EFFECT OF TAUROCHOLIC ACID ON OIL-WATER EMULSIONS

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Linxiao Chen

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Professor Terri A. Camesano
Advisor
Professor Destin Heilman
Advisor
L'Ecole Nationale Supérieur des Industries Chemique
Nancy, France Project Centre

Abstract –

Oil-water emulsions allows for the solvating of compounds that are normally insoluble in aqueous solutions. These emulsions have numerous applications, both consumer and scientific. This project, conducted at L'Ecole Nationale Supérieur des Industries Chemiques (ENSIC), seeks to determine the effects of taurocholic acid, a biological emulsifier produced by the gall bladder, on emulsions and emulsion stability. Two emulsion properties, interfacial tension force and emulsion droplet size was measured while all aspects of emulsion composition – continuous phase, oil phase and surfactant concentration, were varied.

Acknowledgements -

This project would have not been possible to complete without the help and support of Professors Terri Camesano and Destin Heilman. I would also like to thank Emmanuelle Marie and Alain Durand, both of whom were of immense help with not only the completion of my research at ENSIC but also in overcoming the language barrier in the lab.

Executive Summary –

The Ecole Nationale Supérieur des Industries Chemiques (ENSIC) in Nancy, France, in collaboration with Mahidol University of Thailand seek to elucidate the catalytic activity of the lipase enzyme, which aids in the digestion and degradation of fatty acids within the human body, and how certain emulsion characteristics affect the enzyme's activity. ENSIC's role in this research endeavour is the characterisation of emulsions with various compositions, the focus of this report. The results will then be shared with researchers in Thailand where the biochemical portion of this research project is being carried out. The primary focus of this report is the effect of taurocholic acid, a biological surface acting agent, on emulsion characteristics. Two emulsion qualities were used to determine its effects; interfacial tension forces and emulsion droplet diameters. In addition to varying surfactant concentrations, emulsion compositions were also varied in this study.

Interfacial tension measurements were carried out using tensiometer via the deNoüy Ring method wherein a small platinum ring is carefully lifted away from the interface and the force acting upon the ring is measured as the tension force. Three continuous phases were examined; pure water, tris-HCl buffer and phosphate buffer. The oil phase was also varied between dodecane and olive oil. Finally, surfactant concentrations between 10^{-7} g/L to 7g/L were studied. Emulsions were created via sonication and their stability and degradation was tracked over the course of fourteen days. As with the tension force measurements, all three variables – continuous and oil phase compositions as well as surfactant concentration were varied. Droplet diameters were measured using a Malvern high performance particle sizer that relies of laser diffraction to determine droplet diameters. For reasons that will be discussed later, other surfactants were tested in the creating and monitoring of olive oil based emulsions.

The results of the interfacial tension force measurements showed that varying continuous phase composition has no noticeable effect on the tension forces of the system. Oil phase composition did have a slight effect; the interfacial tensions of the dodecane systems were greater than those of olive oil based systems. In all cases, a critical micelle concentration, the concentration at which taurocholic acid begins to aggregate, occurs at approximately the same concentration of 1g/L.

Droplet size measurements confirm the results of the tensions measurements that emulsion characteristics are independent of the continuous phases examined. Dodecane emulsions revealed a phenomenon inconsistent with conventional wisdom. An increase in surfactant concentration decreased emulsion stability rather than increasing it. This pattern was present in all continuous phases examined and may be explained by interactions between the nonpolar alkane and the anionic characteristic of taurocholic acid. Olive oil emulsions proved to be unstable at the start as indicated by a broad range of droplet diameters and a very large average droplet size. Initially, it was thought to be due to interactions between the polar, fatty acid based oil and the anionic surfactant. Other surfactants, both nonpolar and cationic in nature were used as well and while emulsion droplet diameters were reduced, the range of droplet sizes detected remained similarly as broad.

Emulsion properties, as expected, are affected most greatly by the concentration of taurocholic acid present in the system, though in the case of dodecane and taurocholic acid, the effect is contrary to expectations. Because the continuous phases examined were all water based, it comes to no surprise that emulsion properties varied independently to changes in continuous phase. In order to properly examine how lipase enzymes perform in terms of emulsion characteristics, it is recommended that future continuation on this research project should focus on the creation of stable emulsions using biologically relevant oils such as olive oil and taurocholic acid.

Table of Contents –

Table of Tables

Table of Figures -

1.0 – Introduction

Oil-in-water suspensions have numerous scientific and consumer applications. Because some functional chemicals may not be soluble in water and instead would require expensive or even dangerous solvents to properly dissolve, emulsions prove to be an effective way of skirting this problem. Emulsions allow for the delivery of these active materials via water, a medium that is both safe and inexpensive. Emulsions also allow for controlled dilutions of compounds such that they may be used at an optimal concentration. For example, pesticides used in the agricultural industry incorporate the use of emulsions to deliver the water insoluble chemicals at very low concentrations. Emulsions allow the pesticides to be sprayed more effectively and evenly over the crops. Emulsions are also employed by the pharmaceutical and cosmetic industries as well. As many drugs currently being produced are water insoluble, emulsion provide a method by which to produce drugs as well as control the dosage. Emulsions also allow for improved aesthetic qualities of drugs and cosmetic products. Finally, emulsions are also exploited by the food industry to produce stable mixtures of fats and oils. Peanut butter, salad dressings and sauces are all examples of edible emulsions.

These suspensions are not inherently stable and will spontaneously separate into two distinct, immiscible layers. Emulsions are stabilised by the use of emulsifying agents or surface acting agents. These surfactants may take on a variety of different chemical and physical properties but they all share several common characteristics. These emulsifying agents all contain both hydrophilic and hydrophobic regions. Surfactants act to stabilise emulsions and slow down its breakdown, allowing oils to remain suspended in water for prolonged periods of time.

Emulsions are also present in biological systems and aid in a variety of biological functions. In the digestion and breakdown of fat, emulsions provide greater surface area on which lipase enzymes may act and digest the fats. Lipases cannot reside within oil droplets and thus can only operate at

the oil-water interface. Bile acids such as taurocholic acid act as emulsifying agents that stabilise the minute droplets that are formed, allowing for optimal enzyme activity. Additionally, these biomolecules may form micelles around lipids and fatty acids, facilitating their transport through the intestinal membrane and into the body.

In studying the activity of lipase enzymes at the oil-water interface, emulsions must be created to simulate the optimal environment in which the lipase enzyme acts. While much research has been performed on the relationship between emulsions and enzyme activity, there have not been many studies that focus on correlating physical emulsion characteristics and lipase functions. The Ecole Nationale Supérieur des Industries Chemiques, working in partnership with Mahidol University in Thailand are seeking to find a relationship between emulsion characteristics and lipase activity. The project detailed in this report seeks to elucidate how various emulsion components – the aqueous and oil phases and surfactant concentrations – affect the tension forces between phases and emulsion droplet sizes. Work describing the catalytic activity of lipase enzymes is being performed at Mahidol University and both universities will use the results from this project to determine how catalytic activity and emulsion properties are related.

2.0 – Background

This section covers all background information necessary for understanding the material presented in this report as well as how it applies to the broader research project at hand. As mentioned in the introduction, the goal of this project is to characterise the effect of taurocholic acid on the formation and stability of oil-water emulsions. ENSIC, in collaboration with Mahidol University in Thailand will use the findings presented here to gain a better insight into how the properties of the surfactant stabilised oil-water emulsions affects the catalytic activity of lipase enzymes.

2.1 – Enzymes

It is first important to understand what enzymes are and to understand the structure and function of lipase specifically. Enzymes are biological catalysts present in all living organisms. Their main purpose is to increase the rate of chemical reactions that occur within the organism. Enzymes have the ability to increase reaction rates by as much as three orders of magnitude. Enzymes increase reaction rates by lowering the activation energy – the minimal energy required for chemical reactions to occur by introducing a different, less thermodynamically demanding route by which the substrate may be converted into the necessary product.

The majority of enzymes are proteins while there are some, ribozymes, whose catalytic activity stems from RNA portions of the molecule. Proteins are large polymeric chains consisting of amino acids. While there are only 20 essential amino acids, the wide range of combinations and sizes mean that these 20 twenty basic building blocks allow for the formation of innumerable enzymes, each with a highly specific function. The amino acids and its ordering within a protein determine the three dimensional structure of the protein and how it functions it its respective environment. For example, transmembrane proteins, those that may cross a cellular membrane will have a region where there will be a large proportion of amino acids with hydrophilic functional

groups as well as a section where there are a large number of amino acids with exposed hydrophobic functional groups. This allows the protein exist in within a phospholipids membrane as well as being exposed to an aqueous environment outside the membrane.

Enzymes exhibit different degrees of specificity. Some, such as α - glycosidase are stereospecific, acting only on specific bonds and stereoisomers. Other enzymes have high substrate specificity, acting only on one specific substrate. Examples include lactase, sucrase, and maltase, which specifically act on the sugars lactose, sucrose and maltose, respectively. Enzymes exhibiting group specificity are specific to structural and bond characteristics. Pepsin and trypsin are both enzymes that hydrolyse peptide bonds between amino acids. The difference is that pepsin processes only those amino acids that have aromatic functional groups like tyrosine and tryptophan whereas trypsin acts on basic amino acids such as lysine and arginine. Finally, there are enzymes that act on a variety of substrates that share similar structures or bond types. These enzymes such as amylase and lipase show the lowest amount of substrate specificity. (Selim)

Because of the complexity of enzymes, there are many factors that affect the rate of enzymatic processes. Basic chemical principles apply; enzyme activity is directly proportional to enzyme concentration and the rate of reaction increases with the substrate concentration asymptotically, reaching and stabilising at a maximal velocity. Temperature also affects enzymatic activity; a rise in temperature leads to a decrease in activation energy but high temperatures outside of the optimal range for the enzyme will lead to a decrease of activity as the proteins will undergo irreversible denaturisation. As with temperature, enzymes have an optimal pH range in which their activity is best. Outside of that range may lead to changes in charges as well as denaturisation. Activity is also dependent upon time and for certain enzymes, the availability and concentration of coenzymes, metal ions and inhibitors. (Selim)

2.1.1 – Lipase

Lipases are a family of enzymes that are responsible for the breakdown of triglycerides. They are found in the digestive tracts of animals and facilitate the absorption and digestion of nutrients. Triglycerides are broken down into free fatty acids and monoglycerides via the hydrolysis of the ester bonds in said lipids. (Stamatis, Xenakis, & Kolisis, 1999)

While the structure and peptide makeup of lipases differ between both species and specific function, they all share certain conserved elements. The most important is the active region of the enzyme. The active region of lipases contains three amino acids that serve as the catalytic core of the enzyme. This functional group consists of serine, histadine and aspergine and is referred to as the catalytic triad. Found in other enzymes such as proteases, this catalytic triad is responsible for the hydrolysis of the substrate.

In order to prevent binding with other substrates and water, the lipase has within it an ingenious mechanism by which it selectively allows substrates into the catalytic core. A portion of the protein forms a flap-like structure that occludes the catalytic triad from exposure to the external aqueous environment. In this closed state, the enzyme shows very low catalytic activity. (Stamatis, Xenakis, & Kolisis, 1999) In contrast, w2hen lipases are introduced to an oil-water interface, it has been shown that their binding affinity to the substrate increases. In other words, lipases exhibit interfacial activation. (Grochulski, et al., 1993) At the presence of such an interface, the protein undergoes a conformational change and the polypeptide loop covering the active site moves away allowing access to the catalytic site. (Verger, 1997)

Aside from its inherent biological function, lipases have many industrial applications as well. Because of their ability to catalyse reactions in fairly mild conditions, without the help of additional cofactors and in low water conditions, lipases have been used in applications ranging from the manufacturing of soaps and detergents to the synthesis of pharmaceutical products. (Stamatis,

Xenakis, & Kolisis, 1999) Because of the numerous applications of this enzyme, and its increased activity at oil-water interfaces, the characterisation of emulsions between organic and aqueous phases becomes necessary.

2.2 – Emulsions

Emulsions are metastable dispersions of one substance within another where both substances are normally immiscible. The most common example of an emulsion is that of mayonnaise, where oil and vinegar are mixed together and stabilised with egg yolk. Normally, when combined, two immiscible liquids will spontaneously form two layers as in the case of oil and water. With the application of mechanical forces, be it vigorous shaking or sonication, one phase, the dispersed phase, will fragment and mix with the other, the continuous phase. Direct simple emulsions consists of oil droplets dispersed in an aqueous phase, whereas indirect emulsions are the opposite; aqueous droplets dispersed in an oil or organic continuous phase. Additionally, double and multiple emulsions may form when droplets of one phase are dispersed in another phase, which in turn are dispersed in yet another phase. Over time, these phases will eventually separate and the emulsion will break down.

2.2.1 – Surface and Interfacial Tension

Surface and interfacial tensions are important properties when discussing the formation of emulsions. Surface tensions stems from the attractive forces of molecules at the surface of a fluid. Specifically, surface tension arises from the asymmetry of attractive forces at the surface. (Jönsson, Lindman, Holmberg, & Kronberg, 1998) Within a liquid phase of a single compound, particles are acted upon by intermolecular forces in all directions, and because all molecules within the system are identical, the forces acted upon a single particle from all directions are equivalent. On the surface,

unequal forces are experienced by surface particles; more force is exerted upon surface particles from the liquid phase than from the vapour phase, resulting in observed surface tensions.

Interfacial tension follows much of the same principles as that of surface tension. In essence, interfacial tension is merely the surface tension that exists at the interface between two immiscible liquids. This difference in cohesion forces between two liquids also affects the miscibility or immiscibility of the liquids. If the cohesion forces are low enough, interfacial tension lowers and the two liquids then become miscible. In addition to unequal cohesion forces, interactions between molecules of the two liquids also affect interfacial tension. For example, a water-octane system exhibits an interfacial tension of 51mN/m while a water-octanol system exhibits an interfacial tension of 8mN/m. This is because the hydroxyl group of the alcohol orients itself towards the water phase, reducing the tension. (Jönsson, Lindman, Holmberg, & Kronberg, 1998) This is the same principle behind the use of surfactants to stabilise emulsions, which will be covered in detail in later sections.

