SURFACTANT SYSTEMS FOR DRUG DELIVERY AND WATER EVAPORATION REDUCTION

By

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To my parents and sisters
who have been my #1 supporters.

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TABLE OF CONTENTS

page

LIST OF TABLES

LIST OF FIGURES

Abstract of Dissertation Presented to the Graduate School
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Surfactant systems are used to enhance the quality of products used in every aspect of life; from food to cosmetics, from pharmaceutics to detergency, and even from oil recovery to chemical mechanical polishing of silicon wafers. In this dissertation, we investigate these systems for drug delivery as well as water evaporation reduction applications.

Microemulsions are excellent candidates as potential drug delivery systems because of their improved drug solubilization, long shelf-life, and ease of preparation and administration. In the present study, Propofol (2,6-diisopropylphenol) was selected as a test drug to form water external microemulsion. Propofol is intravenous general anesthetic drug, having several favorable anesthetic characteristics, including rapid emergence from unconsciousness without drowsiness. Several oil-in-water microemulsions constituting Propofol (oil), biodegradable non-ionic polymers, surfactants and fatty acid salts were formulated. Various properties of these microemulsions, like particle size, stability on dilution, and pH etc. were measured as a function of time, which shows that these systems are thermodynamically stable. Anesthetic studies of these microemulsion systems were done using randomized crossover design in rats and dogs. Rats randomly received propofol either as a microemulsion or conventional macroemulsion

(Diprivan®) to determine endpoints of anesthetic induction and recovery. Pharmacodynamic and Pharmacokinetic properties of Propofol microemulsion were measured by experiments in dogs. In conclusion, Propofol microemulsions caused general anesthesia in rat/dogs similar to that resulting from macroemulsions. The surfactant concentration and type markedly affects the spontaneous destabilization and anesthetic properties of microemulsions.

Retardation of water evaporation is important from number of viewpoints. In this work, we proposed a new approach to reduce the evaporation of water, i.e. multimolecular (duplex) films of micron or sub-micron thickness of oil and surfactants that spontaneously spreads on the water surface and reduce the water evaporation rate. Investigation of the duplex film of Brij93 and hexadecane has shown some promising results that it can decrease the evaporation rate of water as much as 80%. Addition of polymer (Polyvinyl alcohol) in the water beneath this duplex film helps in further reduction of the evaporation rate. Effects of various parameters like concentration of surfactant, different surfactants in hexadecane, and thickness of the film deposited and various polymeric additives on water evaporation through these films have been studied.

CHAPTER 1
INTRODUCTION AND LITERATURE REVIEW

Surfactant systems are used to enhance the quality of products used in every aspect of life; from food to cosmetics, from oil recovery to detergency, and even from pharmaceutics to chemical mechanical polishing of silicon wafers. Here in this thesis, we present studies on two of such system, namely drug delivery by microemulsions and reduction of water evaporation by duplex films of surfactant in oil.

Microemulsions are excellent candidates as potential drug delivery systems because of their improved drug solubilization, long shelf-life, and ease of preparation and administration. Depending on the solubility of drug in water and oil, all three types of microemulsions (i.e. oil-external, water-external and middle-phase microemulsion) can be used for drug delivery. In the present study, Propofol (2,6-diisopropylphenol) was selected as a test drug to form water external microemulsion. Propofol is intravenous general anesthetic drug, having several favorable anesthetic characteristics, including rapid emergence from unconsciousness without drowsiness. Propofol is water insoluble and currently used in a macroemulsion form, which has various side effects. In the present study, several oil-in-water microemulsions constituting Propofol (oil), biodegradable surfactants and polymers were formulated. Various properties of these microemulsions, like particle size, stability on dilution, and pH etc. were measured as a function of time, which shows that these systems are thermodynamically stable. Anesthetic studies of these microemulsion systems were done using randomized crossover design in rats and dogs.

Retardation of water evaporation is of interest from a number of viewpoints such as in ophthalmology wherein people suffering from dry eye syndrome show very high water loss from eyes, nuclear industry wherein radioactive water from spent fuel may escape into the

atmosphere, antiseptic for burned skin victims, water conservation in certain arid and dry parts of world where water is scarce etc. But, despite this need to reduce water evaporation, few efforts have been directed in finding new ways to decrease the loss of water by evaporation. One of the approaches, which are currently being used, is to spread a monolayer of long chain alcohols/acids on top of water to decrease the water evaporation. Over the years various researchers[1, 2] have worked on reduction on evaporation of water by monolayers, but the maximum reduction in evaporation of water achieved by monolayers is only around 50%. Also, problem with monolayers is that they can be easily removed by winds and undergo thermal as well as biological degradation. Another approach is to use a multimolecular film of oil as a means of reducing the evaporation of water. But in this case small amount of oil does not spread so the amount of oil used is very high, which is not economical. In this work, we proposed a new approach to reduce the evaporation of water, i.e. multimolecular (duplex) films of micron or sub-micron thickness of oil and surfactants that spontaneously spreads on the water surface and reduce the water evaporation rate.

The following is the detailed review of colloidal drug delivery system and reduction of water evaporation.

1.1 Colloidal Drug Delivery

The design and development of new drug delivery systems with the intention of enhancing the efficacy of existing drugs is an ongoing process in pharmaceutical research. It is necessary for a pharmaceutical solution to contain a therapeutic dose of the drug in a volume convenient for administration. Of the many types of drug delivery systems that have been developed, one in particular, i.e. colloidal drug delivery system has great potential for the goal in drug targeting.

A few of the most widely examined colloidal drug delivery systems are micelles, microemulsions, macroemulsions, liposomes, and nanoparticles. These colloidal systems are

shown schematically in Figure 1-1. A comparison of physical properties of these colloidal drug delivery systems is given in Table 1-1.

1.1.1 Micelles

A surfactant, or ***surf***ace-***act***ive ag***ent***, is defined as a substance that adsorbs onto surfaces or interfaces of solutions to lower the surface or interfacial tension of the system.[3] The magnitude of the lowering of the surface or interfacial tension depends on the surfactant structure, concentration, and the physico-chemical conditions of the solution (e.g. pH, salt concentration, temperature, pressure, etc.).[3] Surfactants are typically amphiphatic species, meaning that they are made up of a hydrophobic component, referred to as the "tail," and a hydrophilic component, referred to as the "head" group (see Figure 1-2). When placed in solution, surfactant molecules tend to orient in such a way as to minimize the interactions of the hydrophobic "tail" with water in aqueous solutions, or to minimize the interactions of the hydrophilic "head" with oil in organic solvents. This leads to adsorption of the surfactant molecules onto surfaces or at interfaces, and above a certain concentration, known as the critical micelle concentration or "cmc," surfactants form aggregates known as micelles. When placed into aqueous solutions, surfactant molecules will form spherical aggregates at the cmc where the hydrophobic tails are pointed inward and removed from interaction with water molecules by the hydrophilic head groups as shown in Figure 1-2. When placed into organic solutions, surfactant molecules will form reverse micelles with the hydrophobic tails pointed outward (see Figure 1-2).

When the critical micellar concentration, or cmc, is reached, many of the physical properties of the surfactant solution in water show an abrupt change as shown in Figure 1-3. Some of these properties include the surface tension, osmotic pressure, electrical conductivity, and solubilization. The cmc is a measure of the free monomer concentration in surfactant

solutions at a given temperature, pressure, and composition. Mcbain[4] first investigated the unusual behavior of fatty acid salts in dilute aqueous solution at the cmc in the 1910s and 1920s and was followed by Hartley[5, 6] in the 1930s. Other evidence for surfactant aggregation into micelles was obtained from vapor pressure measurements and the solubility of organic molecules in water. The formation of colloidal-sized clusters of individual surfactant molecules in solution is known as micellization.

Normal micelles are optically isotropic and thermodynamically stable liquid solution of water and amphiphile. Micelles have low viscosity, long shelf life, and are very easy preparation method. But, micelles do not tolerate large amount of apolar species because of their very limited capacity to solubilize oil.

1.1.2 Macroemulsions

It is a commonly known fact that oil and water do not mix. However, emulsifying agents, typically surfactants can be added to a mixture of oil and water to promote the dispersion of one phase in the other in the form of droplets. Over the years, emulsions have been defined in a variety of ways. Emulsions are thermodynamically unstable, heterogeneous systems, consisting of at least one immiscible liquid intimately dispersed in another in the form of droplets, whose diameters are generally in the range of 1 - 100 μm.[7] There are two main types of emulsions: oil-in-water emulsions, in which oil droplets are dispersed in a continuous water phase, or water-in-oil emulsions, in which water droplets are dispersed in a continuous oil phase.

The most fundamental thermodynamic property of any interface is the interfacial free energy, or interfacial tension. The interfacial free energy is the amount of work necessary to create a given interface. The interfacial free energy per unit area is a measure of the interfacial tension between two phases. A high value of interfacial tension implies that the two phases are

highly dissimilar in nature. There are many methods available to measure the interfacial tension between two liquids including the Du Noüy ring method, Wilhelmy plate method, drop-weight or drop-volume method, pendant drop method, spinning drop method, and Sessile drop method.[3]

Emulsification involves the generation of a large total interfacial area. Considering that the two phases in emulsions are not miscible, in order to generate this large interfacial area, the interfacial tension must be lowered significantly according to the following equation:

$$W = \gamma \bullet \Delta A \qquad (1\text{-}1)$$

where W is the work done on an interface, γ is the interfacial tension, and ΔA is the change in interfacial area associated with the work W. According to Equation (1-1), when a constant amount of work is applied to generate an interface, ΔA will be large if γ is small, and thus the interface will expand significantly to form smaller emulsion droplets.

As previously mentioned, the primary means by which the interfacial tension is lowered is through the addition of emulsifying agents, usually surfactants. The surfactant molecule also plays a second role in emulsions which is to stabilize the interface for a time against coalescence with other droplets and concomitant phase separation. A large number of methods have been developed to provide the energy needed to achieve complete emulsification in a given system.[7-10]

1.1.2.1 Emulsion droplet size

Emulsions are classified as either water-in-oil (W/O) or oil-in-water (O/W) depending on which phase is continuous and which is dispersed. The dispersed phase in emulsions, whether oil or water, is usually composed of spherical droplets within the continuous phase. These droplets may be nearly monodisperse in terms of droplet size; or they may have a wide size distribution depending on several factors.[7] In most cases, the wider the size

distribution, the less stable is the emulsion. In other words, emulsions with a more uniform size distribution tend to remain stable for longer time while those with wide size distribution will usually undergo Ostwald ripening, a phenomenon where larger droplets grow at the expense of smaller droplets.[11] In general, emulsions with a narrow size distribution and a small mean droplet size tend to exhibit a greater emulsion stability, all other things being equal.[7] The change in the size distribution with time reflects the kinetics of coalescence in emulsions. Depending on the surfactant that is used to stabilize the emulsion, emulsions can have lifetimes ranging from hours to as long as a few years.[7] In general, emulsions exhibiting a higher yield stress tend to show higher emulsion stability and shelf life.[7]

Emulsion droplet size is also related to the method of preparation that is employed to generate the emulsion. This is a result of the relationship between interfacial area and work that is done on the system, according to Equation (1-1). As can be seen in this equation, if the interfacial tension is constant with time, the change in interfacial area is directly proportional to the amount of work that is put into the system. Some emulsion preparation methods provide more energy (work) than others, and thereby lead to smaller droplets and higher interfacial area.

Micellar stability is another factor that affects the droplet size of emulsions.[12] If the micelles are very stable, flux of surfactant monomers to the interface of droplets will be low, resulting in a higher interfacial tension at the droplet surface and a large droplet size will occur as predicted by Equation (1-1). The process of monomer diffusion from the bulk to the oil/water interface is illustrated in Figure 1-4.

1.1.2.2 Viscosity of emulsions

The viscosity, or resistance to flow, of emulsions could be considered as one of their most important properties. This is true from both a practical and a theoretical viewpoint. In a practical sense, certain cosmetic or even food emulsions are only desirable at a specific viscosity (e.g.

lotions, milk, salad dressings, etc.). Manipulation of emulsion viscosity to achieve the desired product specifications is not a trivial matter. From a theoretical perspective, the viscosity measurements can be used to provide insightful information about the structure and possibly the stability of an emulsion. The overall emulsion stability is affected by the following factors:[7, 13]

- Viscosity of the external phase
- Concentration (i.e., volume fraction) of the internal phase
- Viscosity of the internal phase
- Nature of the emulsifiers
- Surface viscoelasticity of the interfacial film formed at the oil/water interface
- Droplet size distribution

1.1.2.3 Emulsion stability

As mentioned previously, one of the most important parameters in emulsification processes is emulsion stability. For example, milk is a natural emulsion of the O/W. If the stability of milk was only a week or two, the milk would have to be shaken vigorously before pouring. However, in this case nature has provided us with a stable emulsion. Another common example is shampoo, another emulsion. It would be inconvenient if the shampoo were not a stable emulsion, since shaking would be necessary. There are also cases where it is necessary to break down unwanted, naturally occurring stable emulsions. Such examples are the W/O type emulsions which build up in oil storage tanks, or the O/W type emulsions that arise in effluent waters.

So it becomes necessary to understand how to develop an emulsion system to be stable or unstable, depending on the needs of the industry. To understand emulsion stability, it is important be cognizant that there are five types of breakdown processes which can occur in emulsions. These are listed in Table 1-2, along with factors that influence that type of breakdown.[14]

1.1.2.4 Coalescence and phase inversion

When two oil drops approach each other, a thin film of the continuous water phase is trapped between the drops. The behavior of the thin film determines the degree of stability of the emulsion, and the rate of thinning of the film determines the time required for the two drops to coalesce (i.e., coalescence rate). When the film has thinned to a critical thickness, it ruptures, and the two drops unite or coalesce to form one larger drop.[15, 16] The rate of film thinning depends on the surface viscosity of the surfactant film adsorbed at the oil/water interface. The film may drain evenly or unevenly depending on the interfacial tension gradient due to adsorbed surfactant.[17] The factors that influence the rate of film thinning between droplets therefore influence the emulsion stability. A summary of all of the factors influencing coalescence of droplets is given in Table 1-3.[18]

Phase inversion can occur in emulsions due to a number of factors. For a given emulsifier concentration, the viscosity of an emulsion gradually increases as the phase volume of the dispersed phase is increased. However, at a certain critical volume fraction ϕ_c, there is a sudden decrease in viscosity, which corresponds to the point at which the emulsion inverts. ϕ_c was found to increase with increasing emulsifier concentration.[19] The sudden decrease in viscosity is due to the sudden reduction in dispersed phase volume fraction. Often ϕ_c is in the range of 0.74, so that upon inversion, the dispersed phase volume fraction reduces from 0.74 to 0.26, thus reducing the viscosity significantly. ϕ_c should theoretically be in the range of 0.74 for spheres of equal radii to be at the maximum packing,[20] but ϕ_c=0.99 was found for paraffin oil/aqueous surfactant solutions[21] and ϕ_c=0.25 was found for olive oil/water emulsions.[22] The phase inversion of emulsions can be brought about by several parameter changes, listed in Table 1-4.

1.1.2.5 Demulsification

As discussed previously in this section, it is not always desirable to have a stable emulsion. Often an emulsion is present in a system in which it is undesirable. One example is the presence of aqueous emulsion droplets dispersed in crude oil. Crude oil is always associated with water or brine in oil reservoirs and also contains natural emulsifying agents, such as resins and asphaltenes. These emulsifying agents form a thick, viscous interfacial film around the water droplets, resulting in a very stable emulsion. Therefore, demulsification is very important in the crude oil industry. Many physical methods have been developed for demulsification, depending on the industrial application. A wide variety of chemical additives for demulsification have been developed in recent years. These additives are all relatively high molecular weight polymers capable of being adsorbed at the O/W interface and displacing the film. The primary advantage of these additives is that they can be added to the system even before emulsion formation, so that they act as inhibitors.

In the petroleum industry, demulsifiers have been considered for breaking the common fuel oil emulsions. In this area, chemical demulsifiers that have been investigated include ultra-high molecular weight polyoxiranes[23] and micellar solutions containing petroleum sulfonates, electrolytes, and cosurfactants.[24] It is evident that there are many methods for demulsification. The nature of the emulsion to be separated is the key factor in determining which method(s) is best for each particular demulsification problem.

1.1.2.6 Surfactant selection for emulsification

Often the selection of surfactants in the preparation of either O/W or W/O emulsions is made on an empirical basis. However, in 1949, Griffin[25] introduced a semi-empirical scale for selecting an appropriate surfactant or blend of surfactants. This scale, termed the hydrophile-lipophile balance (HLB), is based on the relative percentage of hydrophilic to hydrophobic

groups in the surfactant molecules and ranges from 1 to 40. An HLB of 1 represents a surfactant that is highly oil-soluble and an HLB of 40 represents a highly water-soluble surfactant. Surfactants with a low HLB number normally form W/O emulsions, whereas those with a high HLB number often form O/W emulsions.[26] A summary of the HLB range required for various purposes is given in Table 1-5.

The calculation of the HLB number for a given surfactant, as developed by Griffin,[25] is quite laborious and requires a number of trial and error procedures. Simplification methods were later developed by Griffin[25] that applied to certain surfactants. Davies[26] developed a method for calculating the HLB values of surfactants directly from their chemical formulas, using empirically determined numbers. The HLB number can also be determined experimental through several correlations that have been developed. These correlations relate the HLB number to such parameters as the cloud point,[27] water titration value for polyhydric alcohol esters,[28] and the heat of hydration of ethoxylated surfactants.[29]

Another method that may be used to select a surfactant suitable for forming an emulsion is by using the phase inversion temperature (PIT) method. The phase inversion temperature (PIT) is the temperature at which an emulsion experiences phase inversion, as described in a previous section. The PIT of non-ionic emulsifiers has been shown to be influenced by the surfactant HLB number, so the PIT can be used similarly to the HLB number in selecting an emulsifier.[22] The primary distinction is that the PIT is a characteristic property of the emulsion, not of the emulsifying agent.[22] Due to this, the PIT includes the effect of additives on the solvent, the effect of mixed emulsifiers or mixed oils, etc. In other words, the HLB number is actually a function of all of these properties, but only the PIT completely analyzes a given emulsion system. The PIT method is useful because the PIT is a measurable property

which is related to the HLB number. A summary of effects of PIT and droplet stability from different investigations is given below:[22]

- The size of emulsion droplets depends on the temperature and the HLB of emulsifiers
- The droplets are less stable toward coalescence close to the PIT
- Relatively stable O/W emulsions are obtained when the PIT of the system is some 20 to 65°C higher than the storage temperature
- A stable emulsion is obtained by rapid cooling after formation at the PIT
- The optimum stability of an emulsion is relatively insensitive to changes of HLB value or PIT of the emulsifier, but instability is very sensitive to the PIT of the system
- The stability against coalescence increases markedly as the molar mass of the lipophilic or hydrophilic groups increased
- When the distribution of hydrophilic chains is broad, the cloud point is lower and PIT is higher than when there is a narrow size distribution.

The PIT can be measured by the following methods: 1) direct visual assessment,[30] 2) conductivity measurement,[31-33] 3) Differential Thermal Analysis (DTA) or Differential Scanning Calorimetry (DSC),[34] and 4) viscosity measurement.[35, 36] Both the HUB and PIT methods for selecting an emulsifier in a system have been widely used and adapted to meet industry needs.

1.1.2.7 Applications of emulsions

Emulsions are desirable for many different applications because they provide a system having a large interfacial area. Historically, cosmetic emulsions are the oldest class of manufactured emulsions.[7] Emulsions are desirable for cosmetic applications because: 1) they increase the rate and extent of penetration into the skin, 2) they open up the possibility of applying both water- and oil-soluble ingredients simultaneously (e.g. deodorants), and 3) they provide for efficient cleansing.

Emulsions are also widely used for pharmaceutical applications in the form of creams or ointments and as drug delivery vehicles. They are also ideal for use as polishes (e.g. furniture

polishes, floor waxes, etc.) paints, and agricultural sprays. Many foods are manufactured in the form of emulsions including mayonnaise, salad dressings, milk, and margarine. Another industry where emulsion technology is important is the asphalt industry when the principal requirement is the production of water-repellent surfaces. Emulsions are also used as polymerization vehicles to aid in the production of high polymeric materials such as plastics, synthetic fibers, and synthetic rubbers. These are just a few of the many applications of emulsions.

1.1.3 Microemulsions

A microemulsion is a thermodynamically stable, isotropic dispersion of oil and water containing domains of nanometer dimensions stabilized by an interfacial film of surface-active agent(s).[37] The term "microemulsion" originated from Jack H. Schulman and coworkers in 1959,[38] although Hoar and Schulman originally described water-in-oil microemulsions, which they referred to as transparent water-in-oil dispersions, in 1943.[39] As implied above, microemulsions may be of the oil-in-water (O/W) (see Figure 1-5)) or the water-in-oil (W/O) type depending on conditions of the system and system components.

According to Bancroft,[40-42] phase volume ratios are less important in the determination of the microemulsion type that will be formed (i.e., W/O or O/W) than the surfactant characteristics (e.g. HLB). As previously mentioned, the Bancroft rule states that whichever phase the surfactant has a greater affinity for will typically be the continuous phase.

The creation of a microemulsion entails the generation of a huge interfacial area, which, according to the following equation,[43] requires a significant lowering of the interfacial tension (usually << 1 mN/m):[44]

$$W = \gamma \bullet \Delta A \qquad (1\text{-}1)$$

where W is the work performed, γ is the surface or interfacial tension at the air/water or oil/water interface and ΔA is the change in surface or interfacial area. This ultra-low interfacial tension in spontaneously formed microemulsions is achieved by the incorporation of surfactant(s) (typically a surfactant + a cosurfactant, especially when ionic surfactants are used).[45] Figure 1-6 shows the thermodynamic explanation for the behavior of macro- and microemulsions. As can be concluded from the graph, there is an optimum radius for microemulsion systems where the free energy of dispersion becomes negative, thereby making the microemulsion stable and its formation energetically favorable.[46]

Schulman and others first noticed microemulsion systems in 1943 when they observed that the addition of a medium chain-length alcohol made a coarse macroemulsion that was stabilized by an ionic surfactant become transparent.[39] Even then, Hoar and Schulman recognized the important role of a very low interfacial tension in causing spontaneous emulsification of the added water in oil.[39] They concluded that the role of the alcohol is as a stabilizer against the repulsive electrostatic forces that the ionic surfactant head groups would experience.

Schulman and others used a variety of experimental techniques (e.g. X-ray diffraction,[38] ultra-centrifugation,[47] light scattering,[38] viscosimetry,[48] and nuclear magnetic resonance (NMR)[49, 50]) to elucidate some of the characteristics of these microemulsion systems following the groundbreaking work of Schulman and Hoar. These studies were instrumental in providing them with information about the structure, size, and interfacial film behavior of microemulsions. They were able to determine the size of the droplets and they found that the presence of the alcohol within the system led to greater interfacial fluidity.

Later, in 1967, Prince[38] proposed a theory that the formation of microemulsions was due to the negative interfacial tension that results from high surface pressure of the film. Prince

explained this negative interfacial tension based on the depression of the interfacial tension between the oil and water phase that occurs when surfactant is added. The principle behind this theory is described by the series of equations that follow. The surface pressure of the film at the air/water interface, π_{aw}, is defined as:[38, 45]

$$\pi_{aw} = \gamma_o - \gamma_s \qquad (1\text{-}2)$$

where γ_o is the surface tension of the pure surface (without surfactant) and γ_s is the surface tension of the surface with surfactant. In the case where oil is the second phase (oil/water system), the surface pressure of the surfactant film at the oil/water interface, π_{ow}, can be defined as:

$$\pi_{ow} = (\gamma_{o/w})_o - (\gamma_{o/w})_s \qquad (1\text{-}3)$$

where $(\gamma_{o/w})_o$ is the interfacial tension of the "pure" oil/water interface (i.e., in the absence of surfactant) and $(\gamma_{o/w})_s$ is the interfacial tension of the oil/water interface in the presence of surfactant film. Rearrangement of Equation (1-3) gives:

$$(\gamma_{o/w})_s = (\gamma_{o/w})_o - \pi_{ow} \qquad (1\text{-}4)$$

Based on Equation (1-3), for a surfactant film that can generate a very high surface pressure (π_{ow}), the interfacial tension of the surfactant film at the oil/water interface $(\gamma_{o/w})_s$ becomes negative. This is only a transient phenomenon because generation of a negative interfacial tension leads to a negative free energy of formation of the emulsion, which is an unstable situation. This is illustrated by the following equation:

$$\Delta G_{form} = \gamma \Delta A - T\Delta S^{config} \qquad (1\text{-}5)$$

where ΔA is the increase in interfacial area, ΔS^{config} is the configurational entropy of the droplets of the liquid that are formed and T is the absolute temperature.[51] The negative interfacial tension

accounts for the spontaneous increase in interfacial area that occurs in the formation of microemulsions. When a transient (unstable) negative interfacial tension is experienced, the system will seek to stabilize by spontaneously generating new interfacial area, thereby raising the interfacial tension back to acceptable, stable limits. As previously mentioned, in order to form microemulsions, it is required that the concentration of surfactants be greater than that required to reduce the oil/water interfacial tension to zero and to cover the total interfacial area of all dispersed droplets. The transient negative interfacial tension that is generated facilitates the spontaneous break-up of droplets.

The nature of the thermodynamic stability of microemulsions has long been studied. The stability can be attributed to the fact that the interfacial tension is low enough that the increase in interfacial energy accompanying dispersion of one phase in the other is outweighed by the free energy decrease that is associated with the entropy of dispersion.[46] Furthermore, the free energy decrease that accompanies adsorption of surfactant molecules from a bulk phase favors the existence of a large interfacial area and hence plays a major role in stabilizing microemulsions.[46]

One must also understand the role of cosurfactants in microemulsion formulations. The addition of a short-chain alcohol to a surfactant solution to enhance microemulsion formation has long been practiced.[52] This cosurfactant (short-chain alcohol) serves to 1) fluidize the interface, 2) decrease interfacial viscosity, 3) destroy the lamellar liquid crystalline structures, 4) provide additional interfacial area, 5) reduce electrical repulsion between droplets and also between polar head groups of surfactants by acting as charge screeners and decreasing surface charge density, and 6) induce the appropriate curvature changes.[52]

In 1972, Gerbacia and Rosano[53] investigated the formation and stabilization of microemulsions, with emphasis on the role of the cosurfactant (in this case, pentanol). They

suggested that a critical aspect of the mechanism of formation of microemulsions involves the diffusion of the pentanol through the interface. This process has been found to be a necessary, but not sufficient condition for microemulsification. In essence, the alcohol transiently lowers the interfacial tension to zero as it diffuses through the interface, thereby inciting the spontaneous dispersion of one phase into the other. The surfactant then acts to stabilize the system against coagulation and coalescence. NMR data and calculation of free energies of adsorption of the pentanol into the interface have proven that a strong association between the surfactant and cosurfactant is not necessary for microemulsion formation.[53]

The stability of the phases of surfactant in microemulsions results from the competition between entropic and elastic contributions to the free energy.[54] The two intrinsic parameters that ultimately affect the structure of the aggregates existing in solution are the mean bending modulus, κ, and the Gaussian bending modulus, $\bar{\kappa}$. The bending energy, F_b, is directly related to the mean bending modulus and the Gaussian bending modulus, as can be seen below:

$$F_b = \frac{1}{2}\kappa\left(C_1 + C_2 - 2C_0\right)^2 + \bar{\kappa}C_1C_2 \qquad (1\text{-}6)$$

where C_1 and C_2 are the local principal curvatures of the surfactant layer and C_0 is the spontaneous curvature. Bellocq[55] states that the contribution of the bending energy to the total free energy is a crucial determining factor in the type and characteristic size of the structure. In Equation (1-6) above, the first term represents the amount of energy needed to bend a unit area of interface by a unit curvature amount, and the second term is important to the change of the membrane topology and the phase transition.[55] The spontaneous curvature, C_0, is determined by the nature of the interactions between the surface-active molecules; i.e., the competition in packing of the polar heads and the hydrocarbon tails of the surfactant. If the dominant interactions are between the polar heads, then the surfactant orientation will be concave to water

and a water-in-oil microemulsion will be formed, whereas, if the dominant interactions are between the hydrocarbon tails, the surfactant orientation will be convex to water and an oil-in-water microemulsion will be formed.

