.0001	0.6408	0.6408	0.6408	0.6408
11	<.0001	<.0001	1.0000	1.0000	1.0000	1.0000
12	<.0001	<.0001	1.0000	1.0000	1.0000	1.0000
13	1.0000	1.0000	<.0001	<.0001	<.0001	<.0001
14	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Least Squares Means for effect treat*c Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: Dstabrate						
i/j	**27**	**28**	**29**	**30**	**31**	**32**
15	<.0001	<.0001	0.7918	0.7918	0.7918	0.7918
16	<.0001	<.0001	1.0000	1.0000	1.0000	1.0000
17	0.2761	0.2761	<.0001	<.0001	<.0001	<.0001
18	0.9995	0.9995	<.0001	<.0001	<.0001	<.0001
19	1.0000	1.0000	<.0001	<.0001	<.0001	<.0001
20	1.0000	1.0000	<.0001	<.0001	<.0001	<.0001
21	0.1210	0.1210	<.0001	<.0001	<.0001	<.0001
22	1.0000	1.0000	<.0001	<.0001	<.0001	<.0001
23	1.0000	1.0000	<.0001	<.0001	<.0001	<.0001
24	0.9995	0.9995	<.0001	<.0001	<.0001	<.0001
25	1.0000	1.0000	<.0001	<.0001	<.0001	<.0001
26	1.0000	1.0000	<.0001	<.0001	<.0001	<.0001
27		1.0000	<.0001	<.0001	<.0001	<.0001
28	1.0000		<.0001	<.0001	<.0001	<.0001

Least Squares Means for effect treat*c Pr > |t| for H0: LSMean(i)=LSMean(j) Dependent Variable: Dstabrate						
i/j	**27**	**28**	**29**	**30**	**31**	**32**
29	<.0001	<.0001		1.0000	1.0000	1.0000
30	<.0001	<.0001	1.0000		1.0000	1.0000
31	<.0001	<.0001	1.0000	1.0000		1.0000
32	<.0001	<.0001	1.0000	1.0000	1.0000	