Surface tension and interfacial tension may be measured using a variety of methods, one of which is the de Noüy Ring method. Essentially, tension forces are measured as the force required for the ring to break free of the surface or interface of the liquid(s). The force is measured via a tension balance and is given as the sum of the interfacial force and the weight of the ring. The system is zeroed with the ring attached as to exclude it from the force measurement. This method is measurement is preferred due to its simplicity and relative easy but it does suffer several drawbacks. First, the ring needs to be free of any contamination. Secondly, the ring must lie perfectly horizontal; any angular deviation will cause the resulting force measurement to be inaccurate as the force acting upon the ring will be uneven. (Tadros & Vincent, Liquid/Liquid Interfaces, 1983)

2.2.2 – Emulsion Formation

As mentioned earlier, emulsions are formed as a result of some sort of mechanical shearing stress acting upon the system. There are multiple ways of applying said force, the most obvious being through vigorous shaking. This method is simple but will not result in very small droplets. Mechanical atomisers may also be used. The dispersed phase is atomised into the air and the droplets are then included into the continuous phase. Mixing via rotational motion may also be used to introduce simple shear stresses. Finally, ultrasonic waves may be used to create small droplet emulsions. A rod is submerged into the liquid and vibrated at very high frequencies to disrupt the interface and break up larger droplets created by another emulsification technique. (Walstra, 1983) Ultra-sonication is used in this project and specific protocol will be discussed in the methodology section.

2.2.3 – Characterising Emulsions

Emulsions may be characterised in several ways. Emulsion capacity, the amount of dispersed phase that may be effectively emulsified under set conditions. Specifically, because this method does not reveal droplet sizes, emulsion capacity is often determined as a function of time; the amount of time necessary to emulsify a certain amount of the dispersed phase under set conditions. (Walstra, 1983)

Another method involves observing the amount of phase separation that occurs. Phase separation, either naturally occurring through sedimentation or creaming or through mechanical means such as centrifugation is an indirect way of determining droplet size as the rate of sedimentation depends on droplet size. Sedimentation rate also depends on other factors including flocculation and viscosity. (Walstra, 1983)

Droplet size is the most straightforward method of studying emulsions. Droplet sizes may be determined using laser diffraction spectroscopy. The method works by analysing the amount of

diffraction created by the movement of the particles. The intensity and frequency are related to the particle size by Brownian motion which states that smaller particles move at a faster rate than larger ones due to their smaller hydrodynamic radii. The diffusion coefficient is determined through the scattering intensities and then via the Stokes-Einstein equation, the hydrodynamic radius may be calculated.

2.2.4 – Emulsion Destabilisation

Because emulsions are inherently thermodynamically unstable due to the immiscible nature of the two liquids, over a certain period of time, emulsions will separate and a liquid-liquid bilayer will once again form. This breakdown may occur over the course of a few minutes to a few months and some are stable for years. Emulsion breakdown may occur via several mechanisms; creaming, flocculation, coalescence and Ostwald ripening.

2.2.4.1– Creaming

Creaming occurs as the result of density differences between the two liquids. Buoyancy forces and gravitational forces cause the emulsion to separate based on differences in density, causing the lighter of the two components to rise and collect at the surface. The process is the same as that of sedimentation. When an emulsion creams, the individual particles do not aggregate and may be re-dispersed throughout the continuous phase via shaking. While creaming does not truly represent emulsion destruction, it does lead to the formation of larger droplets.

The rate of creaming is described by Stoke's Law (2.1), which defines the movement of a sphere through a fluid environment

$$v = \frac{2gr^2(\partial\rho)}{9n} \tag{2.1}$$

where *v* represents the velocity of sedimentation of a particle with a radius of r. g represents the gravitational constant, ∂rho the difference in density between the dispersed and continuous phases

and n the viscosity of the continuous phase. (Menon & Wasan, 1983) According to Stoke's Law, the rate of creaming may be reduced by decreasing the effective diameter of the dispersed phase particles and by increasing the viscosity of the continuous phase.

2.2.4.2– Flocculation

Flocculation is the aggregation of droplets into clusters without combining; each droplet in the cluster still retains separate properties. Flocculation is caused by interactions between droplets, mainly van der Waals and electrical double layer interactions. While van der Waals forces are generally considered to be short range, the net force between two droplets has a much longer range. Electrical double layer interactions are a result of charge droplet interaction due to the electrical double layers present at the liquid interfaces. (Tadros & Vincent, Emulsion Stability, 1983) The rate of floc (aggregate) formation is governed by the diffusion of the disperse phase through the continuous phase. Flocculation may be either an irreversible or reversible process. Typically, systems with larger droplet diameters, those greater than 1μm, flocculation may be reversible.

Flocculation also has an effect on emulsion creaming. While the increase in particle size due to aggregation increases creaming, the reduction in density difference due to the inclusion of the aqueous phase within the effective radius reduces the rate of creaming. Research performed by Chanamai and McClemens (2000) showed particle sizes dominate and the rate of creaming increases with flocculation. (Chanamai & McClements, 2000)

Additionally, flocculation is the precursor to coalescence. As stated before, when droplets aggregate together into a floc, they retain their individual properties. This is because the droplets do not touch but are instead separated by a thin liquid film of the continuous phase between the two droplets. Flocs coalesce when said film ruptures. (Tadros & Vincent, Emulsion Stability, 1983)

2.2.4.3– Coalescence

As mentioned in the previous subsection, coalescence occurs when two droplets are in contact and the thin film separating the droplets ruptures. The droplets then combine and form a larger droplet. The mechanism governing coalescence occurs in two parts.

First, the film separating the droplets thins. For this to occur, a pressure gradient must exist in the film to allow a flow of fluid in the film. Factors that can affect film thinning include interfacial tension, difference in density, droplet size and type and presence of surfactants at the interface. (Menon & Wasan, 1983) As the film thins, hydrophobic forces cause the film to rupture, leading to the formation of a larger droplet of the dispersed phase within the continuous phase. These larger droplets are more susceptible to creaming.

2.2.4.4– Ostwald Ripening

Emulsions droplet sizes may also increase without coalescence through a phenomenon called Ostwald ripening. Ostwald ripening is the growth of one emulsion droplet at the expense of a smaller one. The driving force behind this transfer of mass is a difference in the chemical potential of the phase within the droplet and indirectly, the curvature of the drops. Fundamentally, Ostwald ripening occurs as a result of the system trying to reduce the free energy by conforming to a state wherein the interfacial area is minimised. (Taylor, 1998)

This process occurs within emulsions where the continuous and dispersed phases are not completely immiscible, thus allowing small amounts of the dispersed phase to solubilise and travel through the continuous phase. In essence, the contents of smaller droplets are transported and deposited into larger droplets, leading to the continued growth of larger droplets and the shrinking and eventual disappearance of the smaller droplets.

2.3 – Surfactants

Surfactant is an abbreviation given to surface-active agents. Like their name says, surfactants are compounds that are active at surfaces and interfaces between immiscible liquids. They have a tendency to absorb at these interfaces due to their chemical properties. Surfactants are amphiphilic in nature, that is to say, they consists of both a hydrophilic, often referred to as the head group, and a hydrophobic tail group. This allows the molecule absorb at the interface and reside in both a hydrophobic and an aqueous environment.

Surfactants are often categorised by the nature of their hydrophilic heads. The heads may either be non-ionic or ionic in nature. Anionic surfactants make up the largest class of surfactants. Carboxylate, sulphate and phosphate based polar groups impart hydrophilic properties to surfactants belonging to this class. Additional properties such as water or oil solubility may be conferred onto the surfactant by counterions present. The most common anionic surfactant is soap, which is the alkali metal salt of fat and oil derived carboxylic acids. Nonionic surfactants typically have a polyether or polyhydroxyl head group that serves as the polar group. They are the second largest class of surfactants and are compatible with other surfactants. Common surfactants in this category include sucrose esters and polyglycerol esters. Cationic surfactants, the third largest surfactant category have positively charged head groups based upon nitrogen's ability to carry a positive charge as an amine or ammonium based compound. While ammonium based compounds are not sensitive to pH changes, due to the need to be in a protenated state, amine based surfactants may only be used in low pH environments. Finally, the smallest class of surfactants are those that have both cationic and anionic regions on the head group. These zwitterionic surfactants possess the most versatility, performing well in a large range of environments.

2.3.1 – Surfactant Effects

The tendency for surfactants to absorb at the oil-water interface has numerous effects on the stability of emulsions. The interfacial free energy present at the boundary between immiscible systems is lowered in the presence of surfactants and represents the driving force for the absorption of surfactants at the interface. The absorbed surfactant, due to having both hydrophobic and hydrophilic regions, reduces the overall free energy of the system and thus reduces the interfacial forces driving the separation of phases seen in oil-water mixtures. The more surfactant absorbed at the interface, the lower the free energy and interfacial tension (as interfacial tension is merely free energy per unit area). (Jönsson, Lindman, Holmberg, & Kronberg, 1998) As additional surfactants are added to the system, the effect on interfacial tension is limited by the formation of micelles in solution. The concentration at which surfactant aggregation begins is referred to as the critical micelle concentration (CMC).

The critical micelle concentration refers to the limiting surfactant solubility in a particular solution. As mentioned above, surfactant concentrations above the CMC will result in aggregation of the molecules into micelles. Micelle formation is governed by hydrophobic interactions, specifically to orient the surfactants such that the hydrophobic tail regions are not exposed to the aqueous environment. The CMC of a surfactant varies and depends on the chemical structure of said surfactant. It decreases with increasing alkyl chain length and is generally lower for non-ionic compounds. When using a surfactant salt, the valency of the counter ion also has a considerable effect on the CMC value of the surfactant. While monovalent ions show no effect, a divalent ion reduces the CMC value by a factor of four. (Jönsson, Lindman, Holmberg, & Kronberg, 1998) The micelles protect oil droplets from an aqueous environment, forming small droplets that are relatively stable. As stated above, the smaller the droplets, the more stable the emulsion.

2.3.2 – Taurocholic Acid

The surfactant of interest in this project is taurocholic acid (Figure 2.1). Taurocholic acid is a derivative of cholic acid conjugated with taurine.

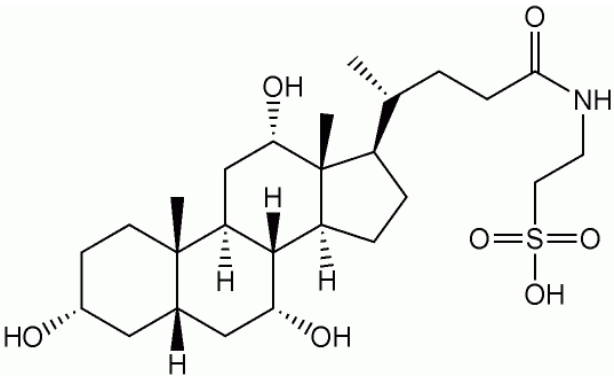

Figure 2.1 – Taurocholic Acid

Taurocholic acid is a biomolecule secreted by the gall bladder. Its function in the body is that of a detergent, to breakdown large fat globules into microscopic scale droplets or emulsions, increasing the surface area available for lipase to act upon.

Taurocholic acid is an anionic surfactant; the sulphate group from the taurine imparts the negative charge onto the molecule at physiological conditions. Additionally, the cholic acid group consists of aromatic rings with various side chains conjugated to it. The side chains are ordered in such a manner that the polar hydroxyl groups are located on one side whereas the nonpolar methyl groups are located on the other, adding to the molecule's surfactant abilities.

2.4 – Applications

Aside from scientific applications, such as the characterisation of interfacial enzyme activity, emulsions and surfactants are used in numerous other applications. By definition, all soaps and detergents are surfactants as they reduce the surface tension of water, making water *wetter* and allowing it to carry away compounds water may not have been able to before. Emulsions are vital to the pharmaceutical industry. As more and more of small molecule therapeutics developed are water

insoluble, emulsions present both an inexpensive and effective way to producing drugs that may deliver the active ingredients in a safe and controlled manner. Similarly in the agricultural industry, pesticides, fungicides and fertilisers are often emulsified to allow them to be dispersed evenly across crops.

Additionally, emulsions are also used widely in the food industry. Salad dressings, peanut butter and chocolate are just three examples of foods where emulsions play a great role. Lecithin found in egg yolks and in soy beans is often the surfactant of choice as it is nontoxic to the human body. In addition to preventing foods from separating, emulsions are often used to control the aesthetic qualities of foods, generally those that are cream based. This focus on aesthetics is also applicable to the cosmetic industry where surfactants are used to not only prevent creams from separating, but also to control the feel and texture of said products, an important factor when marketing the products.

Finally, as with the purpose of this project, emulsions are also used to study the activity of interfacial proteins. In the digestive process, emulsions serve to increase the interfacial surface area available for lipase enzymes to act on, thus increasing their catalytic activity. This project will seek to characterise the properties and stability of emulsions stabilised by taurocholic acid, a naturally occurring surfactant produced by the gall bladder. Tension forces and droplet diameters will be determined using protocol that will be described in the following section.

3.0 – Materials and Methodology

This project focused on elucidating the effect of taurocholic acid on oil-water emulsions. Different aqueous phases were examined as well as different oils. Additionally, the concentration of taurocholic acid was varied. In characterising the effect of this surfactant on oil-water emulsions, surface and interfacial tension tests were performed and droplet sizes were measured and tracked over the course of two weeks. The protocol by which experiments were carried out and the materials used will be covered in this section.