The addition of cosurfactant (short-chain alcohol) can have a profound effect on the curvature. Bellocq reported that there is a very efficient lowering of the bending constant, κ, of the surfactant film with the dilution of the system with short-chain alcohols.[55] This lowering of κ was attributed to thinning of the interface, and this attribution was confirmed with deuterium solid-state NMR.

The chain length of the cosurfactant (alcohol) was found to be critical in microemulsion formation. The chain length determines what types of phases (bicontinuous, lamellar, sponge, vesicle, etc.) will be of importance. These phases play an important role in the type, structure and size of the microemulsion that will be formed.[56] In simplistic terms, a short-chain cosurfactant acts to prevent the formation of or destroy lamellar liquid crystals. Since there is a significant difference between the surfactant chain length and the short-chain alcohol, there is a tail wagging effect due to the fact that the excess hydrocarbon tails have more freedom to disrupt molecular packing through conformational disorder, increased tail motion, and penetration and/or buckling of the chain into the monolayer.[57] This motion of the hydrocarbon tail prevents or disrupts ordering of the molecules and therein prevents formation of or destroys lamellar liquid crystals and enhances microemulsion formation.

It must be noted that cosurfactant addition to microemulsion-forming systems is typically only applicable for ionic surfactant systems. Microemulsions that incorporate non-ionic surfactants may be formed without the need for cosurfactant addition; especially in the case of non-ionic surfactants of the polyethylene oxide adducts (POE). This is because these surfactants

are composed of a homologous series of varying composition and molecular weight,[45] which serves the same purpose of enhancing the interfacial film fluidity. The important factor in the formation of these types of microemulsions is temperature, because this class of materials is solubilized in water by means of hydrogen bonding between the water molecules and the POE chain. Hydrogen bonding is a temperature-sensitive phenomenon, which decreases with increasing temperature. Therefore, the temperature conditions under which a microemulsion is formed are important to the type of microemulsion that is formed. Above a characteristic temperature, which is commonly known as the phase inversion temperature (PIT),[58] the non-ionic surfactant changes its affinity from the water phase to the oil phase. Below the PIT, O/W microemulsions will be formed and above the PIT, W/O microemulsions will be formed.

1.1.3.1 Formation of microemulsions

Now that the some of the basic principles of microemulsions have been discussed, it is easier to understand what conditions must be met for microemulsion formation and why they are required. There are three major factors that must be considered in order to form microemulsions.[22]

First, given the importance of achieving an ultra-low surface tension at the oil/water interface, surfactants must be carefully chosen so that this may be accomplished. Secondly, there must be a large enough concentration of surfactants to stabilize the newly formed interface such that phase separation does not occur. It must be mentioned that the type, structure and characteristics (e.g. Hydrophilic-Lipophilic Balance (HLB), degree of ionization, etc.) of the surfactants may potentially play a major role in determining just how high the surfactant concentration needs to be to stabilize the interface. The third required condition for forming microemulsions is that the interface must be fluid (flexible) enough to facilitate the spontaneous

formation of micro-droplets with a small radius of curvature (50 – 500 Å). That is where cosurfactant structure can become very important.

1.1.3.2 Applications of microemulsions

Microemulsions have a range of industrial applications. They are useful in technologies such as enhanced oil recovery,[59] pharmaceuticals,[60] cosmetics,[61, 62] food sciences,[63] and detergency.[64, 65] Microemulsions have been extensively studied in regards to their use as drug delivery vehicles, and are now gaining attention as possible mediums for use in detoxification of blood to remove free drug from the blood stream of overdose patients.[66]

Drugs that have significant hydrophobic functionality have been shown to partition into the corona (area at the interface) and/or interior core of O/W microemulsions.[66] It is generally accepted that this is largely due to hydrophobic interactions. Recall that the formation of microemulsions leads to the generation of a large interfacial area. It is believed that this large interfacial area facilitates the uptake of relatively large amounts of drug into the microemulsion in a time efficient manner, thereby significantly decreasing the bulk drug concentration.

1.1.4 Nano-emulsions

As early as 1943, Dr. T. P. Hoar and J. H. Schulman[39] discovered that he could prepare transparent water-in-oil dispersions of nanometer size, which displayed stable thermodynamic characteristics (i.e., the water droplets remained stably dispersed indefinitely if left unperturbed, with respect to temperature, pressure, or compositional conditions). Anomalous systems could also be prepared in which oil droplets are dispersed in water. Later, other scientists, including Dr. Stig Friberg,[56] found that they could develop transparent nanometer size dispersions of one medium within another continuous medium, which were not thermodynamically stable, but were kinetically stable (i.e., given time, there will be a breakdown in the stability of the dispersion that will lead to phase separation). Such systems have come to be referred to as nano-emulsions

(thermodynamically unstable systems). Thus, both Schulman's microemulsions and nano-emulsions start in the nanometer range, but the microemulsions are thermodynamically stable and maintain the same size whereas the nano-emulsions coalescence and display an increase in size with time, which ultimately causes phase separation.

To sufficiently comprehend the difference between microemulsions and nano-emulsions, there must be clarification of the nomenclature of the two. As previously mentioned microemulsions is a misleading title considering that their average diameter ranges from 10 to 100 nm. Light scattering and X-ray analysis have indicated that microemulsions are, in fact, coarse mixtures as opposed to molecular dispersions.[44] Nano-emulsions lie within the same size range, but may have diameters that considerably exceed the size of a microemulsion droplet (20-500 nm). The emphasis must be placed on the fact that nano-emulsions are in fact emulsions of nano-size, meaning that they are kinetically stable, (thermodynamically unstable) heterogeneous systems in which one immiscible liquid is dispersed as droplets in another liquid (as emulsions are defined).

Although they are on similar size scales, microemulsions and nano-emulsions have markedly different characteristics (Table 1-6). Nano-emulsions are formally defined as thermodynamically unstable, generally opaque, sub-micron-sized (20 – 500 nm) systems that are stable against sedimentation and creaming.[67] They may have the appearance of microemulsions, but they do not necessarily require as much surfactant concentration in their preparation.[67]

As early as 1981, Rosano *et al.*[53] found that certain microemulsion systems, which they termed "unstable microemulsions", displayed significantly different characteristics from traditional microemulsions. These systems were dependent upon the order of addition of components (i.e., the mixing protocol) and their formation was contingent upon having a large

enough concentration gradient to allow diffusion of amphipathic materials across the oil/water interface.

El-Aasser *et al.*[68] performed studies of a miniemulsification process, which produced O/W miniemulsions (another term for what we refer to as nano-emulsions in this paper) having an average droplet diameter in the size range of 100 – 400 nm. The miniemulsions that they produced were generated by means of a mixed emulsifier protocol, which was comprised of a mixture of ionic surfactant and long-chain fatty acid in concentrations of 1-3 % by weight in the oil phase. They stated four "distinct and significant" differentiating aspects between the necessary conditions of preparation for miniemulsification systems and traditional microemulsion systems, which may also be produced by mixed emulsifier systems:

- Concentration of the mixed emulsifier system: only 1–3 wt% (with respect to the oil phase) is sufficient for the formation and stabilization of miniemulsions, whereas microemulsions typically require 15-30 wt %.
- Droplet size: their miniemulsion droplets range from 100-400 nm in diameter, as opposed to microemulsions, which range from 10-100 nm in diameter.
- Chain length of the cosurfactant (fatty alcohols or acids in this case): miniemulsions require at least a 12-carbon chain length, whereas microemulsions can be prepared with alcohols of shorter chain lengths.
- Order of mixing of the ingredients (mixing protocol): successful production of miniemulsions requires that the ionic surfactant and the fatty alcohol be initially mixed in the water phase for 30 minutes to 1 hour at a temperature above the melting point of the fatty alcohol, prior to the addition of the oil phase, whereas order of mixing does not affect microemulsion formation.

El-Aasser *et al.*[68] performed an array of experiments to help them to better understand the miniemulsification process. They found that the process was a spontaneous phenomenon based on results yielded by both dynamic and static spinning drop experiments. The oddity in this discovery lay in the fact that the measured interfacial tensions were unexpectedly high, ranging from 5 to 15 dynes/cm. They attributed this finding to the formation of emulsion droplets by

diffusion of the oil (in this case, styrene) from drops into the adjacent liquid crystal structure of the mixed emulsifier system. They also stated that the presence of mixed emulsifier liquid crystals, which was confirmed by birefringence observations, significantly improved the emulsification process and led to greater emulsion stability. They concluded that the mechanism of formation of miniemulsions was by swelling of the mixed emulsifier liquid crystals by oil (in this case, toluene). This swelling of the liquid crystalline structure led to its break-up or sub-division to form small emulsion droplets, which were stabilized by the adsorption of the mixed emulsifier complex at the oil-water interface.[68]

Although emulsion stability is generally known to increase with droplet surface charge, the miniemulsions that El-Aasser prepared displayed contradictory behavior; their stability increase corresponded to a reduction in surface charge. These results suggested that the steric component of stabilization was the dominating factor.[68]

The mechanism that has been attributed to nano-emulsion breakdown in a ternary system of brine, oil, and non-ionic surfactant is a 3-stage process.[69] The first and last stages of the droplet growth process were found to be due to Ostwald ripening, whereas the droplet size distribution of the second stage became too broad compared to the expected theoretical distribution (as predicted by the Lifshitz-Slezov-Wagner theory) to be due to Ostwald ripening. Katsumoto *et al.*[69] made this assessment after plotting the cube of the z-average radius, r_z, as a function of time, and obtaining a linear relationship. In addition, their group found that the volume of bound water on miniemulsion droplets plays an important role in obtaining a homogeneous miniemulsion.[70] The storage temperature of the miniemulsion solution and the molecular weight of the surfactant were determined to significantly affect the system's stability: as storage temperature is decreased, the rate of coalescence increases, and as the molecular

weight of the surfactant is increased, the rate of coalescence decreases. The former finding is due to an increase in surface tension because non-ionic surfactants become more water-soluble as temperature is decreased. The latter finding is due to steric effects that become predominant as the surfactant size is increased.

Nano-emulsions may be formed by a few different experimental methods: condensation,[71] low-energy emulsification methods involving phase inversion,[67, 72] or by high energy input during emulsification.[73] Forgiarini *et al.*[67] reinforced the concept that was proposed by El-Aasser[68] concerning the importance of the mixing protocol in the formation of nano-emulsions. They found that they only obtained dispersions of nanometer droplet size when the nano-emulsion was formed via stepwise addition of water to a solution of the surfactant in oil. If other methods of addition were used, the droplet size ranged from 6 – 10 μm.

Izquierdo *et al.* [72] analyzed the formation and stability of nano-emulsions that were prepared by the phase inversion temperature (PIT) method, in which emulsions were formed at a temperature near the PIT and then rapidly cooled to room temperature (25°C) by immersion in an ice bath. They proposed that a change in the interfacial curvature is critical to nano-emulsion formation, and that the presence of lamellar liquid crystals was probably a necessary, but insufficient requirement for preparation of nano-emulsions.[67] They concluded that the key factor for nano-emulsion formation should be credited to the kinetics of the emulsification process.

Nano-emulsions have possible applications as drug delivery vehicles,[73, 74] in drug targeting, as reaction media for polymerization, in personal care and cosmetics, and in agrochemicals. It is also plausible that nanoemulsions may be used in most industries where extraction will ultimately be required. This idea is based on the premise that the nanoemulsions

may be designed so that their stability characteristics will coincide with the requirements of the application.

1.2 Non-Toxic Biomedical and Pharmaceutical Microemulsions

Microemulsions that are to be used for biomedical or pharmaceutical applications must satisfy basic criteria in order to be efficient. Such microemulsions must first and foremost be non-toxic and biodegradable. In recent years microemulsions have been considered as drug delivery vehicles. There are three common means of drug delivery in which microemulsions are incorporated; 1) topical/transdermal delivery, 2) oral delivery, and 3) intravenous delivery. Microemulsions that will be utilized for topical or transdermal applications must have minimal skin irritation and maximum ability for controlled drug flux across the skin. In oral drug delivery, the microemulsions must not be harmful to the gastrointestinal tract and there should be adequate drug adsorption. In intravenous drug delivery, the microemulsions should be stealthy (i.e. able to escape detection as a foreign body by the body's defense systems) and blood-compatible.

1.2.1 Applications

There are many widespread applications for pharmaceutical microemulsions. As mentioned above, drug delivery via microemulsions is an application that is constantly gaining more and more attention among researchers.[75-77] Microemulsions may also be used in reactivity control to manipulate the rates, paths, and stereochemistry of chemical reactions. Other pharmaceutical and biological uses of microemulsions include their use as models for biological membranes, as vehicles for separation and purification of proteins and other biopolymers, and in enzymology. One new area of interest for biological microemulsions is their use for the reversal of drug toxicity in the blood of overdose victims. Each of these applications of pharmaceutical microemulsions is discussed in this review.

1.2.2 Introduction to Pharmaceutical Microemulsions

The two main processes that govern the release of drugs from compartmentalized systems are transfer of drug from the dispersed phase to the continuous phase, and diffusion of the drug through a membrane or interface from the continuous phase to the sink solution.[78, 79] To properly select a suitable microemulsion system for drug solubilization and delivery, it is necessary to have general understanding of the behavior of the drug, both in delivery system as well as in vivo. Other parameters such as aqueous and oil solubility of drug, partition coefficient, droplet size, membrane permeability data across body tissues encountered during delivery,[79] and rate of diffusion in both phases are particularly important. Detailed understanding of the components like surfactant, co-surfactant (if required), and oil making up the microemulsion for desired performance characteristics is required.

As previously mentioned, the major advantages of microemulsion drug delivery systems include:

- Ease of preparation
- Stability
- Ability to solubilize different kind of drugs (oil soluble, water soluble, interface soluble)
- Ability to be filtered
- Ability to encapsulate drugs of different HLB in the same system because the microemulsion can solubilize oil-soluble, water-soluble, and interface-soluble drugs
- Low viscosity

Because of these unique physical properties, microemulsions have attracted a great deal of interest in recent years as drug delivery vehicles.[78]

Specific advantages exist for different types of microemulsion systems. For example, water-in-oil (w/o) microemulsions offers protection of water soluble drugs and sustained release

of water soluble material. Similarly, oil-in-water (o/w) microemulsions present increased solubility, sustained release, and bioavailability of oil soluble material. Middle-phase (or bicontinuous) microemulsion systems provide a concentrated formulation of both oil-soluble and water-soluble drugs. When tailoring a specific microemulsion system for drug delivery, the following requirements must be followed for pharmaceutical microemulsion formulations:

1. All components of microemulsion system must be biocompatible
2. Sufficient drug solubility in microemulsion system
3. Microemulsion system should have long shelf life
4. Rate of release and bioavailability of drug must meet the need of the patient

Few studies have been done on the rate of release of drugs from well-defined micellar structures.

1.2.2.1 Biocompatibility

As previously mentioned, the major disadvantage of using microemulsion formulations as vehicles for drug delivery systems are the higher surfactant concentration (>5% usually) and the possible side effects resulting from the surfactants and co-surfactants required to create these formulations. Most of the microemulsion formulations used for technological applications in the oil recovery, nanoparticle and other industries are composed of surfactants, co-surfactants, and oil incompatible with human physiology. Short chain alcohols other than ethanol are also not biocompatible.

The surfactants used for pharmaceutical microemulsions should be nonirritating. Phospholipids, particularly lecithin, offer a possible nontoxic emulsifier for parenteral use. The cosurfactant should be carefully chosen as well. Most existing short and medium chain alcohols currently used as cosurfactants are useless for pharmaceutical microemulsions because of their toxicity and irritancy. Furthermore, evaporation of these alcohols can destabilize the system, resulting in decrease of formulation shelf life. Therefore, the development of biocompatible

surfactants and cosurfactants for use in pharmaceutical microemulsions is becoming a very important technology to challenge.

1.2.2.2 Solubility and stability

A microemulsion is not an inert vehicle. Adding new components such as drugs to system may affect its phase behavior. For instance, solubilized drug molecules may affect the spontaneous curvature of the surfactant in different ways, either by being incorporated into the surfactant film or by changing the polarities of the polar and/or apolar phases. However, it is important to note that in some oil-in-water microemulsions investigated as vehicles for nanoparticles growth, the solubilization of certain salts was found to actually increase, not decrease, the stability of the microemulsion. Hence, the effect of drug solubilization on microemulsion stability may or may not be a disadvantage in a particular microemulsion formulation.

Drug stability is also an important point. The effect, whether beneficial or detrimental, that the microemulsion will have on the drug must be determined.

1.2.2.3 Bioavailability

Drug bioavailability is another factor that must be considered when choosing a microemulsion. Bioavailability is the fraction or percentage of a dose that reaches the systemic circulation intact when not directly injected into the circulation. By maximizing bioavailability, one maximizes the dose concentration or blood level of the drug. Also, if the drugs being administered are expensive to produce, maximizing bioavailability increases cost effectiveness.

1.2.3 Method of Drug Delivery

All three types of microemulsions, oil-in-water, middle-phase, water-in-oil, can be used as drug delivery vehicles. Each type of microemulsion system can dissolve water-soluble, interface-soluble, and oil-soluble drugs or related compounds. The extent to which each type of compound

is solubilized, however, depends on the microemulsion's structure and composition. Jayakrishnan et al.[80] studied the solubilization by w/o microemulsions and found that the amount of drug incorporated into the microemulsion depends on the concentration of both the surfactants (Brij35/Arlacel 186) and the cosurfactants (short chain alcohols). The limit of solubilization depends on the partition coefficient of the drug in oil and water, and on the relative volume of oil and water in the system. The water-external(o/w) microemulsion presumably can be diluted by the aqueous phase of the stomach and intestine. The oil external (w/o) microemulsion will probably go through an inversion or destabilization upon dilution with stomach and intestinal aqueous phase. Depending on the structure of the surfactant molecules in the microemulsion, the surfactant may enhance the penetration rate or rate of transport of drug through the intestinal wall. It has been reported that the rate of penetration of drug is much faster from microemulsion formulations than from other drug delivery vehicles.

Pharmaceutical microemulsions can be delivered by three routes: parenterally (by injection), orally, and topically (to the skin and eyes). The method by which the drug is delivered dictates the constraints that will be encountered in formulation with regards to solubility, bioavailability, toxicity, and site targetability of the microemulsion/drug system.

1.2.3.1 Parenteral delivery

Microemulsions have advantage over conventional parenteral (injectable) drug delivery systems for a wide variety of reasons. Parenteral administration (especially via the intraveneneous route) of drugs with limited solubility is a major problem in industry because of the extremely low amount of drug actually delivered to a target site. Recently, a number of pharmaceutically acceptable microemulsions for parenteral drug delivery have been done. Microemulsion formulations have distinct advantages over macroemulsion systems when delivered parenterally, because fine particle microemulsions are cleared more slowly than coarse

particle emulsions and, therefore, have a longer residence time in the body. Both o/w and w/o microemulsions can be used for parenteral delivery. The type of microemulsion employed is usually determined by the intended route of delivery as well as the role that the microemulsion will play. Although the literature contains details of many microemulsion systems, few of these can be used for parenteral drug delivery because of toxicity of the surfactant, cosurfactant, and/or oil phases. As already discussed, most short-chain alcohols other than ethanol are not acceptable for parenteral use.

1.2.3.2 Oral delivery

Oral drug delivery is generally preferable to parenteral drug formulations. They are less frightening to children as well as easier to administer to them as well as people with difficulty swallowing solid dosage forms. Microemulsion formulations offer several benefits over conventional oral formulations for oral administration, including increased absorption, improved clinical potency, and decrease drug toxicity. Therefore, microemulsions have been reported to be ideal for oral delivery of drugs such as steroids, hormones, diuretics, and antibiotics.

Pharmaceutical drugs of peptide and protein origins are highly potent and specific in their physiological functions. However, most are difficult to administer orally. With an oral bioavailabilty of most peptides in conventional (i.e., non-microemulsion based) formulations of less than 10%, they are usually not therapeutically active by oral administration. Because of their low oral bioavailability, most protein drugs are only available as parenteral formulations. However, peptides drugs have an extremely short biological shelf life when administered parenterally. Because of this, multiple injections are generally required for parenteral administration of peptide drugs.

The peptide drug by far most frequently studied in relation to oral bioavailability is cyclosporine, an immunosuppressant drug commonly delivered orally to transplant patients to

help prevent organ rejection. A microemulsion-based formulation of this drug, Neoral, has been introduced to replace Sandimmune, a crude oil-in-water emulsion cyclosporine formulation. Neoral is formulated with a finer dispersion, giving it a more rapid and predictable absorption profile and less inter-and intrapatient variability. It was found that no penalty was paid by converting stable renal transplant recipient from Sandimmune to Neoral. In fact, doing so resulted in effective, safe, increased drug exposure, reduced intrapatient variability, potential for reduce chronic rejection and thus greater long-term graft survival. Therefore, microemulsion formulations have much potential in increasing the viability of oral delivery of cyclosporin, and further research can lead to similar results for other peptide drugs.

1.2.3.3 Topical delivery

Topical delivery of drugs can have advantages over other methods for several reasons, one of which is the avoidance of hepatic first-pass metabolism of the drug and related toxicity effects. Another is the direct delivery and targetability of the drug to affected areas of the skin or eyes. Recently, there have been a number of studies in the area of drug penetration into the skin. For example, Gasco et al. studied the transport of azelaic acid, a bioactive substance used for treating a number of skin disorders, from a microemulsion system to abnormal skin. Several groups have been investigating the use of microemulsion formulations as ocular drug carriers. The results of these studies seem promising. It was found that in vitro corneal penetration of indomethacin using microemulsions, for example, was more than 3-fold that of currently available eye drops. Microemulsions were also found to prolong the time of drug release, increasing the duration of time the drug spends in the body.

1.2.4 Pharmaceutical Microemulsions Using Nonionic Surfactants

Interest in nonionic surfactants for pharmaceutical microemulsion formulations is increasing because of their low irritation and high chemical stability. Traditionally, nonionic

surfactants of the polyoxyethylene class have been used in the formulation of these microemulsions.[81] Ho et al.[82] formed microemulsions using polyglycerol fatty acid esters as nonionic surfactants and short-chain alcohols as cosurfactants. From tests such as stability and viscosity measurements, they concluded that an oral insulin delivery system employing microemulsions as carriers is applicable. Jayakrishnan et al.[80] reported the solubilization of hydrocortisone by w/o microemulsions containing a mixture of the non-ionic surfactants brij 35 (Polyoxyethylene 23 lauryl ether) and Arlacel 186 (glycerol monooleate-propylene glycol), isopropanol as cosurfactant, water and n-alkane. The influence of oil soluble surfactant (Arlacel 186) concentration, the oil chain length, and the alcohol concentration on the amount of water solubilized in the w/o microemulsion were studied.

The use of non-ionic surfactant, such as n-alkyl polyoxyethylene ether (CmEn) (where m is hydrocarbon chain length and n is the number of oxyethylene units), to stabilize a microemulsion is particularly attractive because it is generally possible to create a microemulsion without the use of a cosurfactant. This has important advantages from a pharmaceutical viewpoint. First, as already discussed, most cosurfactants are not pharmaceutically acceptable. Second, it is not always possible to dilute a microemulsion containing cosurfactant, whereas microemulsions stabilized only by surfactant appear to be infinely dilutable.[83] Third, nonionic surfactants are generally recognized as the least toxic of the surfactants and are currently used in a variety of pharmaceutical products.

1.2.5 Pharmaceutical Microemulsions Using Anionic Surfactants

Many pharmaceutical microemulsions have been developed recently using anionic surfactants with or without nonionic surfactants. For example, addition of non-ionic surfactants sorbitan monolaurate (Span 20, Arlacel 20) to the anionic surfactant Aerosol OT (AOT) increases the phase boundary of w/o microemulsions of AOT.[84] With hexadecane, the oil phase

the maximum solubilization occurs for a 1:1 weight ratio of sorbitan monolaurate to AOT. This microemulsion formulation is suitable for topical drug delivery application because irritancy caused by the presence of various medium chain length alcohols cosurfactants is absent.

Osborne et al.[85] have shown that sorbitan monolaurate acts as a cosurfactant for the AOT microemulsion system and significantly affects its phase diagram. They have also observed that the mixing time for the waxy AOT solid with viscous sorbitan monolaurate is quite long, but this can be overcome by ethanolic AOT solution, as very low concentrations of ethanol are accepted pharmaceutically because of ethanol's low irritancy and toxicity compared to other alcohols.

Addition of an oil (isopropyl myristate) to the w/o microemulsion system of AOT/water/medium chain alcohol can result in the formation of o/w microemulsions. Trotta et al.[86] produced o/w microemulsion with butanol as cosurfactant, but this formulation has limited applicability because of toxicity of butanol. Garcia-Celema et al.[87] investigated the use of microemulsion composed of Tween 80 (polyoxuethylene-20 sorbitan monooleate) surfactant and isopropyl myristate and isopropyl palmitate for topical application of antifungal drugs clotrimazole, ciclpirox olamine, and econazole nitrate. They reported that antifungal drugs with solubilities ranging from slightly soluble to practically insoluble in both water and oil can be successfully dissolved in a pharmaceutically acceptable ternary microemulsion system. Berthod et al. attempted to use an o/w microemulsion system as a mobile phase for rapid screening of illegal drugs in sports using reverse-phase HPLC. The system heptane/water/sodium dodecyl sulphate (SDS)/n-pentanol was used to quantify 11 drugs in a reversed-phase liquid chromatography column. Now since microemulsion systems have the ability to assay water and oil soluble drugs at once, they have significant potential as drug delivery vehicles.

