The University of Toledo
The University of Toledo Digital Repository

Theses and Dissertations

2010

Pharmaceutically relevant microemulsions with potential topical, ophthalmic, and parenteral applications

Carrie L. Zachar
The University of Toledo

Follow this and additional works at: http://utdr.utoledo.edu/theses-dissertations

Recommended Citation
Zachar, Carrie L., "Pharmaceutically relevant microemulsions with potential topical, ophthalmic, and parenteral applications" (2010). *Theses and Dissertations*. 1025.
http://utdr.utoledo.edu/theses-dissertations/1025

This Thesis is brought to you for free and open access by The University of Toledo Digital Repository. It has been accepted for inclusion in Theses and Dissertations by an authorized administrator of The University of Toledo Digital Repository. For more information, please see the repository's About page.

A Thesis

entitled

Pharmaceutically Relevant Microemulsions with Potential Topical, Ophthalmic, and Parenteral Applications

by

Carrie L. Zachar

Submitted to the Graduate Faculty as partial fulfillment of the requirements of the Master of Science Degree in Pharmaceutical Sciences with Industrial Pharmacy Option

____
Dr. Jerry Nesamony, Committee Chair

____
Dr. Kenneth Alexander, Committee Member

____
Dr. Rose Jung, Committee Member

____
Dr. Frederick Williams, Committee Member

____
Dr. Patricia R. Komuniecki, Dean
College of Graduate Studies

The University of Toledo

May 2010

Copyright 2010, Carrie Lynn Zachar

This document is copyrighted material. Under copyright law, no parts of this document may be reproduced without the expressed permission of the author.

An Abstract of

Pharmaceutically Relevant Microemulsions with Potential Topical, Ophthalmic, and Parenteral Applications

by

Carrie L. Zachar

As partial fulfillment of the requirements of the Master of Science Degree in Pharmaceutical Sciences with Industrial Pharmacy Option

The University of Toledo
May 2010

The purpose of this research was to formulate pharmaceutically relevant microemulsion systems using dioctyl sodium sulfosuccinate (DOSS) as the surfactant. Visually clear microemulsions were identified by titrating mixtures of DOSS and oil with water. The maximum amount of water incorporated in surfactant/oil mixtures was plotted in a ternary phase diagram. Based on the information from phase diagrams DOSS/Ethyl oleate (EO) and DOSS/Crodamol PMP (PMP) mixtures were selected for further studies. Microemulsions were characterized using polarized light microscopy, electrical conductivity, rheology, and dynamic light scattering (DLS). The effects of pH and the addition of acyclovir on the microemulsion-forming compositions were investigated. Anticipating an ophthalmic or parenteral delivery method for the formulations, an aseptic filtration method for sterilization was developed using membrane filtration. This method was validated via filtration and direct inoculation by plating on blood agar and using E. coli as the positive control. The safety of the formulations on NIH 3T3 cells was studied using a neutral red assay and validated using a Bradford protein assay. Dissolution studies were performed on microemulsions containing methylene blue to evaluate the

drug release profile from the microemulsion system. A colorimetric estimation method was used to determine the amount of dye released from the formulation over a 72-hour period. DOSS/EO and DOSS/PMP mixtures were able to emulsify a maximum of 13.7% and 29.7% RO water. No birefringence was observed in the microemulsion formulations. Low electrical conductivity values of approximately 0.05 µS/cm indicated the existence of water-in-oil microemulsions. The conductivity studies demonstrated a "percolation phenomenon" in the formulations when the concentration of water exceeded a threshold value. DOSS/EO microemulsions exhibited Newtonian flow and viscosities of approximately 9 CP. Particle sizes for these DOSS/EO/water and DOSS/PMP/water systems were less than 30 nm in size (mean volume weighted diameter) as determined by DLS. The pH of the buffer influenced the total quantity of buffer that could be incorporated into microemulsion systems. Formulations filtered aseptically were free of bacteria when gram-stained and visualized under a microscope. The filtration method of sterilization was validated by the absence of microbial growth on blood agar plates over a 14-day period. All DOSS/EO microemulsion formulations showed no toxicity to 3T3 cells, except for samples with concentration of 1:15 and Wo values of 10 and 15. Acyclovir showed little effect on microemulsion formulation as similar percentages of acyclovir solution were incorporated into the DOSS/EO and DOSS/PMP mixtures as RO water. In vitro dye release studies demonstrate controlled release of the model drug over a 72-hour time period. Stable microemulsion formulations were prepared using DOSS/EO and DOSS/PMP mixtures. Development and validation of an aseptic filtration method of sterilization shows potential for these formulations to be used as parenteral or ophthalmic preparations. Successful incorporation of an anti-viral drug into the

formulations containing pharmaceutically relevant components also shows potential for various drug delivery applications.

Acknowledgements

I would like to thank Dr. Jerry Nesamony for all of his mentoring and guidance throughout these past two years of my research. He provided me with the resources that allowed me to succeed in this Masters program, while always encouraging me to think independently. Because of him, I am extremely proud of what I have to show for myself after these two years. I am so very grateful, and I feel honored to have been one of his first graduate students. I thank Dr. Kenneth Alexander for the opportunity to pursue my Masters Degree at Toledo and for being a member of my thesis committee. I thank Dr. Rose Jung and Dr. Frederick Williams for all of their assistance with my research, as well as being members of my thesis committee.

I would like to also thank my fellow classmates, Ermias, Bivash, Niraja, and Khushboo, for all of the wonderful experiences we shared. I thank Andrew and Yasmeen for all of their assistance with my research. I could never repay them for all of their help and encouragement. I thank Maki and Dr. Surya Nauli for their help with the polarized light microscopy.

Finally, I thank my parents, family, and friends. I don't think they realize how much their encouragement and support throughout my years of school has meant to me. I would not be where I am today without them. I am tremendously grateful, and extremely blessed to have them all in my life.

Table of Contents

Abstract iii

Acknowledgements vi

Table of Contents vii

List of Tables x

List of Figures xi

1 Microemulsions 1

1.1 Introduction ………………………………………………………... 1

1.2 Characteristics of Microemulsions ……………………………….… 2

1.3 Types of Microemulsions …………………………………………….. 4

1.4 Surfactant Use in Microemulsions ……………………………...… 6

1.4.1 Nonionic Surfactants ………………………………….……..... 7

1.4.2 Ionic Surfactants ……………………………………………. 9

1.4.3 Surfactant Mixtures and Co-surfactants ……………………. 10

1.5 Microemulsion Use …………………………………………………. 13

1.5.1 Advantages and Disadvantages …………………….…...….. 13

1.5.2 Microemulsions in Pharmaceuticals ……………………..…. 14

1.5.3 Other Microemulsion Uses …………………………………. 16

2 Instrumentation 17

2.1 Introduction ………………………………………………………… 17

2.2 Polarized Light Microscopy ………………………………………… 17

2.3 Electrical Conductivity ………………………………………….... 19

2.4 Rheology …………………………………………………….... 20

2.5 Dynamic Light Scattering …………………………………………… 23

3 Materials and Methods 25

3.1 Materials ………………………………………………………… 25

3.1.1 Ethyl Oleate …………………………………………………. 25

3.1.2 Codamol PMP ……………………………………………… 26

3.1.3 Crodamol GTCC …………………………………………… 27

3.1.4 Labrafac Lipophile WL 1349 ……………………………. 29

3.1.5 Oleic Acid ………………………………………………….. 30

3.1.6 Diocytl Sodium Sulfosuccinate …………………………….. 32

3.1.7 Acyclovir …………………………………………………… 35

3.2 Methods 37

3.2.1 Formulation of Microemulsions ………………………….. 37

3.2.2 Buffer and pH Effects ……………………..…………….... 39

3.2.3 Polarized Light Microscopy……………………………….... 39

3.2.4 Conductivity ……………………………………..………. 40

3.2.5 Rheology …………………………………………………. 40

3.2.6 Dynamic Light Scattering……………………………..……. 41

3.2.7 Development of Sterilization Method ……………………. 41

3.2.8 Validation of Sterilization Method ……………………….. 43

3.2.9 Cell Toxicity Studies ……………………………………… 46

3.2.10 Acyclovir Formulations ………………………………….. 46

3.2.11 Dissolution Studies ……………………………………… 47

4 Results and Discussion 48

4.1 Formulation of Microemulsions ……………………………………… 48

4.1.1 Effect of Surfactant Concentration on Microemulsion Formation ……………………………………………………… 52.

4.2 Buffer and pH Effects ………………………………………………... 53

4.3 Polarized Light Microscopy ………………………………………… 54

4.4 Conductivity ………………………………………………………… 57

4.5 Rheology ………………………………………………………….... 61

4.6 Dynamic Light Scattering …..………………………………………… 62

4.7 Development of Sterilization Method………………………………… 64

4.8 Validation of Sterilization Method…………………………………… 65

4.9 Cell Toxicity Studies …………….………………………………… 72

4.10 Acyclovir Formulations …………………………………………….. 74

4.11 Dissolution Studies ..………………………………………………… 75

5 Conclusions 77

References 79

List of Tables

4.1 Percent (%) water incorporation in each of the oils for samples (in triplicate)… 52

4.2 Determined viscosity for various Wo values. ……………………………......... 62

4.3 Summary of results on blood agar plates indicating the presence (+) or absence (-) of growth. ………………………………………………………….. 69

4.4 Summary of results showing absence (-) and presence (T) of toxicity with 1:5, 1:7, 1:10, and 1:15 DOSS/EO microemulsions of varying Wo's. ………... 73

4.5 Percent (%) acyclovir solution emulsified by DOSS/EO and DOSS/PMP mixtures of 1:5, 1:10, and 1:15 (in triplicate). ………………………………… 74

List of Figures

2.1 Schematic of polarizing light microscope. 18

2.2 AR-2000ex rotational rheometer. 20

2.3 Schematic of dynamic light scattering used to measure particle size. 23

3.1 Chemical Structure of Ethyl Oleate. 25

3.2 Chemical Structure of Poly[oxy(methyl-1,2-ethanediyl)], α-(1-oxopropyl)-ω-(tetradecyloxy)-. 26

3.3 Chemical Structures of Glycerides, C8-10. 27

3.4 Chemical Structures of Glycerides, C8-10. 29

3.5 Chemical Structure of Oleic Acid. 31

3.6 Chemical Structure of DOSS. 32

3.7 Chemical Structure of Acyclovir. 35

4.1 Ternary phase diagram representing the microemulsion-forming compositions in DOSS/PMP/water systems. 48

4.2 Ternary phase diagram representing the microemulsion-forming compositions in DOSS/EO/water systems. 49

4.3 Ternary phase diagram representing the microemulsion-forming compositions in DOSS/GTCC/water systems. 49

4.4 Ternary phase diagram representing the microemulsion-forming compositions in DOSS/Labrafac/water systems. 50

4.5 Ternary phase diagram representing the microemulsion-forming compositions in DOSS/Oleic Acid/water systems. 50

4.6 Dependence of pH on percent water incorporated as a clear microemulsion in 1:5 DOSS/EO and DOSS/PMP mixtures. …………………………………….. 53

4.7 Pictures of DOSS/EO Wo=25 without (a.) and with (b.) polarizing filter. ……. 55

4.8 Pictures of DOSS/EO Wo=30 without (a.) and with (b.) polarizing filter. ….… 55

4.9 Pictures of DOSS/EO Wo=195 without (a.) and with (b.) polarizing filter. ….. 56

4.10 Pictures of DOSS/PMP Wo=25 without (a.) and with (b.) polarizing filter. ….. 56

4.11 Pictures of DOSS/PMP Wo=75 without (a.) and with (b.) polarizing filter. ….. 57

4.12 Pictures of DOSS/PMP Wo=95 without (a.) and with (b.) polarizing filter. ….. 57

4.13 Conductivities for microemulsions at various Wo's for DOSS/EO ratios 1:5, 1:10 and 1:15. ………………………………………………………….. 58

4.14 Conductivities for microemulsions from Wo = 2.5 to Wo = 20 for DOSS/EO ratios 1:5, 1:10 and 1:15. ……………………………………………….. 58

4.15 Conductivities for microemulsions at various Wo's for DOSS/PMP ratios 1:5, 1:10 and 1:15. ……………………………………………………………………. 59

4.16 Graph showing viscosity of DOSS/EO microemulsions with Wo's of 2.5, 5, and 7.5 at varying shear rates. ……………………………………………… 61

4.17 Graph of mean volume weighted diameter for microemulsions prepared with DOSS/EO mixtures 1:5, 1:10 and 1:15. …………………………………………… 63

4.18 Graph of mean volume weighted diameter for microemulsions prepared with DOSS/PMP mixtures 1:5, 1:10 and 1:15. ………………………………………….. 63

4.19 Representative picture of test tubes containing filters with the negative control (a), first positive control (b), second positive control (c), Wo = 2.5 (d), Wo = 10 (e), and Wo = 15 (f). Test tubes remained visually the same over the 14 day testing period. ………………………………………………………….. 67

4.20 Representative picture of test tubes from direct inoculation with Wo = 15 (a), Wo = 10 (b), Wo = 2.5 (c), second positive control (d), first positive control (e), and negative control (f). Three layers can be seen in the Wo = 2.5, 10, and 15 samples, and test tubes remained visually the same over the 14 day testing period. …………………………………………………………………….…… 68

4.21 Representative pictures of blood agar plates: direct inoculation of the negative control, Wo = 2.5, and Wo = 10, and filtration of first positive control, second positive control, and Wo = 15. ……………………………..…. 71

4.22 Calibration curve for dissolution studies, where y = 0.0211 + 0.134x. ….…..…. 75

4.23 Release profile of methylene blue from DOSS/EO microemulsion. ………..…. 76

Chapter 1

Microemulsions

1.1 Introduction

Oil and water are immiscible. They separate into two phases when mixed, each saturated with traces of the other component [1]. An attempt to combine the two phases requires energy input to establish water-oil contacts that would replace the water-water and oil-oil contacts. The interfacial tension between bulk oil and water can be as high as 30-50 dynes/cm [2]. To overcome this, surfactants can be used. Surfactants are surface-active molecules. They contain water-loving (hydrophilic) and oil-loving (lipophilic) moieties [3]. Because of this characteristic, they tend to adsorb at the water-oil interface. If enough surfactant molecules are present, they align and create an interface between the water and the oil by decreasing the interfacial tension [2].

An emulsion is formed when a small amount of an appropriate surfactant is mechanically agitated with the oil and water. This results in a two-phase dispersion where one phase exists as droplets coated by surfactant that is dispersed throughout the continuous, other phase. These emulsions are milky or turbid in appearance due to the fact that the droplet sizes range from 0.1 to 1 micron in diameter [3]. As a general rule, the type of surfactant used in the system determines which phase is continuous. If the surfactant is hydrophilic,

then oil will be emulsified in droplets throughout a continuous water phase. The opposite is true for more lipophilic surfactants. Water will be emulsified in droplets that are dispersed throughout a continuous oil phase in this case [4].

Emulsions are kinetically stable, but are ultimately thermodynamically unstable. Over time, they will begin to separate back into their two phases. The droplets will merge together, and the dispersed phase will sediment (cream) [3]. At this point, they degrade back into bulk phases of pure oil and pure water with some of the surfactant dissolved in preferentially in one of the two [2].

1.2 Characteristics of Microemulsions

If a surfactant that possesses balanced hydrophilic and lipophilic properties is used in the right concentration, a different oil and water system will be produced. The system is still an emulsion, but exhibits some characteristics that are different from the milky emulsions discussed previously. These new systems are called "microemulsions". The interfacial tension between phases, amount of energy required for formation, droplet sizes, and visual appearance are only a few of the differences seen when comparing emulsions to microemulsions.

Microemulsions are in many respects small-scale emulsions. They are fragile systems in the sense that certain surfactants in specific concentrations are needed for microemulsion formation [5, 6]. In simplest form, they are a mixture of oil, water, and a surfactant. The surfactant, in this case, generates an ultra-low free energy per unit of interfacial area between the two phases (10^{-3} mN/m), which results from a precise balance between the

hydrophilic and lipophilic nature of the surfactant and large oil-to-water interfacial areas. These ultra-low free energies allow thermodynamically stable equilibrium phases to exist, which require only gentle mixing to form [3, 7]. This increased surface area would ultimately influence the transport properties of a drug [8]. The free energy of the system is minimized by the compensation of surface energy by dispersion entropy. The flexible interfacial film results in droplet sizes that fall in a range of 10-100 nm in diameter for microemulsion systems [2, 3, 5, 9]. Although these systems are formed spontaneously, the driving forces are small and may possibly take time to reach equilibrium [8]. This is a dynamic process. There is diffusion of molecules within the microstructures and there are fluctuations in the curvature of the surfactant film. These droplets diffuse through the continuous phase while kinetics of the collision, merging, and separation of droplets occur [3, 10, 11]. With droplet sizes in the nanometer range, microemulsions are optically transparent and are considered to be solutions [5, 12, 13]. They are homogeneous on a macroscopic scale, but are heterogeneous on a molecular scale [1]. Microemulsions usually exhibit low viscosities and Newtonian flow characteristics. Their flow will remain constant when subjected to a variety of shear rates. Bicontinuous formulations may show some non-Newtonian flow and plasticity [10]. Microemulsion viscosity is close to that of water, even at high droplet concentrations. The microstructure is constantly changing, making these very dynamic systems with reversible droplet coalescence [1].

To study the different properties of microemulsions, a variety of techniques are usually employed. Light scattering, x-ray diffraction, ultracentrifugation, electrical conductivity, and viscosity measurements have been widely used [14]. These are only a few of the

many techniques used to characterize microemulsions. Instrumentation and their application to microemulsions will be discussed in a later chapter.

1.3 Types of Microemulsions

Microemulsions are thermodynamically stable, but are only found under carefully defined conditions [3, 15]. One way to characterize these systems is by whether the domains are in droplets or continuous [16]. Characterizing the systems in this way results in three types of microemulsions: oil-in-water (o/w), water-in-oil (w/o), and bicontinuous. Generally, one would assume that whichever phase was a larger volume would be the continuous phase, but this is not always the case.

Oil-in-water microemulsions are droplets of oil surrounded by a surfactant (and possibly co-surfactant) film that forms the internal phase distributed in water, which is the continuous phase. This type of microemulsion generally has a larger interaction volume than the w/o microemulsions [17]. The monolayer of surfactant forms the interfacial film that is oriented in a “positive” curve, where the polar head-groups face the continuous water phase and the lipophilic tails face into the oil droplets [2]. The o/w systems are interesting because they enable a hydrophobic drug to be more soluble in an aqueous based system, by solubilizing it in the internal oil droplets. Most drugs tend to favor small/medium molecular volume oils as opposed to hydrocarbon oils due to the polarity of the poorly water-soluble drugs. An o/w drug delivery tends to be straightforward when compared to w/o microemulsions. This is the result of the droplet structure of o/w microemulsions being retained on dilution with the biological aqueous phase [17].

Water-in-oil microemulsions are made up of droplets of water surrounded by an oil continuous phase. These are generally known as "reverse-micelles", where the polar headgroups of the surfactant are facing into the droplets of water, with the fatty acid tails facing into the oil phase. This type of droplet is usually seen when the volume fraction of water is low, although the type of surfactant also impacts this as well [17]. A w/o microemulsion used orally or parenterally may be destabilized by the aqueous biological system. The biological system increases the phase volume of the internal phase, eventually leading to a "percolation phenomenon" where phase separation or phase inversion occurs [17]. Oral peptide delivery in w/o microemulsions is still used, however. The hydrophilic peptides can be easily incorporated into the water internal phase and are more protected from enzymatic proteolysis by the continuous oil phase than other oral dosage forms [17, 18]. A w/o microemulsion is best employed, though, in situations where dilution by the aqueous phase is unlikely, such as intramuscular injection or transdermal delivery [17, 19].

When the amount of water and oil present are similar, a bicontinuous microemulsion system may result. In this case, both water and oil exist as a continuous phase. Irregular channels of oil and water are intertwined, resulting in what looks like a "sponge-phase" [3, 20, 21]. Transitions from o/w to w/o microemulsions may pass through this bicontinuous state. Bicontinuous microemulsions, as mentioned before, may show non-Newtonian flow and plasticity. These properties make them especially useful for topical delivery of drugs or for intravenous administration, where upon dilution with aqueous biological fluids, forms an o/w microemulsion [17, 22-25].

1.4 Surfactant Use in Microemulsions

Surfactants are molecules that typically contain a polar head group and an apolar tail. They are surface-active and microstructure-forming molecules with a strong chemical dipole [3]. They can be ionic (cationic or anionic), nonionic, or zwitterionic. Surfactant molecules self-associate due to various inter- and intra-molecular forces as well as entropy considerations. All of these serve to optimize the free-energy overall. For example, when surfactant is mixed with oil and water, they accumulate at the oil/water interface, because it is thermodynamically favorable [17]. The surfactant molecules can arrange themselves in a variety of shapes. They can form spherical micelles, rod-shaped micelles, a hexagonal phase (consisting of rod-shaped micelles), lamellar (sheet) phases, reverse micelles, or hexagonal reverse micelles [17]. At low concentrations of dispersed (internal) phase, spherical, isolated droplets are present in the microemulsions. At higher dispersed phase concentrations, the final structures depend on the interaction between droplets. If they are repulsive, no droplet overlap will be produced due to colliding droplets. If attractive interactions are present, multiple droplets may collide and form other structures [1].

The hydrophilic-lipophilic balance (HLB) of the surfactant can be taken into account to try to rationalize the surfactant's behavior. It is generally accepted that a surfactant with HLB from 3-6 will favor the formation of water-in-oil (w/o) microemulsions, whereas surfactants with HLB from 8-18 are preferred for oil-in-water (o/w) microemulsions [17]. It must be noted, though, that microemulsions are only obtained under certain carefully defined conditions, and the HLB of the surfactant can only be used as a starting point in the selection of components that will form a microemulsion.