3.1 – Preparation

Several solutions must be prepared prior to emulsion creation and analysis. These solutions include the two buffers that will be studied as well as solution used for removing organic residue from sample cells.

3.1.1 – Piranha Solution

Piranha solution is an aptly named highly oxidative solution used to remove any and all organic compounds. The solution is used to clean the sample cells used to determine surface and interfacial tensions. Because any leftover residue in the cells will skew the tensiometer results, the cells must be free of any oils prior the start of the readings. Piranha solution is the preferred method of cleaning glassware in preparation for sensitive experiments because unlike water, it does not leave any mineral residue. One litre of the solution was prepared my mixing sulphuric acid and 30% hydrogen peroxide in a 3:1 ratio. 750ml of sulphuric acid was placed in a two litre beaker kept cold via ice bath. A magnetic stir bar was used to ensure that the solution will be well mixed. 250ml of the 30% hydrogen peroxide was added slowly as the mixing is highly exothermic; care was taken to ensure that the solution does not reach a high temperature. After adding the hydrogen peroxide, the solution was allowed to cool down. Because hydrogen peroxide is photoreactive and will

decompose rapidly in the presence of light, the solution was stored in a brown glass bottle. A cap was placed on the bottle lightly and the bottle was stored inside a hood between uses.

3.1.2 – 0.02M Phosphate Buffer

The phosphate buffer is one of the most important buffers in studying biological systems. The buffer is utilised by cells to maintain physiological pH and in studying the enzymatic activity of lipase this buffer may be used to mimic the environment in which lipase acts.

In preparing 500ml of 0.02M phosphate buffer, 210ml of 0.04ml Na_2HPO_4 and 40ml of 0.04 NaH_2PO_4 are added to 250ml of distilled and deionised water. A hydrate form of Na_2HPO_4 was used – $Na_2HPO_4 \cdot 2H_2O$ – which has a molecular weight of 178.01g/mol. 1.495g of the salt was measured and thoroughly dissolved in 210ml of water. Anhydrous NaH_2PO_4 (molecular weight 119.98g/mol) was used as the second salt component; 0.192g was added to 40ml of water. The solutions were then mixed within a one litre Erlenmeyer flask and 250ml of distilled water was added to bring the final volume of the buffer to 500ml. The pH of the final solution was checked using a glass pH probe to ensure that the buffer pH was 7.5 ± 0.1.

3.1.3 – Tris-HCl Buffer

Tris is the abbreviation for the organic compound tris(hydroxymethyl)aminomethane (Figure 3.1) and is used in the laboratory setting as a buffer to mimic physiological environments.

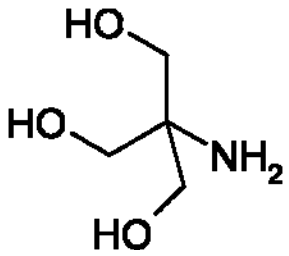

Figure 3.1 – Tris(hydroxymethyl)aminomethane

Tris-HCl is the salt form of the compound and with a pKa value of 8.1, Tris-HCl buffer will be able to maintain the pH of a system between 7.0 and 9.0.

To prepare one litre of the tris-HCl buffer, 0.0339g of tris was combined with 8.7660g NaCl and 0.2058g of $CaCl_2$ within a one litre Erlenmeyer flask. Deionised, distilled water was then added to the flask to make 1L of buffer. In a separate 150ml beaker, 0.833ml 37% HCl was added along with water to make a 100ml solution. Using a glass pH probe, the pH of the buffer was measured while under constant stirring via a magnetic stir rod. HCl was slowly added to the buffer using a Pasteur pipette, waiting between additions for the pH of the buffer to stabilise. HCl was added until the pH of the buffer read 7.5 ± 0.1.

3.2 – Tension Forces

Tension force measurements were performed on Krüss manual tensiometer in order to determine the critical micelle concentration of the system. In order to fully examine the effects of taurocholic acid on emulsions, multiple readings were done while varying the aqueous phase, the oil phase, and the surfactant concentration. Aqueous phases examined include MilliQ filtered water, 0.02M phosphate buffer, and tris-HCl buffer. Laboratory grade extra virgin olive oil (Sigma Aldrich) and dodecane (Sigma Aldrich) served as the oil phases. Taurocholic acid was also obtained from Sigma Aldrich and the following concentrations were examined: 7g/L, 5g/L, 4g/L, 3g/L, 2g/L, 1g/L, 0.5g/L. 0.05g/L, 10^{-3}g/L, 10^{-4}g/L. 10^{-5}g/L, 10^{-6}g/L, and 10^{-7}g/L. All experimental tests are shown in the table below (Table 3.1).

		Surfactant Concentration (g/L)												
		7	5	4	3	2	1	0.5	0.05	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
Water	Dodecane													
	Olive Oil													
Tris-HCl	Dodecane													
	Olive Oil													
Phosphate	Dodecane													
	Olive Oil													

Table 3.1 – Tension Force Measurements – Shaded areas represent combinations of continuous, dispersed and surfactant concentrations tested

Both surface and interfacial tension measurements were performed on all systems; the protocol of each measurement is described below.

3.2.1 – Surface Tension

Glass tensiometer sample cups were first cleaned using the piranha solution. Approximately 40ml of the solution, enough to fill the cups was poured into each sample cup and allowed to sit for a minimum of one hour. The piranha solution was then carefully poured from the cups and any remaining solution in the cups were neutralised with ultra-pure MilliQ water. The cups were then placed into a 50°C oven and allowed to dry for approximately 30 minutes.

The aqueous phase was prepared by measuring out the appropriate amount of surfactant using an analytical balance into a tared gravimetric flask. For higher surfactant concentrations (4-7g/L), a 25ml gravimetric flask was used while for lower concentrations (3-0.05g/L) a 50ml flask was used. Because the analytical balance cannot accurately measure amounts smaller than 0.0001g, in order to prepare aqueous samples with surfactant concentrations lower than 10^{-3}g/L, dilutions must be performed. Starting with a 50ml sample of 10^{-3}g/L aqueous phase, 5ml was taken using a 5ml micropipette and transferred into a clean 50ml gravimetric flask. 45ml of the appropriate aqueous buffer or water was then added to create a solution with a surfactant concentration of 10^{-*4}g/L. This process was repeated to attain the necessary concentrations.

Once the cups have dried and cooled, 20ml of the aqueous-surfactant phase was placed into the sample cups by way of a 5ml micropipette. Said cup was then placed onto the tensiometer. A platinum ring used to conduct the surface tension measurements was flamed over a butane torch to remove any residual compounds before being placed into the tensiometer. The system was zeroed by lowering the ring into the water slowly until the ring is fully submerged – approximately two millimetres below the surface. The surface tension may then be determined by slowly raising the

ring out of the liquid phase and measuring the force the liquid phase is exerting on the ring at the point when the film breaks free from the liquid.

3.2.2 – Interfacial Tension

The protocol for measuring the interfacial tension between oil and aqueous phases resembles the procedure for obtaining the surface tension of liquids. Again, glass sample cups were cleaned using piranha solution and then allowed to dry. The appropriate aqueous phase was prepared in the same manner as done when measuring the surface tensions.

In a cleaned glass sample cup 20ml of the oil (either dodecane or olive oil) being examined was deposited using a 5ml micropipette. This sample will be used to zero the meter before each measurement using the same oil type. The sample cup is placed into the tensiometer and the platinum deNoüy ring was cleaned using acetone and flamed prior to being inserted into the tenisometer. The meter was zeroed by lowering the ring into the oil and calibrating the meter such that the force reads zero. The ring was then removed from the oil, cleaned, flamed and reinserted into the meter. 20ml of the aqueous phase was placed into a second cleaned sample cup and placed into the tensiometer and the ring was lowered into the liquid, about two millimetre deep. 10-15ml of the oil phase was slowly deposited on top of the aqueous phase such that two distinct layers were formed while being careful not to disturb the ring. During runs wherein dodecane was used as the oil phase, 10ml was used whereas in those where olive oil served as the oil phase, 15ml of the oil was layered above the aqueous phase. The system was then allowed to rest and settle for approximately 30 minutes before a reading was made. Similar to the surface tension test, the interfacial tension is read at the point when the aqueous film is torn from the ring as it rises through the oil layer.

3.3 – Droplet Size

The second series of experiments focused on tracking the deterioration of an emulsion over the course of two weeks. Akin to the surface and interfacial tension measurements, emulsion compositions were varied by changing the surfactant concentration and the type of aqueous and oil phases. In such a way, the effect of each element may be compared and correlations may be elucidated from these results. The various compositions for dodecane emulsions are presented in the table below (Table 3.2).

	Dodecane Dispersed Phase											
Continuous Phase	Water				Tris-HCl				Phosphate			
Surfactant Concentration (wt %)	0.5	1.0	2.0	4.0	0.5	1.0	2.0	4.0	0.5	1.0	2.0	4.0

Table 3.2 – Tested Dodecane Emulsion Compositions

Emulsions utilising olive oil were performed as well and the compositions are detailed in the following table (Table 3.3).

Surfactant	Concentration (wt%)	Aqueous Phase	Aqueous Amount (g)	Oil Amount (g)
Taurocholic Acid	2.0	Water	18	2
		Tris-HCl	18	2
		Phosphate	18	2
	4.0	Water	19	1
	2.0	Water	19.5	0.5
	1.0	Water	19.5	0.5
	0.5	Water	19.5	0.5
Modified DexP	6.0	Water	19	1
	4.0	Water	19	1
	2.0	Water	19	1
Dodecycltrimethylammonium	2.0	Water	19	1
Gum Arabic	2.0	Water	19	1
		Tris-HCl	19	1
		Phosphate	19	1

Table 3.3 – Tested Olive Oil Emulsion Compositions

3.3.1 – Emulsion Formation

Stable emulsions are formed via sonication of an oil-water mixture. An emulsion whose hydrophobic phase consists of dodecane is created in a 90:10 mixture of aqueous and hydrophobic phases. In a tared sample vial, taurocholic acid and the appropriate aqueous solution are added such

that the final weight of the solution is 18g. In a separate sample vial, 1.8g of dodecane is weighed out and to it, 0.2g of hexadodecane is added such that the final mass of the oil mixture is 2.0g. The oil mixture was added to the aqueous phase and a small magnetic stir bar was placed inside the sample vial. The vial was placed on a magnetic plate and stirred vigorously for a minimum of one hour. The vial was then removed from the plate and the stir bar was removed from the solution.

Preparation of emulsions utilising olive oil as the hydrophobic phase was similar to the water-dodecane emulsions but rather than consisting of a 90:10 mixture of the two phases, the ratio between the hydrophilic and hydrophobic phases were changed, ranging between 90:10 (18g:2g) and 97.5:2.5 (19.5g:0.5g).

The sample vial containing the mixed solution was placed onto the sonicator and clamped in place such that the sonicating bar is submerged half way down in the liquid. It is important to make sure that the bar touches neither the bottom of the vial nor the sides as the vibrations produced during sonication will cause the vial to shatter. An ice bath was used to keep the vial cool; the process of sonication produces an exorbitant amount of heat. The sonicator was set to run in one second intervals (one second on, one second off) at half power for 120 seconds. These emulsions may then be analysed and stored.

3.3.2 – Droplet Sizing

Emulsions were characterised via droplet size using a Malvern High Performance Particle Sizer (HPPS). A protocol was first created on the machine and set to run three iterations spaced five seconds apart from the conclusion of the previous run. For analysis purposes the aqueous phase was set to "water" and the absorbance and refraction of the dispersed phase (the oil) was set to "Dodecane," a preset option. The Dodecane option was also used when the dispersed phase was olive oil as the two have similar absorbance and refraction settings. Additionally, these settings play no major role in calculating particle sizes and polydispersity index.

Once the protocol has been created and selected, the samples may be loaded into the machine. Using a clean Pasteur pipette, several drops of an emulsion were transferred into a cuvette; both disposable polystyrene and reusable quartz crystal cuvettes were used. The cuvette was then filled with the complementary aqueous phase (i.e. water was used for the water-based emulsion) using another clean Pasteur pipette. The contents of the cuvette were diluted by removing a large portion of its contents and disposing of it and additional pipette-fulls of the aqueous phase was added. The dilution was repeated until the solution in the cuvette adopted a very pale milky colour. The sides of the cuvette were then wiped down with a Kim-Wipe prior to being placed into the machine. Settings were checked prior to starting the run. Afterwards, the plastic cuvettes were disposed of properly while the quartz crystal cuvettes were cleaned with MilliQ water and dried with acetone.

4.0 – Results and Discussion

Emulsions were characterised as per the protocols described in the previous section. The data gathered from the performed experiments were collected and plotted in order to show qualitatively the effects of varying each parameter. The data was analysed and the results are discussed below.

4.1 – Surface Tension

Surface tension measurements for the surfactant aqueous phases were collected and plotted in the graph below (Figure 4.1).