1.2.6 Pharmaceutical Microemulsions Using Phospholipids and Cholesterol

Phospholipids, particularly lecithin are very good surfactant for microemulsion formulation because of their very low toxicity.[88] But, problem in using lecithin as surfactant for microemulsion is that lecithin is slightly too lipophilic to spontaneously form zero mean curvature lipid layer needed for balanced (middle-phase) microemulsion. Also, it is necessary both to adjust the HLB of the lecithin, as well as to destabilize the lamellar liquid crystalline phase that have a strong tendency to form in these systems. HLB adjustment is usually done by adding short chain alcohols that makes the polar solvent less hydrophilic. Aboofazeli[88] have reported the phase properties of lecithin/propanol/water/n-hexadecane systems. Around 2-3% lecithin is the minimum amount required to form a microemulsion at all mixing ratios. The propanol concentration should be in the range 10-15 wt% of the aqueous solvent, with the concentration decreasing slightly with increase in oil content. They have also reported the phase properties of water/lecithin/alcohol/isopropyl myristate system where alcohols were n-propanol, isopropanol, n-butanol, s-butanol, t-butanol and n-pentanol. Major factor influencing the phase behavior of lecithin based microemulsions is the nature and mixing ratio of the cosurfactant used. It is possible to produce a large clear isotropic region extending over a large range of oil and water composition, called balanced microemulsion, by using very high amounts of ethanol (60-80% w/w aqueous phase) as cosurfactant in a lecithin-based system. Other workers have used both o/w and w/o lecithin microemulsions as vehicles to deliver a range of drugs.[86, 89-91]

Considerable interest has been shown in the use of microemulsion drug delivery systems composed of phospholipids for parenteral delivery of lipophilic drugs. Microemulsion systems containing phospholipids are rapidly captured after intraveneous injection by the reticuloendothelial system(RES) organs such as liver and the spleen, and by inflammatory cells.

This can be advantageous in treating diseases localized to the RES182 or to macrophages. But, accumulation of drugs in the liver and spleen could be hazardous in case of other drugs.

Microemulsions also provide unique advantages as opthalmological carrier system, as no impairement of visibility is encountered. Surfactant-containing multicomponent systems for ocular application have been developed and characterized. Kriwet et al.[92] studied the relationship between the colloidal structures of a topical formulation and the drug release in vitro as well as the influence of the microstructure on the stratum corneum drug permeability. They found that phospholipids are able to interact with the structures of the stratum corneum if they are applied as a microemulsion, as opposed to liposomal formulations and other investigated systems. Gasco et al.[93] compared the effectof topical administration of timolol via microemulsion with aqueous solutions. Microemulsion based delivery with lecithin resulted in better concentration-time profile (3.5 times) than the aqueous timolol solutions. It is quite evident that phospholipids and cholesterol based microemulsions have great potential as drug delivery systems, primarily because of their biocompatibility. Such systems can become significant players in the future of targeted drug delivery applications.

1.2.7 Pharmaceutical Microemulsions Using Sugar based Surfactants

Sugar based surfactants microemulsion formulation have potential applications because of their low toxicity, biocompatibility, and excellent biodegradability. They offer an attractive alternative to more convenient ethylene oxide (EO)-based nonionic polymers.[94] Sugar based surfactants are prepared from natural and renewable resources that allow scientists to change structural combinations which have modulating surfactant properties. Also, the phase behavior of this class of non-ionic surfactants is much less influenced by temperature than the phase behavior of typical EO-based nonionic surfactants.[95, 96] Bolzinger et al.[97, 98] investigated the relationship between microetructure and efficacy of a sucrose ester based microemulsion as a drug delivery

system. Sucrose esters are biodegradable surfactants whose hydrophilic and lipophilic properties can be adjusted by varying fatty acid chain lengths.

1.2.8 Pharmaceutical Microemulsions for Drug Detoxification

Drug overdose is a major health care problem all over the world. A number of widely used drugs can cause life-threatening toxicities and are without antidotes. Microemulsion systems that can sequester drugs may offer a solution to this problem. Intravenous injections of such systems can potentially treat overdoses by adsorbing/absorbing drug molecules, thereby, reducing the free drug concentration in blood and tissues.

Varshney et al.[99] have studied various pluronic based o/w microemulsion system for reducing the free concentration of the local anesthetic bupivacaine in normal saline. They observe that both the molecular nature and concentration of the constituents in the microemulsion significantly affect extraction efficiency of microemulsions. Extraction was markedly increased by addition of fatty acid sodium salts due to greater oil/water interface area, increased coulombic interaction between bupivacaine and fatty acids sodium salt, and greater surface activity. Pluronic F127 based microemulsions extracted bupivacaine more efficiently than microemulsions synthesized using other Pluronic surfactants (L44, L62, L64, F77, F87, F88, P104).

1.3 Retardation of Water Evaporation

Water, is one of the main necessity of life. We all know the whole process of water cycle in which it evaporates from sea/river etc., and then condense in upper atmosphere to form clouds and finally rains. There are several instances where we want to reduce the evaporation of water such as in ophthalmology wherein people suffering from dry eye syndrome show very high water loss from eyes, nuclear industry wherein radioactive water from spent fuel may escape into the atmosphere, from fruits and vegetables to increase their shelf life, water conservation in certain

arid and dry parts of world where water is scarce etc. To emphasize the severity of the problem, around 180 million people worldwide suffers from dry eye including 10 million Americans, and nearly 50% of the world population will be living in areas, having water shortage by 2050. So, there is a great need to develop methods to reduce the water loss by evaporation.

The idea of spreading a layer of a hydrophobic (oily) substance on top of water is an old one, dating back to Benjamin Franklin's experiments of damping of water waves in 18th century. Based on the same concept, in early 20th century, Hedestrand[1] and Rideal[2] tried to demonstrate the effect of monolayers on the evaporation rate of water on which they were spread. Early experiment on water evaporation by various researchers demonstrated that a monolayer can decrease the evaporation rate of water. In 1943, Irving Langmuir and Vincent Schaefer[100] substantiated Eric Rideal's[2] pioneer finding that monolayers of long chain alcohols were effective suppressants of the evaporation of water. They measured evaporation rates of water by spreading monolayer, over the surface of water in a film balance, with a flat container with a permeable bottom supporting a solid desiccant $CaCl_2$. Australian researchers, in particular William Mansfield[101] and R.G. Vines, after preliminary laboratory experimentation, then developed successful methods for applying such monolayers on the surface of the large water reservoirs to control evaporation.

In 1952, La Mer and his co-workers[102-106] continued Langmuir's work in an extensive series of laboratory investigations in which the properties to be sought in a monolayer for evaporation control were investigated in detail. The primary aim of his research was to gain knowledge of the molecular mechanism involved in the retardation of evaporation, and understanding of the permeability of films, by studying the influence of molecular architecture. This was accomplished through study of rate of evaporation, in terms of the state of compression

of the film, and the temperature dependence, by means of Arrhenius equation. He used the method of Langmuir and Schaefer[100] for measuring the evaporation of water through monolayers. This method for measuring the rate of evaporation is satisfactory when properly modified, for investigating the influence of the molecular architecture of the molecules composing the monolayer. It gives a quantitative measure of the resistance to evaporation, uncomplicated by motion of air above the monolayer or by any other disturbances.

Benzene and Petroleum ether (Hexane) have been used for many years as volatile solvent for the less soluble long chain molecules. These solvents facilitate the rate of spreading. However, it has been shown by Archer and La Mer[105, 106] that Langmuir's method of spreading the film, by using benzene as spreading solvent and then compressing to the desired surface pressure, yields results that are difficult to reproduce. This is because the rate of spreading of various acids, alcohols is strongly dependent upon the chain length of the molecule. For example, $C_{22}OH$ spreads 2400 times lower than $C_{14}OH$. In the case of long chain solutes, it is the solvent molecules of the solution, which supply the driving force for spreading.[107] They carry the nonspreading solute molecules along, leaving, on evaporation of the solvent, a monolayer of supposedly pure solute molecules.

In 1970's it has been demonstrated conclusively that a compressed, molecularly oriented monolayer will be effective in reducing evaporation of water. Different properties like, evaporation resistance, temperature effects, heat transfer through monolayers have been studied for various monolayers.[108-110] Also, in our laboratory we have shown the importance of molecular packing[111, 112] achieved by using mixed surfactant monolayers and pKa of fatty acids[113] in reduction of evaporation of water.

1.3.1 Duplex Films

Over the years various researchers have worked on water evaporation by monolayers, but till now, maximum reduction in evaporation using monomolecular film of fatty acids or alcohols was around 50%. Moreover, the problem with monolayers is that they can be easily removed by winds and undergo thermal as well as biological degradation.[114] Under such circumstances multimolecular (duplex) films of oil and surfactants would be ideal. Figure 1-7 shows the difference between monolayer and duplex film.

Heymann and co-workers[115, 116] in 1940s had used multimolecular films of oil as a means of preventing or reducing the evaporation of water from exposed water surfaces in arid climates. Using such multimolecular films of paraffin oil and neutral oil from vertical retort tar, they showed a significant reduction in evaporation of water. They found that films of paraffin oil of 1 to 2μm in thickness to which suitable spreading agents have been added would reduce the evaporation by 50 to 60%. Reductions up to 85% were obtained with 1μm films of certain high boiling fractions of neutral oil of vertical retort tar. Powell and co-workers[117] have calculated rate of evaporation of water through surface films of long chain oils. However, other than that not much literature is found in this area to the best of our knowledge.

1.3.2 Spreading of Oil on Water Surface

A quantity of oil placed upon a water surface will spread out by surface-tension forces if the spreading coefficient (S) is positive. This is the net surface tension available to drive the spreading.[3]

$$S = \sigma_w - \sigma_f^0 \qquad (1.7)$$

Where σ_f^0 is the total tension of the oil film at the spreading origin. In the past, σ_f^0 has always been assumed to equal $\sigma_o + \sigma_{ow}$, the sum of the oil-air and oil-water tensions. If the

deposited volume is small enough, gravity will not be an important spreading force, and the dominant resistance to spreading will be the viscous drag exerted on the oil film by the underlying water.[118, 119]

Many oils, including the heavier hydrocarbons, have negative spreading coefficients and will not spread on water.[120] They will spread, however, if they contain a sufficient concentration, C of surfactant, which reduces to the extent that S becomes positive. So, to form a duplex film of surfactant in oil S should be positive, as shown in below (Figure 1-8).

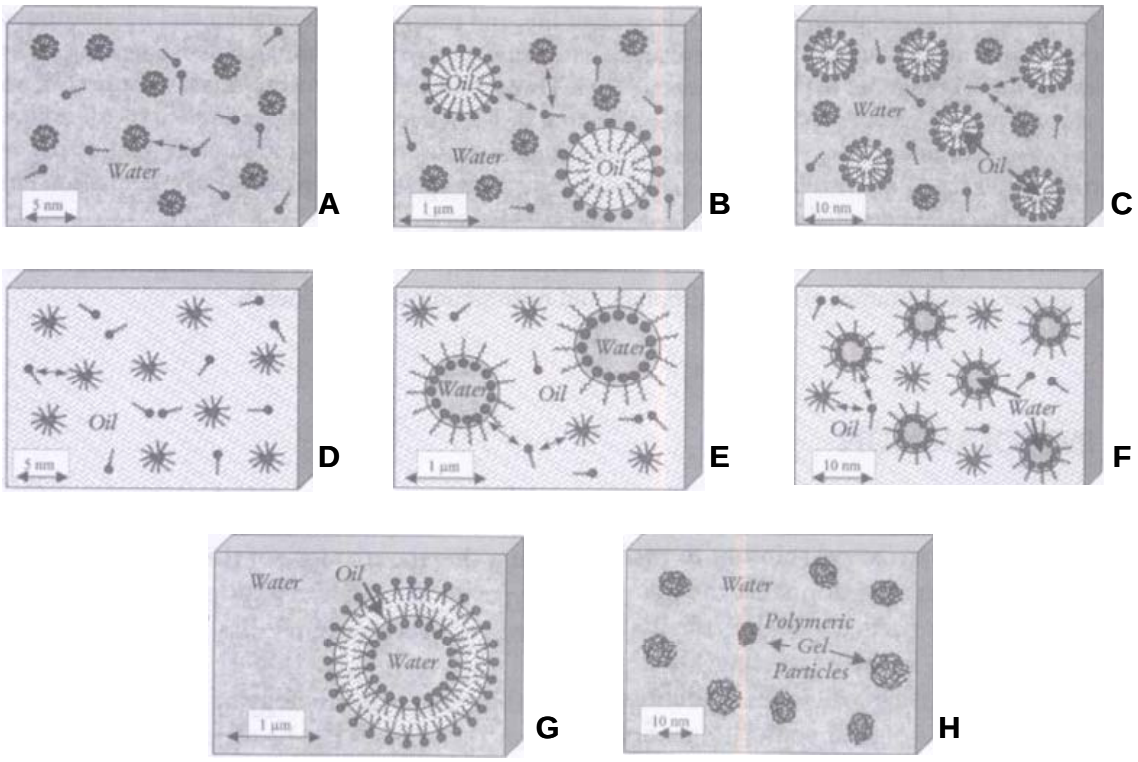


Figure 1-1. Various types of colloidal drug delivery systems. A) Normal Micelles. B) O/W Emulsion. C) O/W Microemulsion. D) Reverse Micelles. E) W/O Microemulsion. F) W/O Microemulsion. G) Liposomes. H) Soft Nanoparticles.

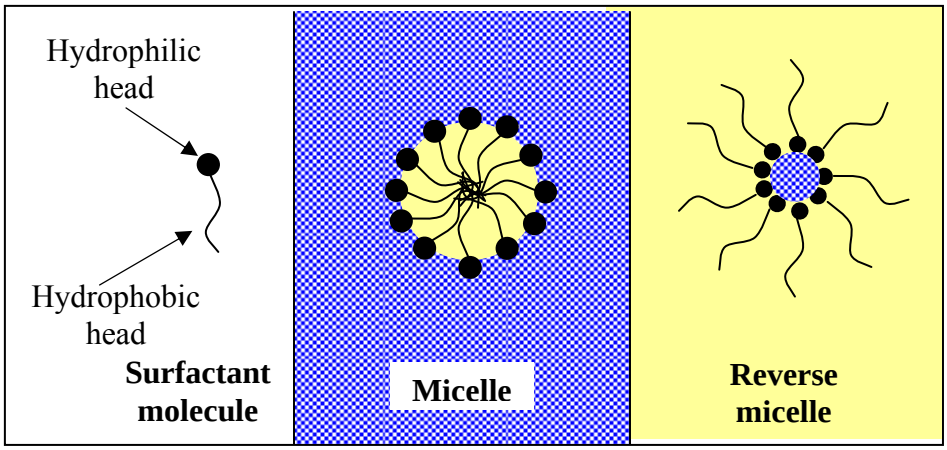


Figure 1-2. Schematic diagram of a surfactant molecule, micelle, and reverse micelle.

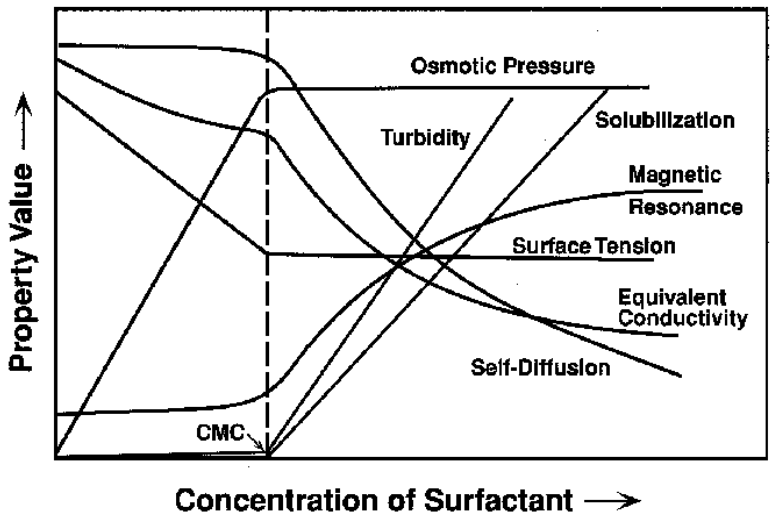


Figure 1-3. Properties of surfactant solutions showing abrupt change at the solution critical micelle concentration (cmc).

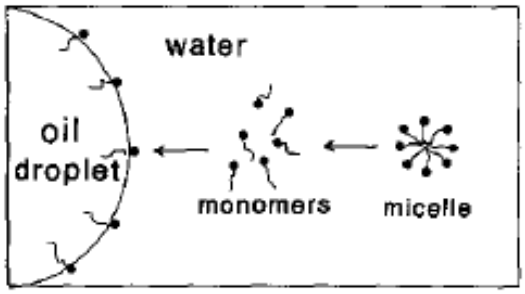


Figure 1-4. Schematic diagram of the adsorption of surfactant monomers from the bulk to the oil/water interface during emulsion formation.

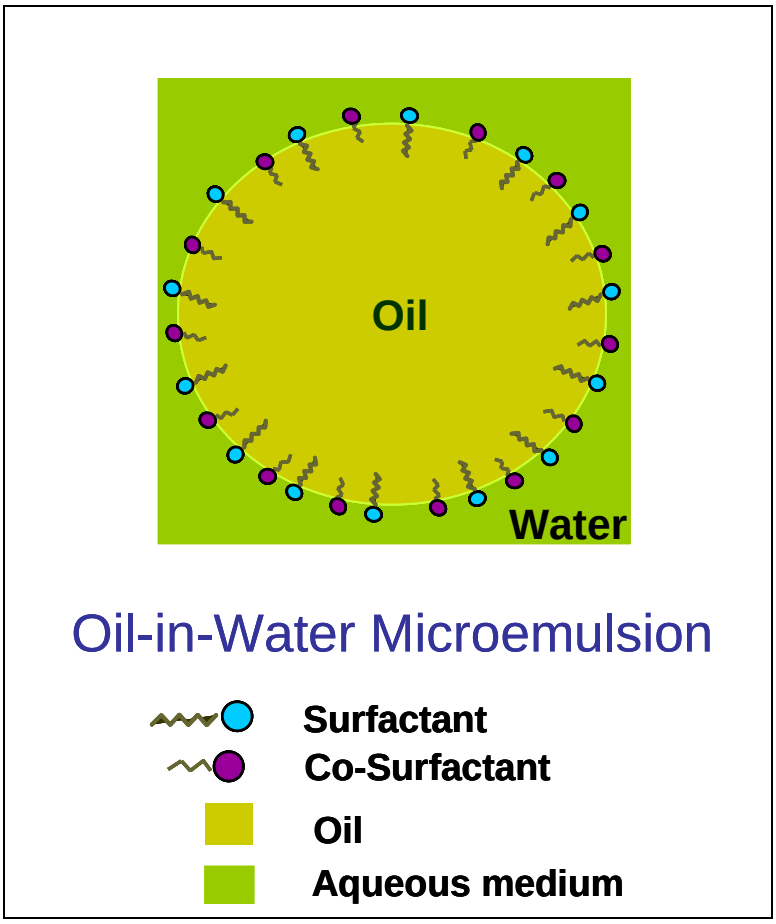


Figure 1-5. Schematic diagram of an oil-in-water (O/W) microemulsion.

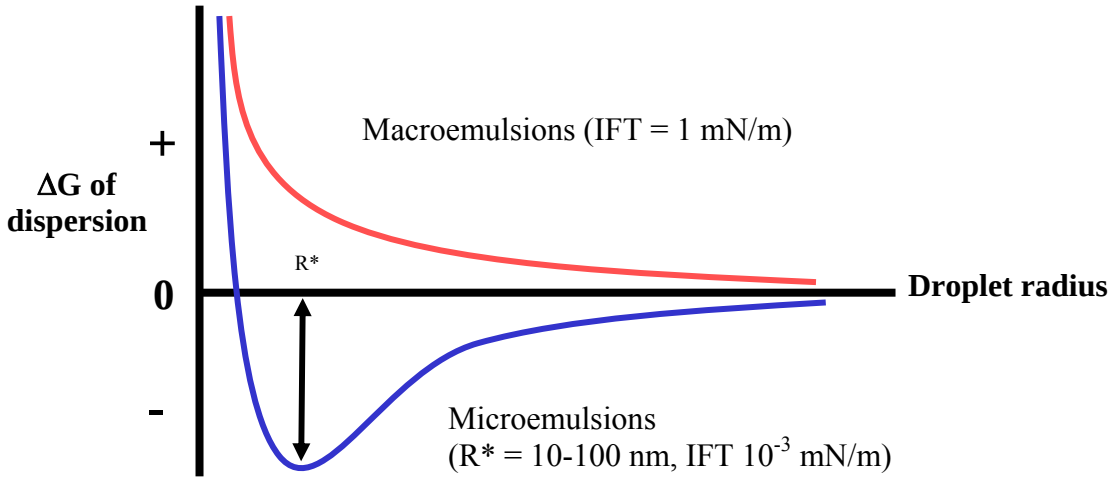


Figure 1-6. Thermodynamic explanation for behavior of macroemulsions and microemulsions.

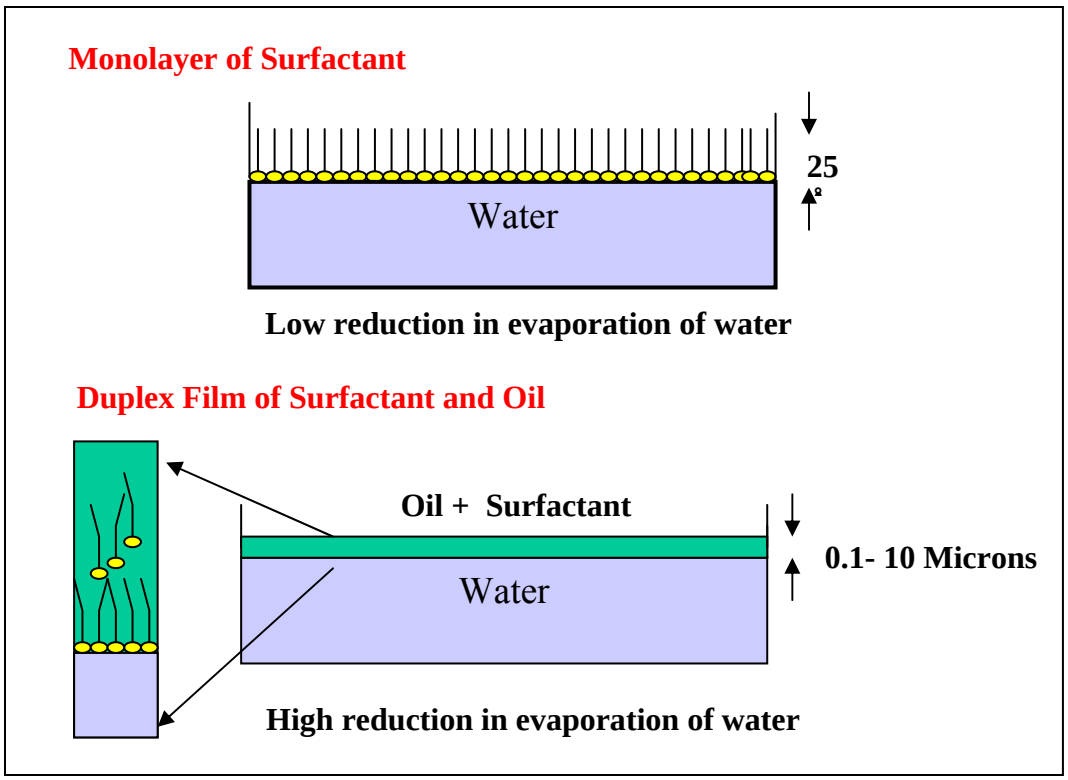


Figure 1-7. Difference between monolayer and duplex film.

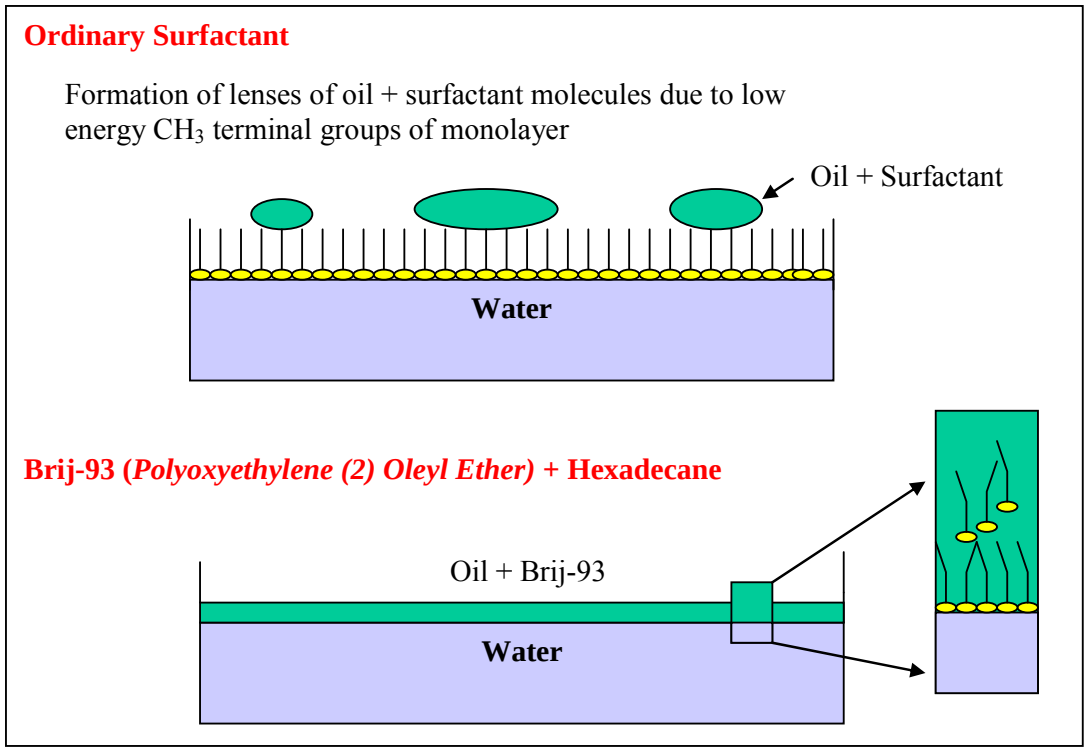


Figure 1-8. Problems in forming uniform duplex film.

Table 1-1. Physical characteristics of various drug delivery systems

Delivery System	Advantages	Disadvantages
Micelles	Low Viscosity Small droplet size Easy Preparation Long shelf-life	Low solubilization Potential toxicity of surfactant
Microemulsions	High solubility of drug Small droplet size Easy preparation Long shelf-life	Large amount of surfactant Drug solubility influenced by environmental conditions Potential toxicity of surfactant
Emulsions	Small amount of surfactant High solubility of drug into carrier	High viscosity Instability Short shelf life Large droplets
Vesicles and liposomes	Made from lecithin and cholesterol Also present in the body	High viscosity Difficult to prepare Often disintegrate once administered
Nanoparticles	Long storage life In vaccinations Slow degradation in body	Limited solubility of drug Difficult to prepare Difficult to control size Polymers which represent constituents are usually not bioacceptable

Table 1-2. Types of breakdown processes occurring in emulsions

Breakdown Type	Description
No change in droplet size (or size distribution)	Buildup of an equilibrium droplet concentration gradient within the emulsion. This phenomenon results from external force fields, usually gravitation, centrifugal, or electrostatic, acting on the system. "Creaming" is a special case in which the droplets collect in a concentrated layer at the top of an emulsion.
No change in basic droplet size, but with buildup of aggregates of droplets in the emulsion	This process is called "flocculation" and results from the existence of attractive forces between the droplets.
Flocculated droplets in an aggregate coalesce to form larger droplets	This process also occurs when creaming or sedimentation results in a close-packed array of droplets and these droplets coalesce. The limiting state is the complete separation of the emulsion into two immiscible bulk liquids.
Average droplet size increases due to the two liquids forming the emulsion being not totally immiscible	This process does not involve actual coalescence of droplets, but rather the transfer of dispersed phase across continuous phase after solubization occurs. If the emulsion is polydisperse, larger droplets will form at the expense of smaller droplets due to the difference in chemical potential for different size droplets (Ostwald ripening). In principle, the system will tend to an equilibrium state in which all the droplets have combined and are one large droplet, or a separated phase.
Emulsion type inverts from W/O to O/W type	This is a questionable "breakdown" process since essentially another emulsion is formed. The inversion process can be brought about by numerous parameter changes, which will be discussed in detail later in this section.