Another method used to relate the type of surfactant to the structures it forms is through the critical packing parameter (CPP). This, like HLB, is an empirical approach since there are many other factors that impact the final structures found in microemulsions. The CPP is a measure of the surfactant's preferred geometry, and therefore can be used to predict the type of structure that possibly will be formed. The CPP can be calculated by dividing the partial molar volume of the hydrophobic part of the surfactant by the product of the optimal headgroup area and length of the surfactant tail [17]. Surfactants that are "cone-shaped" where the tailgroup or headgroup is much larger than the other will tend to accumulate at curved interfaces resulting in micelles. Surfactants that are more "block-shaped" where tailgroup and headgroup are similar in size and the CPP values are close to one tend to form worm-like micelles or lamellar structures. Values of CPP greater than one indicate that the headgoups are much larger, resulting in w/o microemulsion systems. The opposite is true for CPP values less than one. They generally produce o/w microemulsion systems. Values for CPP around one indicate the possible formation of lamellar phases [17].

Regardless of the surfactant chosen for the microemulsion formulation, it must be able to lower the interfacial tension to an extremely small value. This aids the dispersion process, providing a flexible film that readily surrounds droplets of the internal phase while still having appropriate lipophilic character to provide a curvature at the interfacial region [8].

1.4.1 Nonionic Surfactants

Most nonionic surfactants are structurally similar to ionic surfactants, except for the fact that with ionic surfactants, the headgroup is uncharged. Because there are no electrostatic

charges from the headgroups, the interactions between these nonionic headgroups are dominated by steric and osmotic forces [26]. Cosurfactants are generally not needed to form microemulsions with nonionics. This is due to the fact that pure specimens of nonionic's usually are made up of mixtures of slightly varying chain legth [27]. Ethoxylated alcohols are the most common nonionic surfactants [3]. These alcohols contain a wide-ranging degree of ethoxylation, where ethylene oxide is added to fatty acids to make them more water-soluble. They are considered "amphiphiles", with an oil-loving hydrocarbon tail group and a water loving ethoxylated alcohol group [3]. Nonionic surfactants show good biological acceptance [28]. They are able to form microemulsions that are insensitive to pH and electrolyte concentration [8, 17, 29]. Examples of nonionic surfactants include polyoxyethylene surfactants, such as Brij 35, or sugar esters, such as sorbitan monooleate (Span 80). Polyoxyethylene sorbitan monooleate (Tween 80) and polyoxyethylene sorbitan monolaurate (Tween 20) appear safe and acceptable for oral and parenteral use [17, 28]. Polysorbates are partial fatty acid esters of sorbitol and its anhydrides copolymerized with approximately 20, 5 or 4 moles of ethylene oxide for each mole of sorbitol and its anhydrides. These vary in size due to a mixture of molecules and are considered hydrophilic nonionic surfactants. Sorbitans are partial esters of sorbitol and its mono and dianhydrides with fatty acids. These are considered lipophilic nonionic surfactants [28]. Nonionic surfactants that contain sugar hydrophilic groups, such as alkylpolyglucoside surfactants, and sucrose ester surfactants are very hydrophilic and form temperature-insensitive microemulsions with the addition of alcohol [3]. Alkanol amides and polyamines are the primary nitrogen-based nonionic surfactant types [26].

1.4.2 Ionic Surfactants

The use of ionic surfactants can be fairly limited in general pharmaceutical dosage forms. A large majority of ionic surfactants do not form balanced microemulsions without the addition of another component. These additives are required because the head group of the ionic surfactants are generally substantially more hydrophilic than poly(ethylene oxide) moieties. The salts or co-surfactants shift the overall HLB into the optimal range for microemulsion formulation [3]. The addition of co-surfactants will be discussed in a later section.

Ionic surfactants can be cationic, anionic, or zwitterionic. Cationic surfactants generally fall into the class of quaternary ammonium alkyl salts. Alkylammonium halides and tetra-alkylammonium halides are the most numerous in this class. Alkyl ammonium halides are excellent hydrogen bond donors and interact strongly with water. The most well known examples from the cationic surfactant class are hexadecyltrimethyl-ammonium bromide (CTAB) and didodcecylammonium bromide (DDAB). Although less numerous, phosphorous can be quaternarized with alkyl groups to create alkyl phosphonium cationic surfactants as well [26].

Alkali alkanoates, also known as soaps, are the most common anionic surfactants. The anionic charge in these surfactants comes from the ionized carboxyl group. This type is the most well understood surfactant when it comes to their structure and function [26]. Dioctyl sodium sulfosuccinate (DOSS) is the most widely studied anionic surfactant. It has twin tails and is a particularly good stabilizer of w/o microemulsions [17]. Other important classes of anionic surfactants include alkyl sulfates, alkyl ether sulfates, alkyl

sulfonates, aryl sulfonates, methylester sulfonates, α-olefinsulfonates, and sulfonates of alkylsuccinates. The three most important anionic groups in all of these surfactants being the carboxylate, sulfate, and sulfonate groups [26].

Zwitterionic surfactants, which contain both negatively and positively charged groups, form microemulsions upon the addition of co-surfactants. Phospholipids, such as lecithin, are common zwitterionic surfactants. Unlike other ionic surfactants, which are somewhat toxic, these show excellent biocompatibility. This is most likely due to the fact that lecithin is obtained naturally from soybean or egg, which contains diacylphosphatidylcholine as the major constituent [3, 17]. Another important class of zwitterionic surfactants to note is the betaines, such as alkylbetaines, amidoalkylbetaines, and heterocyclic betaines [26].

1.4.3 Surfactant Mixtures and Co-Surfactants

More often than not, one surfactant, whether nonionic or ionic, is not sufficient to form a microemulsion or does not result in an optimal microemulsion-forming region. Combinations of surfactants or sometimes co-surfactants are required for the optimal formation of a microemulsion. The term "co-surfactant" can describe any component that aids the primary surfactant in microemulsion formulation. "Co-surfactant" can refer to a second surfactant being used, but may also refer to a low-molecular-weight amphiphile, such as an alcohol [3].

Two different nonionic surfactants can be mixed together. Mixing a more lipophilic nonionic surfactant with a more hydrophilic nonionic surfactant can result in the exact

HLB needed to form a microemulsion. The two surfactants can be mixed in varying ratios to determine the ideal combination of the two, which results in the largest microemulsion-forming region. Mixtures of nonionic surfactants can be seen in commercial products and can sometimes be regarded as a single component (a pseudo-component) in the microemulsion system [3].

Ionic surfactants can be combined with nonionic surfactants, or higher molecular weight ethoxylated alcohols. These mixtures have synergistic effects, which allow them to be applied to many things. The most popular advantage to these mixtures is the fact that they result in temperature insensitive microemulsions [3]. Generally, ionic and nonionic surfactants react oppositely with increasing temperature. Ionic surfactants show a hydrophilic shift with increasing temperature, while nonionic surfactants exhibit a lipophilic shift. Therefore, when mixed together in a particular ratio, the two will cancel each other out, resulting in a temperature insensitive microemulsion formulation [3].

Frequently, single chain surfactants are not able to reduce the surface tension to the ultra-low levels required for microemulsion formulation. Short and medium chain alcohols, such as butanol, pentanol, ethanol, isopropanol, or propylene glycol, are commonly added as "co-surfactants" [3, 15, 17]. These co-surfactants help to further reduce the surface tension and fluidize the surfactant film, which increases the entropy of the system leading to its thermodynamic stability. Co-surfactants increase the flexibility of the surfactant film around the microemulsion droplet [17, 30, 31]. The co-surfactant molecules distribute themselves between the oil, water, and oil/water interface. The relatively small co-surfactant molecules ultimately get mixed in with the surfactant molecules at the

oil/water interface. This releases the bending stress and allows for easier dispersion [32]. These alcohols increase the fluidity of the hydrocarbon tails of the surfactant. This allows greater penetration of the oil into the surfactant monolayer. As the chain length of the alcohol increases, the flexibility of the film decreases. Alkanols introduce more disorder into the interfacial film since their chain length is much different from the surfactant molecules. Molecules move laterally as the interfacial film spontaneously fluctuates [10]. As an added benefit, alkanols added to ionic surfactants serve to reduce the repulsive forces between the charged head groups of the surfactant molecules. In the case of lecithin microemulsions, an alcohol must be added as a co-surfactant to disrupt the lamellar structures, which characterize its biological behavior allowing for the formation of a microemulsion [17].

Incorporation of co-surfactants can expand the microemulsion-forming region, but this may come at a cost [30]. The requirement of a medium-chain alcohol as a co-surfactant may cause other problems. Many of these alcohols can be irritating to the biological system, especially with chronic use. There are significant toxicity issues with these chemicals, which may prevent microemulsions containing them from being used pharmaceutically [17, 33]. Solubility of the alcohols in microemulsion formulations becomes an issue as well. Most alcohols tend to be more soluble in the aqueous phase of o/w systems than the primary surfactant. Because of this, as the system is diluted, the co-surfactant partitions more in the water-phase and reduces the amount of co-surfactant present at the interface. This destabilizes the droplets, and ultimately the microemulsion system itself. Short chain amines and alkanoic acids are also suitable co-surfactants, but these prove to have similar toxicity issues to the alcohols [17].

1.5 Microemulsion Use

Microemulsions have many inherent properties, which make them interesting for a variety of applications. They are thermodynamically stable theoretically allowing for infinite shelf-life, are translucent, and contain small microstructures [3]. To date, microemulsion use shows great potential in a wide variety of areas including enhanced oil recovery, cutting oils, drug delivery, detergency, and lubrication [15].

1.5.1 Advantages and Disadvantages

As with all things, microemulsions have advantages and disadvantages. Because of the ease of microemulsion preparation, drugs that are thermo-labile are easily incorporated without the risk of degradation [34]. In drug delivery, microemulsions ultimately increase the surface area of drugs, which improves their solubilization and permeation behavior. They are shown to increase solubility and bioavailability of Class II and IV drugs of the biopharmaceutical drug classification system. Class II drugs have high solubility but low permeability and Class IV drugs have low solubility and low permeability [7, 35, 36]. Plasma concentration profiles and drug bioavailability have been shown to be more reproducible in microemulsion formulations [7, 17]. The rate of penetration of drug is much faster from microemulsion systems than from other drug delivery vehicles, while having controlled drug release rates, slow degradation, and target specificity [34]. Microemulsions have a higher solubilization capacity for both hydrophilic and hydrophobic compounds than simple micellar solutions. Because of their thermodynamic stability, they are more favorable than regular emulsions or suspensions since

microemulsions can be manufactured with very little energy input and have a long shelf life [7].

Microemulsions have some disadvantages as well. Formation of microemulsions generally requires large amounts of surfactants and/or co-surfactants. All of these at high concentrations are generally irritating, if not slightly toxic, to the biological system [29, 34]. Many outside factors, such as temperature and pH, influence the stability of microemulsions as well. The effects of these factors must be examined for each potential formulation.

1.5.2 Microemulsions in Pharmaceuticals

Microemulsions have shown great potential in the area of pharmaceuticals. They can be applied to a wide variety of dosage forms including oral, topical, ocular, parenteral, periodontal, buccal, and nasal formulations. Oral delivery offers the opportunity to deliver peptide and protein drugs [7, 9, 37, 38]. Usually when peptides and proteins are delivered orally, they are degraded in the GI and are not therapeutically active. Delivery of these molecules using microemulsions, though, increases their bioavailability [34]. Currently, an oral cyclosporine formulation called Neoral® is on the market. A soft capsule is administered which contains an oil solution of drug and surfactant, which is converted to an o/w microemulsion when it comes in contact with the aqueous stomach environment. The main issue that is faced with cyclosporine delivery is poor intestinal absorption due to high molecular weight and lipophilicity [38, 39]. Microemulsions have given this drug more rapid and reproducible absorption with less inter- and intra-patient variability [34, 38, 40].

Microemulsions have the ability to enhance transdermal drug delivery [22, 30, 31, 33, 36, 41-43]. Using a transdermal route instead of oral eliminates systemic side effects, avoids first pass metabolism, and maintains plasma drug levels for a longer period of time. The main reason more topical formulations are not available is due to the problem that arises from the barrier that human skin provides [36, 39]. The outer layer of skin, or the stratum corneum, is made up of keratin-rich dead cells embedded in a lipid matrix, which makes it highly impermeable. Microemulsions have high solubilizing power, which significantly affect this layer of the skin, allowing for increased drug permeation [39]. Microemulsions favor drug partitioning into skin by modifying the thermodynamic activity of the drug. This, along with altering the stratum corneum, allows for better drug delivery [31]. There is a possibility for irritation with microemulsions used transdermally. The volatile water component can evaporate over time leaving an oil/surfactant solubilizing layer that may irritate the skin [33, 36, 39].

Ocular delivery of microemulsions shows great promise as well. Many properties inherent to microemulsions, such as low viscosity, transparency, and thermodynamic stability, prove to be very advantageous when it comes to this type of dosage form. Similarly to other routes of administration, microemulsions increase the water solubility of certain drugs and enhance absorption into the eye [34, 44]. This could ultimately lead to decreased number of applications.

Other miscellaneous pharmaceutical uses for microemulsions include parenteral, nasal, buccal and periodontal. Microemulsions possess low viscosity, and because of this characteristic, make them ideal for injections and intravenous use [34, 45-47]. The fine

particles of microemulsions are cleared slower from the body than regular emulsions, allowing for increased residence time of the drug at the target site [48]. As with other microemulsion dosage forms, delivery of a microemulsion nasally increase bioavailability [34]. Buccal delivery seems to be promising for drugs that are easily biotransformed [49]. All of these are only a few of the many ways microemulsions can be used pharmaceutically.

1.5.3 Other Microemulsion Uses

Microemulsions show potential in the areas outside of the pharmaceutical industry. The cosmetic industry is such an example. The transparent nature of microemulsions makes them ideal for cosmetics. They possess low viscosity and are easily absorbed by the skin [6]. Because the systems contain an oil phase and a water phase, water-soluble and oil-soluble components can be added together in the same formulation easily [50]. The thermodynamic stability of microemulsions proves to be an important factor in this industry as well. Stable formulations allow for long shelf lives of the products. Microemulsions can be applied in this industry as cleaners, hair products, perfumes, gels, and skin care products, to name only a few [50].

The thermodynamically stable microemulsions prove to be useful media for chemical reactions, due to the fact that they can solubilize both oil and water components as well as have a large internal interface [1, 14, 51]. They can serve as artificial blood substitutes, or can be used as models for biological membranes [34, 52, 53]. In short, microemulsions possess many characteristics that are ideal for multiple and versatile applications.

Chapter 2

Instrumentation

2.1 Introduction

A variety of techniques can be used to characterize microemulsions. In the present study, microemulsions were characterized using dynamic light scattering, polarized light microscopy, electrical conductivity, and rheology. A description of the instrumentation and its application to evaluate microemulsions will be discussed in this chapter.

2.2 Polarized Light Microscopy

Placing a polarizer in front of the condenser lens of a microscope produces plane-polarized light. This type of light vibrates in one plane only. The direction of polarization does not change when reflected from an optically isotropic surface, which has no birefringence [1]. The wave theory of light states that light waves oscillate at right angles to the direction, which the light travels in space. If that light is passed through a polarizer, the light oscillates in one plane only [2].

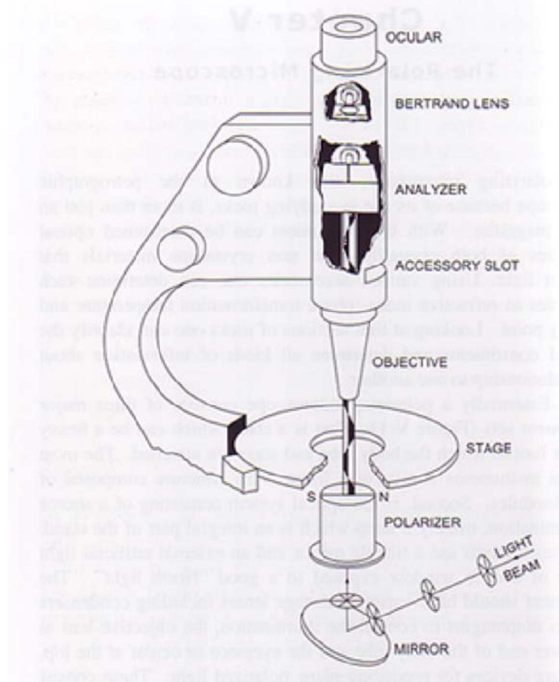

Figure 2.1 Schematic of polarizing light microscope [3]

A polarizing light microscope (Figure 2.1) contains two polarizing filters: the polarizing lens, which is fixed, and an analyzer lens. When non-polarized light is passed through the polarizing lens, only light that vibrates parallel is transmitted as plane-polarized light. This is then directed towards the analyzer lens [4]. Only light that is polarized will pass into the ocular. Polarizing light microscopy can distinguish between isotropic and anisotropic materials. It can be used to differentiate between microemulsions and liquid crystals, since birefringence is not found in microemulsions, which are isotropic systems [5-7].

2.3 Electrical Conductivity

Conductivity is the ability of a material to conduct an electric current through it. The more ions that are present, the more conductive the material. These ions are what carry the electrons. Conductivity probes contain a cathode and an anode. A constant voltage is passed between the two electrodes, and the magnitude of the current that results is ultimately determined by the solution's conductivity. Conductivity (G), the inverse of resistivity (R), is determined from the voltage (E) and current (I) values according to Ohm's Law, which is illustrated in Equation 1 [8].

$$G = \frac{I}{R} = \frac{I\ (\text{amps})}{E\ (\text{volts})} \qquad \text{Eq. 1}$$

Conductivity is useful in the determination of which phase is continuous in the microemulsion. The conductance is very different in o/w, w/o and bicontinuous microemulsion systems [9-12]. When water is the continuous phase, conductivity approaches that of the aqueous media. Conductivity, on the other hand, is very low in w/o microemulsions, due to the fact that the water, which is a better conductor than oil, is in droplets while the oil is continuous. When compared to w/o microemulsions, bicontinuous systems possess significantly higher conductivity. In this case, the charge carriers are transported through the continuous "channels" that are formed in a way similar to an aqueous electrolyte solution [13].

When starting with a w/o microemulsion, conductivity is extremely low. As the volume of water increases, though, conductivity increases as well. This continues with added water until a threshold volume of water is added. Once this happens, a “percolation” effect is observed, where the conductivity suddenly, significantly increases. This is known as the percolation threshold. With water weights between the percolation threshold and about 50% (w/w) of water, it is thought that the bicontinuous system is in existence with channels of oil and water throughout the system. Past 50% (w/w) water, conductivity is shown to decrease slightly, which is consistent with the formation of an o/w microemulsion [10, 14].

2.4 Rheology

Rheometers are used to study the flow properties of a variety of materials. Rotational rheometers, which are the type generally used for microemulsion characterization, is a type of rheometer in which shear is produced by a drag-flow between a moving part and a fixed one (Figure 2.2).

Figure 2.2 AR-2000ex rotational rheometer

These rheometers are composed of a temperature controller, a test geometry, a magnetic induction motor, an angular displacement measurement device, an electronic system to measure/control the torque, a mechanical frame, and a computer-based processing unit. The test geometries used include plate-plate, plate-cone, and concentric cylinder [15]. There are two basic types of rotational rheometers: controlled strain and controlled stress. In a controlled strain rheometer, one of the fixtures oscillates about its axis to produce the oscillatory shear motion. The torque on the stationary disk is then measured. In a controlled stress rheometer, an oscillatory torque is applied to one fixture, and its displacement is monitored [16]. Controlled strain instruments have an advantage at higher frequencies, while controlled stress instruments have the advantage at lower frequencies. This is because in a controlled stress rheometer, the motor current is controlled and the inertia of the instrument will cause this to be different from the actual stress of the sample at high frequencies [16].

Rotational rheometers measure rheological properties under transient and steady state conditions. They are very versatile as many different types of tests can be carried out. This includes dynamic tests (oscillation), flow test (rotation), creep test, temperature sweep, torque sweep, frequency sweep, and time sweep [15]. Dynamic tests include increasing the force at a fixed rate and generating a dynamic stress-strain curve [17]. In a flow test, one of the plates is subjected to a continuous rotational motion. Viscosity measurements are taken as a function of shear rate under realistic continuous flow. A special test in which the fluid is subjected to a stress step (stress controlled mode) is known as a creep test. The strain variation during a period of time is registered, and the viscoelastic behavior is reflected. Temperature sweeps involve the analysis of a sample

under a certain fixed frequency with a temperature program. Torque sweeps help to identify the linear viscoelastic region for a sample. A frequency sweep is used to obtain a viscosity curve. A sweep of frequencies is performed from low to high values under isothermal conditions. Finally, in a time sweep analysis, a sample is evaluated at a given frequency during a period of time, while the change in viscosity is recorded [15].

Rheological properties and viscosity can provide information about the microstructure of microemulsions. Both o/w and w/o microemulsions exhibit Newtonian flow, where there is no change in shear stress across a range of shear rates [5]. Bicontinuous formulations may exhibit non-Newtonian flow behavior and plasticity, which can be fairly complex. Under high and low shear, these systems may show distinct features that may be attributed to the presence of a microstructure unique to bicontinuous systems.

Viscosity describes the interactions between microstructures in microemulsions. A behavior similar to that of conductivity is seen with viscosity as well. Viscosity of o/w microemulsions is usually close to that of water, even at high droplet concentration, probably due to reversible droplet coalescence [13]. In w/o systems, the increase in viscosity with rising water concentration has been attributed to the clustering and shape changing of the internal phase droplets into channels of bicontinuous nature. Viscosity reaches a maximum at the transition point between o/w and w/o microemulsions, and then decreases as the microemulsions revert back to droplets instead of channels. As water becomes the more continuous phase, oil droplets become more disperse [10]. These disperse droplets have less influence on the viscosity of the microemulsion system.