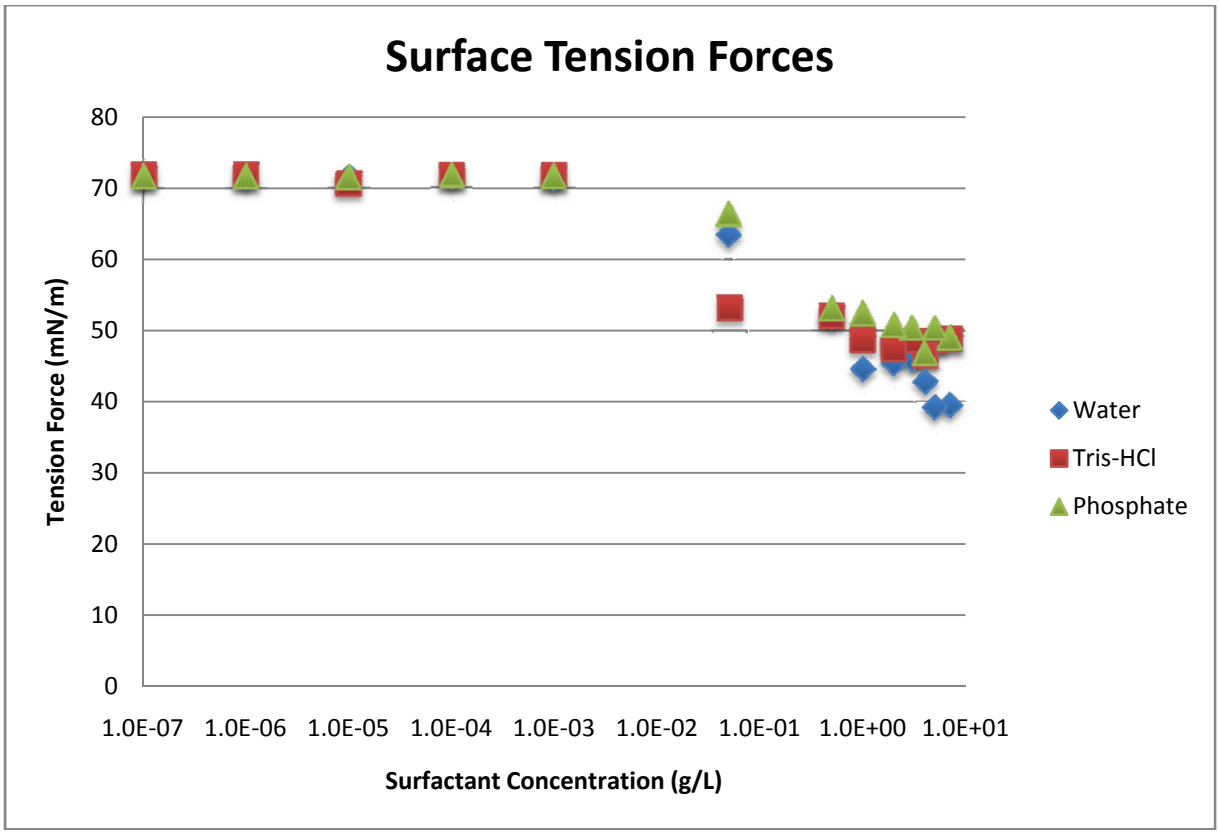

Figure 4.1 – Surface Tension Forces

As shown by the data plotted on the graph, the behaviour of the surfactant on the differing aqueous phases is fairly similar. At lower concentrations of surfactant, the surface tension of the liquids remained constant at approximately 71.7mN/m, the surface tension of water under standard

conditions (25˚C). At a surfactant concentration of 0.001 g/L, the surface tension of the aqueous phase begins to decrease at a significant rate. At around 1 g/L, the rate of decrease slows down for all solutions and the surface tension plateaus. At this point, additional surfactant has no affect on the surface tension of the solutions; the critical micelle concentration has been reached.

In solutions with higher surfactant concentrations, the phosphate buffer and tris buffer remain at approximately 50mN/m. The surface tension of water at surfactant concentrations between 1g/L and 3g/L are slightly lower, at around 45g/L. At higher concentrations of taurocholic acid, the surface tension of pure water drops again to around 40g/L. This second decrease in surface tension is unexpected, as the critical micelle concentration has already been achieved. Any additional surfactant would aggregate and should have no affect on the surface tension of said liquid. This additional drop in surface tension could be the result of contaminants in the solution or on the deNoüy ring. Because these measurements are very delicate, any contaminants will have a significant effect on the results of the measurement.

The difference in surface tensions at and above critical micelle concentration between water and the buffers may be explained by the presence of other salts in the buffer. Because taurocholic acid is introduced into solution as a sodium salt, the salts within the buffer may hinder the dissolution of the sodium taurocholate. From the data, it appears that the type of salt has no effect on surface tension, merely its existence. Regardless, all three buffers show the same critical micelle concentration and from data, it appears it is independent of buffer type so long as the solvent is water.

4.2 – Interfacial Surface Tension

Data collected of interfacial tensions between dodecane oil and water based solutions show that aqueous solution does not have a substantial effect on the surface tension and the critical micelle concentration of the mixture as shown in the plot below (Figure 4.2).

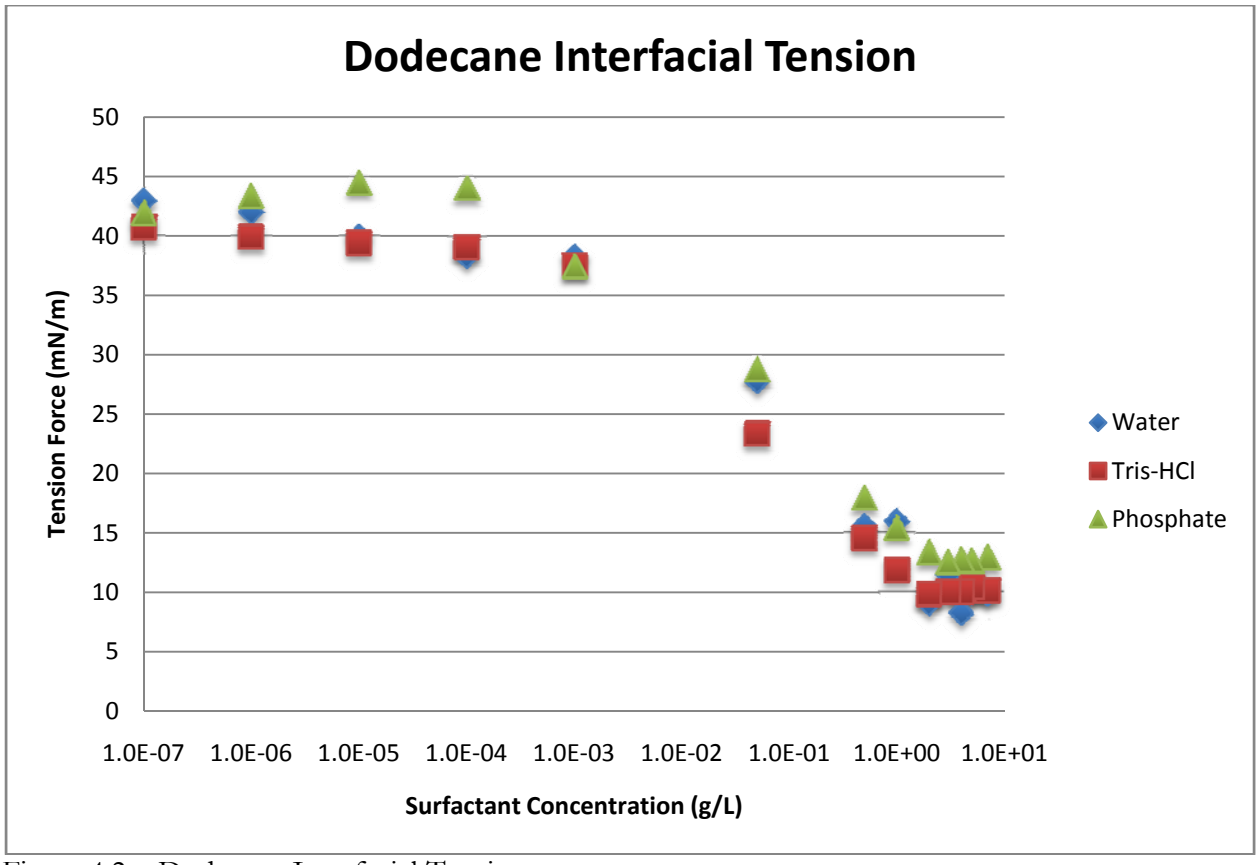

Figure 4.2 – Dodecane Interfacial Tension

The interfacial tension between surfactant-free aqueous phases and dodecane is approximately 43g/L and remains fairly stable at lower concentrations of taurocholic acid. As with the results of the surface tension measurements, interfacial tension begins to decrease at a surfactant concentration of 0.001g/L. Between 0.001g/L and 2g/L, the tension force decreases significantly to 11mN/m. The critical micelle concentration is achieved at 2g/L at which point the interfacial tension remains constant. From the experiments performed, it is apparent that the surfactant behaves similarly in both phosphate and tris buffers as well as in ultra-pure water.

Systems wherein olive oil is used as the hydrophobic phase show behaviour similar to dodecane-based systems. The graph below shows the effect of taurocholic acid on the interfacial tension of various binary systems in which olive oil serves as the oil phase (Figure 4.3).

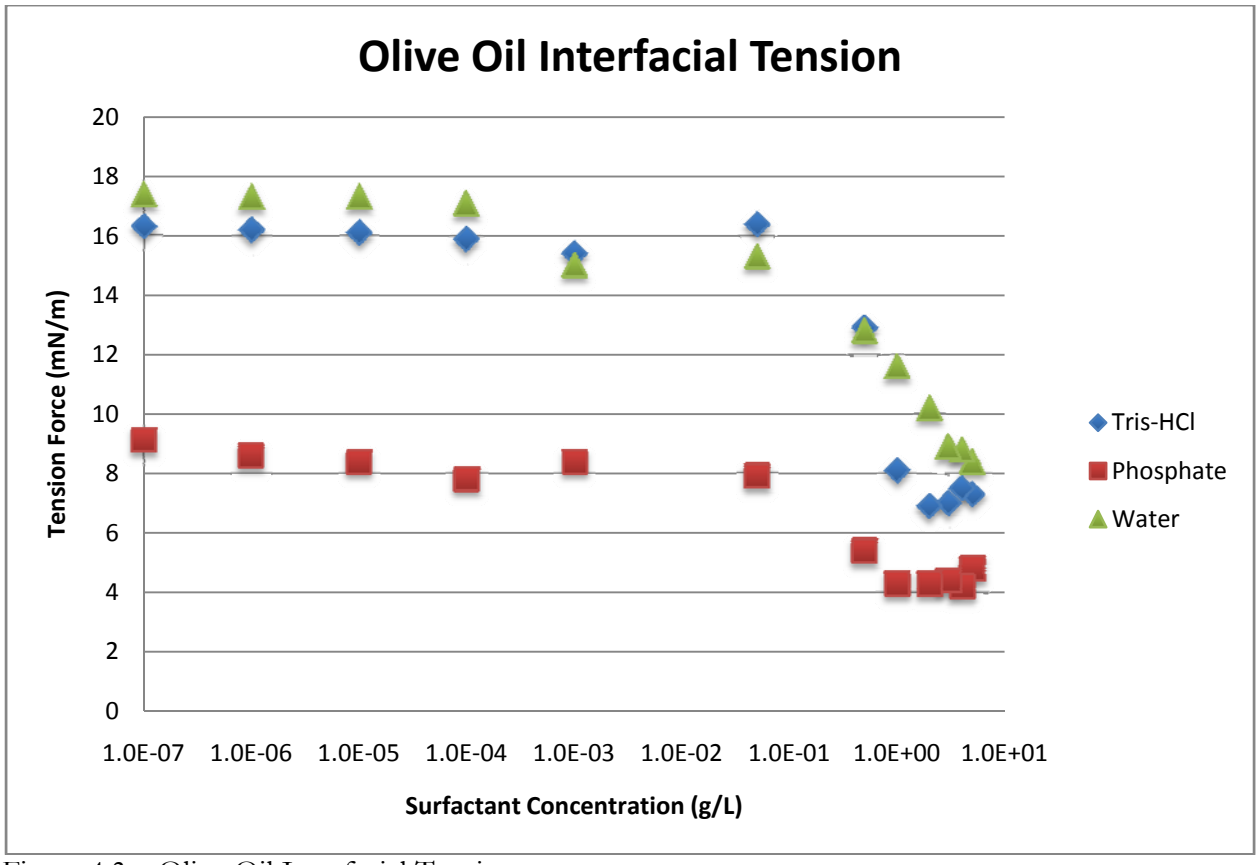

Figure 4.3 – Olive Oil Interfacial Tension

Initial surfactant-less interfacial tensions are considerably lower than those of dodecane systems. For water and tris buffer, interfacial tension at low surfactant concentrations is approximately 17mN/m while phosphate buffer yielded a tension of about 8.5mN/m. Unlike the dodecane systems, all three aqueous phases did not show similar tensions. Additionally, the relationship between aqueous phase and tension forces do not resemble those attained from surface tension measurements. This may be a result of the different interactions that occur at the interface of each binary system. The difference in interfacial tension measurements may also be a result of errors in measurements or contamination of the buffer; in all the measurements performed, changing the aqueous phase resulted in no considerable change in measured tension forces except for the results of the phosphate buffer and olive oil system. Because these measurements are very delicate, any

minute errors, be it contamination or non-uniform formation of film on the deNoüy ring would lead to deviations in the results.

Varying the concentration of surfactant present in the aqueous phases produces the similar effects in all aqueous phases, as expected. Interfacial tension forces begin to decline for all aqueous phases at 0.05g/L. Contrasting from dodecane tension tests and surface tensions results, the CMC for water, tris and phosphate buffers are not equivalent. The results show that the phosphate buffer achieves CMC at the lowest concentration, 1g/L. Tris buffer shows a CMC value of 2g/L while the interfacial tension of water does not level out until the surfactant concentration reaches 3g/L. The variance in CMC values may be the result of the presence of salt cations in solution. The salt shields the charges of surfactant head groups, allowing micelle formation at lower concentrations.

These results indicate that interfacial tension forces are substantially lower than surface tension forces at similar conditions. This may be explained by the fact that there is a greater disparity in intermolecular forces acting upon molecules located at the interface between the atmosphere and aqueous phases than between the oil and aqueous phases. Because of the distances between molecules in the gas phase, the intermolecular forces preset are very weak in comparison to those that exist between molecules in liquid phases due to the molecules' proximity to each other.

These measurements show that the addition of surfactant has a considerable effect on the tension forces between the two phases of the binary system. Concentrations of taurocholic acid above the CMC value do not reduce tension forces as the surfactant begins to aggregate. This aggregation is important in forming micelles. Coupled with the reduced interfacial tension between phases, this phenomenon plays an important role in the stabilisation of emulsions.