Table 1-3. Factors influencing the stability of emulsions

Factor	Description of Effect
Physical nature of the interfacial film	For mechanical stability, a surfactant film with strong lateral intermolecular forces and high film elasticity is desired. A mixture of two or more surfactants is preferred over a simple surfactant (i.e., lauryl alcohol + sodium lauryl sulfate).
Electrical barrier	Significant only in O/W type emulsions, because of conductivity in continuous phase. In the case of nonionic emulsifying agents, charge may arise due to adsorption of ions from the aqueous phase. The repulsion or attraction can be influenced by changing the thickness of the double layer, which is described below.
Viscosity of the continuous phase or of emulsions	A higher viscosity reduces the diffusion coefficient of the dispersed droplets, resulting in reduced frequency of collision and lesser coalescence. Viscosity can be increased by adding natural or synthetic thickening agents. Viscosity also increases as the number of droplets increases; so many emulsions are more stable in concentrated form than when diluted.
Size distribution of dispersed droplets	Uniform size distribution is more stable than an emulsion with the same average droplet size but having a wider size distribution.
Phase volume ratio	As volume of dispersed phase increases, stability decreases Phase inversion can occur if dispersed phase volume is increased enough.
Temperature	Usually, as temperature increases, emulsion stability decreases because of increased frequency of collision.
Steric barrier	Addition of polymer that adsorbs at interface can influence stability. Polymer chains can prevent coalescence due to bulkiness, but they can also enhance flocculation and decrease stability.

Table 1-4. Parameters that affect phase inversion in emulsion and the effect they have

Parameter	Effect on phase inversion
Order of phase addition	W → O + emulsifier → W/O O → W + emulsifier → O/W
Nature of emulsifier	Bancroft's Rule - Making the emulsifier more oil soluble tends to produce a W/O emulsion and vice- versa
Phase volume ratio	Oil/Water Ratio increased in an O/W emulsion →W/O emulsion and vice-versa, as described above in the text
Phase in which emulsifying agent is dissolved	If surfactant can be dissolved at least partially in either water or oil Bancroft's Rule - If surfactant is dissolved in water→ O/W emulsion
Temperature	Depends on the surfactant and its temperature dependence. If emulsion is O/W type with polyoxyethylenated nonionic surfactant, phase inverts to W/O with increase in temperature due to increased hydrophobicity of the surfactant.
Addition of electrolytes	Strong electrolytes (polyvalent Ca) added to O/W (stabilized by ionic surfactant) → inversion to a W/O type Because of decrease in double layer thickness around oil droplets, droplets coalesce and become the continuous phase.

Table 1-5. A summary of HLB ranges and their application

HLB Range	Application
3 to 6	W/O emulsifier
7 to 9	Wetting agent
8 to 18	O/W emulsifier
13 to 15	Detergent
15 to 18	Solubilizer

Table 1-6. Microemulsions vs. Nano-emulsions

Characteristic	Microemulsions	Nano-emulsions
Stability	Thermodynamically stable	Thermodynamically unstable
Droplet size	10 – 100 nm	20 – 500 nm
Surfactant concentration	Usually require 10 – 30 wt% surfactant	Can be formed with 4 – 8 wt % surfactant
Formation	Independent of mixing protocol	Dependent of mixing protocol

CHAPTER 2
PREPARATION OF PROPOFOL MICROEMULSIONS

2.1 Introduction

Much interest had been recently generates in the pharmaceutical and biomedical fields for the development of microemulsions as a drug delivery system. Microemulsions are transparent, thermodynamically stable, isotropic, low-viscosity dispersions consisting of micro domains of oil and/or water stabilized by interfacial film of surface active molecules. Such microemulsions contain droplets with the dimension in the range of 10-100 nm and, hence appear transparent like single phase liquid. Microemulsions provide a highly attractive carrier system for the delivery of drugs because of high total interfacial area due to their small droplet size, their thermodynamic stability as well as their spontaneous formation. The usefulness microemulsions as a drug carrier system stems from their ability to incorporate hydrophilic or hydrophobic drugs which can be protected from direct contact with the body fluids and tissues and can be delivered slowly over a period of time with the decrease in hyper-sensitivity reactions and pain during administration.

The active substance used in this study is propofol (2,6-diisopropylphenol) (Figure 2-1). Propofol is a popular intravenous general anesthetic agent due to several favorable characteristics of this drug. These properties include an anti-emetic effect and rapid emergence from unconsciousness with minimal residual drowsiness.[121-124] However, a primary drawback of propofol is this drug's extreme lipophilicity that necessitates dispersion in soybean macroemulsions to produce white, opaque formulations. This solvent requirement potentially causes several adverse drug outcomes including bacterial growth leading to postoperative infection, severe pain in a majority of patients with peripheral intravenous injection, inclusion of egg products, and others.[125, 126] Alternative formulations yielding similar pharmacodynamic characteristics as the conventional formulations, but without the associated liabilities, would be

useful to enhance patient safety and comfort. Recently, efforts have been made to achieve these goals using other lipid solvents and concentrations, cyclodextrin formulations, microemulsions technology, and prodrug techniques that depend on native enzymes such as alkaline phosphatase to metabolize a parent compound (i.e., phosphono-2,6 diisopropylphenol) to the active drug molecule (i.e., propofol).[127-131]

To address these drawbacks of conventional propofol formulations, we hypothesized that this agent could be associated with biocompatible surfactants to form transparent, colorless, thermodynamically stable, low viscosity, oil-in-water microemulsions with droplets having a 10 50 nm diameter. Thus, instead of regarding propofol's extreme lipophilicity as a hindrance to be overcome, the lipophilicity of propofol (which exists as an oil at room and physiological temperatures) was leveraged to construct the physical core of the microemulsions. Therefore, propofol served dual roles as both the lipid oil core of the microemulsion and the pharmacodynamically active agent to cause anesthesia. In addition, we hypothesized that the spontaneous destabilization of the microemulsion nanodroplets to release propofol could be selectively altered by modifying the concentration and nature of the associated surfactants with resultant changes in latency to anesthetic induction. That is, we hypothesized that the pharmacodynamic and pharmacokinetic properties of propofol microemulsions could be modified by varying the nature and concentration of surfactants. To examine these hypotheses, here, we first determined the physical limitations of possible propofol microemulsions using a variety of propofol and surfactant concentrations of these systems. Second, we measured several parameters of anesthetic induction and emergence in animal model receiving the experimental and conventional formulations of propofol using a randomized, crossover design in rats and dogs (Chapter 3).

2.2 Methods and Materials

2.2.1 Materials

Propofol was obtained from Albemarle Corporation (Baton Rouge, LA). Purified Pluronic, nonionic coblock polymers consisting of polyethylene and polypropylene monomers, was purchased from the BASF Corporation (Florham Park, NJ). Tween, Brij, Mrij, Polyethylene Glycol (PEG), Sodium salts of fatty acid (C8, C10, and C12) were supplied by Sigma Chemical Co. (St. Louis, MO).

2.2.2 Synthesis of Propofol Microemulsions

Microemulsions were prepared by combining propofol (0-100 mg/ml) with surfactant and co-surfactant in normal saline (0.90 mg/ml NaCl) bulk media. Most of the data in this study correspond to pluronic microemulsions which consist of purified pluronic 68 (0-70 mg/ml), and a fatty acid salt (0-12.5 mg/ml) in normal saline (0.90 mg/ml NaCl) bulk media. Water was ultra-purified using a water purification system (Nanopure, Barnstead/Thermolyne, Dubuque, IA) to provide a minimal electrical resistance of 18.2 MΩ. Following agitation with a magnetic stirrer, these components combined to form clear, colorless microemulsions with adjustment of pH to 7.40 using either HCl or NaOH. All experimental formulations were stored under a nitrogen headspace. To characterize the dimension of the individual droplets, the effective droplet size of the nanoparticles was measured by the dynamic light scattering method using a submicron particle sizer analyzer (90Plus, Brookhaven Instruments Corporation, Holtsville, NY) as previously described.[132]

2.2.3 Viscosity Measurements

A Paar Physica US-200 rheometer with cone and plate geometry was used to measure the viscosity of the microemulsions. Viscosity measurements were performed at 25 °C temperature, and the sample temperature was controlled within 1 °C using water as the heat transfer fluid. In

all experiments, a cone of the radius 4.3cm with cone angle 0.5 degree was used. The shear viscosity (η) of the microemulsions of different compositions was measures as a function of shear rate (η) at 25 °C.

2.2.4 Stability Evaluation of Microemulsions

The stability test of propofol microemusions were carried out at room temperature over a period of two and half years. The changes in the droplet size distribution, viscosity, pH and percentage of oil separation were determined and the results were used to evaluate the stability of these systems.

2.2.5 Stability on Dilution of Microemulsions

The stability on dilution test of propofol microemusions were carried out at room temperature by adding 100µl of saline solution to 10 ml microemulsion in every five minutes. The endpoint was the loss of transparency or in other words onset of turbidity.

2.2.6 Oxidation Studies

Oxidation of propofol in microemulsion as well as macroemulsions were done by LC/MS analyses using a Perkin-Elmer PE 200 LC system and an API 4000 triple quadrupole mass spectrometer equipped with an APCI source (Applied Biosystems, Foster City, CA). Samples (25 µl injection volume) were separated on a C18 column (Xterra TM RP18, ODS 5 µm 250 mm × 4.6 mm i.d., Waters) with a guard cartridge (4 mm × 3 mm i.d., Phenomenex, Torrance, CA) prior to the C18 column.

2.3 Results

2.3.1 Microemulsion Synthesis and Characterization

Microemulsions of propofol were formulated using varying concentrations of propofol, purified pluronic, and fatty acid salt in a normal saline bulk media in order to construct a phase diagram of these systems (Table 2-1). Depending on the concentrations of surfactants, propofol

concentrations of 12-15 mg/ml or less produced microemulsions. In contrast, propofol concentrations exceeding 20 mg/ml with these concentrations of surfactants formed opaque, white macroemulsions similar to conventional propofol formulations. For purposes of this study, the final concentration of propofol in the microemulsions was selected to be 1% (10 mg/ml), a concentration equal to that in the conventional commercially available macroemulsion formulation (Figure 2-2).

Subsequently, we more closely investigated the interaction of the concentration and types of surfactants and co-surfactants necessary to form microemulsions (or macroemulsions) while holding the concentration of propofol constant at 10 mg/ml (Figure 2-3, 2.4). At the greatest concentration of purified pluronic 68 (70 mg/ml), no fatty acid co-surfactant was required to create microemulsions of propofol as shown in the binary diagram (Figure 2-3). However, as the concentration of purified pluronic 68 decreased, the concentration of the fatty acid surfactant necessary to form a microemulsion increased. In addition, the nature of the fatty acid significantly affected its concentration of fatty acid salt necessary to create a microemulsion. Thus, the concentration of fatty acid salt required to formulate propofol as the oil core of a microemulsion was markedly diminished by lengthening the carbon chain of the co-surfactant fatty acid from 8 to 10 or 12 carbon atoms at any concentration of purified pluronic 68 and 127. Minimal-to-no differences were noted for microemulsions constructed with C10 or C12 with respect to formation of a microemulsion or a macroemulsion.

The measured particle sizes for various formulations of the microemulsions varied from 11.9 to 47.7 nm (Figure 2-5). In general, as the concentration of purified pluronic 68 was increased, the diameter of the particles also increased irrespective of the carbon length of the

fatty acid cosurfactant. No change was noted in the dimension of the microemulsion droplets containing after two and half years of storage at room temperature (Table 2-2).

We also investigated the relative stability of these propofol microemulsions to dilution with normal saline. That is, we determined the volume of the saline diluent necessary to break the microemulsion into a macroemulsion (Figure 2-6). The volume of diluent required to cause these changes increased with lengthening of the carbon chain in the fatty acid salt. This increase in the diluent volume was most noticeable for the C12 propofol microemulsions that required approximately a six-fold dilution to break the microemulsion.

Propofol in air oxidize to its dimers and trimers, which are yellow in color. And thus becomes yellow over a period of time. The exact mechanism of oxidation to its dimers and trimers is still debated by the scientists. But everyone agrees to the fact that this yellow color development is because of oxidation. To remove the problem of oxidation, we have done two things. First, we kept all the microemulsion samples under nitrogen so that without the oxygen required for oxidation, the oxidation time period is increased. Figure 2-7 represents the gas chromatography data of propofol microemulsion system purged with nitrogen. As we see from the data, nitrogen purged from the system does not remove any propofol from the microemulsion system.

Second, we added chelating agent EDTA (Ethylenedinitrilotetraacetic acid) to which chelates various ions present in the system and thus reduces the oxidation process. Figure 2-8 (a) represent the mass spectroscopy of propofol in microemulsions as compared to pure propofol (Figure 2-8(b)). As we see from MS/GC results the concentration of propofol dimmer (quinine) is much lower in microemulsion system than macroemulsion formulation.

The viscosity values of the microemulsions did not change with time upon (Table 2-3). But the effect of concentration of fatty acid salt on pH as well as conductivity of the micoemulsion was significant (Figure 2-9, 2-10). As sodium salt concentration is increased, the pH, conductivity of the microemulsions also increased due to increase in the ionic surfactant concentration. This data of pH, conductivity is before adding HCL and NaOH to change the pH of the microemulsion system to 7.4 and tell us the amount of acid/base required to change the pH of the system.

2.4 Discussion

Propofol is an organic liquid that exists as oil at room temperature with minimal aqueous solubility. This insolubility is both a fundamental property of propofol and a significant problem for purposes of drug delivery. To address this issue, propofol was formulated in a castor bean oil macroemulsion in the early 1980s, but this solvent was subsequently abandoned due to its propensity to cause anaphylaxis.[133, 134] Instead, a soy bean oil macroemulsion was selected as an alternative emulsification agent to deliver propofol.[135] Currently, these white, opaque macroemulsions (Diprivan®, Baxter PPI Propofol®) contain: propofol (10 mg/ml), soybean oil (100 mg/ml), glycerol (22.5 mg/ml), egg lecithin (12 mg/ml), and a preservative (0.05 mg/ml disodium edetate or 0.25 mg/ml sodium metabisulfite). The requirement that propofol be dissolved in 10% soybean oil with associated surfactant has caused a number of liabilities including support of bacterial growth, addition of egg products that is objectionable to vegans, the necessity of preservatives, and modulation of the inflammatory pathways.[126, 136]

The need for a suitable alternative formulation is evidenced by past and ongoing research into other types of propofol delivery systems. Several investigators have reported favorable results using formulations composed of medium- and long-chain triglyceride macroemulsions to deliver propofol at varying concentrations of 10-60 mg/ml.[128, 137, 138] In addition, propofol has

been complexed with sulfobutylether 7-β-cyclodextrin molecules to form clear, colorless formulations that are stable at a wide range of temperatures (4-50°C), a clear benefit compared to macroemulsion technology, and that have pharmacokinetic properties similar to the conventional drug delivery system.[129, 139] Finally, pro-drug methods have been exploited to develop phosphono-2,6-diisopropylphenol, an agent that is metabolized by native alkaline phosphatase to generate the active agent, propofol, and two byproducts, formaldehyde and phosphate.[140] In this report, we demonstrate that microemulsion methods represent another suitable technology to deliver propofol intravenously with anesthetic parameters similar to the commercially available macroemulsions.

Microemulsions systems are useful for parental administration of drugs. In this study various microemulsion formulations given in Table 2-1 were employed to investigate the physiochemical stability over a period of two and half years. Various concentrations of surfactant solutions (1% to 20%) in 0.9% saline were employed to solubilize propofol. Some microemulsion developed yellow color in time due to oxidation of propofol to its dimers and trimers. But as seen in Figure 2-8 the amount of one of the dimer (quinone) is much lower in microemulsion than in macroemulsion formulation. This suggest us that microemulsion system being smaller dynamic system keep the propofol droplets separated from the ions present in the system longer and thus reducing the oxidation process.

2.4.1 Microemulsion Technology for Drug Delivery

Microemulsions have been previously used to deliver several drugs by the oral and transcutaneous routes. Currently, an oral microemulsion of cyclosporine is available for prevention and treatment of transplant rejection of solid organs.[141] In addition, a number of other drugs have been formulated in microemulsions for oral (e.g., paclitaxel, heparin) and transdermal (e.g., apomorphine, estrogen) delivery.[142-145] Fewer drugs have been delivered using an

intravenous route, but include trans-retinoic acid, flurbiprofen.[146] Unlike other oil-in-water microemulsions wherein the active drug is dissolved in an oil excipient, in the present study propofol acted in two different, complementary roles in the present investigation. That is, the need for an excipients oil for propofol dispersal was obviated by the fact that propofol is itself an oil at room and physiological temperatures. Therefore, propofol could serve not only as the active pharmacodynamic agent, but also could exist as the physical platform for the preparation of these microemulsions. By doing so, the need for additional excipients oils (e.g., soybean or castor bean oil) is eliminated along with the potential for these excipients to nourish bacteria. Although the we hypothesize that propofol microemulsions will not support bacterial growth to the same extent as macroemulsions, additional investigations are needed to test this thesis.

The kinetics of general anesthesia with the propofol microemulsion should be favorable compared to the commercially available propofol macroemulsion. Whereas the time for general anesthesia (defined by the protocol as loss of leg withdrawal to a pinch) should be higher in the case of microemulsion as they have extra surfactant at the interface, because of which it takes longer time for the drug to come out in blood. This short delay is favorable when compared to pro-drug technologies that rely on metabolism, a phenomenon that may vary significantly within any patient population.[147] The differences in induction times between experimental groups reported herein may be caused by differential release of propofol from the individual droplets into the blood. That is, the different propofol nanoparticles have markedly different stabilities against dilution by blood based on the emulsifier structure and concentration selected for the formulation. In general, a microemulsion is thermodynamically stable at equilibrium. One can destabilize a microemulsion by significantly changing pressure, temperature, or chemical compositions. The last variable can be changed simply by diluting a microemulsion with saline

or blood. It is well recognized that the formation of microemulsions requires an ultra low interfacial tension (e.g., ~10^{-3} mNewton/m) at the oil/water interface. Upon dilution with saline, the interfacial tension will increase substantially as the emulsifier molecules (i.e., both pluronic 68 and fatty acid salt molecules) desorb from the droplet surface. This event will markedly increase the interfacial tension at the droplet surface and ultimately destabilize the microemulsion, with release of the active pharmaceutical core (i.e., propofol). However, each emulsifier film around the microemulsion droplet has inherent molecular packing and, hence, stability. The extent of dilution required to destabilize a microemulsion represents its inherent stability. Thus, a microemulsion requiring greater dilution for destabilization indicates that its emulsifier film has a greater stability. Figure 2-7 suggests that the C12 fatty acid salt microemulsions are the most stable as they require the greatest dilution to become a turbid macroemulsion. It is likely; however, that microemulsion destabilization is affected by more than just dilution in vivo as the surfactant can go to various other places in vivo dilution than in vitro. Further understanding and selectively modifying these destabilization rates by use adapting surfactant type and concentrations alludes to the possibility of controlling release times of active pharmaceuticals from nearly immediate (e.g., propofol) to longer times (e.g., chemotherapeutic or antifungal agents).

Since propofol is water insoluble and causes severe pain on injection, it has been formulated as a 1% solution in a fat emulsion containing 10% soybean oil consisting of long chain triglycerides (LCT). However, about half of patients still experience moderate to severe pain on injection.[148, 149] Thus, several methods have been devised to prevent this pain, for example injection into a larger vein, use of various drugs (aspirin, fentanyl, alfentanil, metoclopramide, nitroglycerin, prilocaine, pethidine),[150-154] and co-administration with either

lidocaine or nafamostat mesilate (a kallikrein inhibitor),[149, 155] of which the most effective and common are use of a larger vein and mixing with lidocaine.

Regarding one of the mechanisms of injection pain, recent studies demonstrated generation of the bradykinin caused by propofol and its inhibitory effect of lidocaine and nafamostat mesilate, so that these two drugs are considered to decrease the pain by reducing the plasma bradykinin levels.[149, 156] Bradykinin is produced by contact between the lipid solvent for propofol and the plasma kallikrein-kinin system, and results in modification of the injected vein, such that the propofol in the aqueous phase has easy access to the free nerve endings of the vessel, causing aggravation of the pain.[156] The reason why propofol causes pain on injection was studied by Ohmizo[150] using nafamostat mesilate, a kallikrein inhibitor, as follows: the lipid solvent (long chain triglycerides (LCT)) for propofol activates the plasma kallikrein-kinin system during injection, generating bradykinin that causes hyperpermeability of the vessel and thus dilates the injected local vein. As a result, there is increased contact between the aqueous phase propofol and the free nerve endings of the vessel, resulting in aggravation of propofol-induced pain.[156] When bradykinin generation is reduced by the pharmacological effect of nafamostat mesilate, the propofol-induced pain is decreased. Alternatively, the decrease in pain when using long chain triglycerides/ medium chain triglycerides (LCT/MCT) propofol is considered to be attributed to the lipid solvent that decreases the propofol concentration in the aqueous phase.[137] Association between the aqueous phase propofol concentration and injection pain has also been reported in some studies.[137, 157] Thus, it is possible that a reduction in either bradykinin generation or the aqueous phase propofol concentration decreases the pain on injection with propofol. In addition, it has recently been reported that use of 10% long and medium chain triglycerides (LCT/MCT) mixed at a 1:1 ratio in the carrier emulsion, as an alternative to LCT, reduces pain without any

changes in the pharmacokinetics and pharmacodynamics. It was suggested that this can be attributed to a decreased concentration of the aqueous phase propofol.[127, 158] According to the above reports, reduction of bradykinin generation or propofol concentration in the aqueous phase may be the main reasons for reduced pain on injection with propofol emulsified in LCT/MCT. Although a decrease in the aqueous phase propofol in this emulsion has been demonstrated, [150] the effect on bradykinin generation has not been examined. Apart from the pain, it has been demonstrated that complement (C3a) is activated by LCT.[159] Although the clinical implication of this complement activation is unclear, it may be important, because it has been associated with pulmonary edema.[159]

One benefit of slightly delayed propofol release due to these differential stabilities may be reduced pain on injection. That is, we hypothesize that the concentration of aqueous propofol in peripheral veins will be sufficiently low so that minimal-to-no pain will be experienced during induction. If the relaxation rate is sufficiently long to ensure nanoparticle integrity from the time of injection in a peripheral vein to the time they enter the central circulation (e.g., 10-15 s), then no pain should be caused during injection. This hypothesis is based on the observations of Seki and colleagues[160] who noted that patients with propofol administered through a central venous catheter do not experience pain on injection whereas those patients injected via a peripheral intravenous catheter have a 53-76% incidence of pain.

2.4.2 Selection of Surfactants

The nonionic and ionic surfactants used to formulate propofol microemulsions were carefully selected not only for their ability to combine with the drug to form stable microemulsions, but also because both surfactants have been previously injected intravenously into humans without reported adverse incidents. Justification for choosing these surfactants is presented subsequently.

2.4.3 Nonionic Surfactants

The selection of purified pluronic 68, an ethylene oxide propylene oxide block copolymer, was justified based on number characteristics related to chemical and medical requirements. First, this surfactant allowed rapid and reproducible preparation of propofol microemulsions that possessed good stability over time. In general, microemulsions are thermodynamically stable and hence exhibit infinite shelf life. These formulations are destabilized upon dilution with blood or plasma or saline beyond its tolerance limit. Second, purified pluronic 68 has been previously administered intravenously in large doses to humans without any apparent adverse effect. For example, this surfactant was infused to patients suffering chest crisis due to sickle cell disease in an attempt to reduce the duration and severity of the crisis.[161] Although not efficacious to treat chest crisis at large doses of (e.g., 88g), no apparent untoward effects were noted in the subjects. Third, this surfactant is primarily (>95%) excreted by kidneys and does not stimulate or inhibit human cytochrome P450 systems.[162] In fact, others have hailed this coblock surfactant as a model surfactant for pharmacological use.[162]

2.4.4 Ionic Surfactants

Sodium caprylate was chosen as the best ionic surfactant for the following reasons. First, the surfactant allows reliable synthesis of propofol microemulsions with particle diameters of <50 nm range. Second, sodium caprylate is already present in many blood products (e.g., human albumin) currently in clinically use to stabilize proteins.[163, 164] Third, the smaller length of the carbon backbone reduces the risks of cellular injury associated with fatty acids with longer carbon chains such as sodium oleate that is used to cause lung injury as a model of acute respiratory distress syndrome.[165] These data suggest that sodium caprylate is the best choice currently available for additional investigations using microemulsions of propofol, although the

information from use of C10 and C12 allowed us to understand how surfactant selection can affect the pharmacokinetic and pharmacodynamic properties of propofol microemulsions.

2.4.5 Propofol Anesthetic Action

Propofol is a 2,6-diisopropylphenol with sedative-hypnotic properties used for general anesthesia or as a sedative during surgeries and intense care.[166] Because of its slight solubility in water, the drug is formulated as an emulsion for clinical use. It is highly lipophilic and distributes extensively in the body. Due to the short duration of its anesthetic effects propofol is also utilized for anesthesia maintenance in association with narcotics, benzodiazepin derivatives and inhalatory agents. Its mechanism of action is uncertain, but it is postulated that its primary effect may be potentiation (enhancement) of the GABA-A receptor, possibly by slowing the channel closing time.[167]

Gamma-aminobutyric acid (usually abbreviated to GABA) is the major inhibitory neurotransmitter found in the in the vertebrate central nervous system (CNS), whose action is produced by its selective interaction with at least two classes of GABA receptors, namely GABAA and GABAB receptors..[167] While GABAB receptors are members of the G-proteinlinked receptor superfamily and are coupled with K+ and Ca2+ channels, GABAA receptors are ligand-gated ion channels coupled to an integral chloride channel.[168] Propofol induces central depression by means of a potent agonistic effect on the GABAergic transmission, mediated by binding to the GABAA receptor, and by reducing the metabolic activity in the brain. GABAA receptors are composed of a number of phylogenetically related subunits (α1-6, β1-4, γ1-3, δ, ε, ρ1-3), that coassemble to form a pentameric structure which contains a central Cl^- channel.[169] Indeed, a number of distinct classes of drugs (benzodiazepines and benzodiazepine-like compounds, beta-carbolines, steroids, barbiturates, alcohols, picrotoxin,

tertbutylbicyclophosphorothionate (TBPS)) exert their effects by interacting with specific modulatory sites on this receptor.[168, 169] One of the major outcomes of the recent research in the field of general anesthetics is the observation that a large number of chemically unrelated anesthetics possess the common ability to influence the function of GABAA receptors.[170] There is evidence that propofol also exerts a presynaptic action, leading to an accumulation of g-aminobutyric acid (GABA) in the synaptic cleft and contributing to its hyperpolarizing action in postsynaptic receptors, which are coupled to chlorine channels.[170] Recent research has also suggested the endocannabinoid system may contribute significantly to propofol's anesthetic action and to its unique properties.