2.5 Dynamic Light Scattering

One of the most important characterizations of microemulsions is particle size. This can be determined with the use of dynamic light scattering (DLS). Dynamic light scattering is also known as photon correlation spectroscopy. Small samples of solution (less than 1mL) are needed for the measurement and measurements are made rapidly which are a few of the advantages of this type of particle size analyzer [18]. In simplest terms, a laser is passed through a filter directly onto the scattering medium. The scattered light is then detected by the detector and translated to an autocorrelator [19]. A schematic of DLS can be seen in Figure 2.3.

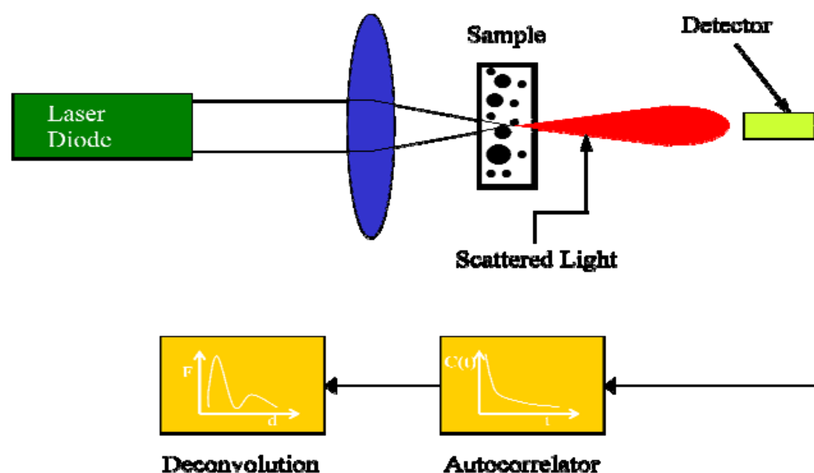

Figure 2.3 Schematic of dynamic light scattering used to measure particle size

An important concept related to DLS is the utilization of Brownian motion of particles in a medium. Particles are known to diffuse in a solution spontaneously. Colloidal particles in a solution move randomly and erratically as a result of inter-particle collisions. This erratic movement is known as Brownian motion [8]. DLS uses this phenomenon in estimating the particle size. As the particles randomly diffuse in solution, the size of the particles can ultimately be determined by how rapidly they diffuse through the medium.

The smaller the particle is, the more rapidly it diffuses through the solution. As the randomly diffusing particles pass through the path of the laser, they scatter the laser light. The linear diffusion rate can be determined by analyzing the changes in the intensity of the scattered light in one direction over time. Common detectors used are the photomultiplier (PMT) detector or the avalanche photodiode (APD) detector. The detector signal creates a correlation function in the instrument's autocorrelator from an intensity vs. time profile. The correlation function is an exponentially decaying function that is related to characteristic decay times. These in turn are related to diffusion coefficients that can be converted into particle radius using the Stokes-Einstein equation, which is illustrated in Equation 2.

$$D = \frac{k_B T}{6\pi \eta r} \quad \text{Eq. 2}$$

This equation takes into consideration the diffusion coefficient (D), Boltzmann's constant (k_B), the absolute temperature (T), and viscosity (η) to calculate the radius of the particle.

Microemulsions as a definition have particle sizes of less than 100 nm. DLS is a reliable method to detect particle sizes in this range. It is a very sensitive instrument, though, and samples must be free of all impurities. To eliminate the possibility for incorrect readings due to impurities that are present, microemulsion samples can be centrifuged, without phase separation. This separates out the unwanted particles while leaving the microemulsion droplets to continue to diffuse throughout the solution. DLS is best applied to systems with a narrow particle size distribution such as microemulsions [20].

Chapter 3

Materials and Methods

3.1 Materials

3.1.1 Ethyl Oleate

CA Index Name: 9-Octadecenoic acid (9Z)-, ethyl ester

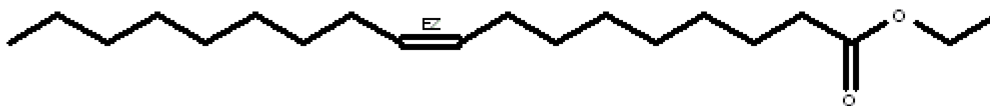

Figure 3.1 Chemical Structure of Ethyl Oleate

Other Names: 9-Octadecenoic acid (Z)-, ethyl ester; Oleic acid, ethyl ester (6CI,8CI); Crodamol EO; Esterol 123; Ethyl (Z)-9-octadecenoate; Ethyl cis-9-octadecenoate; Ethyl oleate; Nikkol EOO; Nofable EO 99

Molecular Formula: $C_{20}H_{38}O_2$

Molecular Weight: 310.51 g/mol

Physical State: Colorless to light yellow oily liquid

Boiling Point: 190°C

Melting Point: -32°C

Specific Gravity: 0.87

Ethyl oleate is primarily used as a vehicle for intramuscular injections. It is used as a solvent for drugs that are formulated in biodegradable capsules for subdermal implantation [1]. Ethyl oleate is known for being used in microemulsions for the delivery of cyclosporine [2]. It is a suitable solvent for steroids and lipophilic drugs, and has properties similar to almond and peanut oils, but is less viscous than fixed oils and more rapidly absorbed by body tissues [3].

3.1.2 Crodamol PMP

CA Index Name: Poly[oxy(methyl-1,2-ethanediyl)], α-(1-oxopropyl)-ω-(tetradecyloxy)-

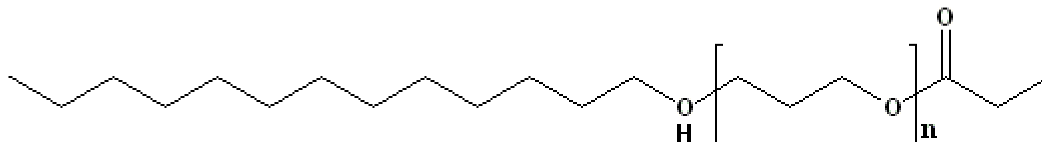

Figure 3.2 Chemical Structure of Poly[oxy(methyl-1,2-ethanediyl)], α-(1-oxopropyl)-ω-(tetradecyloxy)-

Other Names: Crodamol PMP; Polypropylene glycol 2-myristyl ether propionate

Molecular Formula: $(C_3H_6O)_nC_{17}H_{34}O_2$

Physical State: Colorless oily liquid

Boiling Point: N/A

Melting Point: N/A

Specific Gravity: 0.88

Solubility: Insoluble in water

Poly[oxy(methyl-1,2-ethanediyl)], **α**-(1-oxopropyl)-**ω**-(tetradecyloxy)- (PMP) has excellent emollient properties, which makes it a suitable replacement for mineral oil in many formulations. It is a thin liquid, which has good spreading properties and is an excellent solvent.

3.1.3 Crodamol-GTCC

CA Index Name: Glycerides, C8-10

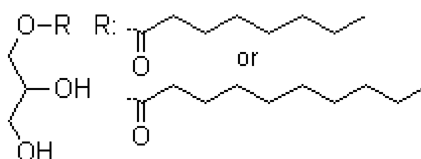

Figure 3.3 Chemical Structures of Glycerides, C8-10

Other Names: Akoline MCM; Akomed E; Arlamol M 812; C8-10 glycerides; Campul MCM; Capric/caprylic triglyceride; Capric/caprylic triglycerides; Caprylic/capric triglyceride; Caprylic/capric triglycerides; Captex 300; Captex 300 Low C6; Captex 300EP; Captex 335; Captex 355; Coconad MT; Coconard MT; Crodamol GTCC; Crodamol PC-DAB 10(S); Delios 325; Delios SK; Delios V; Delios V MCT oil; Delios VK koscher; Dermol M 5; Estasan 3575; Estasan GT 8-60; Estasan GT 8-65; Estol 1527; Estol 3601; Estol 3603; Ethox 2156; Labrafac CCTG; Labrafac LIPO WL 1349; Labrafac Lipophile; Labrafac Lipophile WL 1349; Labrafac WL 1349; Lexol GT 865; Liponate GC; Lumulse CC 33K; Miglyol 810; Miglyol 810N; Miglyol 812; Miglyol 812N; Miglyol 812S; Myritol 312; Myritol 314; Myritol 318; Myritol 325; Neobee M 5; Neobee

O; Neobee Oil M 5; Neoderm TCC; Nikkol Triester F 810; O.D.O.; Panacet 810; Panacete; Panacete 810; Radia 7104; Rofetan GTCC; Rylo TG 50; Sefol 880; Stepan 108; Stepan-Mild GCC; Sun Crystal; Surfac MCTG; Tegosoft CT; Triester F 810; Triglycerides, C8-10; Velsan CCT

Physical State: Colorless, thin, slightly yellow, oily liquid

Boiling Point: N/A

Melting Point: 0°C

Specific Gravity: 0.945

Solubility: Practically insoluble in water, miscible with long-chain hydrocarbons and triglycerides, soluble in acetone, ethanol and isopropanol

GTCC is a vehicle that is stable to oxidation. GTCC has good solvent properties. It contains medium chain triglycerides that can be used as a lipid nutritional source in oral and parenteral supplements. These medium-chain triglycerides consist mainly of completely saturated fatty acids, which give rise to its very low viscosity. GTCC has very low reactivity with sensitive pharmaceutical additives. These medium-chain lipids are absorbed into the portal system upon ingestion and are metabolized in the liver. They are transported as free fatty acids bound to albumin [4]. GTCC is used in many ways in pharmaceutical and healthcare settings which include the following: a solvent for active compounds in topicals, a water resistant topical vehicle, a dispersing aid, a melting point modifier in rectal suppositories, a mineral oil replacement, a crystallization inhibitor for oral solutions, a fill material for capsules, a plasticizer, a dispersion vehicle for colorants, a polishing aid, a tablet lubricant, a lipid source in nutritional products, and a parenteral vehicle [5].

3.1.4 Labrafac Lipophile WL 1349

CA Index Name: Glycerides, C8-10

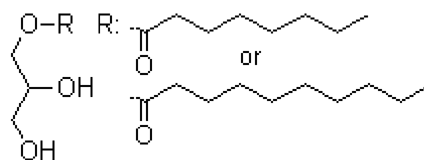

Figure 3.4 Chemical Structures of Glycerides, C8-10

Other Names: Akoline MCM; Akomed E; Arlamol M 812; C8-10 glycerides; Campul MCM; Capric/caprylic triglyceride; Capric/caprylic triglycerides; Caprylic/capric triglyceride; Caprylic/capric triglycerides; Captex 300; Captex 300 Low C6; Captex 300EP; Captex 335; Captex 355; Coconad MT; Coconard MT; Crodamol GTCC; Crodamol PC-DAB 10(S); Delios 325; Delios SK; Delios V; Delios V MCT oil; Delios VK koscher; Dermol M 5; Estasan 3575; Estasan GT 8-60; Estasan GT 8-65; Estol 1527; Estol 3601; Estol 3603; Ethox 2156; Labrafac CCTG; Labrafac LIPO WL 1349; Labrafac Lipophile; Labrafac Lipophile WL 1349; Labrafac WL 1349; Lexol GT 865; Liponate GC; Lumulse CC 33K; Miglyol 810; Miglyol 810N; Miglyol 812; Miglyol 812N; Miglyol 812S; Myritol 312; Myritol 314; Myritol 318; Myritol 325; Neobee M 5; Neobee O; Neobee Oil M 5; Neoderm TCC; Nikkol Triester F 810; O.D.O.; Panacet 810; Panacete; Panacete 810; Radia 7104; Rofetan GTCC; Rylo TG 50; Sefol 880; Stepan 108; Stepan-Mild GCC; Sun Crystal; Surfac MCTG; Tegosoft CT; Triester F 810; Triglycerides, C8-10; Velsan CCT

Physical State: Colorless viscous liquid

Boiling Point: > 150°C

Melting Point: N/A

Specific Gravity: 0.93 – 0.96

Insoluble in water

Soluble in ethanol, chloroform, methylene chloride, and vegetable oils

Labrafac is a non-rancidable fluid that has solubilizing properties. It is a vehicle used in both oral and topical formulations. Generally, it is used as an excipient for soft gelatin capsules, as an anti-sticking agent for tablets, and as an oily phase for W/O or O/W emulsions. It is made up of capric and caprylic triglycerides. Medium-chain triglycerides have a wide variety of uses orally, parenterally, and topically. They can be used as a base for oral emulsions of drugs that are unstable in aqueous media. They have also been shown to enhance intestinal absorption [6]. Medium-chain triglycerides can act as fillers and lubricants in capsules and sugar-coated tablets. In combination with long-chain triglycerides, they can be used for total parenteral nutrition IVs. These nutritional agents can be used for diseases involving the malabsorption of fats. Medium-chain triglycerides offer many advantages over long-chain triglycerides. This includes better spreading properties, no impedance of skin respiration, good penetration and emollient properties, no visible film on skin surface, good compatibility with other components and drugs, good solvent properties, and good stability against oxidation [5].

3.1.5 Oleic Acid

CA Index Name: 9-Octadecenoic acid (9Z)-

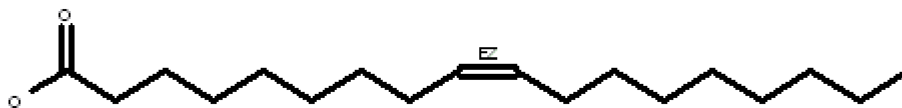

Figure 3.5 Chemical Structure of Oleic Acid

Other Names: 9-Octadecenoic acid (Z)-; Oleic acid (8CI); 9-Octadecenoic acid, (Z)-; 9-cis-Octadecenoic acid; 9Z-Octadecenoic acid; B 115; Clear FRAC EF; Crodacid O-P; Crossential O 94; D 100; D 100 (fatty acid); Edenor ATiO5; Edenor FTiO5; Emersol 205; Emersol 211; Emersol 213NF; Emersol 214NF; Emersol 233; Emersol 6313NF; Extra Oleic 80R; Extra Oleic 90; Extra Oleic 99; Extra Olein 80; Extra Olein 90; Extra Olein 90R; Extra Olein A 1981; Industrene 105; Lunac O-CA; Lunac O-LL; Lunac O-P; Lunac O-V; Lunac OA; NAA 35; NAA 38; Neo-Fat 92-04; Oleine 7503; Pamolyn 100; Priolene 6204; Priolene 6906; Priolene 6907; Priolene 6928; Priolene 6930; Priolene 6933; Vopcolene 27; Wecoline OO; Z-9-Octadecenoic acid; cis-9-Octadecenoic acid; cis-Oleic acid; cis- 9-Octadecenoic acid; 9-cis-Octadecenoic acid; 9-cis-Oleic acid

Molecular Formula: $C_{18}H_{34}O_2$

Molecular Weight: 282.46 g/mol

Physical State: Pale yellow oily liquid

Boiling Point: 360°C

Melting Point: 16.3°C

Specific Gravity: 0.895

Insoluble in water

Oleic acid is used as an emulsifying agent in food products and topical pharmaceutical formulations, mostly as a penetration enhancer. It can be used to improve the bioavailability of poorly water-soluble drugs in tablets, or as part of the vehicle for soft gelatin capsules. Oleic acid can be radiolabeled with ^{131}I and ^{3}H and used in medical imaging as well [5].

3.1.6 Dioctyl Sodium Sulfosuccinate (DOSS)

CA Index Name: Butanedioic acid, 2-sulfo-, 1,4-bis(2-ethylhexyl) ester, sodium salt (1:1)

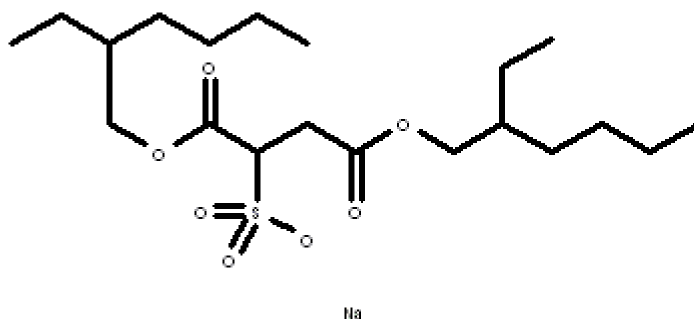

Figure 3.6 Chemical Structure of DOSS

Other Names: Aerosol OT-B (6CI); Butanedioic acid, sulfo-, 1,4-bis(2-ethylhexyl) ester, sodium salt (9CI); Succinic acid, sulfo-, 1,4-bis(2-ethylhexyl) ester, sodium salt (8CI); 05035TX; 1,4-Bis(2-ethylhexyl) sodium sulfosuccinate; AOT; AOT 100; AOT I; Acrosol OT 100; Adekacol EC 4500; Adekacol EC 8600; Aerosol A 501; Aerosol AOT; Aerosol GPG; Aerosol OT; Aerosol OT 100; Aerosol OT 70; Aerosol OT 70PG; Aerosol OT 75; Aerosol OT 75E; Aerosol OT 75PG; Aerosol OT 94; Aerosol OT-A; Aerosol OT-S; Airrol CT 1; Airrol CT 1L; Airrol OP; Alcopol O; Alkasurf SS-O 75; Alphasol OT; Astrowet 608; Astrowet O 70PG; Astrowet O 75; B 80; Berol 478; Bis(2-ethylhexyl) S-

sodium sulfosuccinate; Bis(2-ethylhexyl) sodiosulfosuccinate; Bis(2-ethylhexyl) sodium sulfosuccinate; Bis(2-ethylhexyl) sulfosuccinate sodium salt; Carabon DA 72; Celanol DOS 65; Celanol DOS 75; Chemax DOSS 70PG; Colace; Comfolax; Complemix; Constonate; Coprol; Coprola; Correctol Stool Softener Laxative; DESS; DOSS; DOSS 70; DSS; Defilin; Di(2-ethylhexyl) sulfosuccinate sodium salt; Di-2-ethylhexyl sodium sulfosuccinate; Dialose; Dioctlyn; Dioctyl; Dioctyl sodium sulfosuccinate; Dioctyl sulfosuccinate sodium; Dioctyl sulfosuccinate sodium salt; Dioctyl-Medo Forte; Dioctylal; Diomedicone; Diosuccin; Diotilan; Diovac; Diox; Disonate; Disponil SUS-IC 875; Disposaject; Docusate sodium; Doxinate; Doxol; Drewfax 007; Dulcivac; Dulsivac; Duosol; E 480; Emcol 4500; Empimin OP 70; Empimin OT 75; Eurowet PG; Freetex OT; Gemtex SC; Gemtex SC 40; Genopur SB 1970J; Geropon DOS; Geropon SDS; Geropon SS-O 75; Geropon WT 27; Humifen WT 27G; Isoprotanc; KM 10-1610; Karawet DOSS; Konlax; Koremul 290; Kosate; Lankropol 4500; Lankropol KO 2; Laxinate; Leveling Agent T; Leveller T; Lipal 860K; Lipal 870P; Lumiten I-RA; Lutensit A-BO; Lutensit A-BOS; M 75; Mackanate DOS 75; Manoxol; Manoxol OP; Manoxol OT; Marlinat DF 8; Mervamine; Miconate DOS; Modane Soft; Molatoc; Molcer; Molofac; Monawet M 085P; Monawet MO 65-150; Monawet MO 70; Monawet MO 70E; Monawet MO 70R; Monowet MO 70R; Monowet MO-E 75; NK-EP 70G; Neocol P; Neocol SW-C; Neocol YSK; Neopelex OTP; Nevax; Newcol 290M; Newcol 291EG; Newcol 291M; Newcol 291PG; Newkalgen EP 60P; Newkalgen EP 70G; Nikkol OTP 100; Nikkol OTP 100S; Nikkol OTP 70; Nikkol OTP 75; Nissan Rapisol; Nissan Rapisol A 30; Nissan Rapisol A 80; Nissan Rapisol A 90; Nissan Rapisol B 07; Nissan Rapisol B 30; Nissan Rapisol B 80; Nissan Rapisol B 90; Nonit; Norval; OT; OT 100; OT 70; OT

75; OT 75 (surfactant); OT 75E; OTP 100; OTP 75; Obston; Octowet; PAV 1019; Pelex OT; Pelex OT-P; Pentex 99; Persol KMN 3; Pionin A 51B; Rapisol; Rapisol A 30; Rapisol A 80; Rapisol A 90; Rapisol B 30; Rapisol B 80; Rapisol B 90; Regutol; Revac; Rewopol SBDO 70; Rikakoru M 75; Rikasafu G 30; SBO; SN-Wet OT 70; SV 102; Sanmorin OT; Sanmorin OT 70; Sanmorin OT 70N; Sanseparer 100; Secosol DOS 70; Sobital; Sodium 1,2-bis(2-ethylhexyloxycarbonyl)-1-ethanesulfonate; Sodium 1,4-bis(2-ethylhexyl) sulfosuccinate; Sodium bis(2-ethylhexyl) sulfosuccinate; Sodium bis(ethylhexyl) sulfosuccinate; Sodium di(2-ethylhexyl) sulfosuccinate; Sodium di(ethylhexyl) sulfosuccinate; Sodium dioctyl sulfosuccinate; Sodium docusate; Softil; Soliwax; Solovet; Solusol; Sorpol 5050; Spilon 8; Succinate STD; Sulfimel DOS; Sulfosuccinic acid bis(2-ethylhexyl)ester sodium salt; Sulfosuccinic acid di-2-ethylhexyl ester sodium salt; Sunnol LDF 110; Surfonic DOS 75PG; TKB 20; Talosurf; Tex-Wet 1001; Triton GR 5; Triton GR 5M; Triton GR 7; Triton GR 7M; Triton GR 7ME; Vatsol OT; Velmol; Warcowet 060; Waxsol; Wetaid SR; Yal

Molecular Formula: $C_{20}H_{37}SO_7Na$

Molecular Weight: 444.57 g/mol

Physical State: White waxy solid

Melting Point: Decomposes

Specific Gravity: 1.1

Easily soluble in methanol, diethyl ether, and glycerin

Partially soluble in hot water

Very slightly soluble in cold water

Butanedioic acid, 2-sulfo-, 1,4-bis(2-ethylhexyl) ester, sodium salt (DOSS) is an ionic surfactant. It is generally used as an emulsifying, wetting, dispersing, or solubilizing agent for external use. Therapeutically, DOSS is used as a stool softener. Its surface-active characteristics allow for the management of constipation [7-9]. DOSS has also been reported to increase intestinal absorption of drugs [10].