4.3 – Droplet Size Measurements

4.3.1 – Dodecane Emulsions

Droplet sizes of emulsions created as per the protocol described were measured and followed over a course of fourteen days. Continuous phases with surfactant concentrations ranging between 0.5 to 4.0 weight per cent were examined; at these concentrations, the concentration of taurocholic acid exceeds the critical micelle concentration of the surfactant.

Results of the droplet sizing were plotted to compare how the choice of aqueous buffer affected the degradation of the emulsion over the length of the experiment. The compositions of continuous phases examined were 0.5, 1.0, 2.0 and 4.0 weight per cent taurocholic acid. Error bars present on the graphs are derived from the polydispersity index reported by the particle sizer, which is the range of droplet sizes that were measured by the machine. The z-average size calculated by the particle sizer as the mean particle diameter as determined by the intensity of refracted light.

The graph below represents the breakdown of emulsions with 0.5 weight per cent taurocholic acid (Figure 4.4).

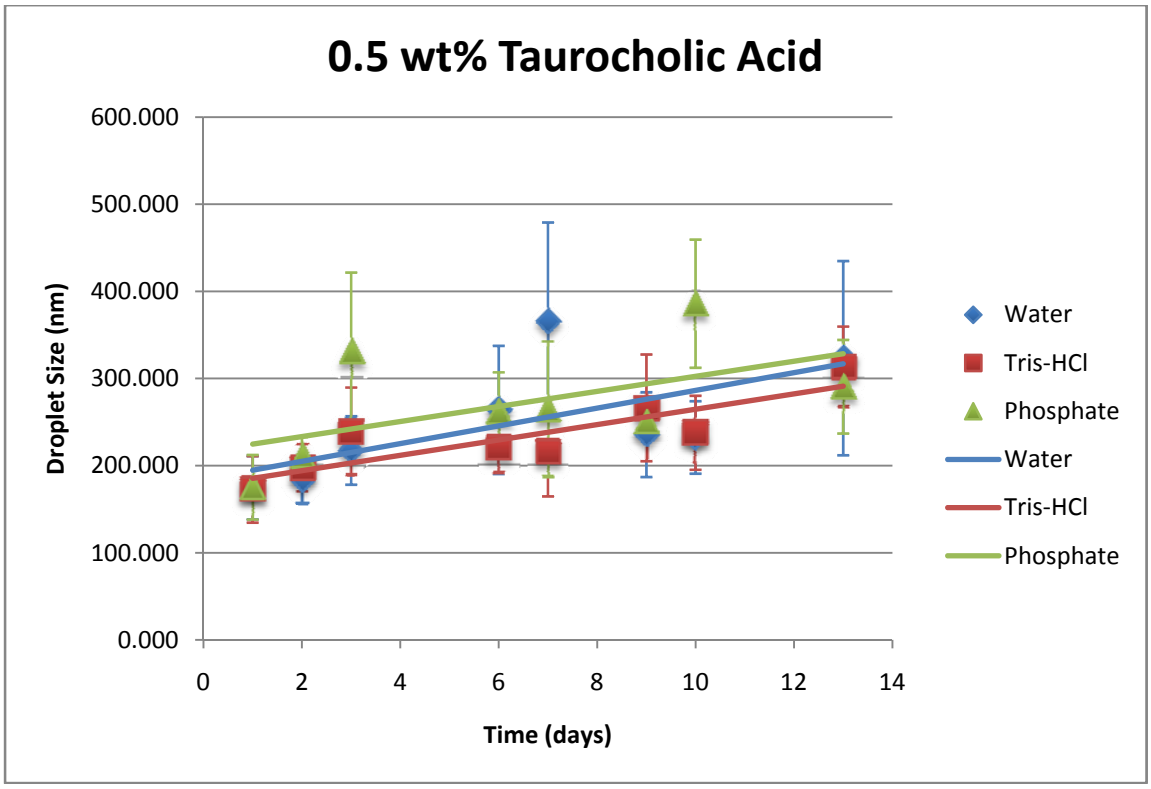

Figure 4.4 – 0.5 Weight Per Cent Taurocholic Acid

Varying the continuous phase appears to have no significant impact in the breakdown of the emulsion over the course of two weeks. A line was fitted to the data. There are present a few outliers which have comparably higher polydispersity indices, resulting in a larger error the particle diameter measurement. Because the high performance particle sizer calculates average particle diameters by measuring light refraction, stray particles, dust or air bubbles may skew the results.

Emulsions consisting of 1.0, 2.0 and 4.0 weight per cent surfactant show the same behaviour as those with 0.5 per cent surfactant (Figures 4.5-7).

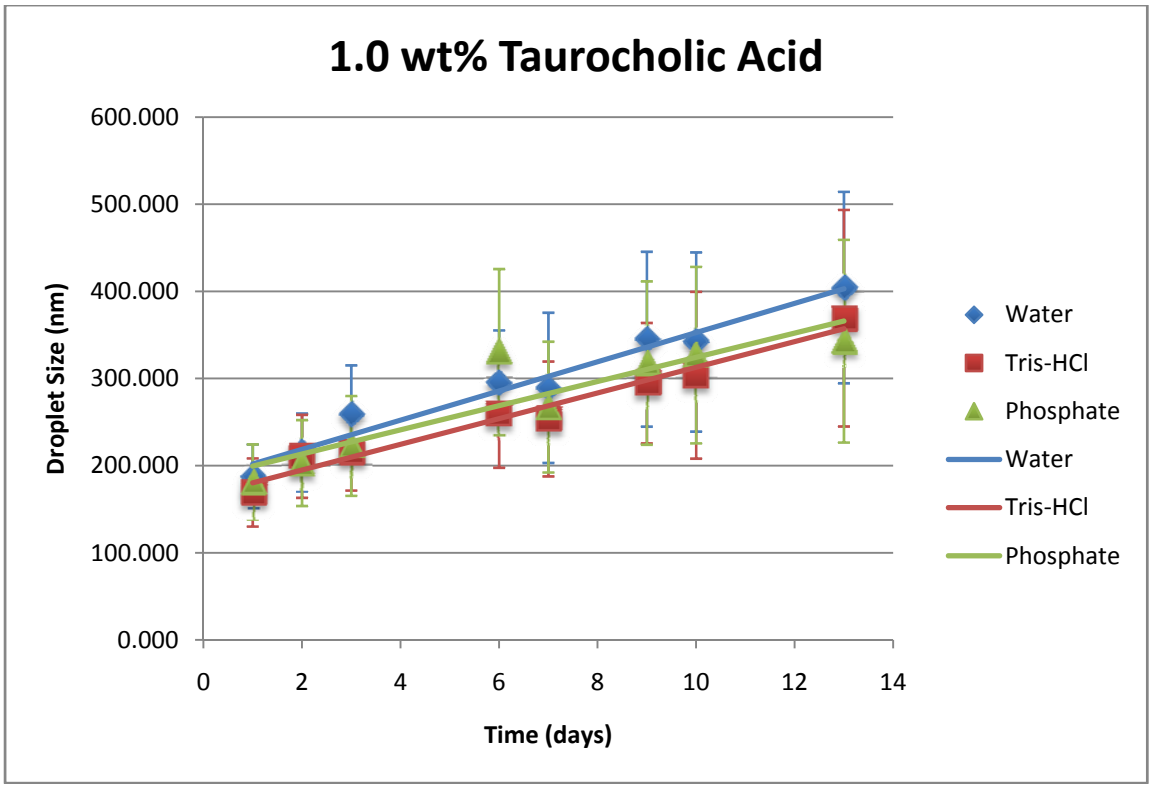

Figure 4.5 – 1.0 Weight Per Cent Taurocholic Acid

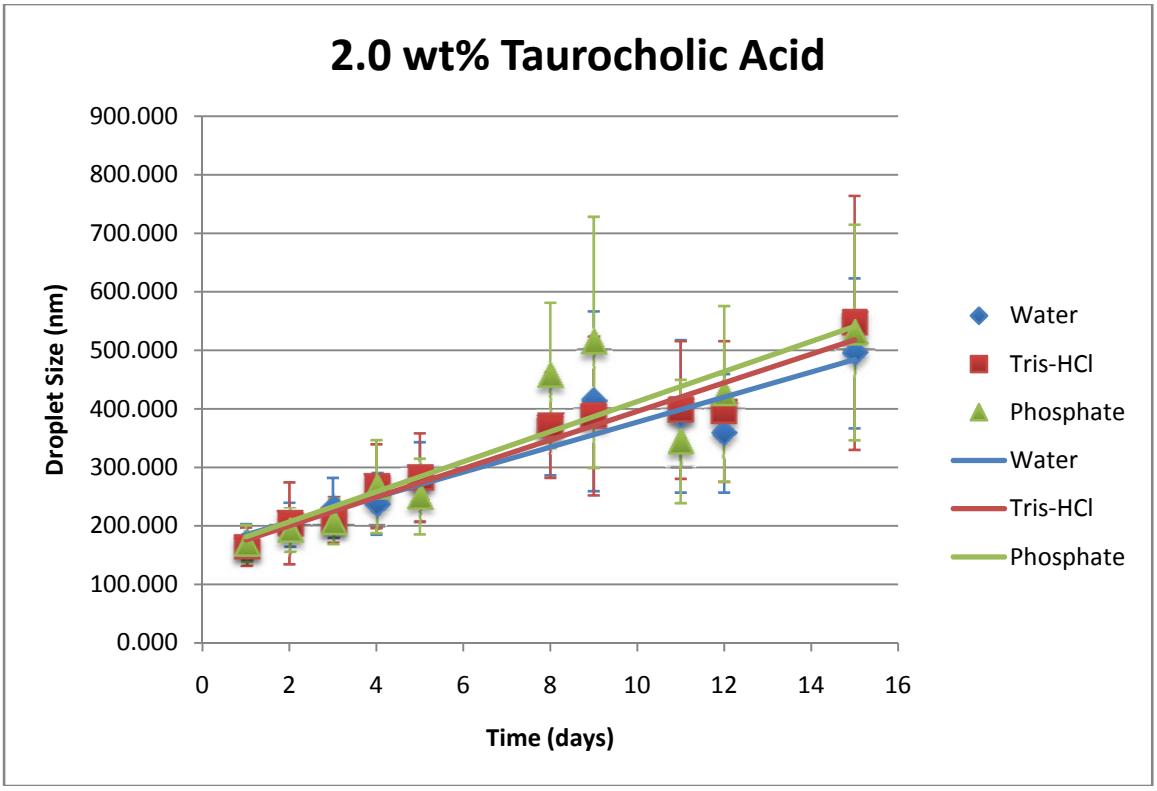

Figure 4.6 – 2.0 Weight Per Cent Taurocholic Acid

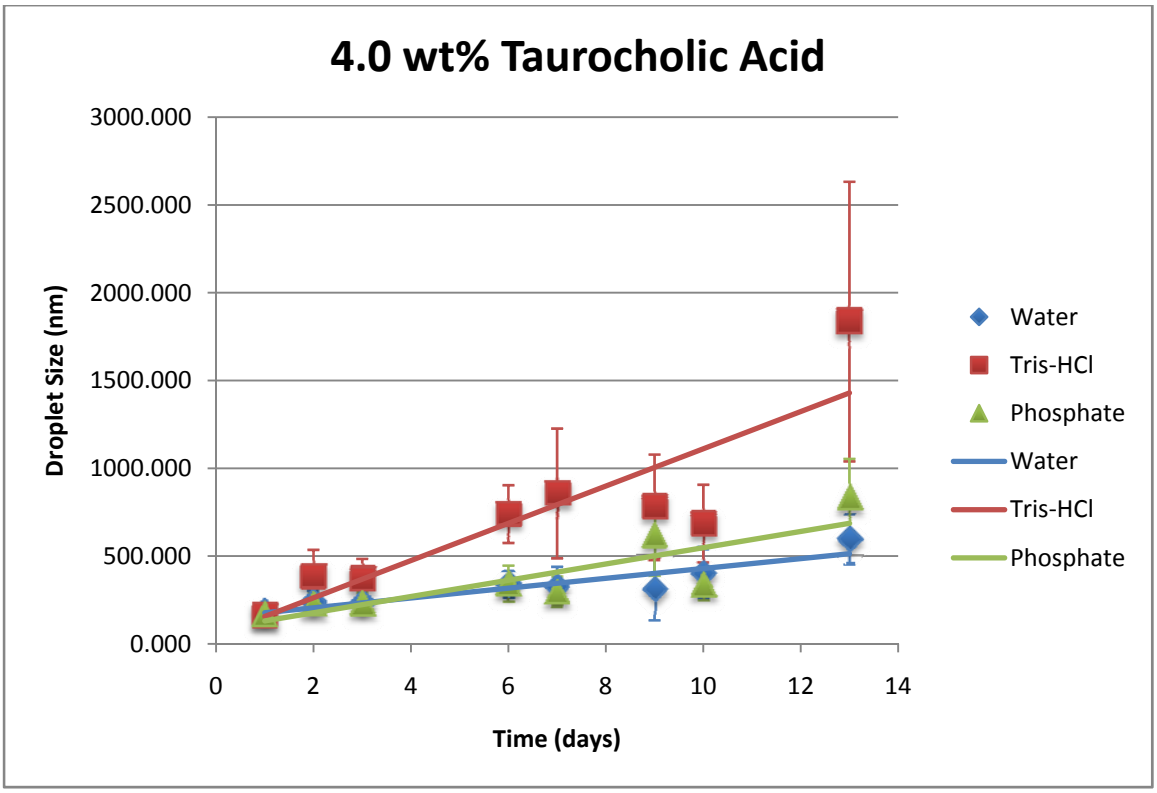

Figure 4.7 – 4.0 Weight Per Cent Taurocholic Acid

This further demonstrates that the aqueous phases examined have no distinct effect on the particle size of the emulsions. Earlier droplet size measurements are more precise; the polydispersity index is much lower than towards the end of the measurements. This is due to the destabilisation of the emulsion; as the emulsion breaks down, droplet sizes do not change uniformly and it becomes more difficult to achieve measurements with low polydispersity. At day 13, the tris buffer emulsion with 4.0 per cent taurocholic acid shows a droplet size of 1836nm but the large polydispersity index reported results in an error of almost ±800nm.