During induction, propofol decreases the systolic and diastolic blood pressure by approximately 20-30 percent with minimal change in heart rate; apnea is also common. The cardiovascular and respiratory effects of propofol, however, should not cause major concern in otherwise healthy patients. By virtue of its pharmacokinetic profile, the drug lends itself to continuous infusion for maintenance of anesthesia. When used as the main anesthetic agent, it produces satisfactory anesthesia with rapid recovery and without major adverse effects in healthy individuals. In continuous infusion propofol can be used as an alternative to inhalation anesthetics.[171]

Propofol permeates the cell membrane at very high rates and has a very high affinity for tissues so that its apparent volume of distribution, depending on the conditions and kind of tissue, can exceed several folds as compared to the aqueous space. Since it interacts intensely with membranes and proteins, the drug also exerts many metabolic effects, including impairment of energy metabolism. Studies of interactions of propofol with tissues, esp. those ones occurring

at the interface between membranes and proteins are likely to reveal new details about the general mode of action of the drug as well as about the mechanism of its anesthetic action. Propofol is highly protein bound in vivo and is metabolised by conjugation in the liver. Its rate of clearance exceeds hepatic blood flow, suggesting an extrahepatic site of elimination as well. The elimination half-life of propofol has been estimated to be between 2–24 hours. Propofol is extensively metabolized by the liver prior to its elimination by the kidney. However, its duration of clinical effect is much shorter because propofol is rapidly distributed into peripheral tissues, and its effects therefore wear off considerably within even a half hour of injection. This, together with its rapid effect (within minutes of injection) and the moderate amnesia it induces makes it an ideal drug for IV sedation.

2.5 Conclusions

Here, we report the successful preparation, characterization, and use of propofol microemulsions for anesthesia in rat similar to that caused by propofol macroemulsions. Formulation of propofol microemulsions was achieved although no excipient oil (e.g., soybean or castor bean oil) was used because propofol exists as an oil and can serve as its own core of an oil-in-water microemulsion. Thus, propofol was used both to solubilize itself and as an active pharmaceutical. The differential diluent volumes in vitro based on changing the concentration and type of surfactant alludes to the possibility of selectively modifying the pharmacokinetic and pharmacodynamic properties of propofol, and potentially other lipophilic drugs.

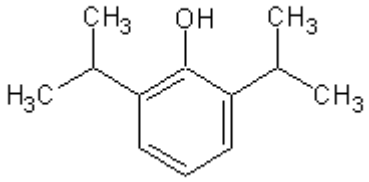


Figure 2-1. Propofol.

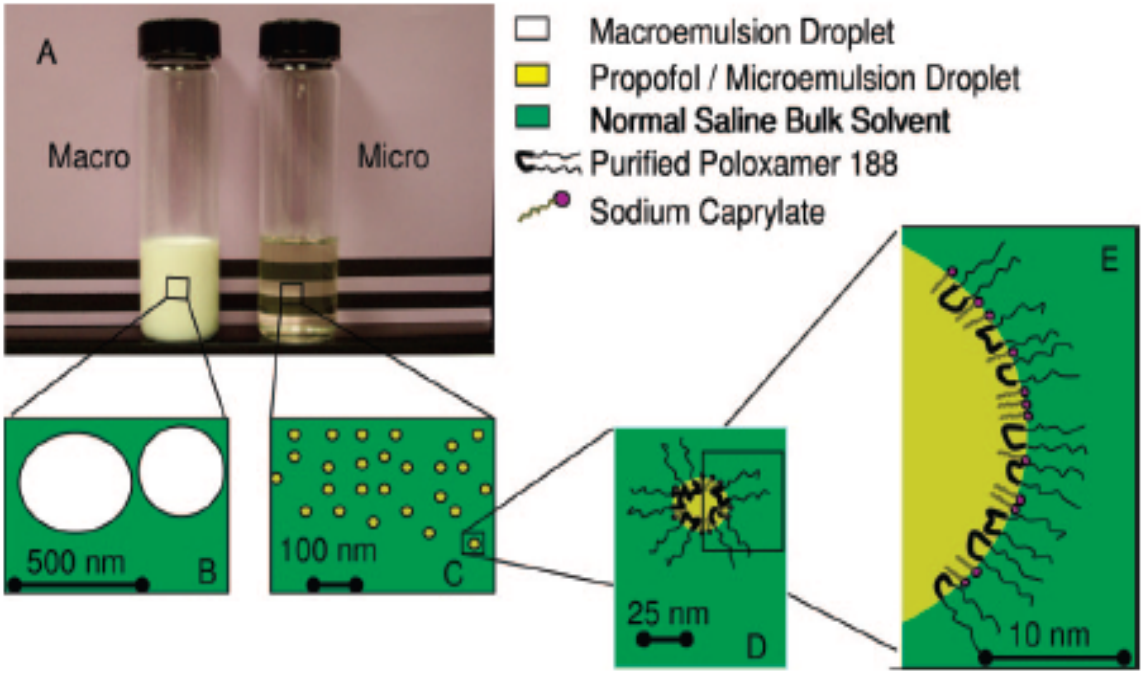


Figure 2-2. Schematic diagram of an oil-in-water (O/W) microemulsion.

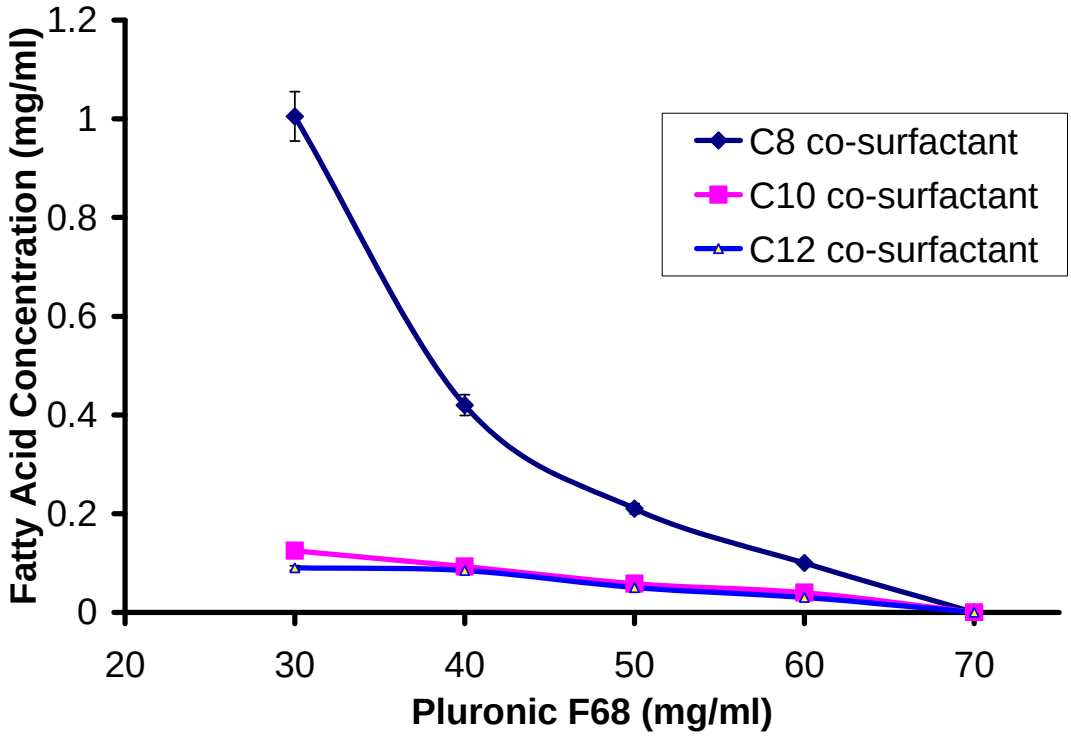


Figure 2-3. Binary diagram noting the concentrations of purified pluronic 68 and the co-surfactant fatty acids necessary to form propofol microemulsions in a bulk media of normal saline. The concentration of propofol was constant at 10 mg/ml. For a given fatty acid cosurfactant, the region above the line is a microemulsion whereas the area below the line represents a macroemulsion formulation.

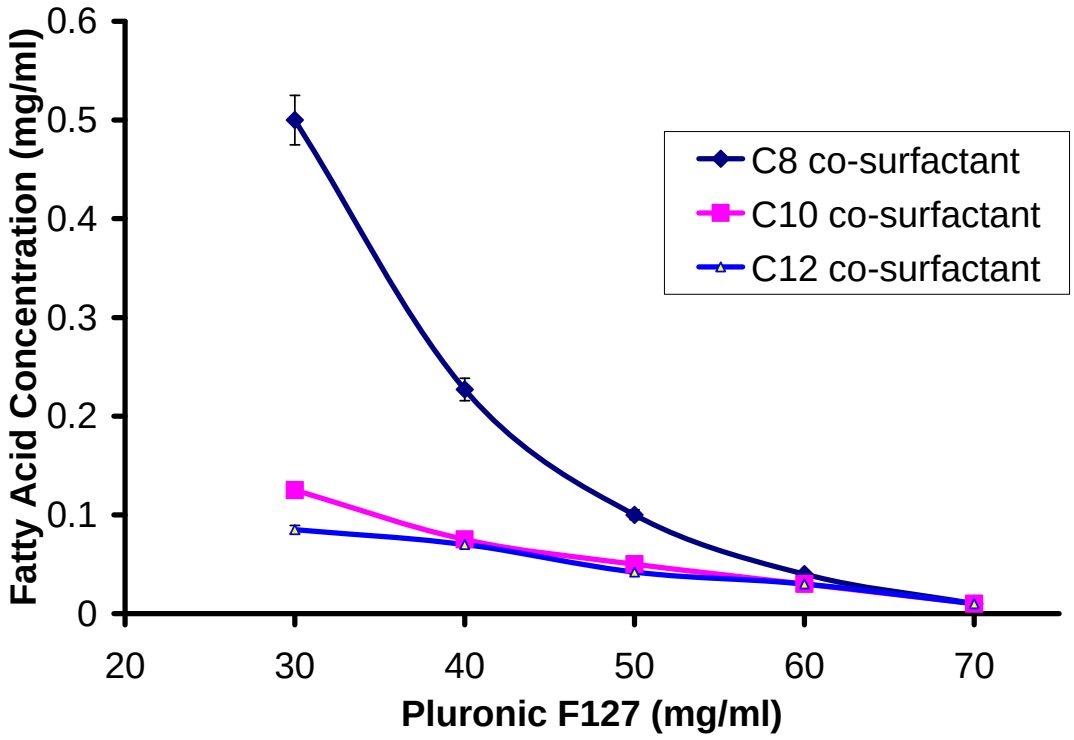


Figure 2-4. Binary diagram noting the concentrations of purified pluronic 127 and the co-surfactant fatty acids necessary to form propofol microemulsions in a bulk media of normal saline. The concentration of propofol was constant at 10 mg/ml. For a given fatty acid cosurfactant, the region above the line is a microemulsion whereas the area below the line represents a macroemulsion formulation.

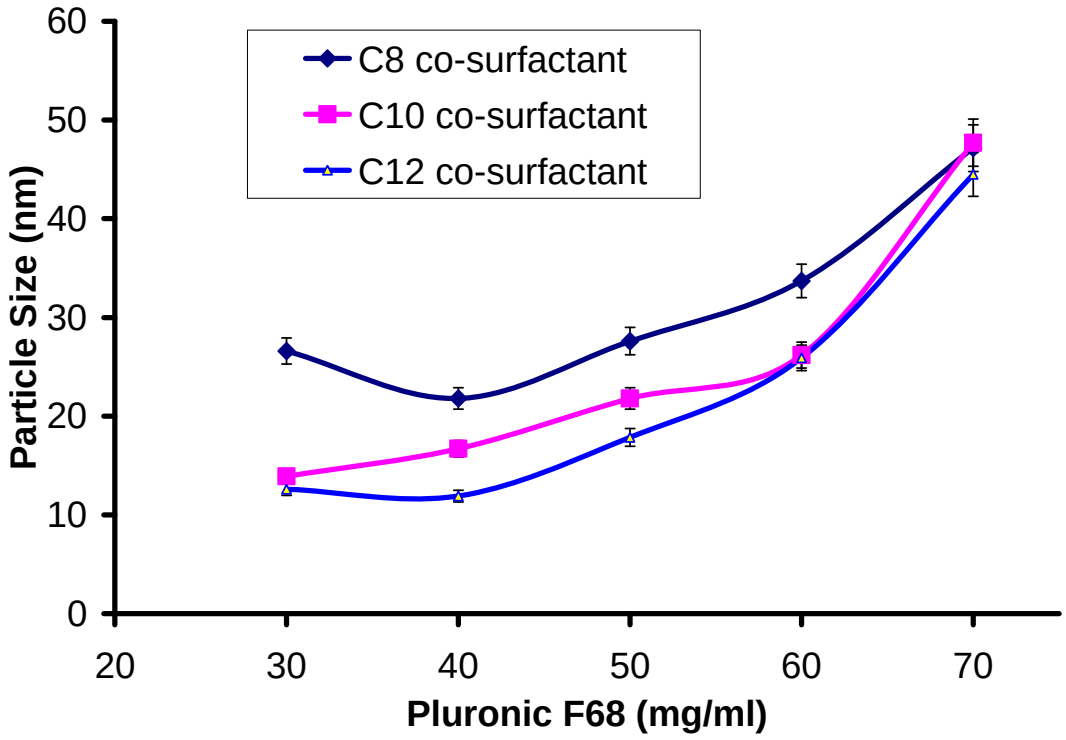


Figure 2-5. Effects of purified pluronic 68 concentration and fatty acid chain length on nanodroplet diameter for propofol microemulsions. In these systems, the concentration of propofol was held constant at 1% (10 mg/ml) and the minimum necessary amount of fatty acid salt was added to formulate propofol microemulsions.

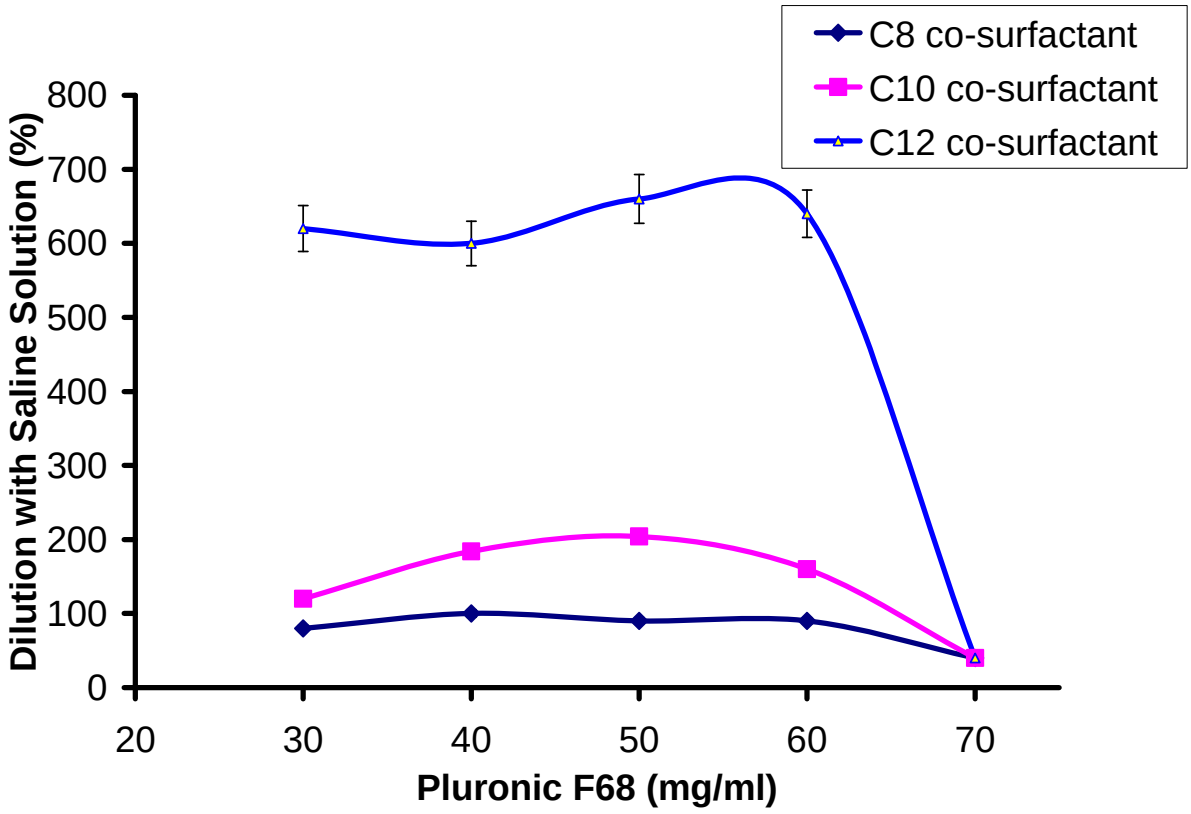
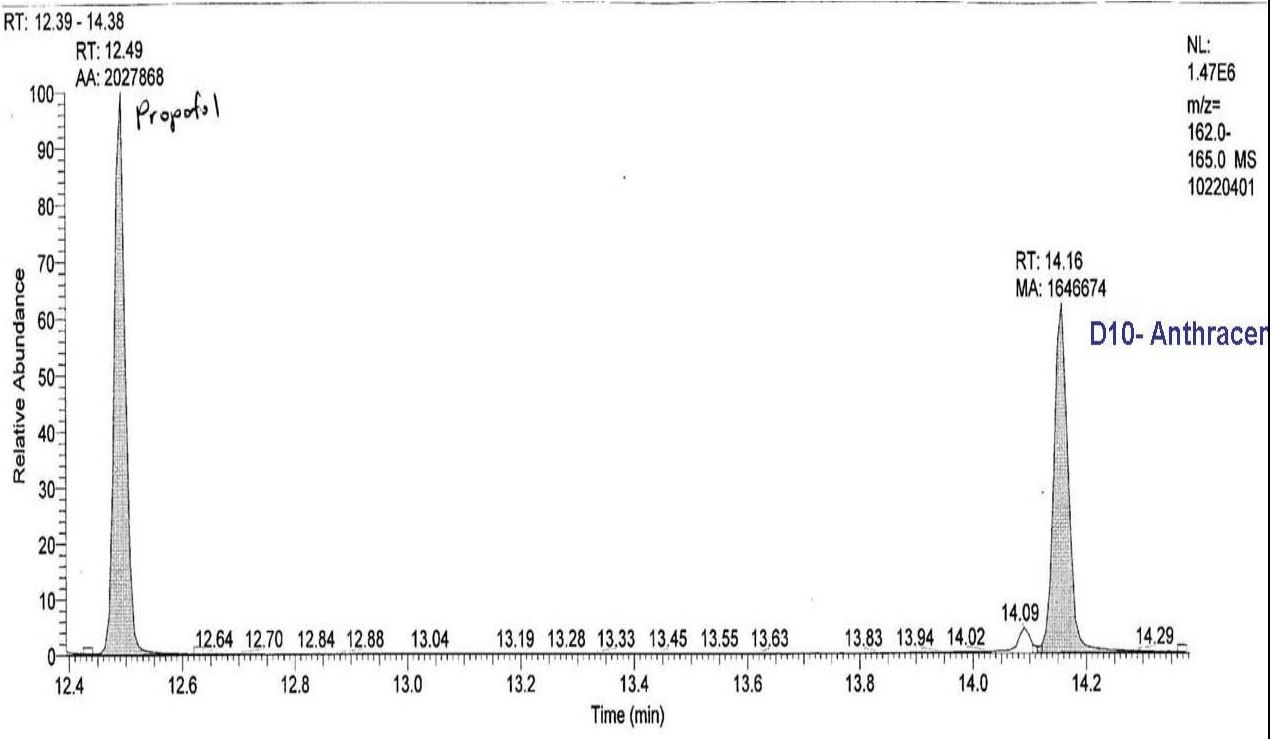
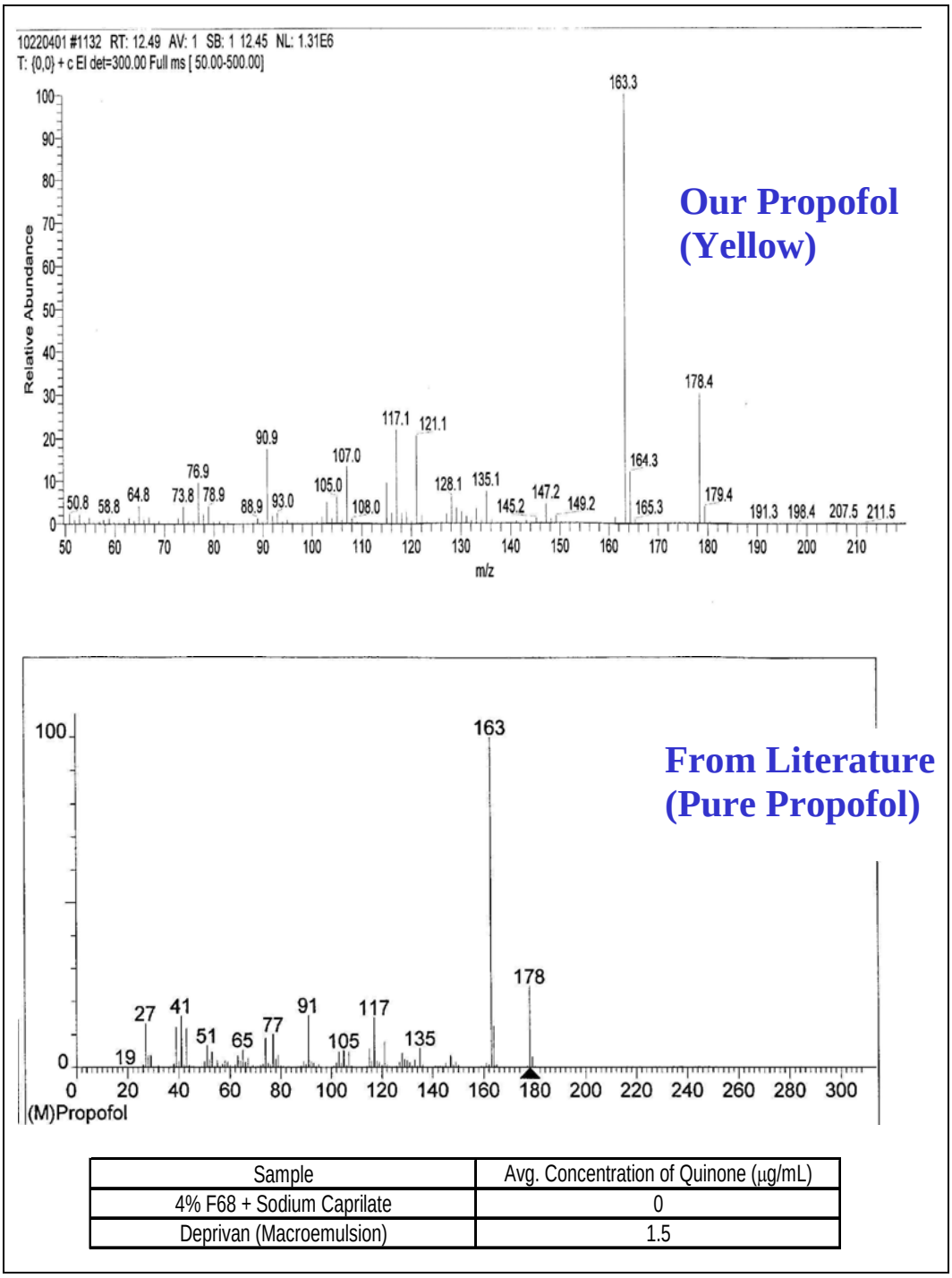


Figure 2-6. Effect of dilution on propofol microemulsions formulated using various concentrations of purified pluronic 68 and several fatty acid salts with variable carbon chain length. Shown are the relative volumes of 0.9% NaCl diluents that caused the microemulsions to break into macroemulsions (i.e., a transition from a transparent to a turbid state). The concentration of propofol in the undiluted microemulsions was constant at 1% (10 mg/ml).



Effect of Purging on Propofol Content of Microemulsion	
Sample	4% F68 + Sodium Caprilate (Microemulsion)
	Avg. Concentration (μg/mL)
1. No Purging	66.5
2. N_2 Purging	65.6

Figure 2-7. Gas chromatography of propofol microemulsion.



Sample	Avg. Concentration of Quinone (μg/mL)
4% F68 + Sodium Caprilate	0
Deprivan (Macroemulsion)	1.5

Figure 2-8. Mass spectroscopy of propofol.

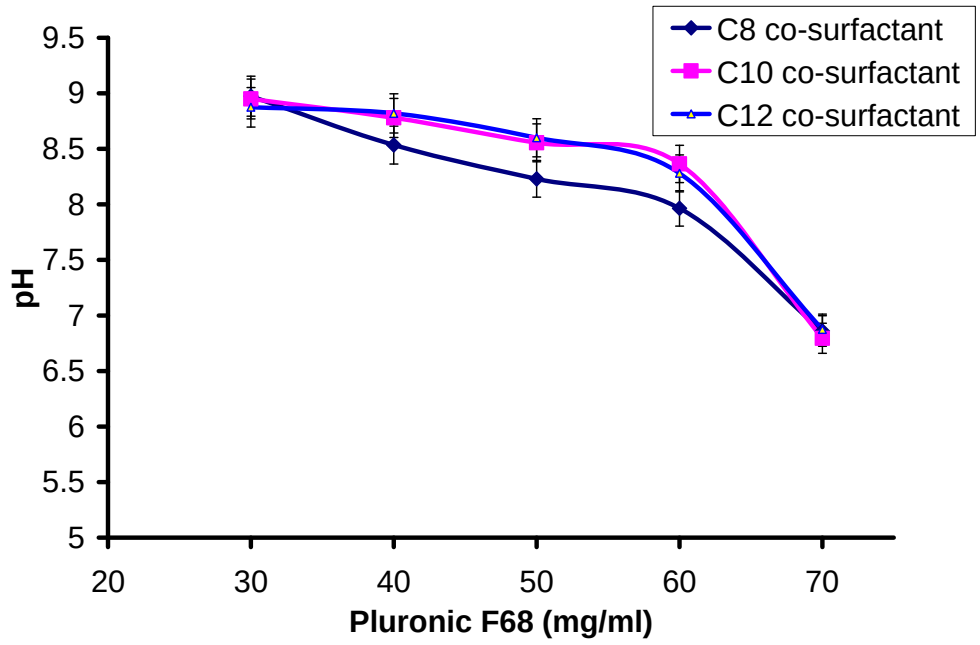


Figure 2-9. The pH of F68 microemulsion.