3.1.7 Acyclovir

CA Index Name: 6H-Purin-6-one, 2-amino-1,9-dihydro-9-[(2-hydroxyethoxy)methyl]-

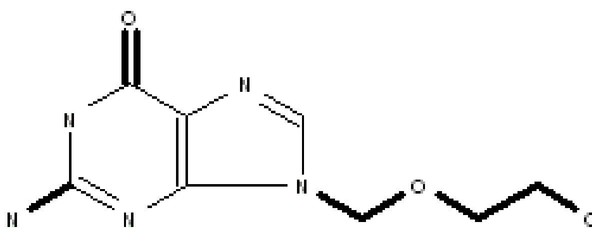

Figure 3.7 Chemical Structure of Acyclovir

Other Names: 9-(2-Hydroxyethoxymethyl)guanine; ACV; Acicloftal; Aciclovir; Acyclo V; Acycloguanosine; Acyclovir; Avirase; BW 248U; BioVir I; BioVir II; Cargosil; Gerpevir; Herpevir; Lovir; NSC 645011; Novirus; Poviral; Rouz-Aciclovir; Vipral; Virorax; Virovir; Wellcome 248U; Zovirax; Zyclir

Molecular Formula: $C_8H_{11}N_5O_3$

Molecular Weight: 225.2 g/mol

Physical State: White solid crystalline powder

Boiling Point: N/A

Melting Point: 257°C

Solubility in Water: 1.3 mg/ml

Solubility in Methanol: 0.2 mg/ml

Acyclovir is a synthetic nucleoside analog, which is active against most species in the herpesvirus family. It is most active against herpes simplex virus I (HSV-I), half as effective against herpes simplex virus II (HSV-II), and one tenth as effective against varicella zoster virus (VZV) and Epstein-Barr virus (EBV). Acyclovir is least active against cytomegalovirus (CMV) and human herpes virus (HHV-6) [11, 12]. It is a virustatic agent, meaning it decreases but does not completely prevent recurrences or long-term natural history of the disease [8]. Acyclovir is indicated for treatment of genital herpes simplex, herpes simplex labialis, herpes zoster, acute chickenpox in immunocompromised patients, herpes simplex encephalitis, acute mucocutaneous HSV infections in immunocompromised patients, herpes simplex keratitis, herpes simplex blepharitis, and Bell's Palsy [11].

Acyclovir has poor oral bioavailability at approximately 10-20%. With a water solubility of 1.3 mg/mL and octanol/water partition coefficient of 0.018, it has very low GI absorption[13]. It is marketed as tablets, topical cream, IV injection, and ophthalmic ointment. Because of such low bioavailability and poor solubility, if a high concentration is needed, IV administration is used. For oral doses, peak plasma concentrations are achieved after 1-2 hours. 9 to 33% is protein bound. Renal excretion accounts for 62 to 91% of elimination. Half-life is 2.5 hours but may be as long as 19.5 hrs in renal failure. It is excreted renally by glomerular filtration and by tubular secretion [8, 11]. Acyclovir has two ionizable groups. The basic group has a pK_a of 2.4, and the acidic group has a pK_a of 9.2 [14].

It is an acyclic guanine nucleoside analog that lacks a 3'-hydroxyl on the side chain. Acyclovir acts by incorporating into viral DNA and competitively inhibits the DNA polymerase. After entering the body, it is selectively converted to acyclo-guanosine monophosphate by viral thymidine kinase. It is then further phosphorylated into its active triphosphate form, acyclovir-triphosphate, by cellular kinases. Acyclovir-triphosphate is what inhibits viral DNA polymerase by incorporating into DNA primer strand during viral DNA replication leading to chain termination due to its lack of the 3′-hydroxy group. Its incorporation into DNA causes chain termination and formation of an inactive complex with the viral DNA polymerase.

Its selectivity of action depends on interaction with two distinct viral proteins: HSV thymidine kinase (HSV-TK) and DNA polymerase. Cellular uptake and initial phosphorylation are facilitated by HSV-TK. Affinity of acyclovir to HSV-TK is 200 times greater than for the mammalian enzyme. Cellular enzymes, which are 40 to 100 times greater in HSV infected cells than normal cells, convert the monophosphate to triphosphate and compete for endogenous deoxyguanosine triposphate (dGTP). Through suicide inactivation, the terminated DNA template containing acyclovir binds the viral DNA polymerase and leads to irreversible inactivation. Uninfected mammalian cell growth is unaffected by high concentrations (>50ug/mL) of acyclovir [11].

3.2 Methods

3.2.1 Formulation of Microemulsions

Microemulsions are formulated by mixing various components such as oil, surfactant, co-surfactant (if needed), and water in various proportions. The concentration of various

mixtures may be plotted in a ternary-phase diagram or a pseudo-ternary phase diagram to identify compositions that form visually clear microemulsions. Formulations were prepared using ethyl oleate (EO), Crodamol GTCC® (GTCC), Labrafac Lipophile WL 1349®, oleic acid, and Crodamol PMP® (PMP). Appropriate quantities of docusate sodium (DOSS) and oil (w/w) were weighed out on a PB303-S/FACT balance (Mettler Toledo) and were mixed overnight to form mixtures of weight rations 1:5, 1:10 and 1:15. Once completely dissolved, aliquots were taken from the surfactant/oil stock solution and titrated with water [15-18]. An aliquot of the surfactant/oil mixture was weighed in screw-cap test tube. Reverse osmosis (RO) water was added drop-wise and the weight was recorded after the addition of each drop. After recording the weight, samples were vortexed for 15 seconds at 1600 rpm and examined for visual clarity. Visual observations were performed for each addition of water. When turbidity that did not disappear after vortexing was observed the sample was stored at room temperature for 24 hours. If the turbidity disappeared after the 24 hour equilibration time more water was added drop wise and the procedure repeated till turbidity appeared. Addition of water was stopped when the turbidity was stable beyond 24 hours. The total amount of water incorporated into a stable microemulsion was determined by summing the water increments until the appearance of turbidity. These weights of water, surfactant and oil were transformed into relative percentages, and these percentages were plotted on a ternary phase diagram using Sigmaplot® software. Surfactant/oil mixtures were prepared in concentrations of 1:4 and 1:7 for EO and PMP. These were prepared in the same manner as previously described, titrated similarly with water, and the data obtained were used in the ternary phase diagrams.

3.2.2 Buffer and pH Effects

Buffer and pH effects on the amount of water incorporated into the microemulsion were examined using a method similar to formulation of microemulsions with RO water. Acetate buffers (pH = 4, 5 and 6) and phosphate buffers (pH = 7, 8, 9, 10 and 11) as well as DOSS/EO and DOSS/PMP in a surfactant/oil ratio of 1:10 were used in the experiment. Aliquots of the surfactant/oil mixture were weighed on an analytical balance and titrated with the various buffer solutions. Each surfactant/oil mixture was titrated with each of the 8 buffers. All experiments were done in triplicate. Maximum percent buffer incorporated was plotted vs. pH for each of the two, surfactant/oil combinations.

3.2.3 Polarized Light Microscopy

Samples were examined under polarized and non-polarized light. Surfactant/oil mixtures of DOSS/EO and DOSS/PMP in concentrations of 1:15 were used. An aliquot of the surfactant/oil mixture was taken and a sufficient amount of water was added to achieve the desired water/surfactant molar ratio (Wo = [H_2O]/[DOSS]) [19, 20]. The desired Wo's were 25, 75, and 95 for PMP, and 25, 30 and 195 for EO. A drop of the sample was placed on a slide and observed using a 40x objective on a Nikon model TiU coupled with photometric Coolsnap EZ 20 MHz monochrome camera, and was controlled by MetaMorph software. Each sample was observed and pictures taken under normal optics with and without a polarizing filter to determine if the samples exhibited optical birefringence.

3.2.4 Conductivity

Conductivity studies were performed using a Mettler Toledo Seven Multi with InLab 741 conductivity probe. The probe had a cell constant of 0.102919 cm^{-1} with a range from 0.001 µS/cm to 500 µS/cm. Samples were prepared (in triplicate) of DOSS/EO and DOSS/PMP ratios of 1:5, 1:10, and 1:15. Water was added, using an analytical balance, to achieve various desired Wo's ranging from 2.5 to 95. The conductivity probe was placed in approximately 10 mL of sample and conductivity measured. After measurements, the probe was rinsed with RO water, soaked in approximately 200 mL fresh RO water for five minutes, rinsed again with RO water, dried, and then used for next sample. This cleaning procedure was performed after each sample reading.

3.2.5 Rheology

Rheology experiments were performed on an AR-2000ex (TA Instruments). Microemulsion samples with Wo = 2.5, 5, and 7.5 were prepared using 1:10 DOSS/EO and RO water. Geometries of the instrument were 60-mm 1-degree Al cone with Peltier plate, and recessed rotor with concentric cylinder jacket. Temperature was controlled at 20°C with the Peltier plate and concentric cylinder jacket. Dynamic and steady flow testing were performed on the samples. Dynamic frequency sweep was performed at 20°C from 10 to 0.1 rad/sec with an oscillatory stress of 0.01 Pa. Dynamic time sweep was performed at 20°C, 0.4 rad/sec, and an oscillatory stress of 0.01 Pa. Steady state flow was performed at 20°C from 0.1 to 1E5 µN-m, 1000 sec^{-1} maximum, then 1000 to 0.1 sec^{-1}.

3.2.6 Dynamic Light Scattering

Dynamic light scattering (DLS) experiments were performed to determine particle size of the microemulsions. DOSS/EO and DOSS/PMP microemulsions of 1:5, 1:10, and 1:15 concentrations with various Wo values were prepared. Microemulsion samples were transferred into 6x50 mm Durex Borosilicate Glass Culture Tubes (VWR Scientific Products) and centrifuged for five minutes at 23°C and 5000 rpm using an Eppendorf 5430R centrifuge. Culture tubes were then placed in the Nicomp 380 ZLS (Particle Sizing Systems, Santa Barbara, CA) and particle size determined. Three cycles were run at 30 minutes per cycle for each sample, with a channel width of 5.5 µSec, temperature of 23°C, and 90° scattering angle. Mean volume weighted diameter was then calculated from the three runs for each sample.

3.2.7 Development of Sterilization Method

An aseptic filtration method was developed for the DOSS/EO microemulsion systems that show potential to be applied to other microemulsion systems. Microemulsions show great potential to be used as an ophthalmic dosage form. Microemulsions can increase the solubility of a wide variety of drugs and enhance that drug's absorption in the eye [21]. Many inherent properties of a microemulsion (low viscosity, transparency, thermodynamic stability, etc.) prove to be very advantageous for ophthalmic administration [22]. Currently, CMV retinitis in AIDS patients is treated with chronic antiviral therapies (due to the drugs' virustatic abilities) including intravenous injections, intravitreal injections, or intraocular implants. This proves to be a difficult and not an ideal method to deliver the drug [23, 24]. An ocular microemulsion dosage form of the

drug could prove useful in a situation such as this. Sterility of a product is required for ocular use, so an aseptic filtration method was developed for the DOSS/EO microemulsion system. Due to the relatively small amounts of water in the microemulsions, the samples couldn't be sterilized by autoclaving. A filtration method was therefore developed for the formulations due to the low viscosity and ease of filtration without the use of elaborate equipment.

All glassware used were packaged appropriately, autoclave tape applied on the wrap, and were autoclaved at 15 psi and 121°C for 15 minutes. Mueller Hinton (MH) Agar plates were then prepared by dissolving the dehydrated agar in RO water followed by autoclaving. After autoclaving and cooling slightly, aseptic technique was used to transfer approximately 20 mL MH Agar solution into presterilized monoplates. The MH Agar cooled in the plates, which were then refrigerated until ready to use. When ready for use, the MH Agar plates were equilibrated to room temperature (RT) by maintaining at RT for approximately 30 minutes.

Microemulsions were prepared using 1:5 DOSS/EO and a sufficient amount of sterile water for injection to achieve Wo's of 2.5, 10, and 15. The surfactant/oil mixture was measured out and sufficient water was added using an analytical balance. The solution was vortexed for 15 seconds at 1600 rpm and allowed to equilibrate for 24 hours at room temperature. After system equilibration, 2 mL of the sample was withdrawn into a sterile syringe in a laminar hood and approximately 100 μL passed through a 0.2 μm Nalgene® syringe filter (25 mm surfactant free cellulose acetate membrane) directly onto the plate. After allowing absorption into the agar for approximately one hour in the laminar hood,

plates were then closed and placed in an incubator at 35°C. The plates were then checked daily for any growth. This was repeated in duplicate.

Small squares of the cloudy portions of MH agar were cut out and placed in test tubes containing MH broth. Samples of the microemulsions were also directly introduced into test tubes containing MH broth. All tubes were vortexed and incubated at 35°C. Samples were taken from the tubes after approximately 24 hours, were gram stained, and examined under a microscope for bacteria.

EO alone as well as the 1:5 DOSS/EO mixture were plated on MH Agar plates. 50 μL of unfiltered EO, unfiltered DOSS/EO, filtered EO, and filtered DOSS/EO were plated in a laminar hood and incubated at 35°C. The EO and DOSS/EO samples were filtered using the same syringe filter as the microemulsion samples. The plates were checked daily for signs of growth. This experiment was done in duplicate.

3.2.8 Validation of Sterilization Method

Validation of the sterilization method was done using direct inoculation and membrane filtration. Fluid thioglycollate medium (FTM) and tryptic soy broth (TSB) were prepared, and used for direct inoculation and membrane filtration. All tests were carried out under aseptic conditions in a laminar airflow hood.

For direct inoculation, there was a negative control, two positive controls, and three samples. The negative control contained 10 mL of the uninoculated medium. The positive controls were prepared using E. coli. E. coli ATCC 25922 was cultured on an MH Agar plate and incubated at 35°C for approximately 24 hours. Bacterial colonies on the plate

were swabbed and placed into a test tube containing 5 mL of sterile saline. The bacterial concentration was determined using a Spectronic 20 Genesys Spectrophotometer (Spectronic Instruments) at 625 nm. Sterile saline was used as a blank, and the sample absorbance was compared to a 0.5 McFarland Standard (1.5 CFU/mL). Serial dilutions were performed to achieve dilutions of 10^{-4} and 10^{-8} CFU/mL. The first positive control contained 9.9 mL of the medium and 100 μL of the 10^{-4} dilution. The second positive control contained 9.9 mL of the medium and 100 μL of the 10^{-8} dilution. The three samples test tubes contained 9 mL of the medium and 1 mL of the sample that had been passed through a 0.2 μm Nalgene® syringe filter (25 mm surfactant free cellulose acetate membrane) attached to a syringe. The microemulsion samples were identical to the formulations used in method development involving sterilization by aseptic filtration (1:5 DOSS/EO microemulsions with Wo = 2.5, 10, and 15). FTM samples were incubated at 35°C. One set of TSB samples was incubated at 35°C, and another set of TSB samples was incubated at room temperature (approximately 20°C). This totaled 18 test tubes for direct inoculation.

For membrane filtration, there was a negative control, two positive controls, and three samples. The negative control contained 10mL of uninoculated medium. A Millipore® 25 mm glass microanalysis vacuum filter holder with fritted glass support filtration apparatus was assembled. The receptacle for the fluid to be tested was held to the fritted glass support with an anodized aluminum spring clamp. A No. 5 stopper attached the apparatus to a 125 mL filter flask with a vacuum pump connection. Millipore nitrocellulose membrane filters of 0.22 μm pore size were placed on the support base and 500μL of the sample was placed in the receptacle. Formulations identical to those used in

direct inoculation were used for membrane filtration. The sample was filtered by pipetting the sample onto the filtration unit placed, and vacuum was applied to facilitate filtration. After the sample passed through the membrane completely, the membrane was washed twice with sterile water. Filters were removed from the apparatus, cut into 3 pieces of approximately equal surface area and placed into test tubes containing 10mL of medium (FTM, TSB at 35°C, and TSB at room temperature). There were two positive controls. For the first positive control, 200 μL of the 10^{-4} E. coli dilution was added to 1.8 mL of sterile saline in a test tube. The membrane filter was first wet with sterile saline, and then 1mL of the first positive control solution was placed into the receptacle to be filtered. After the solution filtered through, the membrane was washed with sterile water twice. The filter was then removed and treated similarly as with the microemulsion samples described earlier. The same procedure was followed for 200 μL of the second positive control, as with the first positive control. This totaled 18 test tubes for membrane filtration.

Microemulsion samples of 400 μL were withdrawn from each of the 36 tubes for direct inoculation and filtration on Days 0, 7 and 14. These samples were transferred into Eppendorf microcentrifuge tubes and then spiroplated onto blood agar plates using a SpiralBiotech Autoplate 4000. Test tubes containing TSB were vortexed before samples were withdrawn for spiroplating. Samples from test tubes containing FTM were not vortexed and taken from the bottom of the tube below the pink layer for spiroplating. Samples were manually loaded from the Eppendorf tubes into the machine stylus. The stylus then plated the samples, and was put through a washing phase to be washed with bleach and sterilized water.

3.2.9 Cell Toxicity Studies

3T3 cells (mouse embryonic fibroblast tissue) were subcultured in DMEM media containing 5% fetal bovine serum and penicillin/streptomycin. Cells were then transferred to a 96-well plate and incubated for 24 hours. Microemulsion samples were prepared with Wo's of 2.5, 5, 10 and 15 using DOSS/EO concentrations of 1:5, 1:7, 1:10, and 1:15. After cells had incubated for 24 hours, 100μL of microemulsion was applied to each well, followed by 100μL of media. After 24 hours, cell viability testing was performed via a neutral red assay [25]. Finally a Bradford protein assay was performed on the cells [26].

3.2.10 Acyclovir Formulations

Acyclovir was incorporated into the microemulsion systems. 4 mM solutions of acyclovir were prepared by weighing an appropriate amount of the solid in a 5 mL volumetric flask. The solid acyclovir was dissolved by adding approximately 4.5 mL RO water to the flask and placed in an incubator overnight at 30°C to increase the rate of dissolution. Once completely dissolved, the solution was made up to the 5mL mark with RO water. Aliquots of the 1:5, 1:10, and 1:15 DOSS/EO and DOSS/PMP mixtures were weighed on an analytical balance. The DOSS/oil mixtures were then titrated with the acyclovir solution following the same procedure as the formulation of microemulsions with RO water and buffers. All experiments were done in triplicate. Maximum percent acyclovir solution incorporated was compared to maximum percent RO water incorporation for each formulation.

3.2.11 Dissolution Studies

Methylene blue was used to study the release in microemulsion systems. A calibration curve was first prepared using a 0.02 M stock solution of methylene blue. Five methylene blue dilutions were prepared in test tubes containing 2.5, 5, 7.5, 10, and 15 μL of the stock dye solution. Then, to each of the tubes, a sufficient volume of RO water was added to achieve a final volume of 4 mL. The tubes were vortexed, and the absorbance was then determined for each of the test tubes at 668 nm using a Spectronic 20 Genesys Spectrophotometer (Spectronic Instruments) with RO water as a blank. The absorbance was plotted versus concentration of dye (μmol/mL) to obtain a calibration curve.

A 1M methylene blue solution was prepared for the microemulsion formulations by dissolving 3.739 g of methylene blue powder in 10mL of RO water. An aliquot of the methylene blue solution was added to an appropriate quantity of 1:10 DOSS/EO mixture taken in a test tube, and the tube was vortexed. The sample was transferred into a Spectra/Por® Biotech Cellulose Ester Dialysis Membrane, molecular weight cut-off of 100,000Da (Spectrum Laboratories, Rancho Dominguez, CA), clamped on the ends to prevent microemulsion spillage. The filled dialysis membrane was submerged in 1 L of RO water containing a magnetic stirrer and kept in a water bath at 37°C. Samples of 4 mL were withdrawn from the RO water, transferred to test tubes, and replaced with fresh RO water at various time points. The absorbance of the samples was determined at 668 nm using a Spectronic 20 Genesys. The concentration of methylene blue (μmol/mL) was determined from a previously prepared calibration curve. Methylene blue concentration was plotted versus time to create a release profile. Experiments were done triplicate.

Chapter 4

Results and Discussion

4.1 Formulation of Microemulsions

Surfactant/oil mixtures were prepared (w/w) in ratios of 1:5, 1:10, and 1:15 using five oils, which include EO, PMP, GTCC, Labrafac Lipophile, and oleic acid. Water was added drop-wise to the surfactant/oil mixtures using the titration method and visually inspected to determine the amount of water able to be incorporated into each formulation. Phase diagrams were constructed to visualize the microemulsion forming regions. These can be seen in Figures 4.1 –4.5.

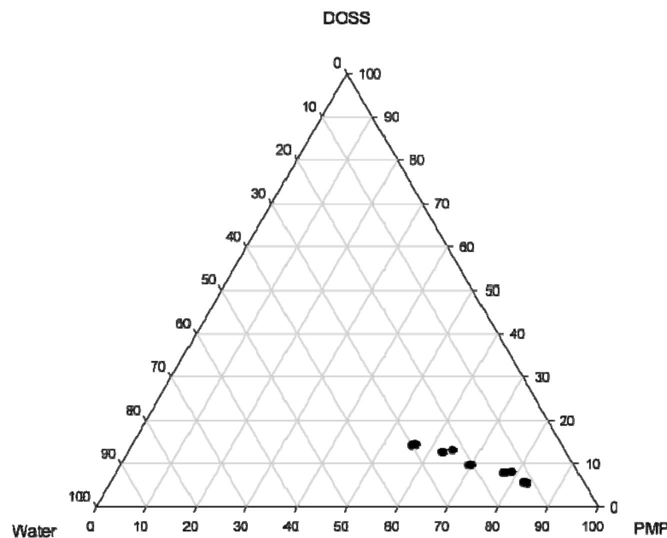

Figure 4.1 Ternary phase diagram representing the microemulsion-forming compositions in DOSS/PMP/water systems.