The results of the emulsion droplet size tracking further confirm that aqueous phase type has very little effect on the behaviour of emulsions as both buffers utilise water as the solvating agent. While these emulsions do not appear to vary in relation to the type of aqueous phase used, the concentration of surfactant does affect the destabilisation of the emulsion.

As the graphs presented below show, increasing the concentration of surfactant decreases the stability of the emulsion (Figures 4.8-10).

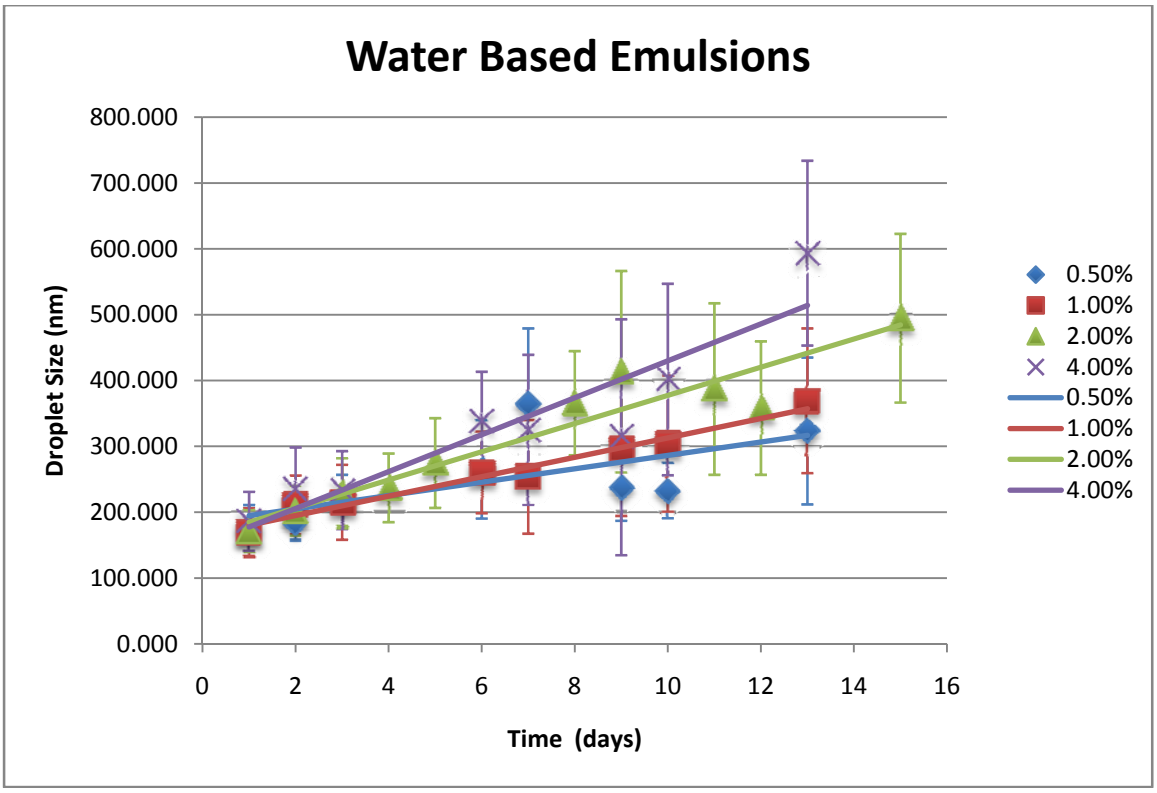

Figure 4.8 – Water Based Emulsions

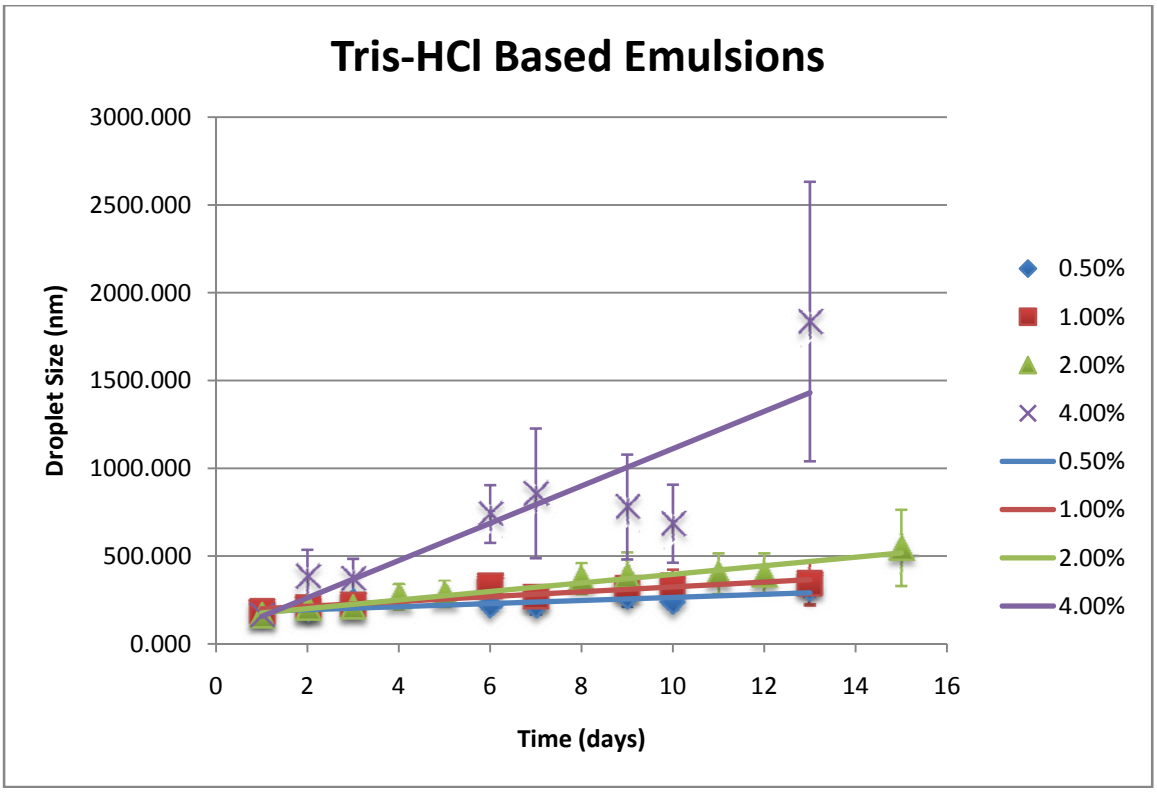

Figure 4.9 – Tris-HCl Based Emulsions

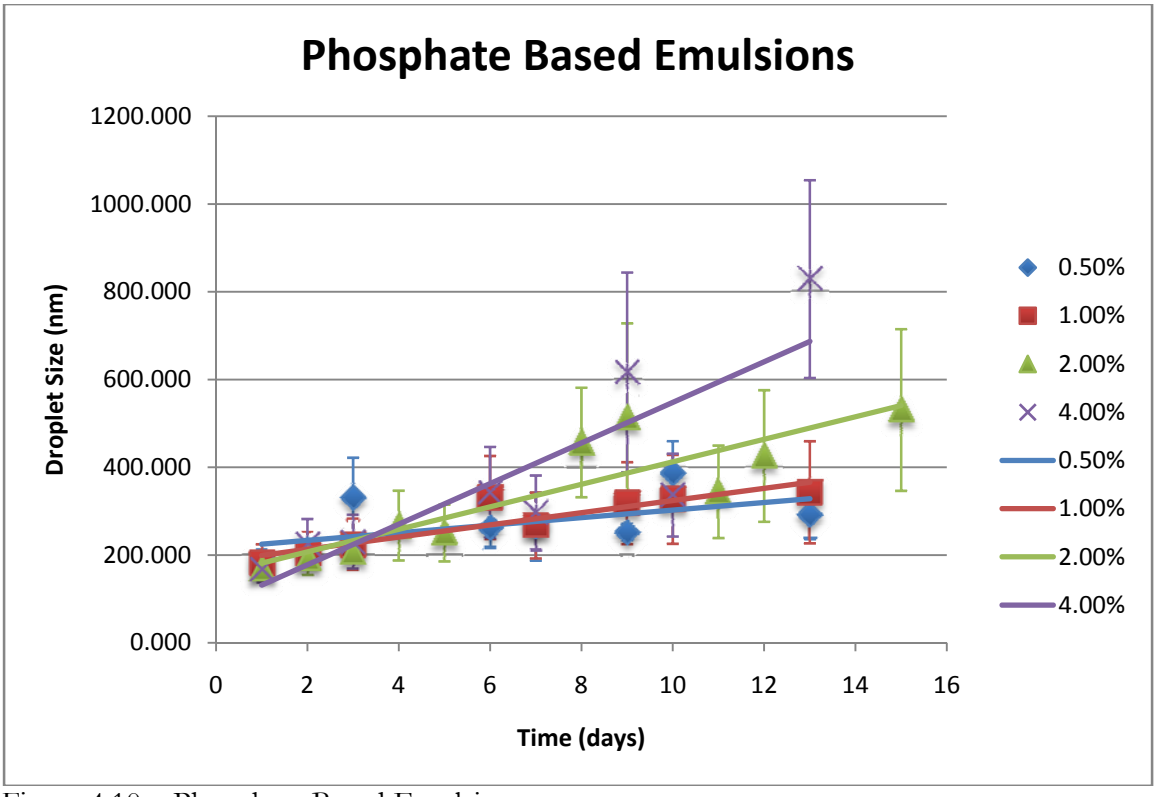

Figure 4.10 – Phosphate Based Emulsions

In all three cases, emulsion droplet sizes are initially equal but as time increases the emulsions begin to destabilise and those containing higher amounts of surfactant show greater destabilisation as indicated by larger droplet sizes. The behaviour observed is contrary to conventional thought that the addition of more surfactant should further stabilise an emulsion. The behaviour observed may be the result of interactions between nonpolar oils such as dodecane and anionic surfactants including taurocholic acid. A study on emulsion stability performed by Osipow, Birsan and Snell (1957) reported comparable results. The emulsions studied in the paper used n-tetradecane as the hydrophobic phase in a continuous phase using sodium lauryl sulphate and sodium cetyl sulphate surfactants. n-tetradecane and dodecane are both straight chain hydrocarbons and share many of the same chemical properties. Most importantly, the two alkanes are both non-polar oils. Additionally, the surfactants used by Osipow, Birsan and Snell share similar functional groups with the taurocholic acid used as a surfactant in the emulsions discussed in this report. Taurocholic acid, sodium lauryl sulphate and sodium cetyl sulphate are all anionic surfactants containing a sulphate group. Their study suggests that greatest emulsions stability is greatest at CMC concentrations and decreases as surfactant concentration is increased, as demonstrated by the data presented.

4.3.2 – Olive Oil Emulsions

Creating a stable olive oil emulsion proved significantly more difficult than in creating an emulsion with dodecane. Initial droplet size measurements performed on the three aqueous phases following the original protocol revealed that the emulsions were very unstable (Table 4.1).

Olive Oil Emulsions 2.0 wt% Taurocholic Acid		
Continuous Phase	PDI	Z Average Diameter (nm)
Water	0.144	2549
	0.284	4167
	0.459	6104
Tris-HCl Buffer	1	7235
	1	13100
	1	16200
Phosphate Buffer	1	7843
	1	6705
	0.934	8889

Table 4.1 – 2.0 wt% Olive Oil Emulsion
18g Continuous, 2g Oil Phase

Droplet sizes for olive oil emulsions are orders of magnitude larger than those observed in the olive oil emulsions. These larger droplets lend to the greater instability of the olive oil emulsions, which can be seen by the large fluctuations in droplet sizes between consecutive measurements. The droplet sizes for 2.0 weight per cent taurocholic acid in water shows this clearly; droplet sizes over three consecutive measurements show an increase from 2549nm to 6104nm over the short amount of time required for the measurements to be taken. In contrast, dodecane emulsions in water do not show a three-fold increase in droplet size over the course of fourteen days. These inconsistencies are also present with both buffer solutions. In addition, the polydispersity index calculated were extremely high; most results have a PDI of over 0.5, many reported a PDI value of 1.0 meaning that the droplets sizes measured by the apparatus are highly inconsistent. Higher concentrations of taurocholic acid yielded similar results. The polydispersity index value of these measurements also correlates to a broad distribution of droplet sizes.

Reducing the oil to water ratio of these emulsions does not reduce the range of droplet sizes detected by the particle sizer; polydispersity indices of these measurements still remain high. Reducing the amount of oil in the emulsion does have an effect on the consistency of the size measurements. In an attempt to elucidate a relationship between surfactant concentration and

emulsion stability, emulsions composed of 19.5g aqueous phase and 0.5g oil phase was used. While the polydispersity index of these measurements still correlate to a broad distribution, the initial droplet sizes of these emulsions do show a trend (Table 4.2, 4.3).