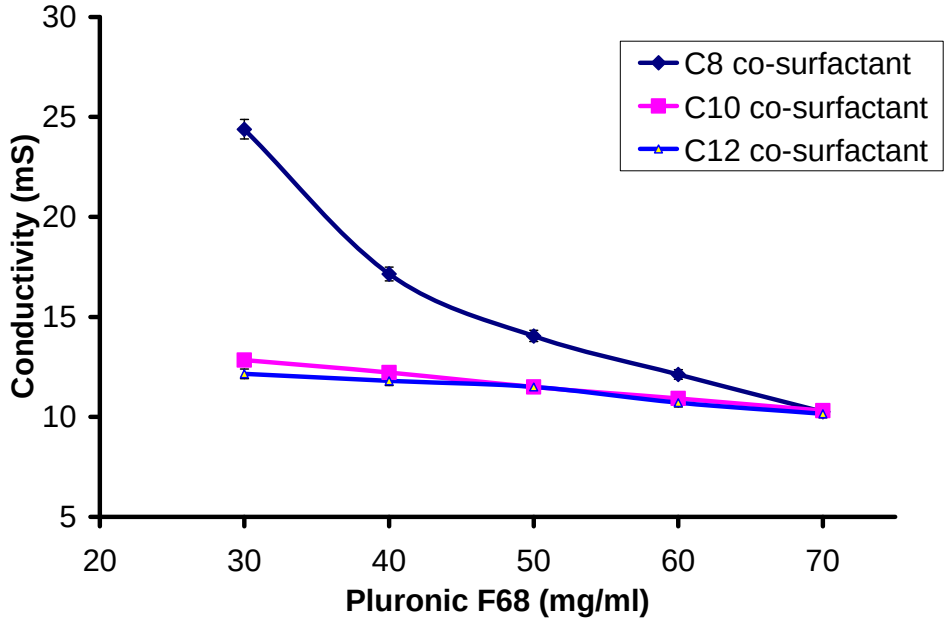


Figure 2-10. Conductivity of F68 microemulsion.

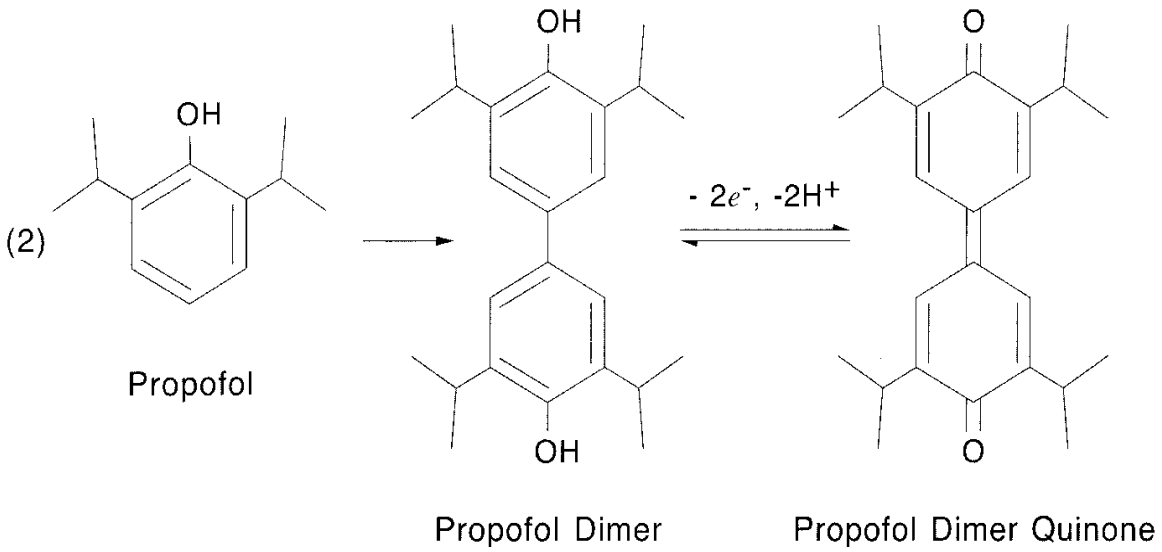


Figure 2-11. Formation of propofol dimer and propofol dimer quinone in propofol emulsions.

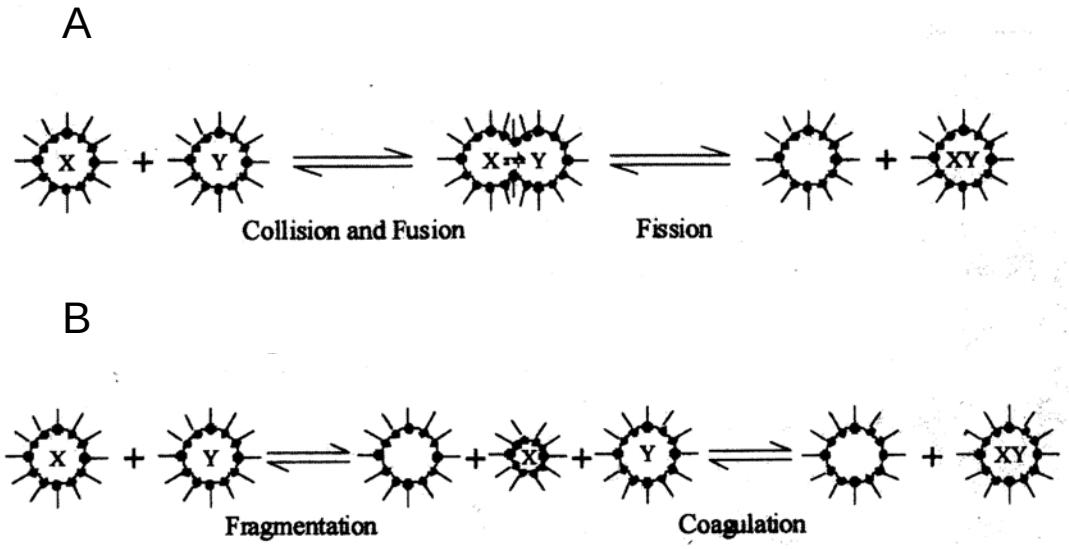


Figure 2-12. Dynamic behavior of microemulsions. A) Collision with merging/breakdown. B) Fragmentation/coagulation.

Table 2-1. List of propofol microemulsion

S. No.	Surfactant	Co-surfactant
1	Pluronic 68	Sodium Octanoate, Sodium Decanoate Sodium Dodecanoate
2	Pluronic 77	Sodium Octanoate, Sodium Decanoate Sodium Dodecanoate
3	Pluronic 88	Sodium Octanoate, Sodium Decanoate Sodium Dodecanoate
4	Pluronic 108	Sodium Octanoate, Sodium Decanoate Sodium Dodecanoate
5	Pluronic 127	Sodium Octanoate, Sodium Decanoate Sodium Dodecanoate
6	Tween 20	Polyethylene Glycol 200, Polyethylene Glycol 400, Polyethylene Glycol 600
7	Tween 40	Polyethylene Glycol 200, Polyethylene Glycol 400, Polyethylene Glycol 600
8	Tween 60	Polyethylene Glycol 200, Polyethylene Glycol 400, Polyethylene Glycol 600
9	Tween 80	Polyethylene Glycol 200, Polyethylene Glycol 400, Polyethylene Glycol 600
10	Brij Series	Tween 20, Tween 40, Tween 60, Tween 80
11	Span Series	Tween 20, Tween 40, Tween 60, Tween 80
12	Polyoxy Fatty acid Esters	Polyethylene Glycols 200, Polyethylene Glycols 400
13	Tween 60	No cosurfactant required
14	Tween 80	No cosurfactant required
15	Polyethylene Glycol 400	No cosurfactant required
16	Polyethylene Glycol 600	No cosurfactant required

Table 2-2. Size of propofol microemulsions

Microemulsion	Droplet Size (nm)	Droplet Size (nm) (2 years old)
Pluronic 68(5%)/Sodium Octanoate	27.6	28.4
Pluronic 68(5%)/Sodium Decanoate	25.4	25.6
Pluronic 68(6%)/Sodium Dodecanoate	21.8	25.4
Pluronic 127(4%)/Sodium Octanoate	34.1	34
Pluronic 127(5%)/Sodium Decanoate	29.5	30.6
Pluronic 127(6%)/Sodium Dodecanoate	24.5	25

Table 2-3. Viscosity of propofol microemulsions at 25 °C

Microemulsion	Viscosity (cps) 10 (1/S)	Viscosity (cps) 100 (1/S)	Viscosity (cps) 1000 (1/S)
Pluronic 68(5%)/Sodium Octanoate	1.25	1.19	1.15
Pluronic 68(5%)/Sodium Decanoate	1.25	1.19	1.14
Pluronic 68(5%)/Sodium Dodecanoate	1.27	1.19	1.15
Pluronic 127(5%)/Sodium Octanoate	1.42	1.39	1.36
Pluronic 127(5%)/Sodium Decanoate	1.42	1.38	1.37
Pluronic 127(5%)/Sodium Dodecanoate	1.42	1.39	1.37

CHAPTER 3
ANESTHETIC PROPERTIES OF PROPOFOL MICROEMULSIONS

3.1 Introduction

Propofol's is a unique compound compared to the other intravenous anesthetics. Its lone ionizable functional group, the hydroxyl, has a pKa of 11, which renders it unsuitable for forming salts in solution. The remaining portion of the molecule, the benzene ring and isopropyl side groups, are highly lipophilic. The result is a molecule with a poor water miscibility (146 mg/L).[172] So, on an average human being with 4L of blood, assuming the blood solubility is the same as water can solubilize 0.584 gm of free propofol in blood assuming propofol solubility in blood is the same as water. But in human system, even though the dose of propofol is 200 mg/L to 400 mg/L for induction and maintenance of anesthesia.[173] Free concentration of propofol in blood is 25 mg/L,[173] because propofol being hydrophobic compound partition or attaches to various lipophilic compounds as well as other biosurfaces in the blood. Thus, the aqueous phase of blood never reaches to its saturation limit by propofol. The concentration of propofol in blood can be considered as the transient phenomenon coupled with its quick partitioning into hydrophobic components of the blood (e.g. red blood cells, membranes, hydrophobic proteins). Also, propofol being hydrophobic is highly protein bound in vivo[173]. High lipophilicity (logP 4.16)[174] of propofol means that good miscibility can only be achieved in lipophilic substances or organic solvents. Because vehicles for clinical delivery of anesthetics should be devoid of sedative and anesthetic properties, as well as toxic side effects, nearly all small molecular weight organic solvents into which propofol is freely miscible are not useful.

However, propofol can also be combined with biocompatible surfactants to form transparent, colorless, and thermodynamically stable, oil-in-water microemulsions of nanometer diameter without the need for excipient oils such as soybean oil as already discussed in previous

chapter. In these microemulsions, propofol serves the physical role as the core of the particles since this drug exists as oil at room temperature, and as the active pharmaceutical agent to cause general anesthesia.

To examine anesthetic properties, we measured several parameters of anesthetic induction and emergence in rats receiving the experimental and conventional formulations of propofol using a randomized, crossover design. Previously, we have shown that these microemulsions can be formed using various non-ionic and ionic surfactants and have physical properties similar to those of a propofol macroemulsion, the conventional formulation. In addition, the surfactant type and concentration used to formulate these propofol microemulsions should affect the pharmacokinetic properties in animals.

However, the limited blood volume (e.g., ~50 ml) of the rat compared to that volume (e.g., ~30 ml) necessary to measure plasma propofol concentrations repeatedly precluded determining pharmacokinetic properties. Therefore, a large animal model (i.e., dog) study was conducted in using a randomized, crossover design in order to compare the pharmacokinetics of a propofol microemulsion with those of a macroemulsion. In addition, several pharmacodynamic effects were noted including anesthetic induction and emergence times, possible alteration of erythrocytes, leukocyte, and platelet cell populations, and potential changes in the coagulation indices.

3.2 Methods and Materials

3.2.1 Materials

Propofol was obtained from Albemarle Corporation (Baton Rouge, LA). Purified pluronic F68, a nonionic coblock polymer consisting of polyethylene and polypropylene monomers, was purchased from the BASF Corporation (Florham Park, NJ). Sodium salts of fatty acid (C8, C10, and C12) were supplied by Sigma Chemical Co. (St. Louis, MO).

3.2.2 Synthesis of Propofol Microemulsions

Microemulsions for rat studies were prepared by combining propofol (0-100 mg/ml) with purified Pluronic F68 (0-70 mg/ml), and a fatty acid salt (0-12.5 mg/ml) in normal saline (0.90 mg/ml NaCl) bulk media. Water was ultra-purified using a water purification system (Nanopure, Barnstead/Thermolyne, Dubuque, IA) to provide a minimal electrical resistance of 18.2 MΩ. Following agitation with a magnetic stirrer, these components combined to form clear, colorless microemulsions with adjustment of pH to 7.40 using either HCl or NaOH. All experimental formulations were stored under a nitrogen headspace. To characterize the dimension of the individual droplets, the effective droplet size of the nanoparticles was measured by the dynamic light scattering method using a submicron particle sizer analyzer (90Plus, Brookhaven Instruments Corporation, Holtsville, NY) as previously described.[132]

Microemulsion for dog studies were prepared by combining propofol (10 mg/ml) with purified Pluronic F68 (50 mg/ml), and a fatty acid salt (2.1 mg/ml) in a normal saline (0.90 mg/ml NaCl) bulk media. Water was ultra-purified using a water purification system (Nanopure, Barnstead/Thermolyne, Dubuque, IA) to provide a minimal electrical resistance of 18.2 MΩ. Following agitation with a magnetic stirrer, these components combined to form clear, colorless microemulsions. We then adjusted the pH to 7.40 using either HCl or NaOH. The experimental formulation was kept under a nitrogen head to delay oxidation of propofol. Before administration, the propofol microemulsion was filtered through a 0.45 μm sterile filter, but the macroemulsion ((Diprivan®, AstraZeneca Pharmaceuticals, Wilmington, DE) was not filtered.

3.2.3 Rat Experiments

All experimental protocols involving the use of animals were reviewed and approved by the University of Florida Institutional Animal Care and Use Committee. Sprague-Dawley rats (350-500 g; either sex) were purchased from Charles Rivers Laboratories (Wilmington, MA).

Before delivery of animals to the investigators by the vendor, rats underwent catheterization of the left femoral vein with subcutaneous tunneling to an exit site between the scapulae. Thus, rats were supplied by the vendor with pre-implanted, heparinbonded, femoral vein catheters and did not require additional instrumentation at the time of the experiments that might require sedation or anesthesia that could confound interpretation of results. Rats were caged singly in order to avoid damage to the catheter by cage mates. They were allowed unlimited access to food and water with a 12:12 hour light:dark cycle. On the day of experimentation, each rat was weighed. Thereafter, the central venous line of each rat was easily accessed in conscious, unrestrained rats. All catheters were aspirated until blood was observed and then flushed with 0.5 ml of normal saline. Subsequently, rats entered the animal experimental protocol.

3.2.3.1 Animal experimental protocol

Rats were randomized to receive either 1) an experimental propofol microemulsion (n=6 per microemulsion) after filtration through a 200 nm pore filter in order to ensure sterility or 2) a conventional macroemulsion of propofol in a soybean-based solvent (Diprivan, AstraZeneca Pharmaceuticals, Wilmington, DE). In both cases, the formulation was infused at a rate of 10 mg/kg/min propofol via a microprocessor-controlled syringe pump (sp2000i, World Precision Instruments, Sarasota, FL) in order to avoid varying rates of infusion associated with manual injection that could potentially confound anesthetic induction time parameters. The endpoints of anesthetic induction were total drug dose, time to loss of exploratory behavior (i.e., stunned), time to loss of righting reflex, time to loss of lash reflex to gentle stroking (i.e., canthal reflex), and time to loss of reflexive withdrawal of the leg following a great toe pinch every 10 s by a metal clamp. In these experiments, the metal clamp was rubber shod to avoid tissue damage during the experiment. Following loss of withdrawal, the drug infusion was discontinued. Endpoints of anesthetic recovery were return of the withdrawal response to a toe pinch, recovery

of spontaneous eye blinking, recovery of a sustained head lift, and recovery of the righting reflex. Following the first anesthetic, each rat was recovered for at least 14 days before enrollment in the experimental limb opposite the original assignment. Thus, each rat was anesthetized with both experimental and conventional propofol formulations with random initial assignment and with a 14 day interval before crossover between the formulations. Following a 14 day period of observation after the participation in the crossover limb, the experiment was ended.

3.2.3.2 Statistical analysis

Measurements are reported as mean±standard deviation. Statistical analysis was performed with SigmaStat 3.1 (Systat Software, Inc., Point Richmond, CA). Prior to parametric testing, the assumption of normality was validated using the Kolmogorov-Smirnov test with Lilliefors' correction. For normally distributed data, one-way repeated measures analysis of variance (factor: propofol formulation) was used to test for overall statistical significance followed by Bonferonni post-hoc pairwise testing, when appropriate, with two-tailed protection to analyze multiple comparisons between different formulations of propofol. For nonparametric data, Friedman repeated measures analysis of variance on ranks was used followed by Bonferonni testing with two-tailed protection when appropriate. $P<0.05$ was considered to be statistically significant.

3.2.4 Dog Experiments

The Institutional Animal Care and Use Committee of Calvert Laboratories (Olyphant, PA) approved the study protocol prior to investigation. Treatment of animals was in accordance with the conditions specified in the Guide for the Care and Use of Laboratory Animals. Ten purpose-bred and experimentally naive beagles (Marshall Bioresources, North Rose, NY) aged 7-8 months and weighing 7.0-8.9 kg were identified by ear tattoo. All animals had access to certified

canine diet (Teklad, Harlan, Indianapolis, IN) up to 400 g/day. Tap water was available ad libitum to each animal via an automatic watering device. Study animals were acclimated to their housing for a minimum of five days prior to entering the experimental protocol. After being fasted for 16-20 hours, dogs were weighed and prepared for the experiment. Peripheral intravenous catheters were inserted in the right and left leg veins for intravenous administration of propofol formulations and the collection of whole blood samples, respectively.

3.2.4.1 Animal experimental protocol

On the day of experimentations, dogs (n=10) were randomized to receive either the propofol microemulsion (n=5) or macroemulsion (n=5) intravenously at a rate of 10 mg/kg/min via a microprocessor-controlled syringe pump in order to obviate potential confounding influences caused by variable administration rates from manual injection. During the infusion, the endpoint of anesthetic induction was the time necessary to cause the loss of reflexive withdrawal of the leg following a toe pinch every 15 sec with a rubber-shod clamp. After loss of withdrawal of the leg, the infusion was discontinued. The total drug dose to cause induction was the mass of propofol required to cause loss of leg withdrawal. Thereafter, the subject was observed for recovery of the withdrawal to a toe pinch. Also, the investigators measured the heart rate (Pagewriter, Hewlett Packard Company, Palo Alto, CA), indirect peripheral blood pressure (9301V, Cardell Veterinary), and respiratory rate at the following time points: pre-dose, immediately post- induction at one minute, and every five minutes thereafter until dogs emerged from anesthesia.

The electrocardiogram was examined for arrhythmias. In addition to these parameters, animals were observed for apnea or other signs of respiratory distress. Body temperature was measured with a thermometer and was maintained using a heating pad placed under the animal.

Following this anesthetic, dogs were allowed to recover and then returned to the kennel. The dogs were allowed to recover and were then returned to the kennel.

After the first set of experiments, dogs were recovered for at least 7 days before being crossed over to receive the formulation in the opposite limb of the study. Thus, dogs previously enrolled to receive microemulsion (or macroemulsion) were switched to receive the opposite formulation. Following anesthetic induction in the opposite limb of the investigation, dogs were recovered for a least 7 days before the investigation was terminated.

3.2.4.2 Blood sample acquisition and processing

During these experiments, blood samples were taken for two purposes. First, samples were secured before induction and at times 1, 5, 10, 15, 20, 25, and 30 min after induction (or until the dog emerged from anesthesia) to measure the propofol concentration in order to study pharmacokinetics of these propofol formulations. The blood samples were acquired from a second intravenous cannula placed in vein on a leg contralateral to that used for propofol injection. These blood samples (1-2 ml) for assessment of propofol concentration were stored in non-additive blood tubes and centrifuged at 3,000 revolutions/min. The resultant supernatant was aspirated and stored at -20 ºC until thawed for measurement of propofol concentrations as described subsequently (see Measurement of Plasma Propofol Concentration). Second, blood samples were acquired to determine the effects of the formulations on the hemogram, prothrombin time, activated partial thromboplastin time, and fibrinogen concentrations. These blood samples (4 ml) were collected pre-induction, immediately post- induction when concentration of propofol and excipients would be expected to peak in the plasma, and during recovery of anesthesia. In all cases, the maximum amount of blood collected was limited to 1% of the body weight over a period of two weeks.

3.2.4.3 Measurement of plasma propofol concentration

Plasma propofol concentrations were measured using a method previously reported by Dennis and coauthors.[175, 176] Briefly, the mobile phase was passed through a 0.22 μm nylon filter (Millipore, Bedford, MA) and degassed by sonication. Stock solutions (10 mg/ml) of propofol and thymol, the internal standard, were prepared in methanol and stored at 4 °C. Vials were filled with nitrogen after opening to prevent drug oxidation. Standard solutions of propofol (2, 20, and 200 μg/ml) were made by further dilution of the stock solution with methanol. Quality control samples (10, 100 and 1000 ng/ml) were prepared by adding 50 μl of appropriate propofol standard solution to 10 ml of blank plasma. These samples were vortexed and kept frozen in 1 ml aliquots at -20 °C until analysis.

For calibration curves, blank plasma samples (1 ml) were spiked with 25 μl of the appropriately diluted standard solutions to final propofol concentrations of 5, 10, 50,100, 500, 1000 and 2000 ng/ml. Control samples containing no added propofol were also prepared. SPE cartridges were activated with 1 ml of methanol and washed with 1 ml of water. Plasma samples (1 ml) were diluted with 1 ml of phosphate-buffered saline after adding 20 μl of thymol (50 μg/ml) as an internal standard, acidified with 20 μl of phosphoric acid, vortexed, and loaded onto activated cartridges. The cartridges were washed three times (1 ml of water followed by 1 ml of 1% KHCO3 in acetonitrile:water (1:9), followed by 1 ml of acetonitrile:water (2:3)) and dried in a low vacuum for 30 sec. Solutes were eluted with 1 ml of methanol without vacuum. Vacuum was used briefly to remove remaining solvent in the cartridge. Solutes were vortexed and transferred to autosampler vials for analysis. LC/MS/MS analyses were performed using a Perkin-Elmer PE 200 LC system and an API 4000 triple quadrupole mass spectrometer equipped with an APCI source (Applied Biosystems, Foster City, CA). Samples (25 μl injection volume) were separated on a C18 column (Xterra TM RP18, ODS 5 μm 250 mm × 4.6 mm i.d., Waters)

with a guard cartridge (4 mm × 3 mm i.d., Phenomenex, Torrance, CA) prior to the C18 column. The isocratic mobile phase consisted of methanol and 0.05% aqueous ammonium hydroxide solution (98:2) at a flow rate of 1 ml/min. The deprotonated precursor molecular ions were selected and fragmented by nitrogen gas collision in the Q2 region with a collision energy of −45 eV. The resulting mass spectra were acquired in full scan mode from m/z 100–200. The most abundant product ion at 161 for propofol and the ion at 133 for thymol were selected for multiple reaction monitoring (MRM) quantitation. Calibration curves were constructed by plotting the ion abundance peak area ratios (propofol/IS) as a function of plasma propofol concentration. These data were then fitted with an unweighted least squares regression analysis to the equation: $y = ax + b$, where a defines the slope and b the intercept of the ordinate. The propofol concentrations of unknown samples were calculated using the results of the regression analyses.

3.2.4.4 Statistical and pharmacokinetic analysis

Measurements are reported as mean±standard deviation. Statistical analysis was performed with SigmaStat 3.1 (Systat Software, Inc., Point Richmond, CA). Prior to parametric testing, the assumption of normality was validated using the Kolmogorov-Smirnov test with Lilliefors' correction. For normally distributed data with two groups, the paired t-test was used. For normally distributed data with more than two groups, one-way repeated measures analysis of variance (factor: propofol formulation) was used to test for overall statistical significance followed by Bonferonni post-hoc pairwise testing, when appropriate, to analyze multiple comparisons between different formulations of propofol. For nonparametric data, Friedman repeated measures analysis of variance on ranks was used followed by Bonferonni testing when appropriate. $P<0.05$ was considered to be statistically significant.

Pharmacokinetic analysis of each dog's blood concentration versus time profile was performed using the data analysis program WinNonLin (Scientific Consulting, Inc., USA)

according to Knibbe and colleagues.[128] The following bi- and tri-exponential equations were used in order to describe the blood concentration-time profiles after injection to determine the best fitted relationship:

$$C(t) = C_1 \bullet e^{-\lambda_1 \bullet t} + C_2 \bullet e^{-\lambda_2 \bullet t} \quad (3\text{-}1)$$

$$C(t) = C_1 \bullet e^{-\lambda_1 \bullet t} + C_2 \bullet e^{-\lambda_2 \bullet t} + C_3 \bullet e^{-\lambda_3 \bullet t} \quad (3\text{-}2)$$

where C(t) is the blood concentration of propofol at time t, C1, C2 and C3 are the coefficients and λ1, λ2, λ3 the exponents of the equation. Statistical analysis of pharmacokinetic data was performed using analysis of variance (ANOVA) with a statistical software package (Epistat v3.0, T.L. Gustafson, Wound Rock, TX, USA).

3.3 Results

3.3.2 Anesthetic Properties in the Rat

Two separate series of experiments were performed to determine the anesthetic properties of propofol microemulsions in rats. In the first set, the concentration of the nonionic surfactant purified Pluronic F68 was primarily changed and compared to the macroemulsion control to cause anesthesia in rats. In the second set, the carbon chain length of the fatty acid was increased (C8, C10, C12) to determine if this modification affected anesthetic induction and emergence times compared to the macroemulsion control.

3.3.2.1 Modification of nonionic surfactant concentration

In the first series of experiments, rats were administered propofol microemulsions with different concentrations of the nonionic surfactant purified Pluronic F68 (3%, 5%, and 7%) or they received the conventional macroemulsion. Twenty-three rats were enrolled in this protocol, but five rats completed only one limb of this protocol due to retraction of the catheter under the skin during the recovery interval from the first limb of the study. Data from these five rats (n=3

in microemulsion groups, n=2 macroemulsion group) were removed from the study. The remaining rats (n=18) completed the crossover study and were anesthetized with these microemulsions (n=6 per group) and with the macroemulsion control (n=18). All rats experienced rapid general anesthesia without apnea and recovered for at least 14 days without apparent injury. Following the 14 day observation period after crossover, the experiment was terminated. The summary data from these induction experiments are presented in Figure 3-1 and Table 3-1. All microemulsions required greater doses for anesthetic induction as defined by loss of leg withdrawal to a toe pinch compared to the required dose of macroemulsion (P=0.005, <0.001, and <0.001 compared to 3, 5, and 7% purified Pluronic F68 microemulsions, respectively). In addition, the time to onset of anesthesia caused by the 5% purified Pluronic F68 microemulsions was significantly longer than that caused by the macroemulsion for stunning (P<0.001), loss of the righting reflex (P<0.001), and loss of the lash reflex (P=0.01). For emergence parameters, the 3% microemulsion caused more rapid emergence than the macroemulsion control as assessed by return of righting (P= 0.03), but not for return of leg withdrawal (P=0.71), sustained headlift (p=0.10), or lash reflex (P=0.40). Finally, rats emerged more rapidly following anesthesia as assessed by return of leg reflex (P=0.01) and return of the lash reflex (P=0.01) with the 7% microemulsion compared to the 3% microemulsion.