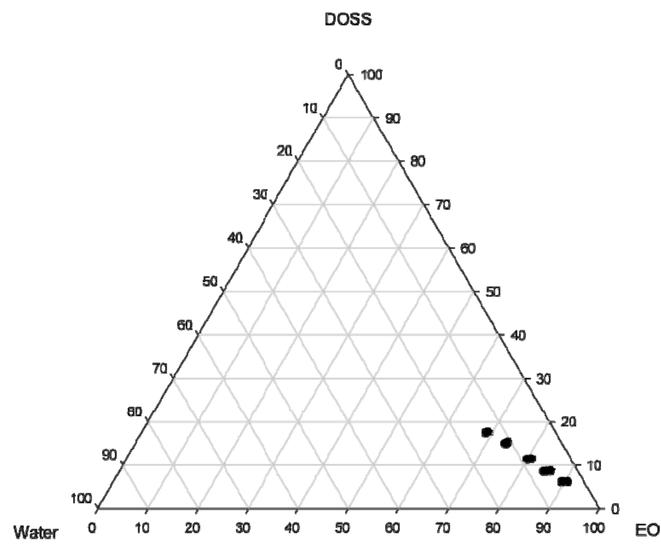

Figure 4.2 Ternary phase diagram representing the microemulsion-forming compositions in DOSS/EO/water systems.

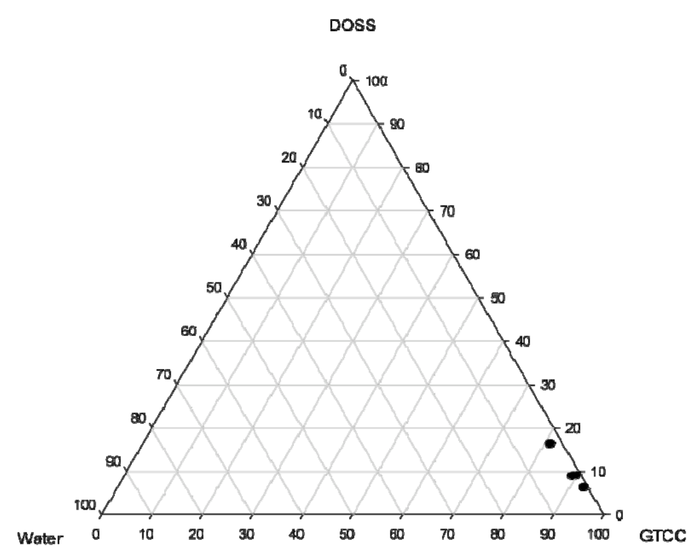

Figure 4.3 Ternary phase diagram representing the microemulsion-forming compositions in DOSS/GTCC/water systems.

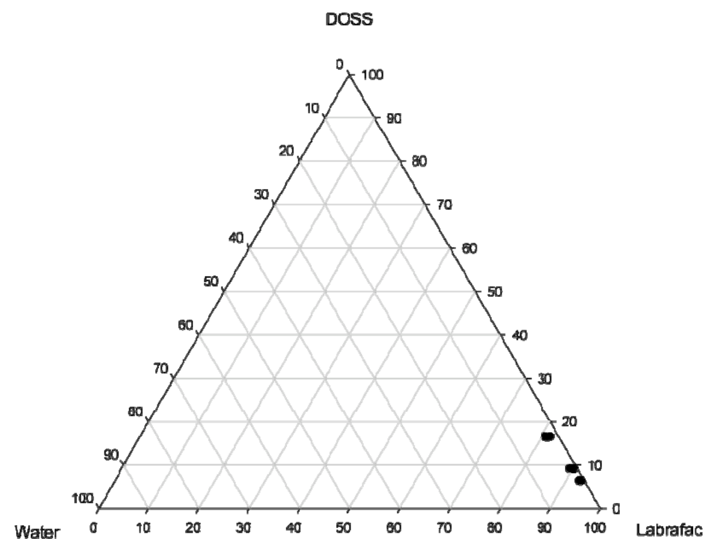

Figure 4.4 Ternary phase diagram representing the microemulsion-forming compositions in DOSS/Labrafac/water systems.

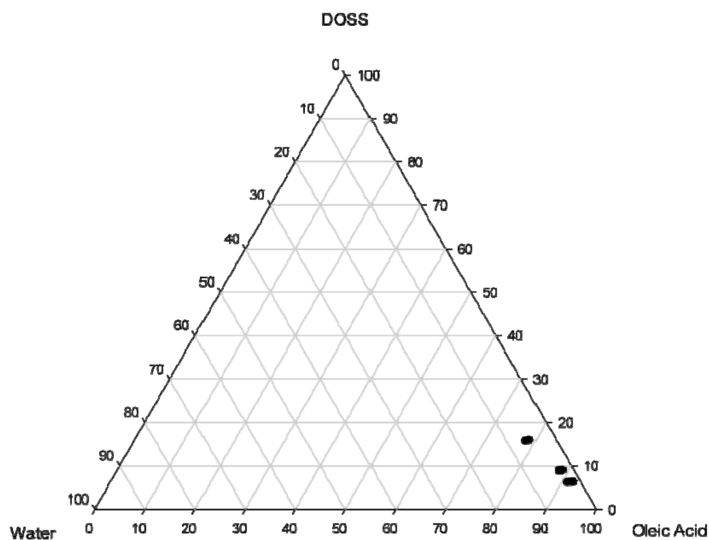

Figure 4.5 Ternary phase diagram representing the microemulsion-forming compositions in DOSS/Oleic Acid/water systems.

Systems containing GTCC, Labrafac, and Oleic acid as the oil showed fairly low incorporation of water to form visually clear microemulsion systems. Because of this, they were not selected for further investigation. The PMP and EO containing systems showed the most potential for microemulsion formation, due to their ability to emulsify a sufficient amount of water (>10% for over half of all surfactant/oil ratios with EO and PMP) while still remaining as a visually clear and homogenous microemulsion. Because of this, these two surfactant/oil systems were chosen for further characterization and drug incorporation.

Knowledge of the hydrophobicity or polarity of the oil can help in determining its microemulsion-forming abilities. Oils can be assigned an equivalent alkane carbon number, or the oil's polarity can be measured by quantifying its miscibility with water alone [1-3]. When mixing the oil and the water, relative volumes added may change if the oil in itself is partly miscible with water. If the oil contains any lipophilic surface-active components, an emulsion may result, even without the addition of a surfactant. EO may be an example of this. Crodamol PMP is not only considered an oil, but it is a good solvent as well. In some formulations, Crodamol PMP is used in part as an emulsifier [4]. Its ability to incorporate such large amounts of water in the microemulsions could be due to the fact that along with adding a surfactant to the system, the oil itself had inherent emulsifying properties. Oleic acid is also used in combination with other components as an emulsifying agent as well. This may explain its ability to incorporate more water than the two triglyceride formulations, GTCC and Labrafac, which do not contain any lipophilic surface-active components. Depending on the size of the oil, relative to the hydrophobic chain of the surfactant, the oil may penetrate into the surfactant tails of the

interfacial monolayer. Smaller oil molecules would position themselves among the surfactant tails, which would increase the effective surfactant hydrophobe volume, effecting the CPP [5].

4.1.1 Effect of Surfactant Concentration on Microemulsion Formation

Surfactant/oil systems of various concentrations were prepared using all the oils tested earlier. The ratio of DOSS/oil systems used in this study include 1:4, 1:5, 1:7, 1:10, and 1:15. All surfactant/oil systems were titrated with water to determine the microemulsion forming regoin. Table 4.1 represents the maximum percent of water incorporated inot different surfactant/oil systems forming a clear and homogenous microemulsion.

Table 4.1 Percent (%) water incorporation in each of the oils for samples (in triplicate).

Oil	1:4	1:5	1:7	1:10	1:15
EO	13.7 ± 0.3	11.1 ± 0.3	8.5 ± 0.5	6.2 ± 0.7	4.0 ± 0.6
PMP	29.7 ± 0.4	23.9 ± 1.3	20.6 ± 0.2	14.3 ± 0.9	11.7 ± 0.3
GTCC	n/a	2.6 ± 0.3	n/a	1.6 ± 0.6	0.9 ± 0.1
Labrafac	n/a	2.3 ± 0.5	n/a	1.1 ± 0.4	0.9 ± 0.1
Oleic Acid	n/a	5.9 ± 0.4	n/a	2.7 ± 0.3	2.1 ± 0.6

Ethyl oleate was the only system in which a visual turbidity appeared while being titrated with water. After some equilibration time, this turbidity would disappear and a clear microemulsion was observed. During titration with water, as the concentration of water came close to the border, requirement for an equilibration time to observe a clear microemulsion decreased. As with all other formulations, once the maximum amount of water that could be emulsified was added, the sample appeared turbid, and did not clear

after 24 hours of equilibration. Because of the observed turbidity at Wo's before the microemulsion border was reached, EO microemulsions were prepared 24 hours in advance before any other tests were performed. This ensured that an equilibrated system was subjected to subsequent characterizations.

4.2 Buffer and pH Effects

The effect of pH on the amount of water emulsifies into a microemulsion was investigated using various buffers. DOSS/EO and DOSS/PMP in a ratio of 1:10 were titrated with buffers of varying pH, and the maximum amount of buffer incorporated into a clear microemulsion was determined (Figure 4.6).

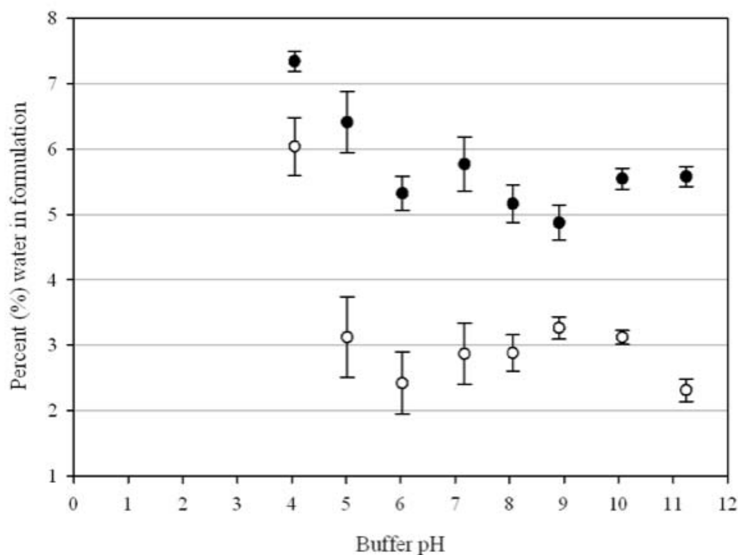

Figure 4.6 Dependence of pH on percent water incorporated as a clear microemulsion in 1:5 DOSS/EO (•) and DOSS/PMP (◦) mixtures

As a general trend, the surfactant/oil mixtures emulsified less buffer than RO water. 1:10 DOSS/EO was able to emulsify 6.2% RO water, and the same ratio of DOSS/PMP could emulsify 14.3% RO water. However, the DOSS/EO mixture was able to incorporate almost 1% more buffer than RO water when titrated with pH 4 acetate. The amount of pH 5 acetate buffer able to be emulsified was almost equal to that of RO water. In DOSS/EO mixtures, buffers from pH 6 to pH 11 significantly reduced the amount of water incorporated as a clear microemulsion.

The total amount of aqueous buffer incorporated into a clear microemulsion in DOSS/PMP mixture was less than 6.5% for buffers of pH 4 to 11. DOSS being an ionic surfactant may possibly interact with salts present in the buffers reducing its ability to properly orient in the interfacial region [6]. The interfacial film may be increasing in disorder, as can sometimes be seen with co-surfactants, due to the presence of the buffer salts resulting in a lessened ability to emulsify water [7]. This phenomenon may be attributed to the reduced amount of water emulsified into a clear microemulsion in these surfactant/oil mixtures.

4.3 Polarized Light Microscopy

Microemulsions are optically isotropic colloidal dispersions that exhibit no birefringence under polarized light, so samples that exhibit no birefringence suggests that they are microemulsions [8]. DOSS/EO and DOSS/PMP in a ratio of 1:15 (surfactant:oil) were used for the polarized light microscopy studies. Compositions that formed visually clear and stable microemulsion represented within the microemulsion forming region in the ternary phase diagram were selected in this study. Similarly, compositions at the

boundary region of microemulsion area and beyond the microemulsion region were also used in the study. Microemulsions possessing Wo values of 25, 30 and 195 were prepared in DOSS/EO mixtures. Sample with Wo = 25 exhibited no birefringence under polarized light, indicating that it was a microemulsion (Figure 4.7).

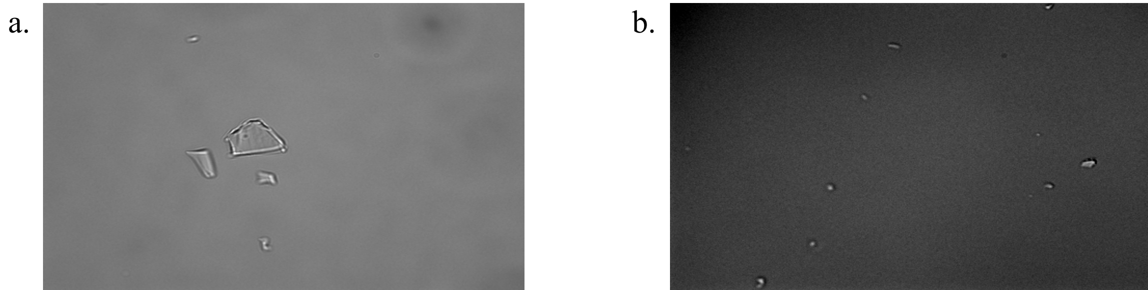

Figure 4.7 Pictures of DOSS/EO Wo=25 without (a.) and with (b.) polarizing filter

Microscope pictures of Wo = 30 with and without polarized lens shows limited structural delineations indicating that the formulations contained structures other than droplets. These formulations represent compositions that are leaving the microemulsion forming region in the ternary phase diagram (Figure 4.8). Contrasting the earlier result was the Wo = 195 sample that exhibited a droplet structure visible under normal and polarized light. This indicates formation of a macroemulsion at this composition (Figure 4.9).

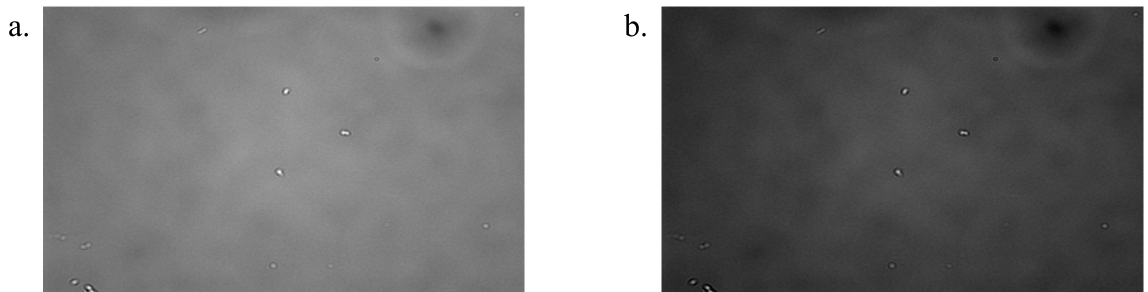

Figure 4.8 Pictures of DOSS/EO Wo=30 without (a.) and with (b.) polarizing filter

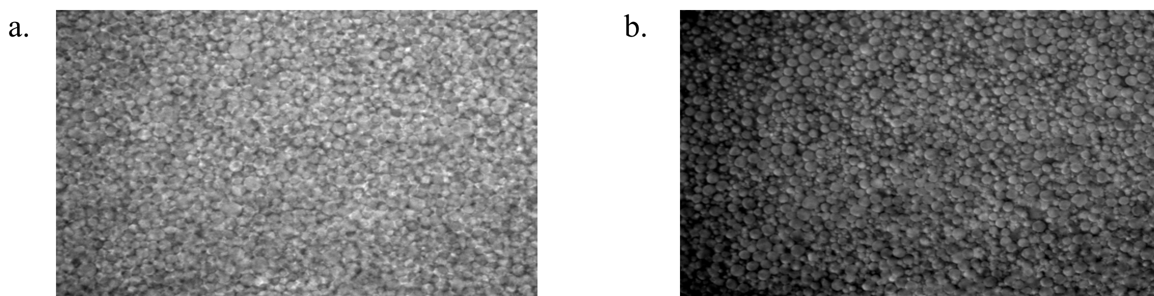

Figure 4.9 Pictures of DOSS/EO Wo=195 without (a.) and with (b.) polarizing filter

Microemulsions prepared in DOSS/PMP mixtures incorporated larger quantity of water when compared to DOSS/EO mixtures. Microemulsions possessing Wo values of 25, 75, and 95 were prepared, representing samples that were within the microemulsion region, at the microemulsion border, and beyond the microemulsion region respectively. When observed under the microscope, DOSS/PMP samples with Wo values of 25 and 75 showed no birefringence through a polarizing filter and represent microemulsions (Figures 4.10 and 4.11). The formulation with a Wo of 95 exhibited bicontinuous structures under the polarized lens (Figure 4.12), indicating that it was beyond the optically isotropic microemulsion region. Air bubbles in the sample that were trapped under the slipcover are visible in Figure 4.9, and debris that was present can be seen in Figure 4.10.

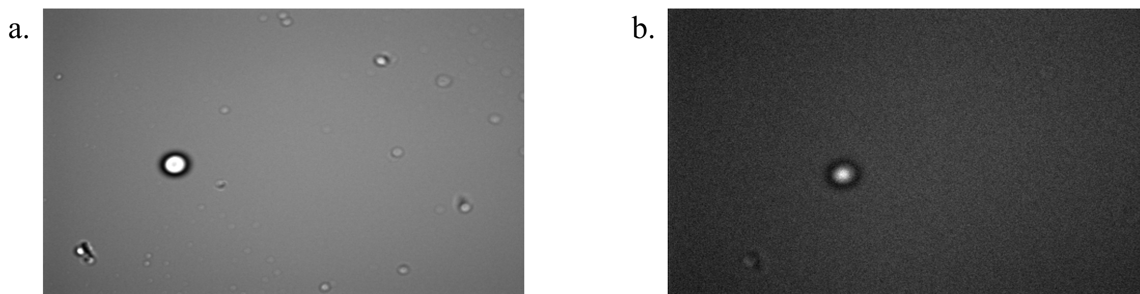

Figure 4.10 Pictures of DOSS/PMP Wo=25 without (a.) and with (b.) polarizing filter

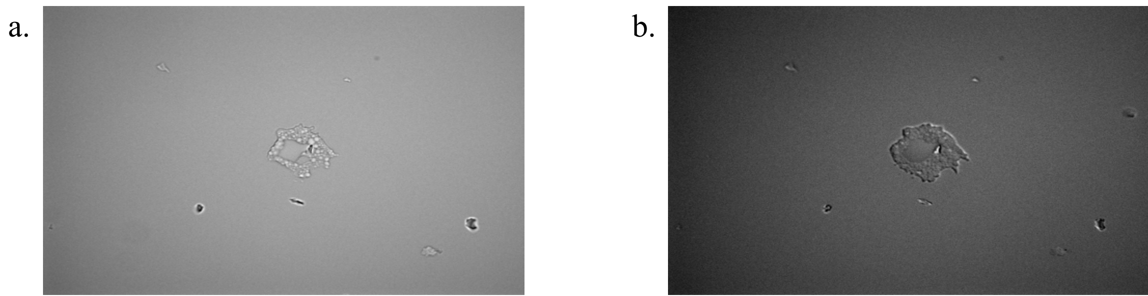

Figure 4.11 Pictures of DOSS/PMP Wo=75 without (a.) and with (b.) polarizing filter

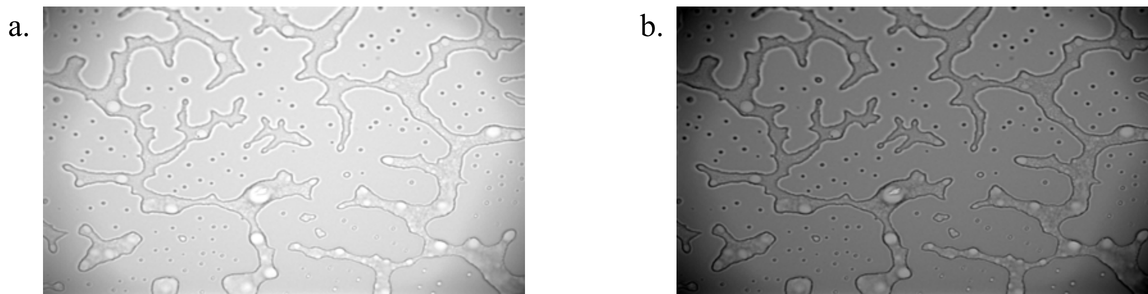

Figure 4.12 Pictures of DOSS/PMP Wo=95 without (a.) and with (b.) polarizing filter

4.4 Conductivity

Electrical conductivity testing was done using DOSS/EO and DOSS/PMP mixtures in ratios of 1:5, 1:10 and 1:15. The amount of water present in the formulations was modulated by varying the Wo value. The observed conductivity values were then plotted as a function of Wo. All formulations demonstrated composition dependent changes in conductivity. Conductivity values of 0.011 μS/cm, 0.033 μS/cm, and 0.122 μS/cm were observed at small Wo's. This indicates that oil, which is known to have low conductivity, is the continuous phase and water droplets surrounded by surfactant constitute the internal phase. A percolation phenomenon is often observed for water-in-oil (w/o) emulsions where the electrical conductivity steeply rises above a certain threshold as the water concentration increases, as if water was becoming the continuous phase [9, 10].