Olive Oil Emulsions 4.0 wt% Taurocholic Acid ,19g Water, 1.0g Oil		
Continuous Phase	PDI	Z Average Diameter (nm)
Water	1	3754
	1	1900
	1	2460

Table 4.2 - 4.0 wt% Olive Oil Emulsion
19g Continuous, 1g Oil Phase

Olive Oil Emulsions 19.5g Continuous Phase, 0.5% Oil Phase		
Surfactant Concentration	PDI	Z Average Diameter (nm)
2.0 %	0.511	4974
	1	3895
	1	2820
1.0 %	1	4984
	1	2946
	1	4828
0.5 %	0.786	9155
	0.401	19000
	1	3946

Table 4.3 – Olive Oil Emulsions
19.5g Continuous, 0.5g Oil Phase

The data shows that the droplet sizes of the emulsions are proportional to the concentration of surfactant in the emulsion. While these emulsions were not followed over time, emulsion stability may be correlated to the initial emulsion droplet size. Because larger droplets tend to cream and coalesce, larger droplets lead to greater instability of the emulsion. At lower surfactant concentrations (0.5 weight per cent), average droplet size of the emulsion is about 8317nm. Increasing surfactant concentration to 2.0 weight percent reduced the initial emulsion droplet size to 3896nm.

Compared to dodecane-based emulsions, the olive oil emulsions show greater instability. This may be due to the difference in the nature of the oil. While dodecane is a straight chain alkane, olive oil consists of a mixture of fatty acids, which are polar lipids with long hydrocarbon tails. Polarity is imparted onto the oil by the carboxylic head group of the fatty acids. Emulsion instability and broad distribution may be caused by the electronegativity of the carboxylic group and the anionic qualities of the surfactant.

To test whether or not this may be the cause, different surfactants were tested. A polymeric surfactant based on dextran was used to determine the effect a non-polar surfactant on emulsion stability and dodecyltrimethylammonium bromide used to determine cationic surfactant effects on emulsion droplet size.

Olive Oil Emulsions Various Surfactants (2.0%), 19g Continuous, 1g Oil Phase		
Surfactant Type	PDI	Z Average Diameter (nm)
Modified DexP	1	1165
	1	1145
	1	1142
Dodecyltrimethylammonium	1	1694
	1	2248
	1	2627

Table 4.4 – Olive Oil Emulsions, Various Surfactants
19g Continuous, 1g Oil Phase

The data above shows that the use of non-polar and cationic surfactants reduce the size of emulsion droplets in comparison to the taurocholic acid but does not differ significantly between each other. While the measured droplet size is smaller, the distribution of droplet sizes remains quite large as indicated by a polydispersity index of 1.

In comparing the results from the dodecane and olive oil emulsions, it is clear that olive oil emulsions prove considerably more difficult to produce. While taurocholic acid may play a small role in the instability of the emulsion, it appears that the oil type – polar or non-polar – is the primary factor in emulsion stability. This is further substantiated by the use of Gum Arabica, a

viscosity-increasing agent, instead of surfactants as a stabilising compound. Results show that the Gum Arabica produces similarly sized droplets as well as a polydispersity index of 1 resulting from highly scattered particle diameters.

5.0 – Conclusions

An analysis of the results obtained from the tension force and droplet size measurements show that the decomposition of emulsions is primarily affected by the concentration of taurocholic acid present in the system and less by the type of continuous phase. The continuous (aqueous) phase had very little effect on the behaviour of the binary system. While the salts present in the buffers may have resulted in slight deviations in both interfacial tension and surface tension forces, the differences were very little and did not appear to adhere to any noticeable model. Because the buffers consist of primarily water, it should be expected that the results from these buffers would be very close to those of the pure water results. Had another hydrophilic liquid been used as the aqueous phase, such as alcohols, then the results may have differed. Critical micelle concentrations were determined for all binary systems and revealed that taurocholic acid begins to aggregate at the interface at a concentration of about 1g/L to 2g/L. The droplet size analyses performed also confirm this conclusion. While taurocholic acid concentrations in the continuous phases were held constant and the phases themselves varied, very little differences were detected in the droplet sizes of the emulsions. The sizes begin to differentiate towards the end of the fourteen-day observation period, but these differences may be attributed to the broader polydispersity as a result of emulsion breakdown.

The concentration of taurocholic acid has a significant impact on emulsion stability, though its effect was unexpected. Contrary to conventional wisdom wherein an increase in surfactant concentration would result in more stable emulsions, increasing taurocholic concentration in the emulsion led to a decline in emulsion stability. This phenomenon may be explained by interactions between the nonpolar dodecane oil and the anionic taurocholic acid. Similar results were obtained from experiments using comparable alkanes and anionic surfactants. Over the course of fourteen

days, emulsions did destabilise as indicated by the increase in both average droplet sizes and the broadening of the droplet sizes measured as a result of various coarsening phenomena, primarily coalescence and Ostwald ripening.

In studying the catalytic activity of lipase, olive oil was used to simulate the fatty acids that might be present for degradation *in vivo*. While interfacial tension force measurements do show significantly lower tension forces between the oil and aqueous phases, it was concluded that olive oil based emulsions were considerably less stable than dodecane emulsions. No stable emulsions were created using ultra-sonication; average droplet sizes were magnitudes larger than those of the dodecane emulsions. Additionally, the range of droplet diameters was very broad as indicated by high polydispersity indices. By examining olive oil emulsions stabilised with non-ionic and cationic surfactants, it was concluded that the nature of the surfactant does affect the droplet diameters of the emulsion. Because fatty acids have an electronegative carboxyl group, by using a non-ionic or cationic surfactant, the droplet diameters were reduced. Regardless, emulsion stability remained low as the average droplet size was calculated from a very broad range of droplet diameters indicated by high polydispersity indices.

While *in vivo* emulsion stability may not play an important role, as the process is transient, a stable and consistent emulsion is vital in studying the enzymatic activity of lipase and its relationship with emulsion droplet diameters in a laboratory setting. Additionally, because of the applications of taurocholic acid as a biological surfactant for commercial use, it is important create stable emulsions that will not decompose on the shelf. Future work on this topic should focus on elucidating the effect of the oil phase on emulsion stability in order to determine where the difficulty in creating an olive oil emulsion arises. One possibility posed is the polar nature of the oil and its interactions with the surfactant. Further experiments must be performed to confirm this hypothesis. Establishing a stable emulsion will prove beneficial in studying the activity of lipase and relating said results with

the physical characteristics of emulsions. In addition, taurocholic acid may be used as a surfactant in many applications, including in food processing. By developing a proper method of establishing emulsions with food grade oils such as olive oil that are stable over the course of weeks or even months, taurocholic acid may be used commercially as a biological surfactant.

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Appendix A –
Surface Tension Force Data

Surface Tension Force Data				
		Continuous Phase		
Taurocholic Acid Concentration (g/L)		Water	Tris-HCl	Phosphate
	7	39.5	48.9	49.1
	5	39.2	48.6	50.4
	4	42.8	46.5	46.9
	3	45.9	48.6	50.4
	2	45.4	47.4	50.8
	1	44.6	48.7	52.5
	0.5	51.9	52.1	53.2
	0.05	63.4	53.2	66.4
	1.00E-03	71.4	71.7	71.7
	1.00E-04	71.5	71.7	71.8
	1.00E-05	71.6	70.6	71.6
	1.00E-06	71.6	71.9	71.7
	1.00E-07	71.7	71.9	71.7
	0	71.5	72.5	72.1

Appendix B –
Interfacial Tension Force Data – Dodecane

Interfacial Tension – Dodecane Oil Phase				
		Continuous Phase		
Taurocholic Acid Concentration (g/L)		Water	Tris-HCl	Phosphate
	7	9.8	10.1	12.9
	5	9.9	10.4	12.6
	4	8.2	10	12.7
	3	11.4	10	12.5
	2	9	9.8	13.3
	1	15.9	11.8	15.4
	0.5	15.5	14.5	17.9
	0.05	27.7	23.3	28.7
	1.00E-03	38.2	37.5	37.4
	1.00E-04	38.3	39.1	44
	1.00E-05	39.9	39.4	44.5
	1.00E-06	42	39.9	43.4
	1.00E-07	42.9	40.7	41.9
	0	42.4	42.7	43.1

Appendix C –
Interfacial Tension Force Data – Olive Oil

Interfacial Tension – Olive Oil Oil Phase				
		Continuous Phase		
Taurocholic Acid Concentration (g/L)		Water	Tris Buffer	Phosphate
	5	8.4	7.3	4.8
	4	8.8	7.5	4.2
	3	8.9	7	4.4
	2	10.2	6.9	4.3
	1	11.6	8.1	4.3
	0.5	12.8	12.9	5.4
	0.05	15.3	16.4	7.9
	1.00E-03	15	15.4	8.4
	1.00E-04	17.1	15.9	7.8
	1.00E-05	17.3	16.1	8.4
	1.00E-06	17.3	16.2	8.6
	1.00E-07	17.4	16.3	9.1

Appendix D –
Emulsion Droplet Size Data
Water/Dodecane

Water/Dodecane Emulsion – 0.5% Taurocholic Acid					
Day	Trial	PDI	PDIW	Mean Count	Z
1	1	0.149	69.29	274	179.4
1	2	0.172	71.17	263	171.4
1	3	0.200	77.38	264	173.1
2	1	0.089	54.49	430	182.8
2	2	0.015	21.98	423	180.7
2	3	0.002	82.88	437	185.3
3	1	0.284	116.50	378	218.5
3	2	0.095	65.36	350	212.4
3	3	0.060	54.48	378	221.5
6	1	0.267	125.80	158	243.3
6	2	0.386	187.10	204	301.2
6	3	0.272	128.90	153	247.4
7	1	0.354	186.00	134	312.4
7	2	0.411	248.70	227	387.8
7	3	0.412	250.30	280	394.9
9	1	0.144	88.90	288	234.2
9	2	0.178	100.00	288	236.8
9	3	0.192	103.30	281	236.0
10	1	0.124	81.17	170	230.3
10	2	0.104	75.62	166	234.5
10	3	0.159	92.59	170	232.4
13	1	0.462	236.40	158	347.6
13	2	0.701	286.60	128	342.3
13	3	0.271	146.10	119	280.4

Water/Dodecane Emulsion – 1.0% Taurocholic Acid					
Day	Trial	PDI	PDIW	Mean Count	Z
1	1	0.157	74.57	316	188.0
1	2	0.160	74.80	324	187.3
1	3	0.148	71.99	325	186.9
2	1	0.209	96.48	263	211.1
2	2	0.168	88.18	268	215.4
2	3	0.148	84.08	270	218.8
3	1	0.230	115.10	145	240.0
3	2	0.221	135.50	177	288.3
3	3	0.137	91.25	146	246.3
6	1	0.155	115.30	226	292.9
6	2	0.137	100.60	219	285.0
6	3	0.239	149.30	229	305.4
7	1	0.354	170.20	142	286.1
7	2	0.363	174.10	142	289.0
7	3	0.349	173.10	142	293.0
9	1	0.404	224.18	314	353.8
9	2	0.299	185.70	298	339.6
9	3	0.315	192.50	294	342.2
10	1	0.379	212.90	337	345.7
10	2	0.350	201.20	331	340.2
10	3	0.357	203.20	330	340.1
13	1	0.193	173.00	156	393.7
13	2	0.338	241.90	175	416.0
13	3	0.366	244.40	160	403.8

Water/Dodecane Emulsion – 2.0% Taurocholic Acid					
Day	Trial	PDI	PDIW	Mean Count	Z
1	1	0.140	63.99	298	171.0
1	2	0.156	67.37	295	170.9
1	3	0.098	63.11	293	169.8
2	1	0.203	91.20	279	202.5
2	2	0.159	79.47	272	199.5
2	3	0.088	59.33	272	201.3
3	1	0.197	103.80	159	233.9
3	2	0.214	103.90	160	224.7
3	3	0.203	104.20	159	231.5
4	1	0.195	105.00	347	237.8
4	2	0.199	105.80	329	237.5
4	3	0.188	102.10	337	235.6
5	1	0.230	129.80	231	270.7
5	2	0.255	134.50	226	266.4
5	3	0.254	144.80	242	287.2
8	1	0.344	192.50	159	328.2
8	2	0.269	196.70	196	379.6
8	3	0.048	85.60	201	388.9
9	1	0.509	292.50	185	410.2
9	2	0.518	283.00	189	393.3
9	3	0.632	345.90	197	435.1
11	1	0.354	231.50	224	288.8
11	2	0.409	274.00	250	438.6
11	3	0.406	276.00	249	433.9
12	1	0.369	221.70	432	365.2
12	2	0.299	193.20	417	353.3
12	3	0.294	192.90	419	356.0
15	1	0.483	354.50	182	510.2
15	2	0.546	359.20	182	486.1
15	3	0.013	55.24	178	488.0

Water/Dodecane Emulsion – 4.0% Taurocholic Acid					
Day	Trial	PDI	PDIW	Mean Count	Z
1	1	0.256	93.31	194	184.2
1	2	0.221	86.90	197	184.6
1	3	0.230	90.51	200	188.8
2	1	0.260	117.80	206	231.0
2	2	0.257	110.00	191	217.2
2	3	0.364	154.50	233	256.0
3	1	0.264	119.10	211	231.8
3	2	0.268	122.90	212	237.3
3	3	0.240	113.40	211	231.5
6	1	0.387	198.20	184	318.8
6	2	0.226	145.10	176	365.1
6	3	0.118	112.70	190	328.0
7	1	0.501	228.90	159	323.4
7	2	0.483	225.60	168	324.7
7	3	0.488	228.90	162	327.5
9	1	0.349	184.70	351	312.7
9	2	0.330	182.00	362	316.7
9	3	0.300	171.00	353	312.3
10	1	0.542	297.60	229	404.4
10	2	0.507	279.10	230	392.1
10	3	0.526	296.10	243	408.2
13	1	0.243	291.90	477	592.5
13	2	0.315	382.50	545	681.7
13	3	0.110	167.90	427	506.5