3.3.2.2 Modification of ionic surfactant concentration

In a separate series of experiments, rats were administered microemulsions with a constant concentration of purified Pluronic F68 (5%), but with ionic surfactants (i.e., fatty acid salts) of increasing carbon chain length (C8, C10, and C12). Twenty-one rats entered this protocol, but three rats did not complete the crossover experiments due to catheter retraction under the skin (n=2 for microemulsion group, n=1 for macroemulsion group) during the 14 day recovery interval between crossover. The information from these three rats was removed from the data set.

The remaining rats in this protocol were infused with the three different propofol microemulsions (n=6 per group) and with the macroemulsion (n=18). As with the first set of animal experiments all rats experienced general anesthesia without apnea and recovered for at least 14 days without apparent injury. After 14 days following the crossover limb, the experiment was ended. The summary data for these experiments are presented in Table 3-2. (Note: the rats listed for the C8 fatty acid salt experimental propofol microemulsion were the same as those noted for purified poloxamer 5% in the Table 3-1 and are presented again in Table 3-2). The dose of propofol necessary to cause anesthesia was greater for microemulsions compared to the macroemulsion. In addition, the length of time to induction was longer for all the microemulsions compared to the macroemulsion for all induction parameters measured. Also, the microemulsion formulated with C10 fatty acid salt required a larger dose to cause anesthesia than for the microemulsions made with C8 ($P<0.001$) or C12 ($P=0.01$) fatty acid salts. For emergence, rats receiving the C12 fatty acid salt microemulsion required a longer time to emerge as assessed by return of leg withdrawal ($P=0.008$), righting reflex ($P=0.002$), and sustained headlift ($P=0.008$) compared to the macroemulsion. Similarly, these rats receiving the C12 microemulsion took longer to emerge than the animals receiving the C8 microemulsion as measured by return of the righting reflex ($P=0.004$) and a sustained headlift ($P=0.004$).

3.3.3 Anesthetic Properties in the Dogs

All dogs (n=10) experienced general anesthesia with subsequent recovery when injected with either formulation of propofol. The mean dose of microemulsion or macroemulsion to cause loss of leg withdrawal was 10.3±1.2 and 9.7±1.6 mg/kg, respectively ($P=0.39$). Although anesthetic induction caused marked changes in the heart rate ($P<0.001$), blood pressure ($P<0.001$), and respiratory rate ($P<0.001$) over time, these changes were similar for animals assigned to receive either the microemulsion or macroemulsion (Figure 3-3, Figure 3.4). There

were no significant differences with respect to group assignment in the heart rate (P=0.62), blood pressure (P=0.81), or respiratory rate (P=0.60) for dogs administered the microemulsion or macroemulsion. Apnea or respiratory distress was not observed. The duration of anesthesia caused by these doses of anesthetic were 1046±275 and 1094±225 sec for the microemulsion and macroemulsion, respectively (P=0.70).

3.3.3.1 Effects on erythrocytes and leukocytes

Data stratified by group assignment (microemulsion, macroemulsion) and time (pre-induction, post-induction, recovery period) for parameters of the erythrocyte (Red Blood Cells) population are presented in Table 3-3. No differences were observed between formulations with respect to indices of erythrocyte populations as noted by the P values in Table 3-3. However, significant reductions in some indices were observed with respect to time. That is, there were decrements in the red blood cell count (P<0.001), hemoglobin concentration (P<0.001), hematocrit (P<0.001), absolute reticulocyte count (P=0.002), and relative reticulocyte count (P=0.030) over time for both groups.

Similar results were observed after measuring the concentration of white blood cells and subtypes (i.e., neutrophils, lymphocytes, eosinophils, mast cells, basophils) as tabulated in Table 3-4. That is, although no differences were noted between formulation types of propofol as noted by the P values in Table 3-4, significant reductions in the total white blood cell concentrations and the concentrations of white cell sub-populations were noted in both groups. However, neither formulation assignment nor time significantly affected the fraction (i.e., differential count) of different white blood cell types (data not shown).

3.3.3.2 Effects on platelets and thrombosis

Neither the microemulsion nor macroemulsion of propofol affected platelet concentration, fibrinogen concentration, prothrombin time, or activated partial thromboplastin time (Table 3-5).

In one dog that received the propofol macroemulsion, the platelet concentration was measured to be 8,000 platelets/ μl in the recovery period although the concentrations in the same dog determined at pre-induction and post-induction were 240,000 and 233,000 platelets/μl, respectively. Following microscopic examination of the specimen, no clumping of platelets was noted. Therefore, this outlying data point was retained and accounts for the increased standard deviation for this group (111,000 platelets/ μl) compared to the deviations for other groups (39,000-50,000 platelets/ μl).

3.3.3.3 Propofol pharmacokinetics

In order to understand the pharmacokinetic profiles of these formulations of drugs, the plasma concentration of propofol was measured at various times after administration of propofol as noted in Table 3-6. After a bolus of propofol at 0 min, a large peak propofol concentration was measured followed by rapid decrements in the concentrations at 1, 5, and 10 min. At 15 and 20 min, increases in the propofol concentrations were observed for both themacroemulsion and microemulsions groups. Notwithstanding these time-related changes, no significant differences in plasma propofol concentrations were noted at 1, 5, 10, 15, or 20 min between the dogs receiving the microemulsion or macroemulsion formulations.

3.4 Discussion

In rat study, kinetics of general anesthesia with the propofol microemulsion were favorable compared to the commercially available propofol macroemulsion. Whereas the time for general anesthesia (defined by the protocol as loss of leg withdrawal to a pinch) during macroemulsion injection was 122-126 s, the latency periods during microemulsion treatment were 166-203 s. This short delay is favorable when compared to pro-drug technologies that rely on metabolism, a phenomenon that may vary significantly within any patient population.[147] The differences in induction times between experimental groups reported herein may be caused by differential

release of propofol from the individual droplets into the blood. That is, the different propofol nanoparticles have markedly different stabilities against dilution by blood based on the emulsifier structure and concentration selected for the formulation. In general, a microemulsion is thermodynamically stable at equilibrium. One can destabilize a microemulsion by significantly changing pressure, temperature, or chemical compositions. The last variable can be changed simply by diluting a microemulsion with saline or blood. It is well recognized that the formation of microemulsions requires an ultra low interfacial tension (e.g., ~10^{-3} mNewton/m) at the oil/water interface.31 Upon dilution with saline, the interfacial tension will increase substantially as the emulsifier molecules (i.e., both poloxamer 188 and fatty acid salt molecules) desorb from the droplet surface. This event will markedly increase the interfacial tension at the droplet surface and ultimately destabilize the microemulsion, with release of the active pharmaceutical

In dog study, we investigated the dose of propofol, micro- or macroemulsions, administered to dogs to induce anesthesia, along with possible effects on blood, the first tissue encountered by the formulations. The measured variables of anesthesia, vital signs, indices of blood cell populations or thrombosis, and plasma concentrations of propofol did not significantly differ between the groups of animals given either the propofol microemulsion or macroemulsion.

3.4.1 Microemulsion Fate Upon Injection

In a macroemulsion or microemulsion, propofol is highly concentrated in the emulsified oil droplets (defined as the discontinuous phase), with only small quantities in the aqueous phase (i.e., continuous phase), the latter of which constitutes the largest volume of the microemulsion or macroemulsion (1% propofol formulation). Upon administration of a propofol-containing emulsion, propofol diffuses across the droplet interface and passes into the bloodstream. Major factors that govern this process for propofol or any lipophilic drug are the drug concentration gradient, the partition coefficient, the drug diffusivity in both phases, and the interfacial area of

the drug-containing oil droplets. Therefore, similar to any drug releasing particle, microemulsions and macroemulsions slow the availability of free drug as compared with drugs administered in solutions in which they are molecularly dissolved.

The drug diffusivity from dispersed phase (oil phase) to continuous phase (water phase) is different in macro and microemulsions. In microemulsions, propofol molecules take longer time to come out of the microemulsions because of the extra barrier of the very low interfacial tension surfactant film. These microemulsions grow in size when they get diluted in the blood, as more and more surfactant leave the oil/water interface for other places in the blood and vein. And after a certain dilution these microemulsion convert to macroemulsion. So, this extra barrier for drug to come out of the microemulsion to the blood slows the availability of free drug in microemulsion than in macroemulsion. Eventually the bioavailability of propofol in blood reaches to its pharmacological limits at which we see the start of the onset of anesthesia, but it would take longer time in the case of microemulsion than in macroemulsion.

The total interfacial surface area is a highly important factor in the rate of drug release from a propofol containing droplet. This in turn is dependent on the size and number of oil droplets resulting from the injection. In a propofol macroemulsions made from identical emulsifiers, if they contain uniform droplets (monodisperse) of 1.0 μm in diameter, the total oil–water surface area, or droplet–aqueous phase interface, would be 0.66 m^2/ml. However, if the particle size were reduced to 0.1 μm, it would have a total oil–water surface area of 27.6 m^2/ml, nearly 42 times greater. The latter allows for a more rapid rate of release of propofol to the blood, or in other case faster anesthesia. Thus, a microemulsion has greater total interfacial area but higher resistance to diffusion across interface. Since experimens showed greater induction

time, it implies that the interfacial resistance must be a dominating factor as compared to total interfacial area.

3.4.2 Propofol Anesthetic Properties

In dog studies, all animals experienced rapid anesthesia as assessed by loss of leg withdrawal with mean propofol doses of approximately 10 mg/kg for both the microemulsion and macroemulsion. This dose of propofol is greater than that previously reported in other studies investigating anesthetic induction in unpremedicated dogs using propofol formulated in a macroemulsion. In those reports, the mean dose of propofol noted ranged from 3.8-6.9 mg/kg with a mean±standard error of the mean calculated from these six studies of 5.8±0.5 mg/kg.[177-179] Because the mean dose observed by the present investigators was greater for the macroemulsion (in addition to the microemulsion), this increase was likely due to differences in the study protocol including a higher rate of propofol infusion (10 mg/kg/min) and use of younger animals (i.e., 8 month old dogs) in the present report. Although we can not identify any studies detailing age-related effects on propofol dose in dogs, previous investigations conducted in rats and humans demonstrate that greater doses are required in younger, compared to older, subjects.[180, 181] For example, Schnider and colleagues observed that the mean plasma propofol concentrations at which 50% of the human participants experienced anesthetic induction were significantly greater (1.88-fold increase) for 25 year old (2.35 μg/ml) compared to 75 year old (1.25 μg/ml) subjects.[181] Likewise, we observed a similar proportional increase in propofol macroemulsion dose (9.7 mg/kg / 5.8 mg/kg or 1.67-fold greater) for younger dogs compared to the mixed age dog populations from the previously reported dog studies.[179] In addition, the duration of anesthetic was remarkably similar to that previously reported for dogs. That is, whereas we observed a mean duration of anesthesia of 18.2 min whereas Watkins and colleagues reported a value of 18 min. Therefore, the pharmacodynamic effects of propofol administered in either

formulation are consistent with previously reported values in dogs and are similar for the microemulsion and macroemulsion preparations. One limitation of this type of investigation is that any conclusions are limited to the dose studied. Extrapolation to other doses and sustained infusion of propofol require additional work and evidence. In addition, any extrapolation of these data to potential human use should be done cautiously because of possible species differences and the need for safety investigations (e.g., dose escalation studies).

3.4.3 Propofol Concentration

The plasma propofol concentrations reported in the present study demonstrated a high peak concentration (25–32 μM) followed by rapid decline. Similar findings for the propofol macroemulsion were noted by Zoran et al.[182], who observed peak concentrations of 2.3 μg/mL (12.9 μM) and 3.29 μg/mL (18.5 μM) in mixed breed dogs and Greyhounds, respectively. Similarly, Cockshott et al.[183] noted peak concentrations of approximately 4 μg/mL (22.4 μM) in specimens obtained from dogs after injection of a 7 mg/kg propofol bolus. The lesser concentrations reported for previous investigations may have been due to the fact that the dogs enrolled in their previous studies received less propofol (5 mg/kg) over a longer time period than did dogs in our investigation. The observation of secondary peaks in plasma propofol concentrations at 15–20 min in our study was unexpected. We had previously hypothesized the existence of a single,large peak concentration of propofol immediately after bolus injection followed by steady decrements. Two possible reasons may account for these secondary peaks in propofol concentrations. First, this finding may be an artifact of the protocol. We stopped sampling blood for propofol concentrations when dogs emerged from anesthesia. Therefore, dogs with the least plasma propofol concentrations were excluded from the summary data at 15 and 20 min. This event would tend to cause the mean propofol concentrations to increase. Second, this phenomenon has been observed by others studying the pharmacokinetics of

propofol in both dogs[182] and humans.[183, 184] For example, Zoran[182] described a secondary peak concentration of propofol in five of eight mixed breed dogs and eight of ten Greyhounds. Similar to the present investigation, they noted that this peak occurred at the time that the dogs emerged sufficiently to achieve a sternal position (i.e., righting reflex). The exact cause of this secondary peak is unknown, but previously suggested reasons include changes in cardiac output associated with movement after anesthesia, influx of propofol from peripheral tissues with loss of venodilation caused by propofol, use of venous (vis-a`-vis arterial) sampling, and changes in blood components due to splenic contraction in dogs associated with the phlebotomy necessary for acquiring multiple blood specimens.[182]

3.4.4 Coagulation

One possible drawback of using intravenous microemulsions to delivery propofol is potential interference by the components of the formulations with the coagulation system. In the present study, we used purified Pluronic F68 as a nonionic surfactant to reduce the interfacial tension between propofol oil droplets and the aqueous environment in order to construct microemulsions. Purified Pluronic F68 is a polymer consisting of poly(ethylene oxide)/poly(propylene oxide) copolymers ((C2H4O)70–(C3H6O)35–(C2H4O)70) and has been thought to possibly modify platelet adhesion. This and related polymers have been used for coating biological implants constructed from various materials and have been shown to reduce platelet adherence, an effect primarily dependent on the number of propylene oxide (C3H6O) residues vis-à-vis ethylene oxide (C2H4O) residues (Note: the number and proportion of the residues are varied by the manufacturers to construct unique polymers).[185, 186] Previously, Morey and colleagues studied human blood using thromboelastography and demonstrated that microemulsions similar to those used in the current investigation reduced platelet function (i.e., thromboelastograph maximal amplitude, elastic modulus) in a concentration-dependent manner,

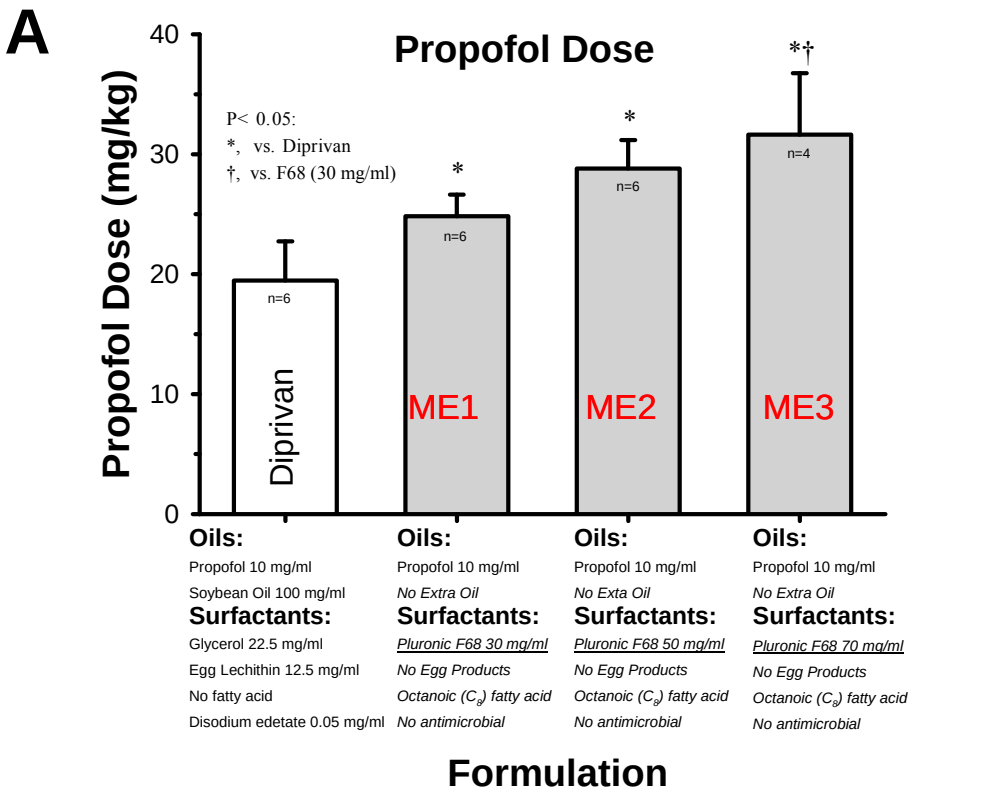
but did not decrease the overall platelet population or detectably interfere with clotting factor function.[187] Furthermore, this effect of the microemulsions was highly correlated (r2=0.94) to that caused by the concentration of the co-block polymer component of the microemulsion, but was not caused by the oil or fatty acid components. In contrast to these in vitro investigations, minimal-to-no effects have been observed during in vivo investigations. For example, Orringer and colleagues hypothesized that high-dose (i.e., 100 mg/kg for 1 hour followed by 30 mg/kg per hour for 47 hours), intravenous infusion use of purified Pluronic F68 as an investigational drug to treat acute vaso-occlusive crisis of human patients with sickle cell disease would reduce the clotting associated with sickled erythrocytes, but observed only mild effects in a pediatric subgroup also receiving hydroxyurea.[161] Likewise, no changes were noted in dogs due to administration of formulations in the platelet count, fibrinogen concentration, prothrombin time, or activated partial thromboplastin time in the present study. Although useful as screening tools, these parameters of coagulation are not sensitive to detect subtle changes in clotting or platelet functions. Therefore, this limitation of the present study should be further addressed with investigations specifically dedicated to observations of possible changes in clotting using sensitive tests (e.g., thromboelastography, platelet contractile force, clot elastic modulus).

3.5 Conclusions

Here, we report the successful use of propofol microemulsions to cause anesthesia in rat similar to that caused by propofol macroemulsions. The differential diluent volumes in vitro and destabilization times in vivo based on changing the concentration and type of surfactant alludes to the possibility of selectively modifying the pharmacokinetic and pharmacodynamic properties of propofol, and potentially other lipophilic drugs. Future work will be aimed towards exploring these pharmacokinetic/pharmacodynamic differences for propofol and other lipophilic agents

and to determining if these propofol microemulsions reduce the liabilities (e.g., stinging on injection, support of bacterial growth) associated with soybean oil-based macroemulsions.

Here, we have shown that propofol formulated as a microemulsion can be used successfully to anesthetize dogs in a way similar to a commercially available propofol macroemulsion. Furthermore, no detectable adverse effect was observed on indices of the hemogram or parameters of clotting.



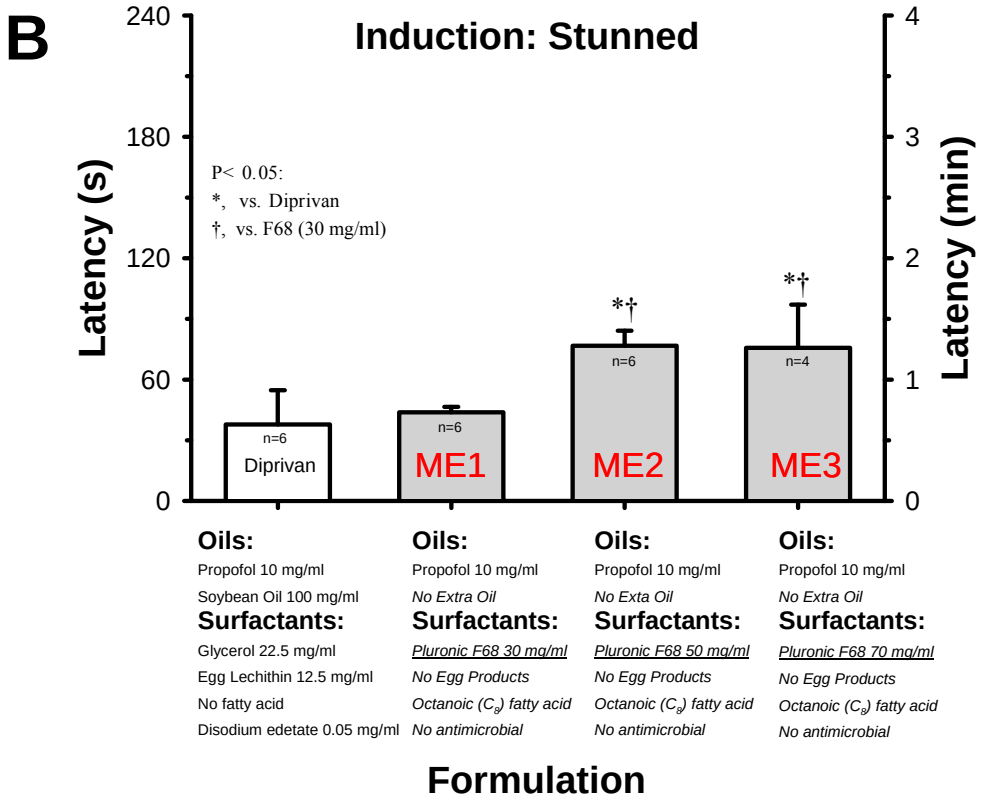


Figure 3-1. Anesthetic induction parameters in Rats. A) Propofol dose. B) Stunned.

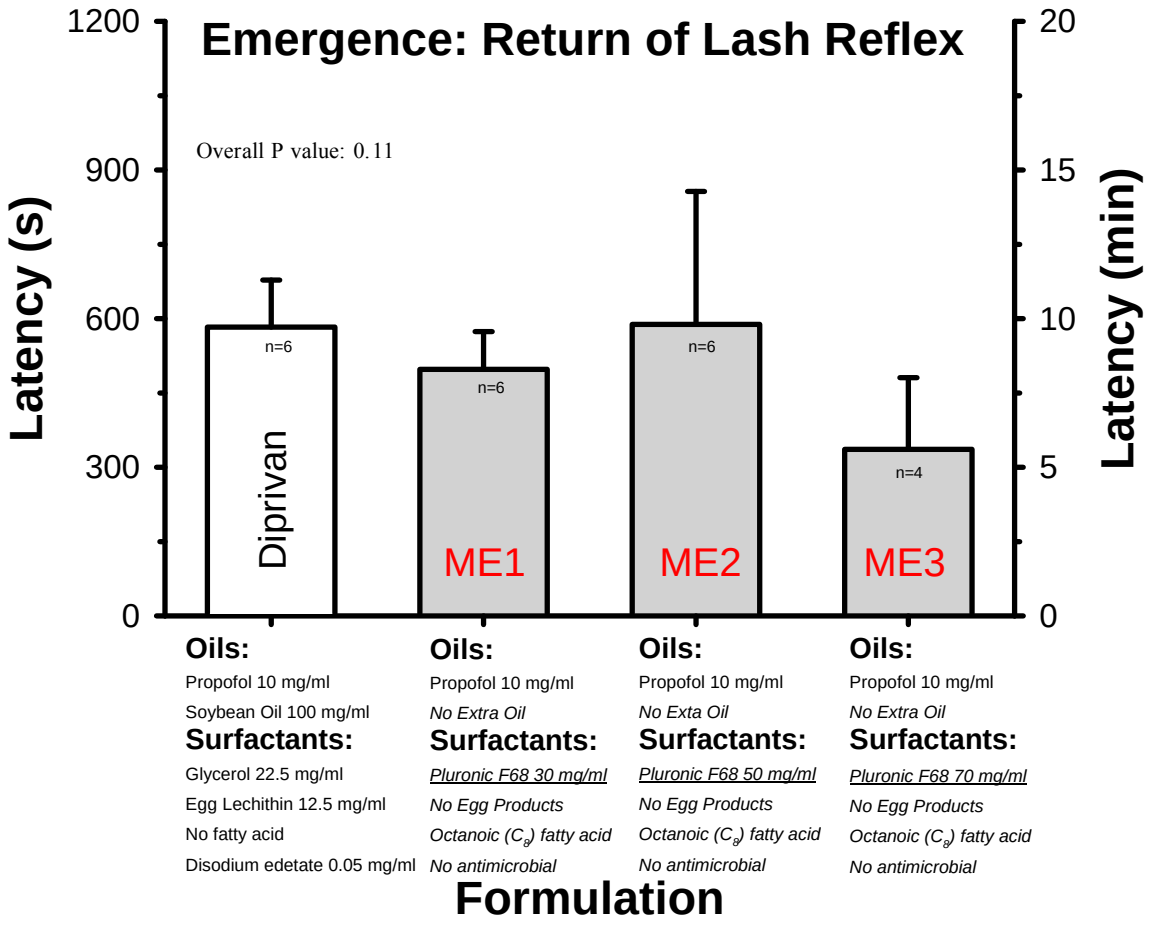


Figure 3-2. Anesthetic emergence in Rats.