This was observed in both DOSS/EO and DOSS/PMP microemulsions at high Wo values (Figure 4.13-4.15).

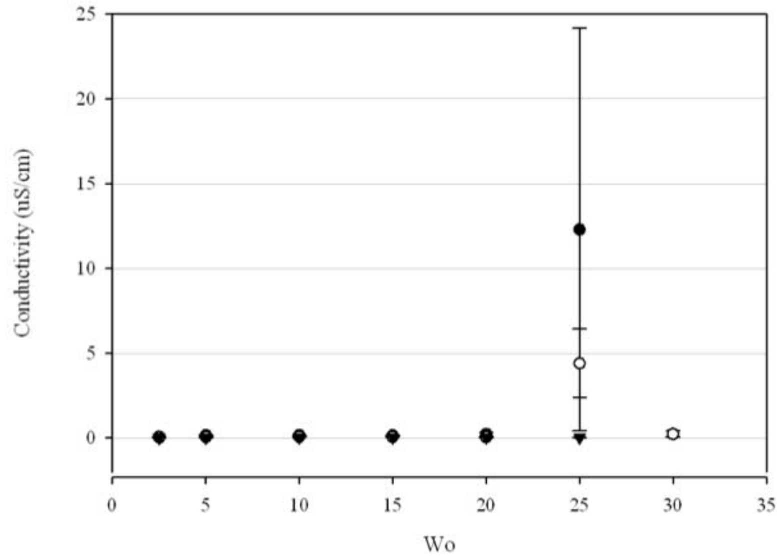

Figure 4.13 Conductivities for microemulsions at various Wo's for DOSS/EO ratios 1:5 (•), 1:10 (◦) and 1:15 (▼).

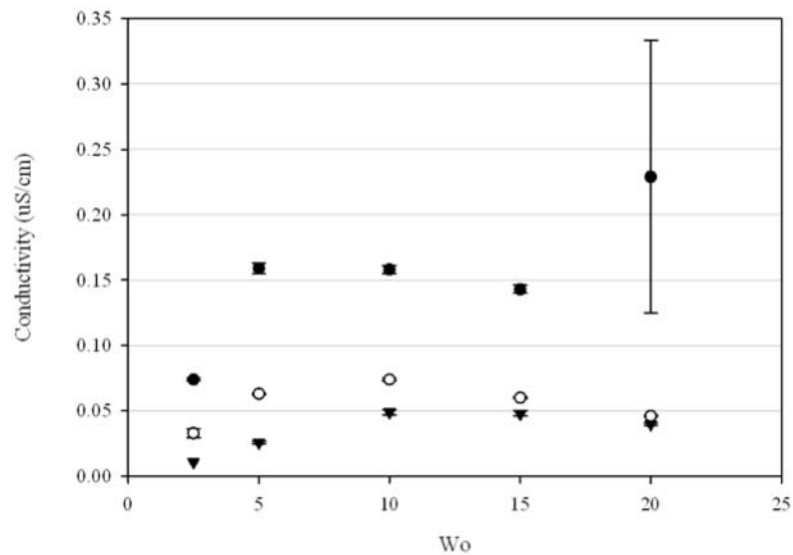

Figure 4.14 Conductivities for microemulsions from Wo = 2.5 to Wo = 20 for DOSS/EO ratios 1:5 (•), 1:10 (◦) and 1:15 (▼).

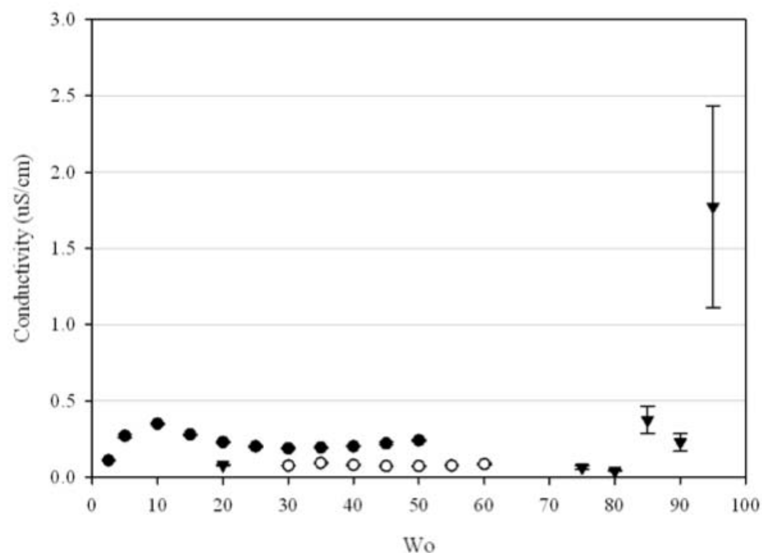

Figure 4.15 Conductivities for microemulsions at various Wo's for DOSS/PMP ratios 1:5 (•), 1:10 (◦) and 1:15 (▼).

A percolation like phenomenon was observed in microemulsions prepared in 1:5 DOSS/EO mixtures as demonstrated by large fluctuations in conductivity values as Wo value changed from 20 to 25. In this surfactant/oil ratio, a Wo value of 20 still represents a microemulsion formulation, but a Wo of 25 is beyond the microemulsion region. In the 1:5 DOSS/EO system, a formulation with Wo value of 25 appeared turbid, further demonstrating that the system had crossed the water-in-oil microemulsion threshold. These conductivity results also correlate with the data obtained from phase diagrams constructed during the microemulsion formulation.

In 1:10 DOSS/EO mixture, a significant increase in conductivity was recorded when Wo changed from 20 (6.7% w/v water) to 25 (8.3% w/v water). The sample containing 6.7% (w/v) water is still in microemulsion region, but 8.3% (w/v) water is outside of

microemulsion region. Visible cloudiness was observed in Wo of 25 and 30 formulations indicating that they are not microemulsions. The Wo 30 formulation prepared in 1:10 DOSS/EO mixture separated into constituent phases while measurements were performed.

In 1:5 DOSS/EO mixture, a change in the Wo value from 2.5 to 25 did not produce significant changes in the measured conductivity values. This indicates that all mixtures with Wo's from 2.5 to 25 are within the microemulsion region. None of the samples showed visible cloudiness or turbidity, further validating the determination that they were all within the microemulsion region.

DOSS/PMP formulations are able to emulsify a greater amount (29.7%) of water as a water-in-oil microemulsion than DOSS/EO formulations, as was seen in the formulation phase diagrams. Because of this, Wo values of up to 100 could be tested for electrical conductivity. A sudden increase in conductivity along with fluctuating values were noted when the Wo reached 95 in a 1:15 DOSS/PMP mixture. The dramatic increase in conductivity correlated with the observation of visual cloudiness in the sample. The percolation phenomenon has been noticed and studied by many [11-13]. W/o microemulsions systems, such as those seen in this research, especially have exhibited this percolation behavior throughout a variety of microemulsion studies [14-16]. The formation of a bicontinuous state has been suggested to aid percolation. Feldman et al. and Mukhopadhyay et al. suggest that the conductivity increase seen in percolation is due to the counterions moving throughout the bicontinuous water channels resulting in an increase in conductivity [17-19].

4.5 Rheology

W/o microemulsions are known to exhibit Newtonian flow properties as well as relatively low viscosities [9, 13, 20-23]. DOSS/EO microemulsion samples with Wo values of 2.5, 5, and 7, exhibited Newtonian flow properties. As shear rate varied, viscosity remained relatively constant. This can be seen in Figure 4.16. This consistent with rheological behavior reported in other microemulsions [21, 24].

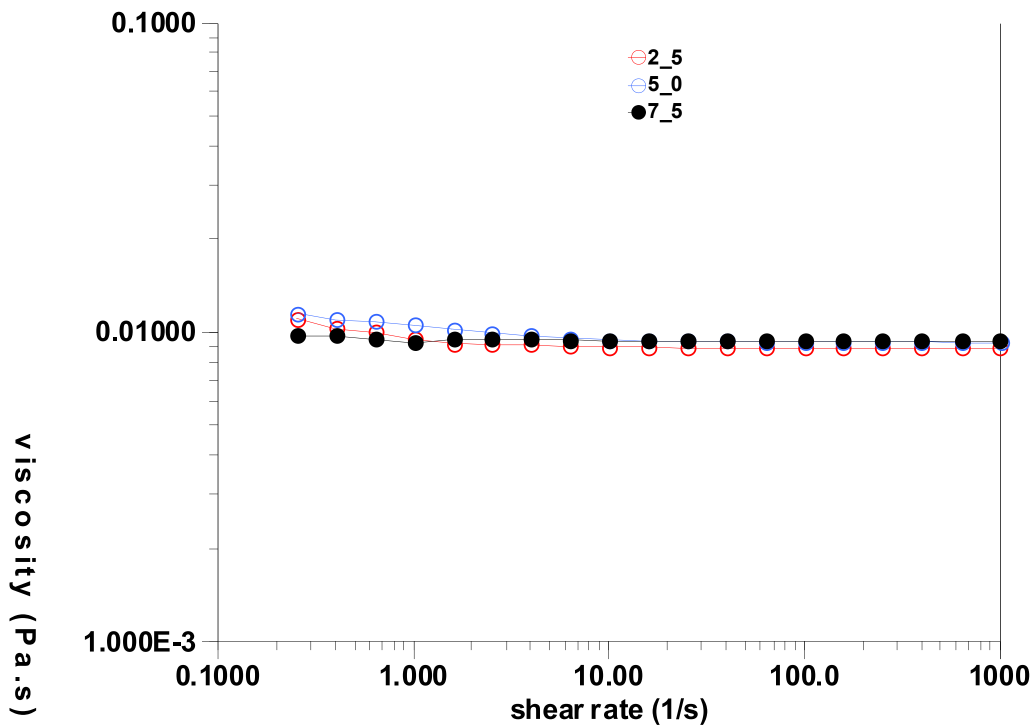

Figure 4.16 Graph showing viscosity of DOSS/EO microemulsions with Wo's of 2.5, 5, and 7.5 at varying shear rates.

Microemulsion samples showed relatively low viscosities, close to that of the oil used, ethyl oleate. Viscosity slightly increased with increasing water concentration, which is

probably due to an increase in the droplet sizes. Viscosities of the microemulsion samples can be seen in Table 4.2.

Table 4.2 Determined viscosity for various Wo values

Wo	Viscosity (Cps)
2.5	9.05
5	9.48
7.5	9.61

Viscosity sheds light on the internal structures of microemulsions [13]. Since viscosity of the systems is affected by these internal structures, the slight increases in viscosity are probably due to the increase in droplet sizes with increasing Wo values. Similar viscosities have been reported for microemulsions in other research [20, 21, 23].

4.6 Dynamic Light Scattering

Particle size was determined for a variety of microemulsion samples. Particle sizes for DOSS/EO and DOSS/PMP microemulsions can be seen in Figures 4.17 and 4.18. Particle sizes for DOSS/EO microemulsions ranged from 1.1nm to 18nm in diameter. For all three concentrations, particle sizes followed the general trend of small particles at low Wo values. As Wo values increase, more water is added to the system, and the particle sizes increase with droplet swelling. This effect of increasing particle size with increasing internal phase concentration has been noted in other research as well [25-27].

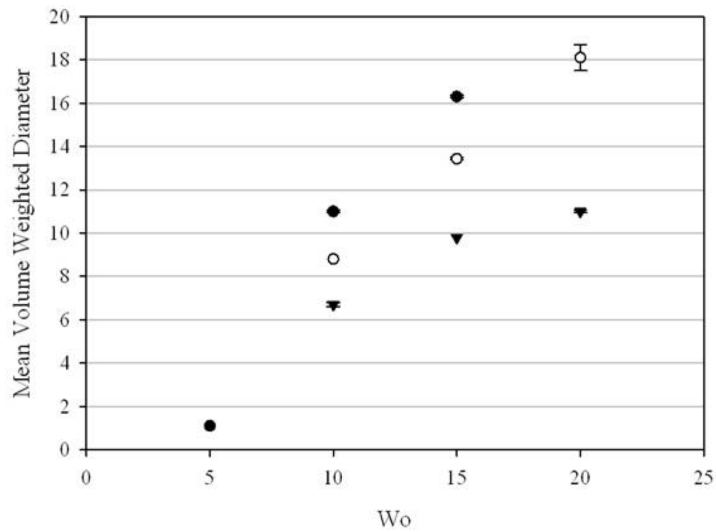

Figure 4.17 Graph of mean volume weighted diameter for microemulsions prepared with DOSS/EO mixtures 1:5 (•), 1:10 (◦) and 1:15 (▼).

Similar results were seen with the DOSS/PMP microemulsion systems. Particle sizes ranged from 6.7 to 26.6 nm in diameter. Larger Wo values were tested for DOSS/PMP formulations since DOSS/PMP mixture could emulsify a larger percent of water.

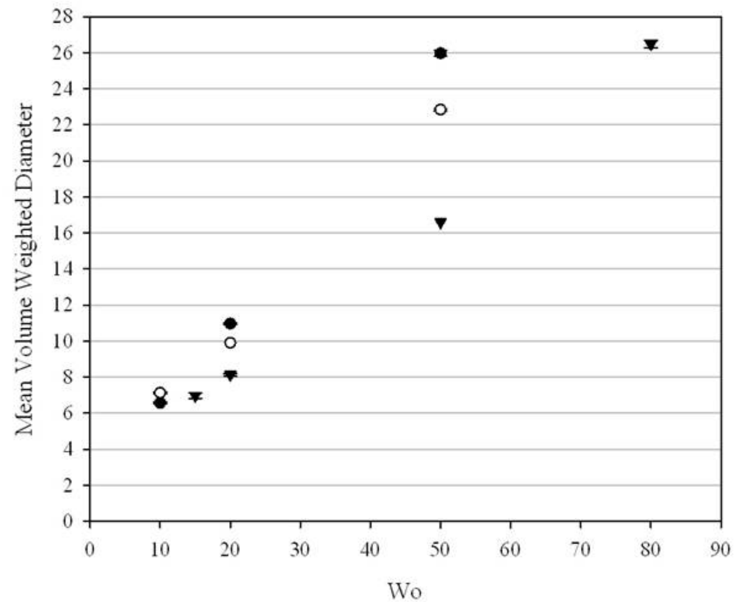

Figure 4.18 Graph of mean volume weighted diameter for microemulsions prepared with DOSS/PMP mixtures 1:5 (•), 1:10 (◦) and 1:15 (▼).

A general trend that was observed for both DOSS/EO and DOSS/PMP systems was that the particle size increased with an increase in surfactant concentration at a particular Wo value. This is most likely due to the fact that with more surfactant in the formulation, a larger volume of the internal phase will be emulsified, ultimately resulting in larger droplets.

4.7 Development of Sterilization Method

An aseptic filtration method of sterilization that can be universally applied to similar microemulsion formulations was developed using DOSS/EO microemulsions that were formulated in this research project. Microemulsions were prepared using 1:5 DOSS/EO and sterile water for injection to achieve Wo's of 2.5, 10 and 15. Microemulsions were withdrawn into a sterile syringe and passed through a 0.22 μm filter directly onto an MH Agar plate in a laminar hood. After allowing the formulation to absorb into the agar, the plates were incubated and checked for microbial growth after 24 hours. Growth is generally indicated by the cloudy appearance of the agar after incubation. All three samples showed white cloudiness on the plates after 24 hours incubation. Upon visual inspection and dissection of the agar, it was determined that the cloudiness was within the agar and not on the surface, where colonies are generally found. The water used in the formulations was commercial sterile water for injection and was therefore ruled out as a possible source of the cloudiness.

Bacteria cannot grow in oil, because water is necessary for their growth [28]. It may be possible, though, for microbes to be dormant within oil, so EO and the DOSS/EO mixture were further examined. To determine the source of the cloudiness, EO and the DOSS/EO

mixture were plated on MH Agar plates. Filtered and unfiltered EO and DOSS/EO mixture were plated on MH agar plates. Four agar plates containing the test liquid were incubated for 24 hours and checked for signs of microbial growth. The plates containing the unfiltered and filtered EO showed no cloudiness. However, the plates containing both filtered and unfiltered DOSS/EO mixtures displayed white cloudiness similar to that seen with the microemulsion samples. This led to the conclusion that DOSS is the source of the cloudiness.

The white cloudiness in the agar plates that contained DOSS could be the result of DOSS precipitation since it is of itself a white, waxy substance that is not very soluble in water, which is a main component of MH agar [29]. As the microemulsions permeate into the agar, it is possible that the emulsion breaks apart allowing the white, waxy DOSS to be visible. To further examine the possibility that the cloudiness was caused by the formulation itself and was not due to microbial growth, filtered microemulsion samples and sections of the cloudy MH agar were investigated. Small squares of the cloudy MH agar from the microemulsion samples and from the DOSS/EO samples were excised and placed into individual test tubes that contained MH broth. Filtered microemulsions were directly introduced into test tubes containing MH broth as well, which turned cloudy upon vortexing. After 24 hours of incubation, samples were taken from each of the test tubes, applied to slides, and gram-stained to determine the presence of bacteria. When all slides were observed under a microscope after gram staining, tiny irregularly shaped objects were visible. These were significantly smaller than bacteria, and were not the shapes of bacteria that are usually seen from gram-staining [30]. This experiment confirmed the identity of the tiny irregularly shaped objects as precipitated DOSS.

4.8 Validation of Sterilization Method

Formulations identical to those used in sterility testing were used in the validation of sterility. Samples were analyzed using a direct inoculation technique as well as filtration, utilizing two different broths according to USP standards. Fluid thioglycollate medium (FTM) is used for the growth and detection of anaerobic bacteria [30-33]. Soybean casein digest medium or tryptic soy broth (TSB) is used for the detection of fungi and aerobes [30, 33].

Samples were filtered through a 0.22 μm filter system under aseptic conditions. As per USP standards, the sample was passed through the filter, and the filter was rinsed with sterile water [33]. The filter was then cut into three pieces and placed in test tubes that contained TSB (stored at 20°C), TSB (stored at 35°C), or FTM (stored at 35°C). The samples stored at 35°C mimicked temperatures close to body temperature which promote the growth of aerobic and anaerobic bacteria. Samples stored at room temperature (20°C) were used for the detection of fungi [30]. A representative picture of the test tubes containing the filters can be seen in Figure 4.19. All filtration test tubes except for the first positive control remained clear throughout the 14 days of the study.

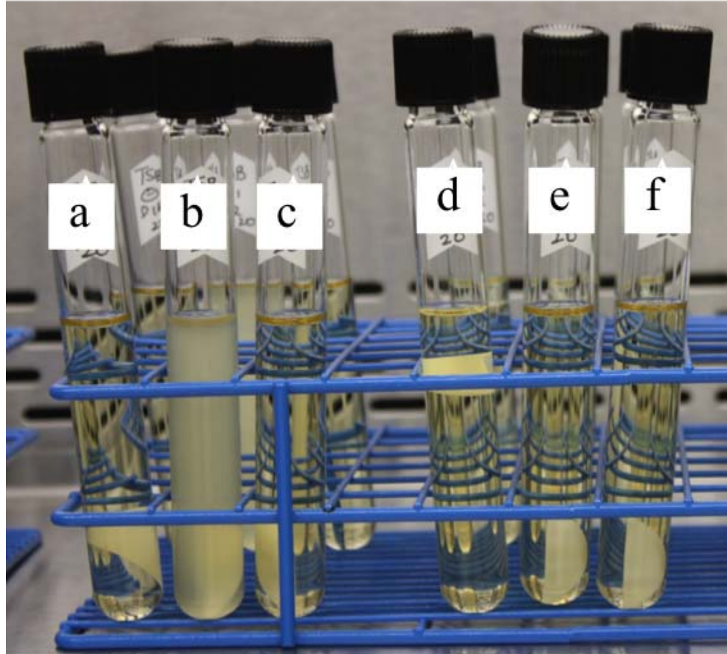

Figure 4.19 Representative picture of test tubes containing filters with the negative control (a), first positive control (b), second positive control (c), Wo = 2.5 (d), Wo = 10 (e), and Wo = 15 (f). Test tubes remained visually the same over the 14 day testing period.

The same samples were directly inoculated into test tubes of media. After sitting, the direct innoculation tubes that contained the microemulsion samples separated into three layers (Figure 4.20). Before samples were taken to plate on the blood agar, the TSB test tubes were vortexed to ensure uniformity. The negative control and second positive control were the only tubes that remained clear throughout the entire 14 day testing period. All others, upon vortexing, were uniformly turbid.

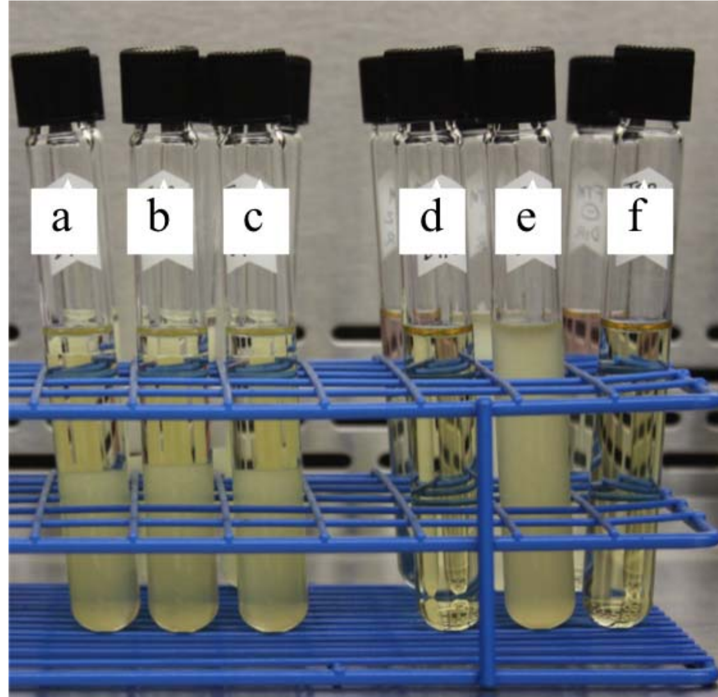

Figure 4.20 Representative picture of test tubes from direct inoculation with Wo = 15 (a), Wo = 10 (b), Wo = 2.5 (c), second positive control (d), first positive control (e), and negative control (f). Three layers can be seen in the Wo = 2.5, 10, and 15 samples, and test tubes remained visually the same over the 14 day testing period.