Appendix E –
Emulsion Droplet Size Data
Tris-HCl/Dodecane

Tris-HCl/Dodecane Emulsion – 0.5% Taurocholic Acid					
Day	Trial	PDI	PDIW	Mean Count	Z
1	1	0.171	70.85	217	171.4
1	2	0.204	77.76	219	172.3
1	3	0.213	80.52	219	174.5
2	1	0.110	66.21	205	199.7
2	2	0.046	41.50	203	193.8
2	3	0.070	51.97	194	196.6
3	1	0.240	114.40	325	233.4
3	2	0.231	113.60	328	236.4
3	3	0.087	73.24	347	248.8
6	1	0.107	71.17	224	217.3
6	2	0.590	55.35	226	227.1
6	3	0.024	39.98	208	214.8
7	1	0.218	101.40	215	217.1
7	2	0.219	99.90	210	213.3
7	3	0.224	102.10	212	215.6
9	1	0.138	90.74	258	244.7
9	2	0.226	129.00	274	270.8
9	3	0.271	148.00	285	283.6
10	1	0.089	70.86	419	236.9
10	2	0.139	88.25	406	236.3
10	3	0.159	95.84	408	240.1
13	1	0.299	172.40	376	315.4
13	2	0.027	53.19	402	325.4
13	3	0.030	51.87	367	299.5

Tris-HCl/Dodecane Emulsion – 1.0% Taurocholic					
Day	Trial	PDI	PDIW	Mean Count	Z
1	1	0.213	79.06	294	171.2
1	2	0.224	79.16	291	167.4
1	3	0.204	76.55	298	169.0
2	1	0.313	118.70	205	212.1
2	2	0.184	88.80	198	207.1
2	3	0.136	78.51	201	213.1
3	1	0.202	96.72	308	215.2
3	2	0.146	81.93	304	214.4
3	3	0.150	83.41	296	215.6
6	1	0.209	117.20	423	256.5
6	2	0.120	90.67	423	261.7
6	3	0.390	162.20	406	259.7
7	1	0.293	137.70	418	254.5
7	2	0.275	131.90	424	251.6
7	3	0.245	126.00	418	254.8
9	1	0.228	140.00	434	293.0
9	2	0.209	133.60	432	292.5
9	3	0.225	141.50	431	298.6
10	1	0.406	196.00	313	307.5
10	2	0.416	195.50	298	303.0
10	3	0.369	182.90	294	301.0
13	1	0.428	245.10	212	374.7
13	2	0.407	223.60	196	350.4
13	3	0.524	277.00	214	382.8

Tris-HCl/Dodecane Emulsion – 2.0% Taurocholic					
Day	Trial	PDI	PDIW	Mean Count	Z
1	1	0.160	64.52	462	161.3
1	2	0.185	69.38	463	161.2
1	3	0.152	65.30	466	167.4
2	1	0.295	110.80	248	203.8
2	2	0.049	146.15	254	207.9
2	3	0.098	163.04	241	201.2
3	1	0.132	77.08	167	212.3
3	2	0.153	81.37	164	207.9
3	3	0.156	80.62	158	204.0
4	1	0.328	153.50	375	268.2
4	2	0.261	134.30	378	262.8
4	3	0.271	141.80	374	272.1
5	1	0.198	123.70	250	277.6
5	2	0.310	156.90	248	281.8
5	3	0.356	172.30	263	288.6
8	1	0.266	190.30	351	369.0
8	2	0.318	213.00	384	377.8
8	3	0.130	132.50	365	367.1
9	1	0.502	275.80	259	389.2
9	2	0.462	261.00	254	383.7
9	3	0.492	271.80	251	387.5
11	1	0.312	225.60	275	403.7
11	2	0.376	244.20	268	398.1
11	3	0.362	235.90	270	392.3
12	1	0.349	231.20	155	391.6
12	2	0.400	250.00	146	395.3
12	3	0.363	240.50	156	399.0
15	1	0.712	464.70	292	550.8
15	2	0.767	511.30	301	583.7
15	3	0.416	326.60	274	506.0

Tris-HCl/Dodecane Emulsion – 4.0% Taurocholic					
Day	Trial	PDI	PDIW	Mean Count	Z
1	1	0.160	64.86	326	162.0
1	2	0.135	60.27	330	164.1
1	3	0.083	47.26	327	163.9
2	1	0.553	283.40	320	381.1
2	2	0.591	275.30	306	358.0
2	3	0.772	360.20	363	410.0
3	1	0.381	221.00	443	358.1
3	2	0.381	231.00	443	374.2
3	3	0.381	233.80	434	378.7
6	1	0.217	321.50	192	689.5
6	2	0.381	317.00	196	837.7
6	3	0.253	347.90	209	692.3
7	1	0.717	679.20	302	802.3
7	2	0.764	747.90	312	855.5
7	3	0.748	790.80	320	914.2
9	1	0.651	675.80	248	837.4
9	2	0.526	533.20	233	735.3
9	3	0.582	584.60	231	766.2
10	1	0.453	471.10	278	700.7
10	2	0.460	461.20	272	680.0
10	3	0.357	402.50	267	673.2
13	1	0.470	1598.00	144	2330.0
13	2	1.000	1794.00	126	1794.0
13	3	1.000	1385.00	120	1385.0

Appendix F –
Emulsion Droplet Size Data
Phosphate/Dodecane

Phosphate/Dodecane – 0.5% Taurocholic Acid					
Day	Trial	PDI	PDIW	Mean Count	Z
1	1	0.193	77.68	245	176.7
1	2	0.153	68.57	247	175.4
1	3	0.192	76.16	239	173.7
2	1	0.058	50.57	240	210.0
2	2	0.012	22.35	227	201.1
2	3	0.074	60.06	244	220.9
3	1	0.510	245.80	168	344.2
3	2	0.247	166.20	163	334.7
3	3	0.195	137.40	145	311.3
6	1	0.322	150.40	152	265.1
6	2	0.080	72.17	146	254.7
6	3	0.038	51.64	147	264.5
7	1	0.305	142.90	163	258.8
7	2	0.343	153.10	162	261.5
7	3	0.377	168.10	176	273.8
9	1	0.053	56.54	141	245.0
9	2	0.031	44.43	185	254.9
9	3	0.004	15.98	183	252.1
10	1	0.002	15.85	166	354.4
10	2	0.287	208.50	176	398.1
10	3	0.287	217.10	268	405.3
13	1	0.190	120.10	110	275.4
13	2	0.103	95.38	121	297.2
13	3	0.127	106.90	111	299.3

Phosphate/Dodecane – 1.0% Taurocholic Acid					
Day	Trial	PDI	PDIW	Mean Count	Z
1	1	0.223	85.10	206	180.0
1	2	0.239	89.83	213	183.8
1	3	0.214	83.28	205	180.2
2	1	0.234	98.270	208	203.3
2	2	0.261	106.800	220	208.9
2	3	0.213	90.940	214	196.8
3	1	0.217	104.40	177	224.1
3	2	0.282	117.60	177	221.3
3	3	0.300	121.90	175	222.6
6	1	0.338	209.80	256	360.5
6	2	0.426	203.60	340	311.8
6	3	0.252	159.90	236	318.4
7	1	0.348	158.50	177	268.7
7	2	0.284	143.90	180	270.1
7	3	0.317	148.10	177	263.0
9	1	0.360	190.30	410	317.4
9	2	0.328	180.10	393	314.6
9	3	0.357	192.10	392	321.5
10	1	0.373	195.80	264	320.8
10	2	0.358	195.70	269	323.9
10	3	0.415	216.30	270	336.0
13	1	0.479	239.90	318	346.6
13	2	0.467	215.70	313	338.1
13	3	0.497	242.50	317	344.0

Phosphate/Dodecane – 2.0% Taurocholic Acid					
Day	Trial	PDI	PDIW	Mean Count	Z
1	1	0.136	62.53	202	169.4
1	2	0.166	67.77	202	166.3
1	3	0.147	65.30	209	170.5
2	1	0.275	79.66	283	190.6
2	2	0.156	76.65	296	194.2
2	3	0.147	73.04	296	190.6
3	1	0.178	86.96	243	206.1
3	2	0.138	76.77	239	206.8
3	3	0.131	74.52	232	206.2
4	1	0.327	146.20	226	255.8
4	2	0.340	156.60	230	268.8
4	3	0.395	173.90	251	276.6
5	1	0.299	136.30	246	249.2
5	2	0.244	121.60	247	246.1
5	3	0.259	129.90	255	255.2
8	1	0.472	339.00	198	493.6
8	2	0.198	206.60	222	487.5
8	3	0.273	203.00	171	388.3
9	1	0.701	407.20	368	486.5
9	2	0.720	438.70	391	517.0
9	3	0.677	442.00	439	537.0
11	1	0.402	214.10	260	337.7
11	2	0.368	212.10	270	349.6
11	3	0.357	206.10	269	345.2
12	1	0.506	310.20	248	435.9
12	2	0.523	307.90	252	425.9
12	3	0.461	281.70	253	415.2
15	1	0.591	401.90	190	522.7
15	2	0.697	439.80	197	526.8
15	3	0.237	263.60	192	542.0

Phosphate/Dodecane – 4.0% Taurocholic Acid					
Day	Trial	PDI	PDIW	Mean Count	Z
1	1	0.155	66.16	411	168.0
1	2	0.180	69.19	418	163.1
1	3	0.132	61.77	418	170.0
2	1	0.283	112.20	367	211.0
2	2	0.313	127.60	383	228.1
2	3	0.199	104.90	405	235.0
3	1	0.259	116.30	200	228.6
3	2	0.286	122.30	196	228.6
3	3	0.315	128.80	191	229.5
6	1	0.237	151.40	162	311.2
6	2	0.461	223.10	145	328.7
6	3	0.427	252.20	192	385.8
7	1	0.365	181.60	205	300.4
7	2	0.297	159.80	195	293.2
7	3	0.322	167.80	195	295.9
9	1	0.460	453.50	224	668.3
9	2	0.603	434.40	204	546.5
9	3	0.569	478.20	244	633.7
10	1	0.361	203.10	158	338.2
10	2	0.278	177.50	158	336.6
10	3	0.309	186.10	150	335.0
13	1	0.82	609.00	396	672.5
13	2	0.181	362.00	407	851.7
13	3	0.157	381.00	531	962.5

Appendix G –
Emulsion Droplet Size Data
Water/Olive Oil

Water (18g)/Olive Oil (2g)			
2.0% Taurocholic Acid			
PDI	PDI Width	Mean Count	Z
0.144	965.8	446	2549
0.284	2222	454	4167
0.459	4133	521	6104
0.747	6095	339	7051
1	2338	328	2338
0.915	9864	337	1.03E+04
0.625	3536	171	4472
1	4061	156	4061
1	4729	158	4729

Water (19g)/Olive Oil (1g)			
4.0% Taurocholic Acid			
PDI	PDI Width	Mean Count	Z
1	3754	275	3754
1	1900	253	1900
1	2460	262	2460

Water (19.5g)/Olive Oil (0.5g)			
2.0% Taurocholic Acid			
PDI	PDI Width	Mean Count	Z
0.511	3556	268	4974
1	3895	273	3895
1	2820	288	2820

Water (19.5g)/Olive Oil (0.5g)			
1.0% Taurocholic Acid			
PDI	PDI Width	Mean Count	Z
1	4984	378	4984
1	2946	376	2946
1	4828	434	4828

Water (19.5g)/Olive Oil (0.5g)			
0.5% Taurocholic Acid			
PDI	PDI Width	Mean Count	Z
0.786	8117	167	9155
0.401	7503	169	1.19E+04
1	3946	163	3946

Water (19g)/Olive Oil (1g)			
6.0% Modified DexP			
PDI	PDI Width	Mean Count	Z
1	982.9	186	982.9
1	926.7	191	926.7
1	859	192	859

Water (19g)/Olive Oil (1g)			
4.0% Modified DexP			
PDI	PDI Width	Mean Count	Z
1	1778	247	1778
1	1752	251	1752
1	1786	251	1786

Water (19g)/Olive Oil (1g)			
2.0% Modified DexP			
PDI	PDI Width	Mean Count	Z
1	2781	410	2781
1	3562	423	3562
1	5142	419	5142
1	3288	44	3288
1	3220	48	3220
1	816.6	98	816.6
1	875.6	101	875.6
1	841.7	102	841.7
1	1165	222	1165
1	1145	223	1145
1	1142	221	1142

Water (19g)/Olive Oil (1g)			
2.0% Dodecyltrimethylammonium bromide			
PDI	PDI Width	Mean Count	Z
1	1694	148	1694
1	2248	156	2248
1	2627	170	2627

Water (19g)/Olive Oil (1g)			
2.0% Gum Acacia			
PDI	PDI Width	Mean Count	Z
0.668	4073	430	4984
0.361	4370	389	7270
0.352	5043	422	8496
0.962	5314	208	5419
1	3940	213	3940
1	3657	220	3657

Appendix H –
Emulsion Droplet Size Data
Tris-HCl/Olive Oil

Tris-HCl (18g)/Olive Oil (2g)			
2.0% Taurocholic Acid			
PDI	PDI Width	Mean Count	Z
1	7235	317	7235
1	1.31E+04	357	1.31E+04
1	1.62E+04	388	1.62E+04
0.485	1.07E+04	174	1.53E+04
0.109	6336	176	1.92E+04
0.23	8059	185	1.68E+04

Tris-HCl (19g)/Olive Oil (1g)			
2.0% Gum Acacia			
PDI	PDI Width	Mean Count	Z
1	2413	116	2413
1	3005	120	3005
1	2536	121	2536

Appendix I –
Emulsion Droplet Size Data
Phosphate/Olive Oil

Phosphate (18g)/Olive Oil (2g)			
2.0% Taurocholic Acid			
PDI	PDI Width	Mean Count	Z
1	7843	438	7843
1	6705	339	6705
0.934	8591	368	8889

Phosphate (19g)/Olive Oil (1g)			
2.0% Gum Acacia			
PDI	PDI Width	Mean Count	Z
0.371	2022	200	3319
0.552	3536	204	4759
1	3343	209	3343