The USP indicates that if the material being tested for sterility renders the medium turbid when directly inoculated, the samples are to be diluted in the medium [33]. This cannot be done for the microemulsion formulations being tested. As was seen when formulation and phase diagram studies were performed, once the microemulsions reached a certain water concentration, the formulations appeared turbid. Since the media used were water based, dilution of the samples would only add more water to the systems and increase their turbidity. Since turbidity of the test tubes was not able to be used as the detection of microbes as per the USP standards, another method was employed.

To detect the presence of microbes, samples from the test tubes were taken and transferred onto blood agar plates. Samples were taken from the filtration and direct inoculation test tubes on days 0, 7, and 14 for plating. After 24 hours incubation at 35°C, the plates could then be inspected for microbial growth (Table 4.3).

Table 4.3 Summary of results on blood agar plates indicating the presence (+) or absence (**-**) of growth.

Day 0	Direct			Filtration		
	FTM	TSB	TSB 20	FTM	TSB	TSB 20
Negative Control	-	-	-	-	-	-
First Positive Control	+	+	+	-	-	+
Second Positive Control	-	-	-	-	-	-
Wo = 2.5	-	-	-	-	-	-
Wo = 10	-	-	-	-	-	-
Wo = 15	-	-	-	-	-	-
Day 7						
Negative Control	-	-	-	-	-	-
First Positive Control	+	+	+	+	+	+
Second Positive Control	-	-	-	-	-	-
Wo = 2.5	-	-	-	-	-	-
Wo = 10	-	-	-	-	-	-
Wo = 15	-	-	-	-	-	-
Day 14						
Negative Control	-	-	-	-	-	-
First Positive Control	+	+	+	+	+	+
Second Positive Control	-	-	-	-	-	-
Wo = 2.5	-	-	-	-	-	-
Wo = 10	-	-	-	-	-	-
Wo = 15	-	-	-	-	-	-

Day 0 showed slight growth only on 4 of the 6 first positive control plates. All samples plated on Days 7 and 14 showed no growth except for the first positive control. Negative control plates were always absent of microbial growth. Only the first positive control showed growth on the plates, as well as turbidity in the test tubes. The second positive control was most likely too low of a bacteria concentration, and therefore did not show any growth. Plates with the microemulsion samples did not show microbial growth. Direct inoculation microemulsion plates showed a transparent ring in the blood agar where the microemulsion sample was most concentrated. It appeared that the microemulsion samples hemolyzed the red blood cells in the agar plates (Figures 4.21b,c). Representative pictures of the blood agar plates can be seen in Figure 4.21a-4.21f.

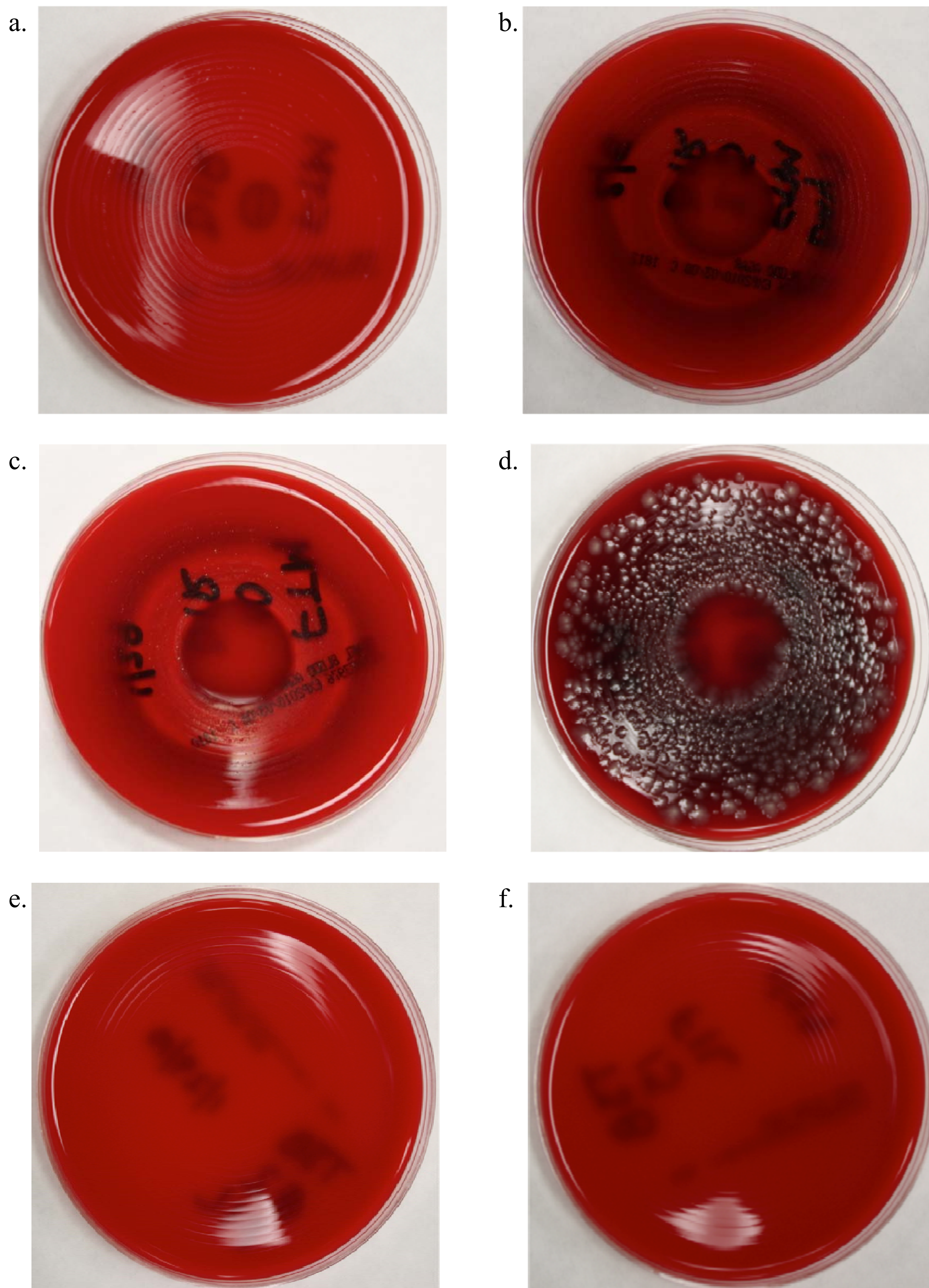

Figure 4.21 Representative pictures of blood agar plates: direct inoculation of the negative control (a), Wo = 2.5 (b), and Wo = 10 (c), and filtration of first positive control (d), second positive control (e), and Wo = 15 (f).

The results obtained from Days 0, 7, and 14 validate the aseptic filtration method for the DOSS/EO microemulsions.

4.9 Cell Toxicity Studies

A neutral red assay was performed on the cells that had been exposed to microemulsions of varying DOSS/EO concentrations and Wo values. In this assay, the dye is absorbed by live cells and stains the mitochondria within. Cells that are not living cannot retain the dye when washed. After being exposed to dye and then washed, all color that remains will be due only to the living cells that absorbed and retained the dye [34]. Absorbance readings were taken for each well. To determine cell toxicity, the absorbance of the wells that were exposed to microemulsion samples were compared to a control well which contained cells without a microemulsion treatment. Treatments were considered to be non-toxic if the absorbance from the cells treated with a microemulsion was similar to or the same as the absorbance from the control well. Along with the neutral red assay, a Bradford protein assay was performed to standardize the absorbance readings. The assay stains proteins so the number of cells present in each well could be determined. This allows you to relate the total number of cells in the well to the absorbance of the live cells. From this, cell toxicity can ultimately be determined when comparing the treated wells to the control well.

An overall summary of the cell toxicity results can be seen in Table 4.4. When compared to the control cells, the only DOSS/EO microemulsion samples that showed toxicity were samples prepared with 1:15 DOSS/EO mixture having Wo's of 10 and 15.

Table 4.4 Summary of results showing absence (-) and presence (T) of toxicity with 1:5, 1:7, 1:10, and 1:15 DOSS/EO microemulsions of varying Wo's

DOSS/EO Ratio	Wo Values			
	2.5	**5**	**10**	**15**
1:5	-	-	-	-
1:7	-	-	-	-
1:10	-	-	-	-
1:15	-	-	T	T

As a general rule, the higher the surfactant concentration, the more water can be incorporated into the system. DOSS/EO mixture in the highest surfactant ratio of 1:4 was able to emulsify the largest amount of water (13.7%) while the lowest surfactant concentration of 1:15 emulsified the lowest (4.0%). Toxicity was seen only in 1:15 DOSS/EO mixtures with Wo values of 10 and 15. The media added in this experiment is water-based. Because of this, when the media is added to the wells, it ultimately results in a net increase in the amount of water present in the internal phase of the formulation. Since the formulations tested were w/o microemulsions the addition of more water to the systems does not constitute a simple dilution. As stated earlier, the 1:15 DOSS/EO mixture emulsifies the least amount of water of all DOSS/EO mixtures. The 1:15 DOSS/EO microemulsion samples with Wo values of 10 and 15 already contained close to the maximum amount of water for that system. When the aqueous media was added over the layer of these formulations in the wells a breach of the percolation threshold can occur. This results in turbidity and increased viscosity of the original microemulsion. Because these two samples were no longer transparent microemulsions after the media was added, and the systems were more viscous, the media may not sufficiently reach the

cells in order to keep them viable. All other formulations and dilutions tested were able to incorporate more water, which allowed them to remain as intact microemulsions even upon addition of the media.

4.10 Acyclovir Formulations

A solution of acyclovir was titrated with DOSS/EO and DOSS/PMP concentrations 1:5, 1:10, and 1:15. Similar trends were seen with the incorporation of acyclovir solution as were seen with RO water. 1:5 DOSS/oil mixtures emulsified the most acyclovir solution followed by 1:10, and then 1:15 with the least. Also, DOSS/PMP mixture emulsified a larger amount of acyclovir solution than DOSS/EO mixture. The percent w/w acyclovir solution emulsified in the respective DOSS/oil mixtures can be seen in Table 4.5.

Table 4.5 Percent (%) acyclovir solution emulsified by DOSS/EO and DOSS/PMP mixtures of 1:5, 1:10, and 1:15 (in triplicate).

	1:5	1:10	1:15
EO	12.99 ± 0.38	7.20 ± 0.17	4.18 ± 0.07
PMP	29.40 ± 0.06	19.55 ± 0.17	14.91 ± 0.42

A slight increase in the quantity of acyclovir solution capable of incorporation in the DOSS/EO and DOSS/PMP mixtures was observed. One possibility for this may be that the drug is slightly surface active in itself. A large number of drug molecules have been shown to be surface active [5, 35].

4.11 Dissolution Studies

The calibration curve obtained for methylene blue standard solutions can be seen Figure 4.22. The equation for the line was calculated to be y = 0.0251 + 53.9272x with an R^2 value of 0.9925.

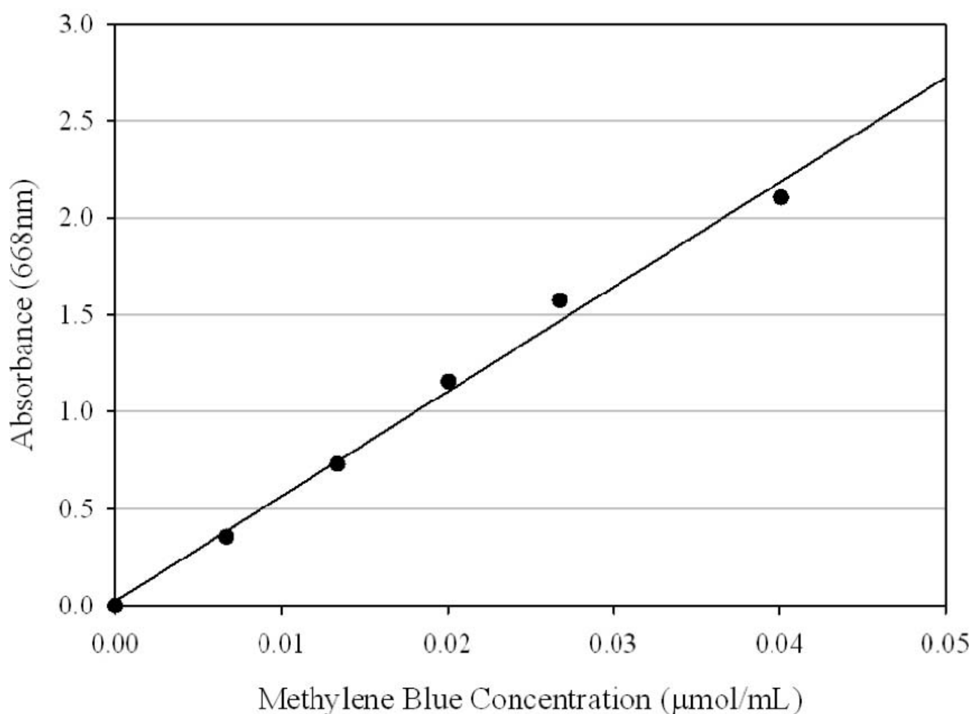

Figure 4.22 Calibration curve for dissolution studies, where y = 0.0251 + 53.9272x.

Absorbances obtained from the release study were substituted into the equation from the calibration curve to determine the concentration of methylene blue present. This was plotted versus time to determine a dye release profile (Figure 4.23).

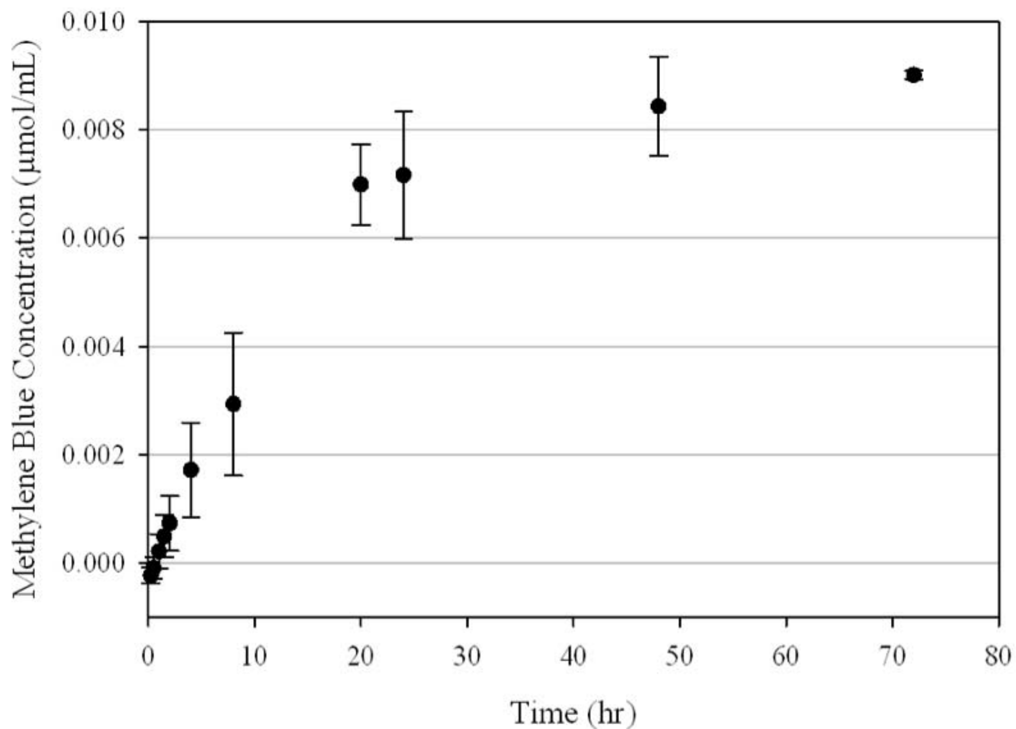

Figure 4.23 Release profile of methylene blue from DOSS/EO microemulsion

Methylene blue release was recorded over a 72-hour time period. Release seemed to begin to plateau between 48 and 72 hours. At 72 hours in the experiment, all three trials released approximately 0.009 μmol/mL of methylene blue. The dissolution profile demonstrates a controlled release pattern for the model drug.

Chapter 5

Conclusions

Microemulsions were formulated with water, diocytl sodium sulfosuccinate, and two oils, EO and PMP. Characterization through conductivity, rheology, polarizing light microscopy, and DLS helped to define the clear, microemulsion forming regions. Microemulsions exhibited Newtonian flow and relatively low viscosities slightly higher than the viscosity of the oil itself. No birefringence was observed in microemulsion-forming compositions. Conductivities were low indicating that the systems were water-in-oil microemulsions. Once a certain concentration of water was added, a percolation phenomenon was observed where the conductivity drastically increased, indicating the water was becoming the continuous phase of the system. DLS measurements indicated that particle sizes were below 30nm for all Wo values in DOSS/EO and DOSS/PMP microemulsion systems.

When compared to RO water, buffer and pH appeared to have an impact of the percent water incorporated in microemulsions. In general, a smaller percentage of buffer was able to be incorporated into both DOSS/EO and DOSS/PMP systems than RO water. An acyclovir solution was successfully incorporated into mixtures of DOSS/EO and DOSS/PMP. The percentage of acyclovir solution able to be emulsified by DOSS/EO and

DOSS/PMP mixtures was slightly higher than RO water. The percent RO water and percent acyclovir solution able to be incorporated into the DOSS/EO and DOSS/PMP microemulsions indicates that these formulations show potential for drug delivery applications.

An aseptic filtration method of sterilization that can be universally applied to similar microemulsion formulations was successfully developed and validated using DOSS/EO microemulsion systems as a model. The sterilization of the microemulsions shows potential to be used in ophthalmic formulations. Preliminary toxicity studies using 3T3 cells lines show little toxicity of the DOSS/EO microemulsion systems.

References

Chapter 1

1. Capek, I., *Radical polymerization of polar unsaturated monomers in direct microemulsion systems.* Adv Colloid Interface Sci, 1999. **80**(2): p. 85-149.

2. Gelbart, W.M. and A. BenShaul, *The "new" science of "complex fluids".* Journal of Physical Chemistry, 1996. **100**(31): p. 13169-13189.

3. Holmberg, K., *Handbook of applied surface and colloid chemistry*. 2002, Chichester ; New York: Wiley.

4. Bankcroft, W.D., *The theory of emulsification.* Journal of Physical Chemistry, 1913. **17**.

5. Langevin, D., *Microemulsions.* Accounts of Chemical Research, 1988. **21**(7): p. 255-260.

6. Sonneville-Aubrun, O., J.T. Simonnet, and F. L'Alloret, *Nanoemulsions: a new vehicle for skincare products.* Adv Colloid Interface Sci, 2004. **108-109**: p. 145-9.

7. Shafiq, S., et al., *Development and bioavailability assessment of ramipril nanoemulsion formulation.* Eur J Pharm Biopharm, 2007. **66**(2): p. 227-43.

8. Shafiq-un-Nabi, S., et al., *Formulation development and optimization using nanoemulsion technique: a technical note.* AAPS PharmSciTech, 2007. **8**(2): p. Article 28.

9. El-Laithy, H.M., *Preparation and Physiochemical Characterization of Diocytl Sodium Sulfosuccinate (Aerosol OT) Microemulsion for Oral Drug Delivery.* AAPS PharmSciTech, 2003. **4**(1): p. Article 11.

10. Moulik, S.P. and B.K. Paul, *Structure, dynamics and transport properties of microemulsions.* Advances in Colloid and Interface Science, 1998. **78**: p. 99-195.

11. Zana, R., *Dynamics of surfactant self-assemblies : micelles, microemulsions, vesicles, and lyotropic phases*. Surfactant science series. 2005, New York: Marcel Dekker/CRC Press.

12. Gerbacia, W. and H.L. Rosano, *Microemulsions. Formation and stabilization.* Journal of Colloid and Interface Science, 1973. **44**(2): p. 242-248.

13. Shinoda, K. and H. Kunieda, *Conditions to produce so-called microemulsions: factors to increase the mutual solubility of oi l and water by solubilizer.* Journal of Colloid and Interface Science, 1973. **42**.

14. Singh, H.N., et al., *Structural description of wate r-in-oil microemulsions using electrical resistance.* Berichte der Bunsen-Gesellschaft, 1983. **87**(12): p. 1115-1120.

15. Giustini, M., S. Murgia, and G. Palazzo, *Does the Schulman's titration of microemulsions really prov ide meaningful parameters?* Langmuir, 2004. **20**(18): p. 7381-4.

16. Giustini, M., et al., *Microstructure and dynamics of the water-in-oil CTAB/n-pentanol/n-hexane/water microemulsion: A spectroscopic and conductivity study.* Journal of Physical Chemistry, 1996. **100**(8): p. 3190-3198.

17. Lawrence, M.J. and G.D. Rees, *Microemulsion-based media as novel drug delivery systems.* Adv Drug Deliv Rev, 2000. **45**(1): p. 89-121.

18. Lee, V.H., *Enzymatic barriers to peptide and protein absorption.* Crit Rev Ther Drug Carrier Syst, 1988. **5**(2): p. 69-97.

19. Gasco, M.R., F. Pattarino, and F. Lattanzi, *Long-acting delivery systems for peptides: reduced plasma testosterone levels in male rats after a single injec tion.* Int J Pharm, 1990. **62**: p. 119-123.

20. Jahn, W. and R. Strey, *Microstructures of microemulsions by freeze fracture electron microscopy.* J. Phys Chem, 1988. **98**.

21. Scriven, L.E., *Equilibrium bicontinuous structures.* Nature (London), 1976. **263**.

22. Liu, H., et al., *Bicontinuous cyclosporin a loaded water-AOT/Tween 85-isopropylmyristate mic roemulsion: stru ctural characterization and dermal pharmacokinetics in vivo.* J Pharm Sci, 2009. **98**(3): p. 1167-76.

23. Bolzinger, M.A., T.C. Carduner, and M.C. Poelman, *Bicontinuous sucrose ester microemulsion: a new vehicle for t opical delivery of niflamic acid.* Int J Pharm, 1998. **176**: p. 39-45.

24. Carlfors, J., I. Blute, and V. Schmidt, *Lidocaine in microemulsion- a dermal delivery system.* J. DIsp. Sci. Technol, 1991. **12**: p. 467-482.

25. Corswant, C.v., P. Thoren, and S. Engstrom, *Triglyceride-based microemulsion from intravenous administration of sparingly soluble substances.* J Pharm Sci, 1998. **87**: p. 200-208.

26. Lange, K.R., *Surfactants : a practical handbook*. 1999, Munich Cincinnati: Hanser Publishers ; Hanser Gardner Publications. xiii, 237 p.

27. Tadros, T.F., *Surfactants*. 1984, London ; Orlando: Academic Press. xii, 342 p.

28. Kibbe, A.H., *Handbook of Pharmaceutical Excipients*. 3rd ed. 2000, London: Pharmaceutical Press.

29. Djordjevic, L., et al., *Characterization of caprylocaproyl macrogolglycerides based microemulsion drug delivery vehicles for an amphiphilic drug.* Int J Pharm, 2004. **271**(1-2): p. 11-9.

30. El Maghraby, G.M., *Transdermal delivery of hydrocortisone from eucalyptus oil microemulsion: effects of cosurfactants.* Int J Pharm, 2008. **355**(1-2): p. 285-92.

31. Junyaprasert, V.B., et al., *Aerosol OT microemulsions as carriers for transdermal delivery of hydrophobic and hydrophilic local anesthetics.* Drug Deliv, 2008. **15**(5): p. 323-30.

32. Hait, S.K. and S.P. Moulik, *Interfacial composition and thermodynamics of formation of water/isopropyl myristate water-in-oil microemulsions stabilized by butan-1-ol and surfactants like cetyl pyridinium chloride, cetyl trimethyl ammonium bromide, and sodium dodecyl sulfate.* Langmuir, 2002. **18**(18): p. 6736-6744.

33. Shakeel, F., et al., *Nanoemulsions as vehicles for transdermal delivery of aceclofenac.* AAPS PharmSciTech, 2007. **8**(4): p. E104.

34. Jadhav, K.R., et al., *Applications of microemulsion based drug delivery system.* Curr Drug Deliv, 2006. **3**(3): p. 267-73.

35. Amidon, G.L., et al., *A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability.* Pharm Res, 1995. **12**(3): p. 413-20.

36. Kogan, A. and N. Garti, *Microemulsions as transdermal drug delivery vehicles.* Adv Colloid Interface Sci, 2006. **123-126**: p. 369-85.

37. Ho, H.O., C.C. Hsiao, and M.T. Sheu, *Preparation of microemulsions using polyglycerol fatty acid esters as surfactant for the delivery of protein drugs.* J Pharm Sci, 1996. **85**(2): p. 138-43.

38. Kovarik, J.M., et al., *Reduced inter- and intraindividual variability in cyclosporine pharmacokinetics from a microemulsion formulation.* J Pharm Sci, 1994. **83**(3): p. 444-6.

39. Malmsten, M., *Microemulsions in Pharmaceuticals*, in *Handbook of Microemulsion Science and Technology*, P. Kumar and K.L. Mittal, Editors. 1999, Marcel Dekker: New York. p. 755-771.

40. Noble, S. and A. Markham, *Cyclosporin. A review of the pharmacokinetic properties, clinical efficacy and tolerability of a microemulsion-based formulation (Neoral).* Drugs, 1995. **50**(5): p. 924-41.

41. Ambade, K.W., et al., *Formulation and evaluation of flurbiprofen microemulsion.* Curr Drug Deliv, 2008. **5**(1): p. 32-41.

42. Baboota, S., et al., *Design, development and evaluation of novel nanoemulsion formulations for transdermal potential of celecoxib.* Acta Pharm, 2007. **57**(3): p. 315-32.

43. Junyaprasert, V.B., et al., *Transdermal delivery of hydrophobic and hydrophilic local anesthetics from o/w and w/o Brij 97-based microemulsions.* Journal of Pharmacy and Pharmaceutical Sciences, 2007. **10**(3): p. 288-298.

44. Fialho, S.L. and A. da Silva-Cunha, *New vehicle based on a microemulsion for topical ocular administration of dexamethasone.* Clin Experiment Ophthalmol, 2004. **32**(6): p. 626-32.

45. Hwang, S.R., et al., *Phospholipid-based microemulsion formulation of all-trans-retinoic acid for parenteral administration.* Int J Pharm, 2004. **276**(1-2): p. 175-83.

46. Moreno, M.A., M.P. Ballesteros, and P. Frutos, *Lecithin-based oil-in-water microemulsions for parenteral use: pseudoternary phase diagrams, characterization and toxicity studies.* J Pharm Sci, 2003. **92**(7): p. 1428-37.

47. Park, K.M. and C.K. Kim, *Preparation and evaluation of flurbiprofen-loaded microemulsion for parenteral delivery.* Int J Pharm, 1999. **181**(2): p. 173-9.

48. Park, K.M., et al., *Phospholipid-based microemulsions of flurbiprofen by the spontaneous emulsification process.* Int J Pharm, 1999. **183**(2): p. 145-54.

49. Agurirre, J.M., et al., J. Oral Pathol. Med., 2004. **33**(7): p. 381-385.

50. Aikens, P.A. and S.E. Friberg, *Microemulsions in Cosmetics*, in *Handbook of microemulsion science and technology*, P. Kumar and K.L. Mittal, Editors. 1999: New York. p. 773-787.

51. Salem, J.K.J., *Kinetics of the Oxidation of Phenylhydrazine by [Fe(CN)6]3- in water-in-oil microemulsions.* Journal of Dispersion Science and Technology, 2006. **27**: p. 795-798.

52. Swarbrick, J. and J.C. Boylan, *Encyclopedia of pharmaceutical technology* . 2nd ed. 2002, New York: Marcel Dekker. 3 v. (xxi, 3032, 64 p.).

53. Ko, J. and D.M. Small, Journal of Lipid Research, 1997. **38**: p. 1544-1552.

Chapter 2

1. Cahn, R.W. and P. Haasen, *Physical metallurgy*. 4th, rev. and enhanced ed. 1996, Amsterdam ; New York: North-Holland. 3 v. (xlix, 2740, A56, S39 p.).

2. Girard, J., *Principles of environmental chemistry*. 2005, Sudbury, MA: Jones and Bartlett Publishers. xxv, 677 p.

3. Croft, W.J., *Under the microscope : a brief history of microscopy*. Series in popular science. 2006, Hackensack, NJ: World Scientific. xiii, 138 p.

4. Hilfiker, R., *Polymorphism in the pharmaceutical industry*. 2006, Weinheim: Wiley-VCH. xix, 414 p.

5. Junyaprasert, V.B., et al., *Transdermal delivery of hydrophobic and hydrophilic local anesthetics from o/w and w/o Brij 97-based microemulsions.* Journal of Pharmacy and Pharmaceutical Sciences, 2007. **10**(3): p. 288-298.

6. Friberg, S., *Micelles, microemulsions, liquid crystals, and the structure of stratum corneum lipids.* J Soc Cosmet Chem, 1990. **41**: p. 155-171.

7. Shah, D.O. and R.M. Hamlin, Jr., *Structure of Water in Microemulsions: Electrical, Birefringence, and Nuclear Magnetic Resonance Studies.* Science, 1971. **171**(3970): p. 483-485.

8. Martin, A.N. and P. Bustamante, *Physical pharmacy : physical chemical principles in the pharmaceutical sciences*. 4th ed. 1993, Philadelphia: Lea & Febiger. ix, 622 p.

9. El-Laithy, H.M., *Preparation and Physiochemical Characterization of Diocytl Sodium Sulfosuccinate (Aerosol OT) Microemulsion for Oral Drug Delivery.* AAPS PharmSciTech, 2003. **4**(1): p. Article 11.

10. Moulik, S.P. and B.K. Paul, *Structure, dynamics and transport properties of microemulsions.* Advances in Colloid and Interface Science, 1998. **78**: p. 99-195.

11. Fialho, S.L. and A. da Silva-Cunha, *New vehicle based on a microemulsion for topical ocular administration of dexamethasone.* Clin Experiment Ophthalmol, 2004. **32**(6): p. 626-32.

12. Lawrence, M.J. and G.D. Rees, *Microemulsion-based media as novel drug delivery systems.* Adv Drug Deliv Rev, 2000. **45**(1): p. 89-121.

13. Capek, I., *Radical polymerization of polar unsaturated monomers in direct microemulsion systems.* Adv Colloid Interface Sci, 1999. **80**(2): p. 85-149.

14. Djordjevic, L., et al., *Characterization of caprylocapr oyl macrogolglycerides based microemulsion drug delivery veh icles for an amphiphilic drug.* Int J Pharm, 2004. **271**(1-2): p. 11-9.

15. Naranjo, A., *Plastics testing and characte rization : industrial applications* . 2008, Munich ; Cincinnati: Hanser. xi, 363 p.

16. Dealy, J.M. and P.C. Saucier, *Rheology in plastics quality control*. SPE books. 2000, Munich Cincinnati, Ohio: Hanser Publishers ; Hanser Gardner Publications. vii, 140 p.

17. Menard, K.P., *Dynamic mechanical analysis : a practical introduction* . 2008, Boca Raton, FL: CRC Press. xix, 218 p.

18. Sutherland, E., et al., *Diffusion in S olutions of Mice lles. W hat does dy namic light scattering measure?* J. Chem. Eng. Data, 2009. **54**: p. 272-278.

19. Berne, B.J. and R. Pecora, *Dynamic light scattering : with applications to chemistry, biology, and physics*. Dover ed. 2000, Mineola, N.Y.: Dover Publications. vii, 376 p.

20. Dahneke, B.E. and D.K. Hutchins, *Characterization of particles of modulated dynamic light scattering. I. Theory.* Journal of Chemical Physics, 1994. **100**(11): p. 7890-7902.

Chapter 3

1. Ory, S.J., et al., *The effect of a biodegradable contraceptive capsule (Capronor) containing levonorgestrel on gonadotropin, estrogen, and progesterone levels.* Am J Obstet Gynecol, 1983. **145**(5): p. 600-5.

2. Kim, C., S. Ryuu, and K. Park, *Preparation and physiochemical characterisation of phase inverted water/oil microemulsion containing cyclosporine A.* Int J Pharm, 1997. **147**: p. 131-134.

3. Howard, J. and J. Hadgraft, *The clearance of oily vehicles following intramuscular and subcutaneous injections in rabbits.* Int J Pharm, 1983. **16**: p. 31-39.

4. Ruppin, D.C. and W.R. Middleton, *Clinical use of medium chain triglycerides.* Drugs, 1980. **20**(3): p. 216-24.

5. Kibbe, A.H., *Handbook of Pharmaceutical Excipients*. 3rd ed. 2000, London: Pharmaceutical Press.

6. Swenson, E. and W. Curatolo, *Intestinal permeability enhancement for proteins, peptides and other drugs; mechanisms and potential toxicity.* Adv Drug Deliv Rev, 1992. **8**: p. 39-92.

7. Budavari, S., *The Merck index : an encyclopedia of chemicals, drugs, and biologicals*. 12th ed. 1996, Whitehouse Station, NJ: Merck. 1 v. (various pagings).

8. Gennaro, A.R. and J.P. Remington, *Remington's pharmaceutical sciences*. 18th ed. 1990, Easton, Pa.: Mack Publishing. xvi, 2000 p.

9. Swarbrick, J. and J.C. Boylan, *Encyclopedia of pharmaceutical technology*. 2nd ed. 2002, New York: Marcel Dekker. 3 v. (xxi, 3032, 64 p.).

10. El-Laithy, H.M., *Preparation and Physiochemical Characterization of Diocytl Sodium Sulfosuccinate (Aerosol OT) Microemulsion for Oral Drug Delivery.* AAPS PharmSciTech, 2003. **4**(1): p. Article 11.

11. Goodman, L.S., et al., *Goodman & Gilman's the pharmacological basis of therapeutics*. 11th ed. 2006, New York: McGraw-Hill. xxiii, 2021 p.

12. Cabri, W. and R. Di Fabio, *From bench to market : the evolution of chemical synthesis*. 2000, Oxford ; New York: Oxford University Press. xvi, 266 p.

13. de Vrueh, R.L., P.L. Smith, and C.P. Lee, *Transport of L-valine-acyclovir via the oligopeptide transporter in the human intestinal cell line, Caco-2.* J Pharmacol Exp Ther, 1998. **286**(3): p. 1166-70.

14. Xu, Q., et al., *Ion-exchange membrane assisted transdermal iontophoretic delivery of salicylate and acyclovir.* Int J Pharm, 2009. **369**(1-2): p. 105-13.

15. Boonme, P., et al., *Characterization of Microemulsion Structures in the Pseudoternary Phase Diagram of Isopropyl Palimate/Water/Brij 97:1-Butanol.* AAPS PharmSciTech, 2006. **7**(2).

16. Junyaprasert, V.B., et al., *Transdermal delivery of hydrophobic and hydrophilic local anesthetics from o/w and w/o Brij 97-based microemulsions.* Journal of Pharmacy and Pharmaceutical Sciences, 2007. **10**(3): p. 288-298.

17. Schmidts, T., et al., *Development of an alternative, time and cost saving method of creating pseudoternary diagrams using the example of a microemulsion.* Colloids and Surfaces A: Physiochemical and Engineering Aspects, 2009. **340**: p. 187-192.

18. Shafiq-un-Nabi, S., et al., *Formulation development and optimization using nanoemulsion technique: a technical note.* AAPS PharmSciTech, 2007. **8**(2): p. Article 28.

19. Salem, J.K.J., *Kinetics of the Oxidation of Phenylhydrazine by [Fe(CN) 6]3- in water-in-oil microemulsions.* Journal of Dispersion Science and Technology, 2006. **27**: p. 795-798.

20. Petit, C., et al., *Characterization of a 4-Component Cationic Reversed Micellar System - Dodecyltrimethylammonium Chloride Hexanol Normal-Heptane and 0.1-M Kcl Solution.* Journal of Physical Chemistry, 1992. **96**(11): p. 4653-4658.

21. Jadhav, K.R., et al., *Applications of microemulsion based drug delivery system.* Curr Drug Deliv, 2006. **3**(3): p. 267-73.

22. Fialho, S.L. and A. da Silva-Cunha, *New vehicle based on a microemulsion for topical ocular administration of dexamethasone.* Clin Experiment Ophthalmol, 2004. **32**(6): p. 626-32.

23. Cheung, T.W. and S.A. Teich, *Cytomegalovirus infection in patients with HIV infection.* Mt Sinai J Med, 1999. **66**(2): p. 113-24.

24. Hodge, W.G., et al., *Once weekly intraocular injections of Ganciclovir for maintenance therapy of cytomegalovirus retinitis: Clinical and Ocular Outcome.* Journal of Infectious Diseases, 1996. **174**: p. 393-396.

25. Borenfreund, E. and J.A. Puerner, *Toxicity determined in vitro by morphological alterations and neutral red absorption.* Toxicol Lett, 1985. **24**(2-3): p. 119-24.

26. Bradford, M.M., *A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding.* Anal Biochem, 1976. **72**: p. 248-54.

Chapter 4

1. Bourrel, M., J.L. Salager, and R.S. Schechter, *A correlation for the phase behavior of nonionic surfactants.* J Colloid Interface Sci, 1980. **75**.

2. Holmberg, K., *Handbook of applied surface and colloid chemistry*. 2002, Chichester ; New York: Wiley.

3. Wormuth, K.R. and E.W. Kaler, *Microemulsifying polar oils.* J. Phys Chem, 1989. **93**.

4. Braun, D.B., *Over-the-counter pharmaceutical formulations*. 1994, Park Ridge, N.J., U.S.A.: Noyes Publications. xi, 459 p.

5. Lawrence, M.J. and G.D. Rees, *Microemulsion-based media as novel drug delivery systems.* Adv Drug Deliv Rev, 2000. **45**(1): p. 89-121.

6. Swarbrick, J. and J.C. Boylan, *Encyclopedia of pharmaceutical technology* . 2nd ed. 2002, New York: Marcel Dekker. 3 v. (xxi, 3032, 64 p.).

7. De Gennes, P.G. and C. Taupin, *Microemulsions and the flexibility of the oil/water interfaces.* Journal of Physical Chemistry, 1982. **86**: p. 2294-2304.

8. Ambade, K.W., et al., *Formulation and evaluation of flurbiprofen microemulsion.* Curr Drug Deliv, 2008. **5**(1): p. 32-41.

9. Langevin, D., *Microemulsions.* Accounts of Chemical Research, 1988. **21**(7): p. 255-260.

10. Hait, S.K., S.P. Moulik, and R. Palepu, *Refined method of assessment of parameters of micellization of surf actants and percolation of w/o microemulsions.* Langmuir, 2002. **18**(7): p. 2471-2476.

11. Scher, H. and R. Zallen, Chem Phys, 1970. **53**.

12. Landauer, R., J. Appl Phys, 1952. **23**.

13. Moulik, S.P. and B.K. Paul, *Structure, dynamics and transport properties of microemulsions.* Advances in Colloid and Interface Science, 1998. **78**: p. 99-195.

14. Friberg, S., I. Lapczynaska, and G. Gillberg, J. Colloid Interf Sci, 1976. **56**.

15. Cametti, C., et al., *Electrical conductivity and percolation phenomena in water-in-oil microemulsions.* Phys Rev A, 1992. **45**(8): p. R5358-R5361.

16. Lagues, M., J. Phys. Lett, 1979. **40**.

17. Feldman, Y., et al., J. Phys Chem, 1996. **100**.

18. Mukhopadhyay, L., P.K. Bhattacharya, and S.P. Moulik, Colloids Surf, 1990. **50**.

19. Chatenay, D., et al., Phys Rev Lett, 1985. **54**.

20. El Maghraby, G.M., *Transdermal delivery of hydrocortisone from eucalyptus oil microemulsion: effects of cosurfactants.* Int J Pharm, 2008. **355**(1-2): p. 285-92.

21. Junyaprasert, V.B., et al., *Transdermal delivery of hydrophobic and hydrophilic local anesthetics from o/w and w/o Brij 97-based microemulsions.* Journal of Pharmacy and Pharmaceutical Sciences, 2007. **10**(3): p. 288-298.

22. Kim, S., et al., *Phase behavior, microstructure transition, and antiradical activity of sucrose laurate/propylene glycol/the essential oil of Melaleuca alternifolia/water microemulsions.* Colloids and Surfaces A: Physiochemical and Engineering Aspects, 2009. **348**(1-3): p. 289-297.

23. Junyaprasert, V.B., et al., *Aerosol OT microemulsions as carriers for transdermal delivery of hydrophobic and hydrophilic local anesthetics.* Drug Deliv, 2008. **15**(5): p. 323-30.

24. Djordjevic, L., M. Primorac, and M. Stupar, *In vitro release of diclofinac diethylamine from caprylocaproyl macroglycerides based microemulsions.* Int J Pharm, 2005. **296**: p. 73-79.

25. Shafiq, S., et al., *Development and bioavailability assessment of ramipril nanoemulsion formulation.* Eur J Pharm Biopharm, 2007. **66**(2): p. 227-43.

26. Shafiq-un-Nabi, S., et al., *Formulation development and optimization using nanoemulsion technique: a technical note.* AAPS PharmSciTech, 2007. **8**(2): p. Article 28.

27. Shakeel, F., et al., *Nanoemulsions as vehicles for transdermal delivery of aceclofenac.* AAPS PharmSciTech, 2007. **8**(4): p. E104.

28. Al-Adham, I.S., et al., *Microemulsions are membrane-active, antimicrobial, self-preserving systems.* J Appl Microbiol, 2000. **89**(1): p. 32-9.

29. Budavari, S., *The Merck index : an encyclopedia of chemicals, drugs, and biologicals*. 12th ed. 1996, Whitehouse Station, NJ: Merck. 1 v. (various pagings).

30. Hugo, W.B., et al., *Hugo and Russell's pharmaceutical microbiology* . 7th ed. 2004, Malden, Mass.: Blackwell Science. x, 481 p.

31. Farber, J.F. and E.B. Seligmann, Jr., *Bacteroides vulgatus, a nonsporeforming anaerobe for testing fluid thioglycollate medium.* Appl Microbiol, 1968. **16**(7): p. 1102-3.

32. Pittman, M., *A Study of Fluid Thioglycollate Medium for the Sterility Test.* J Bacteriol, 1946. **51**(1): p. 19-32.

33. United States Pharmacopeial Convention., *The United States Pharmacopeia : the National Formulary*. 2006, Rockville, Md.: United States Pharmacopeial Convention.

34. Borenfreund, E. and J.A. Puerner, *Toxicity determined in vitro by morphological alterations and neutral red absorption.* Toxicol Lett, 1985. **24**(2-3): p. 119-24.

35. Attwood, D. and A.T. Florence, *Surfactant Systems: Their Chemistry, Pharmacy and Biology*. 1983, London: Chapman and Hall.