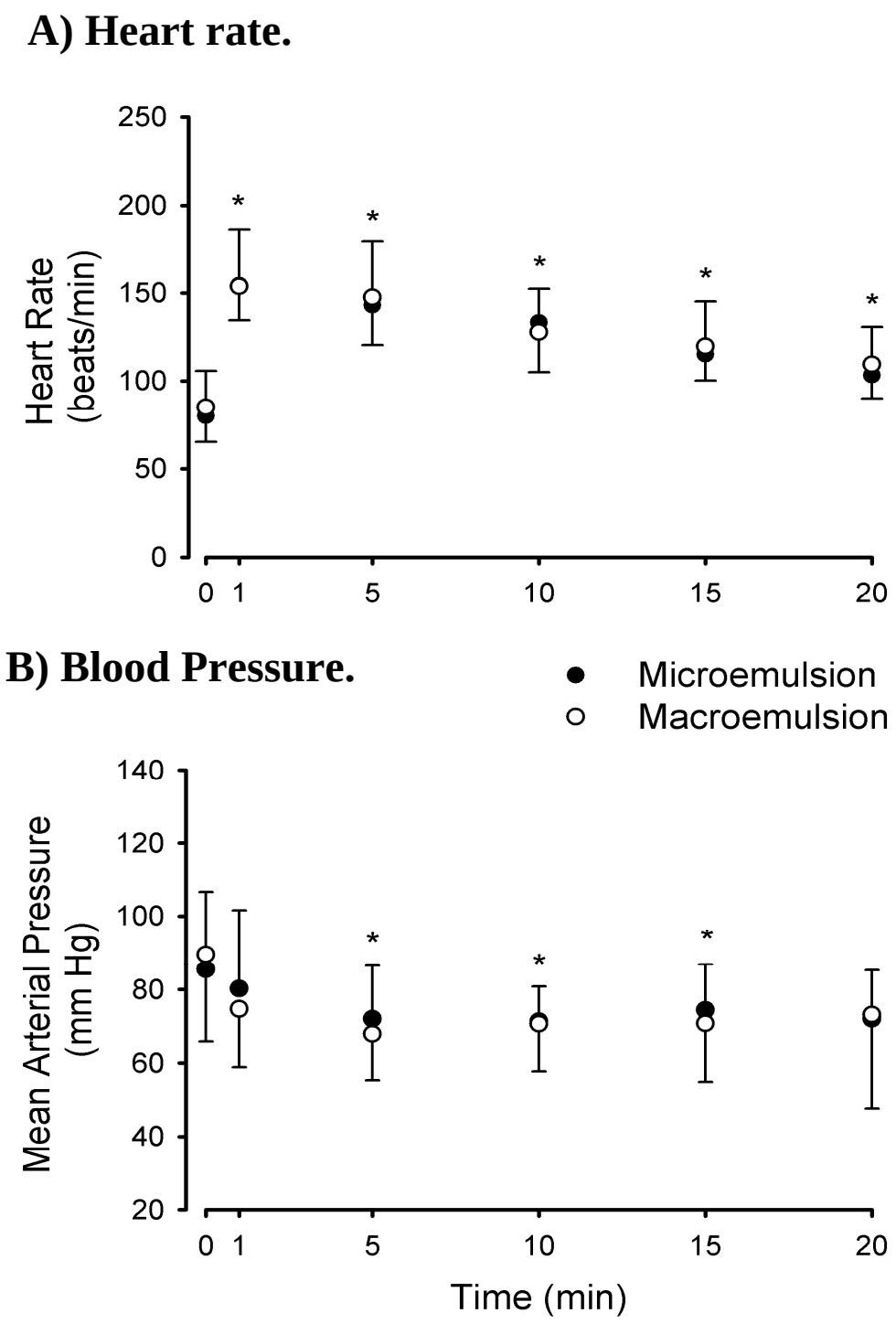


Figure 3-3. Time-dependent effects of a propofol microemulsion or macroemulsion on Dogs. A) Heart rate. B) Mean arterial blood pressure.

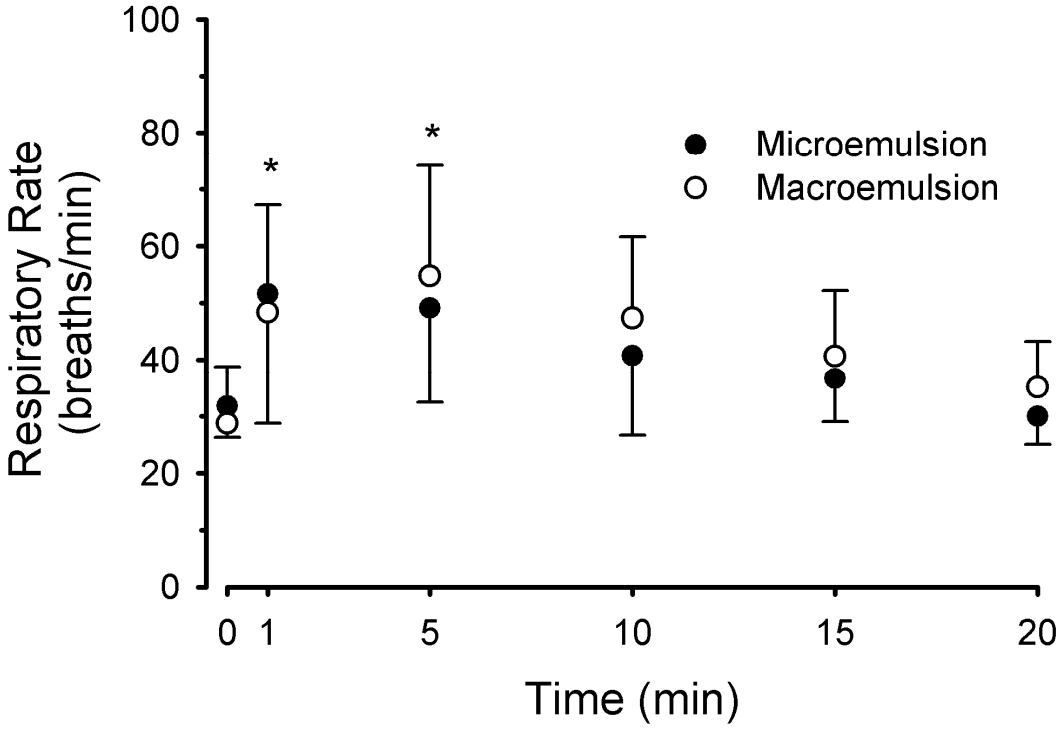


Figure 3-4. Time-dependent effects of a propofol microemulsion or macroemulsion on respiratory rate in Dogs. Data is expressed as mean±standard deviation.

Table 3-1. Dose and latency intervals for anesthetic induction and emergence in rat following intravenous infusion of a propofol macroemulsion formulation and several propofol microemulsion formulations with C8 fatty acid salt and differing purified poloxamer 188 (Pluronic F68) concentrations

Prameter	Propofol Formulation			
	Macroemulsion	Microemulsion		
		3%	5%	7%
N	18	6	6	6
Anesthetic Induction				
Dose (mg/kg)	21.0 ± 4.9	26.6 ± 4.0	30.4 ± 4.5	30.2 ± 4.8
Stunned (s)	38 ± 8	48 ± 8	69 ± 13	64 ± 12
Loss of Righting Reflex (s)	55 ± 15	68 ± 11	91 ± 12	78 ± 15
Loss of Lash Reflex (s)	87 ± 19	93 ± 17	129 ± 24	106 ± 16
Loss of Leg Withdrawal (s)	126 ± 29	160 ± 23	182 ± 26	181 ± 28
Anesthetic Induction				
Return of Leg Withdrawal (s)	375 ± 147	368 ± 77	405 ± 193	274 ± 105
Return of Lash Reflex (s)	306 ± 170	347 ± 84	362 ± 195	230 ± 107
Return of Righting Reflex (s)	681 ± 125	460 ± 64	629 ± 126	581 ± 155
Return of Sustained Headlift (s)	720 ± 105	495 ± 83	644 ± 133	596 ± 143

Data expressed as mean±standard deviation of N number of experiments.

Table 3-2. Dose and latency intervals for anesthetic induction and emergence in rat following intravenous infusion of a propofol macroemulsion formulation and several propofol microemulsion formulations containing with C8, C10, or C12 fatty acid salts and 5% purified poloxamer 188 (Pluronic F68)

Prameter	Propofol Formulation			
	Macroemulsion	Microemulsion		
		C_8	C_{10}	C_{12}
N	18	6	6	6
Anesthetic Induction				
Dose (mg/kg)	20.2 ± 4.0	30.4 ± 4.5	41.9 ± 3.4	33.9 ± 1.6
Stunned (s)	41 ± 9	69 ± 13	69 ± 10	83 ± 12
Loss of Righting Reflex (s)	55 ± 8	91 ± 12	110 ± 13	100 ± 13
Loss of Lash Reflex (s)	90 ± 11	129 ± 24	156 ± 11	142 ± 33
Loss of Leg Withdrawal (s)	122 ± 24	182 ± 26	251 ± 20	203 ± 10
Anesthetic Induction				
Return of Leg Withdrawal (s)	270 ± 140	405 ± 193	318 ± 63	516 ± 176
Return of Lash Reflex (s)	358 ± 193	362 ± 195	334 ± 57	528 ± 240
Return of Righting Reflex (s)	645 ± 104	629 ± 126	712 ± 192	889 ± 175
Return of Sustained Headlift (s)	667 ± 116	664 ± 133	725 ± 197	850 ± 119

Data expressed as mean±standard deviation of N number of experiments.

Table 3-3. Effects of propofol microemulsions (Micro) and macroemulsions (Macro) on parameters of the Red Blood Cell population in Dogs

Prameter	Pre-Induction		Post-Induction		Recovery		Recovery
	Micro	Macro	Micro	Macro	Micro	Macro	
RBC (10^6/μl)	6.04 ± 0.45	6.13 ± 0.43	5.99 ± 0.41	6.07 ± 0.34	5.47 ± 0.71	5.40 ± 0.50	0.41
Hemoglobin (10^3/μl)	14.1 ± 0.80	14.3 ± 0.80	14.0 ± 0.70	14.4 ± 0.50	12.7 ± 1.40	12.6 ± 1.00	0.66
Hematocrit (%)	42.0 ± 2.30	42.7 ± 2.70	41.9 ± 2.00	42.4 ± 1.50	37.9 ± 4.00	37.5 ± 2.80	0.78
MCV (fl)	69.6 ± 2.40	69.7 ± 2.30	70.0 ± 2.50	69.9 ± 2.20	69.6 ± 2.30	69.5 ± 2.20	0.84
MCH (pg)	23.4 ± 0.70	23.3 ± 0.70	23.4 ± 0.80	23.7 ± 0.70	23.3 ± 0.70	23.3 ± 0.80	0.45
MCHC (g/dl)	33.7 ± 0.50	33.5 ± 0.60	33.5 ± 0.40	33.9 ± 0.40	33.5 ± 0.50	33.6 ± 0.40	0.48
Reticulocytes (10^3/μl)	67.3 ± 18.7	73.9 ± 33.8	67.4 ± 17.5	64.3 ± 20.3	53.7 ± 11.5	50.8 ± 12.4	0.97
Reticulocytes (%)	1.12 ± 0.30	1.21 ± 0.50	1.15 ± 0.28	1.06 ± 0.36	1.01 ± 0.22	0.96 ± 0.30	0.88

Table 3-4. Effects of propofol microemulsions (Micro) and macroemulsions (Macro) on the White Blood Cell count and population differential in Dogs

Prameter	Pre-Induction		Post-Induction		Recovery		Recovery
	Micro	Macro	Micro	Macro	Micro	Macro	
WBC (10^3/μl)	8.55 ± 1.04	9.48 ± 2.20	7.24 ± 1.50	8.81 ± 2.23	7.72 ± 1.30	8.15 ± 2.79	0.14
Neutrophils (10^3/μl)	5.03 ± 0.69	5.93 ± 1.41	4.15 ± 1.09	5.36 ± 1.35	4.49 ± 0.96	5.03 ± 1.94	0.11
Lymphocytes (10^3/μl)	2.60 ± 0.71	2.61 ± 0.68	2.37 ± 0.64	2.37 ± 0.72	2.35 ± 0.65	2.24 ± 0.62	0.82
Monocytes (10^3/μl)	0.58 ± 0.17	0.59 ± 0.30	0.44 ± 0.13	0.55 ± 0.29	0.57 ± 0.16	0.57 ± 0.29	0.58
Eosinophils (10^3/μl)	0.19 ± 0.13	0.21 ± 0.21	0.16 ± 0.12	0.17 ± 0.17	0.17 ± 0.11	0.19 ± 0.18	0.47
Basophils (10^3/μl)	0.06 ± 0.03	0.08 ± 0.07	0.05 ± 0.02	0.05 ± 0.05	0.06 ± 0.03	0.05 ± 0.03	0.59

Table 3-5. Effects of propofol microemulsions (Micro) and macroemulsions (Macro) on Platelet population and indices of Thrombosis in Dogs

Prameter	Pre-Induction		Post-Induction		Recovery		Recovery
	Micro	Macro	Micro	Macro	Micro	Macro	
PlAtelets (10^3/μl)	338 ± 43	330 ± 48	319 ± 39	322 ± 50	331 ± 42	299 ± 111	0.55
Fibrinogen (mg/dl)	202 ± 30	219 ± 80	202 ± 23	227 ± 87	204 ± 30	206 ± 81	0.52
PT (s)	8.7 ± 0.4	8.7 ± 0.3	9.0 ± 0.4	9.0 ± 0.4	8.9 ± 0.4	8.9 ± 0.5	0.89
aPTT (s)	10.1 ± 0.6	10.2 ± 0.4	10.3 ± 0.5	10.3 ± 0.4	10.2 ± 0.6	10.1 ± 0.6	0.76

Table 3-6. Plasma propofol concentration after induction of anesthesia in Dogs

Time (min)	Plasma propofol concentration (μM)			
	Microemulsion	n	Macroemulsion	n
-1	<0.05	10	<0.05	10
1	25.2 ± 8.7	10	31.7 ± 14.1	10
5	2.9 ± 1.2	10	3.7 ± 1.9	10
10	1.9 ± 0.6	10	2.1 ± 0.7	10
15	2.3 ± 1.3	9	1.6 ± 0.5	9
20	1.9 ± 1.4	4	2.4 ± 1.1	6

CHAPTER 4
RETARDATION OF WATER EVAPORATION THROUGH DUPLEX FILMS OF SURFACTANT IN OIL

4.1 Introduction

Early experiment on retardation of water evaporation can be traced back in 1924, where Hedestrand[1] tried to demonstrate the effect of monolayers on the evaporation rate of water on which they were spread. In 1943, Langmuir and Vincent Schaefer[100] measured evaporation rates of water by suspending, over the surface of water in a film balance (Figure 4-1), a flat container with a permeable bottom supporting a solid dessicant (calcium chloride). Also, in our laboratory we have shown the importance of molecular packing achieved by using mixed surfactant monolayers and pKa of fatty acids in reduction of evaporation of water. The maximum reduction in evaporation using monomolecular film of surfactants was around 50%. However, the problem with monolayers is that they can be easily removed by winds and also they undergo thermal and biodegradation easily.[114] Under such circumstances multimolecular films of oil and surfactants could be ideal.

Heymann and co-workers[115, 116] in the 1940s had used multimolecular films of oil as a means of preventing or reducing the evaporation of water from exposed water surfaces in arid climates. Using such multimolecular films of paraffin oil and neutral oil from vertical retort tar, they showed a significant reduction in evaporation of water. However, other than that not much literature is found in this area to the best of our knowledge. In our research, we found one such surfactant, Brij-93, after screening various surfactants which can mix with hexadecane and uniformly spread on water surface and effectively reduce the evaporation. The effect of temperature, various polymeric additives, volume of the duplex film deposited and concentration of surfactant in the mixture was studied. The structure of the film at the interface was studied using Brewster Angle Microscopy.

4.2 Methods and Materials

4.2.1 Materials

The surfactant Brij-93 (HLB 4.9) or Polyoxyethylene (2) Oleyl Ether was supplied by ICI, Speciality Chemicals, (Wilmington, DE). Stearic acid, Stearyl alcohol, Poly Vinyl alcohol, Bovine Serine Albumin was purchased from Sigma Aldrich. Hexadecane and anhydrous Calcium Chloride was from Fisher Scientific, (Fair Lawn, NJ). Artificial tear solutions were purchased from local Walmart pharmacy. Water used in all the experiment was de-ionized water, unless specifically mentioned. All experiments were carried out at 23 ± 1 °C unless otherwise stated.

4.2.2 Spreading of Duplex Film

All the experiments were carried on Langmuir film balance (Figure 4-2), having trough of dimensions lxbxh=25x12x1.25 cm3. The trough was filled with 385ml of water each time and the evaporation measurements were carried out on pure water surface first, before spreading of the duplex film. The duplex film was then deposited on top of water by putting some drops of solution (oil+surfactant) using microsyringe on various areas of the trough. Depending on the volume deposited, the average thickness of the film ranged from 4 to 10 micron in thickness.

For the monolayer studies: 1% solution of compound is made in 1:1:3 mixtures of chloroform, methanol and hexane.

4.2.3 Evaporation Studies

A known amount of anhydrous calcium chloride was measured and placed in a petri dish. The petri dish was then covered with nylon cloth, inverted, and placed 3 mm above the surface of a water or water covered with film. The rate of evaporation was measured by the increase in weight of the desiccant, anhydrous $CaCl_2$. The change in desiccant weight was measured every 20 min over a period of 1 hour. To reduce the contribution of air, i.e. moisture of air absorbed by

$CaCl_2$, the Langmuir trough is covered with aluminum foil and increase in weight of the desiccant ($CaCl_2$) was measured every 20 min over a period of 1 hour. This is then subtracted from earlier measurement, to get the actual value of water evaporated from the surface.

4.2.4 Brewster Angle Microscopy Studies

The Brewster angle microscope used was a BAMl from Nanofilm Technologie Gmbh (Gottingen, Germany). A He-Ne laser (p-polarized) was used as the light source. The angle of incidence was initially set to 53' and then adjusted to minimize the reflected intensity of the clean water surface prior to spreading of each film. All photos presented were taken with a rotatable analyzer set at 90' placed in between the reflected signal and the CCD camera. A frame-grabbing program (Videopix, Sun Microsystems) was used to generate photos from videotape and convert them to a computer-readable format.

4.3 Results and Discussion

Evaporation experiments on these films are done using Langmuir film balance. We have studied the effect of following factors on the rate of evaporation for duplex film of Brij 93 and hexadecane.

- Comparison with Monolayers
- Thickness of the Film Deposited
- Concentration of Surfactant
- Various Polymeric Additives
- Stability of the Film
- Structure of this film using Brewster Angle Microscopy

Figure 4-3 shows the comparison of duplex film of Brij 93 and Hexadecane with monolayers of Stearic acid and Stearyl alcohol. Stearic acid monolayer alone does not have significant effect in reduction in evaporation rate. However, when replaced by monolayer of Stearic reduction in evaporation by more than 40%. Duplex film of Brij93 in Hexadecane shows

very high reduction of evaporation, this is due to high resistance provided by interfacial layer of surfactant at water/hexadecane interface as well as hexadecane/air interface.

Figure 4-4 shows the effect of addition of duplex film of Brij-93 and hexadecane on retardation of evaporation. It can be seen that increase in addition of the surfactant plus oil mixture results in retardation of evaporation up to 85%. Not much significant evaporation reduction was observed above addition of 400 microliters or 16-micron thick film. Because the film is not uniform after 16 micron thickness, as the surfactant and oil combination form emulsion droplet rather than continuous film. The diffusion of water across hexadecane oil layer can be described in terms of the equation:

$$J = -mc\frac{d\mu}{dx} \qquad (4\text{-}1)$$

Where, J is net flux/area, m is mobility of water in hexadecane, c is concentration and dμ/dx, the gradient of chemical potential, is the driving force. This equation is a general form of the Nernst-Planck equation. The chemical potential of water (μ) can be expanded in terms of water vapor pressure (p).

$$J = -RTm\frac{c}{p}\frac{dp}{dx} \qquad (4\text{-}2)$$

The diffusion coefficient (D) is defined by RTm, where R is gas constant and T is absolute temperature. For a dilute solution, such as water in hexadecane, the ratio c/p is constant and is termed a solubility coefficient (α). Then:

$$J = -D\alpha\frac{dp}{dx} \qquad (4\text{-}3)$$

Integrating across the hexadecane layer:

$$J = \frac{D\alpha}{\delta}\Delta p \qquad (4\text{-}4)$$

Where, δ is the thickness of the layer. If water is in equilibrium across the interfaces on either side of the hexadecane layer, Δp is the difference in water vapour pressure between the media on either side of the hexadecane layer. As, we see from equation 4-4 water flux is inversely proportional to the thickness of the film, because of which we will see less and less differential decrease in water evaporation rates as we increase the thickness of the film (Figure 4-4).

Figure 4-5 shows that increase in concentration of Brij-93 in hexadecane the retardation of evaporation increases first (till Brij93 conc. 0.025%) after which it remain constant. This can be explained as due to incomplete surface coverage of the oil film on water surface at surfactant concentration lower than 0.025%, further addition of surfactant result in formation of oil in water emulsion. This can be further confirmed using our Brewster Angle Microscopy results wherein one finds clear pattern initially ending up into circular emulsion droplet (Figure 4-6 (c)).

Figure 4-7 shows the effect of different polymers on retardation of water evaporation. 100 microliters of 1 wt. % polymer were first deposited on water trough followed by addition of 100 microliters of duplex film of Brij-93 and hexadecane. Addition of 100 microliters of duplex film on top of a Polyvinyl alcohol (PVA, MW-10,000) film in water retards evaporation by more than 80 % much more than the duplex film of hexadecane + Brij-93 alone (60%). This is due to tight packing of polyvinyl alcohol at the interface between surfactant and oil layer. We further studied the effect of polymers such as Bovine Serum Albumin, Hydroxyethyl cellulose, Hydroxypropyl cellulose, Carboxymethyl cellulose, but none of them show retardation of evaporation. Which further underscore the fact that Polyvinyl alcohol being very surface active polymer crowd the water/hexadecane much more than with the surfactant alone, and thus reducing the effective evaporation of water.

This duplex film is very elastic as seen in Figure 4-8 shows the effect compression/expansion and aging of duplex film on water evaporation. It can be seen deduced that this duplex film (Brij93 + Hexadecane) is very effective in reducing the evaporation of water after one day. These duplex films are much is much more stable than other films, as well as compression/expansion does not reduce its evaporation reduction significantly.

Figure 4-8 shows how commercially available tear solutions reduces water evaporation compared to duplex film. As we see from the graph they do not reduce any water evaporation as compared to duplex film. This proves to the possibility that if we can able to find this type of duplex films in sub-micron size range we would be able to apply them to solve the problem of Dry Eye.

4.4 Conclusion

Brij 93 in Hexadecane spreads uniformly on the water surface forming a Duplex film. Duplex film reduces water evaporation much more in comparison to long chain acids and alcohols. Rate of evaporation of water can be suppressed to as much as 80%, by increasing film thickness. Duplex film is elastic i.e. continuous expansion or compression of the film in a manner akin to the blinking of the eye does not decrease the evaporation reduction efficiency. Addition of surface active polymer PVA further decreases the evaporation rate of water, whereas other polymers do not reduce evaporation rate. This oil/surfactant combination can form an emulsion with water which can be used to reduce evaporation of water. Brewster Angle Microscopy pictures shows that surface structure of the film changes by varying surfactant concentration, volume etc.

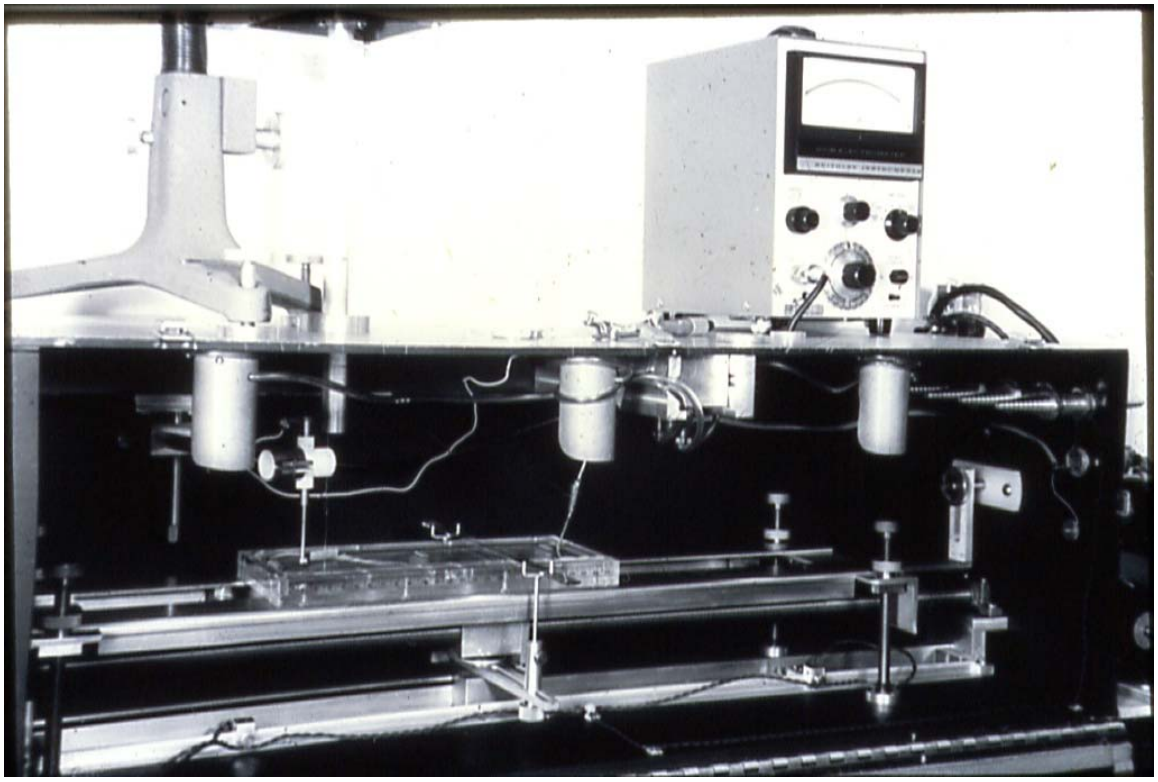


Figure 4-1. Langmuir film balance.

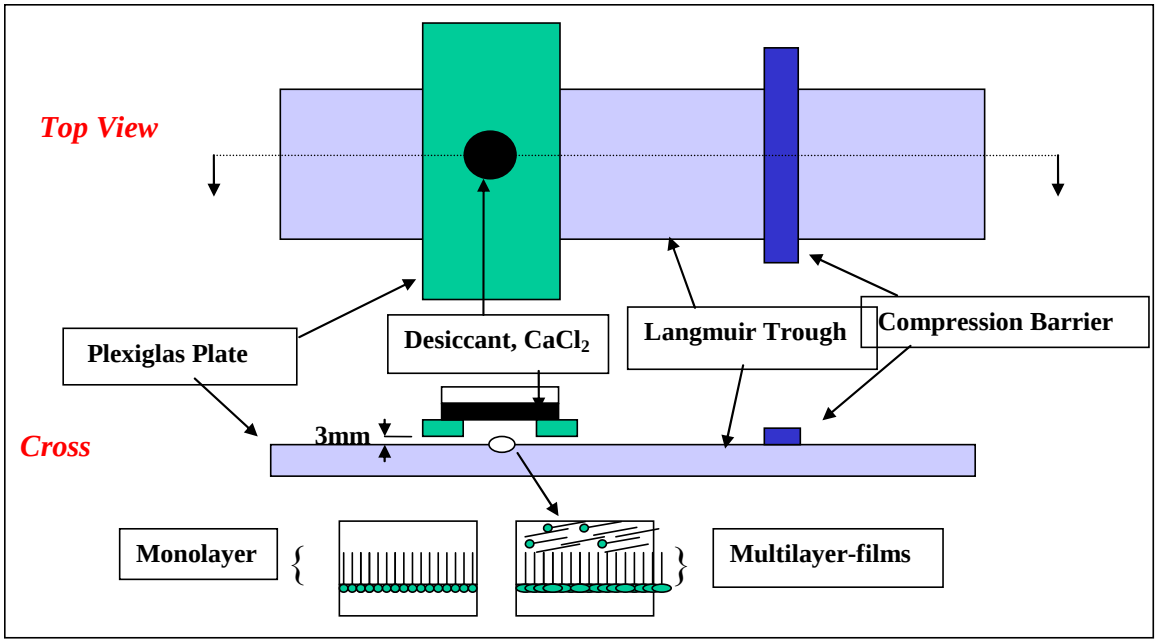


Figure 4-2. Evaporation experiment setup.

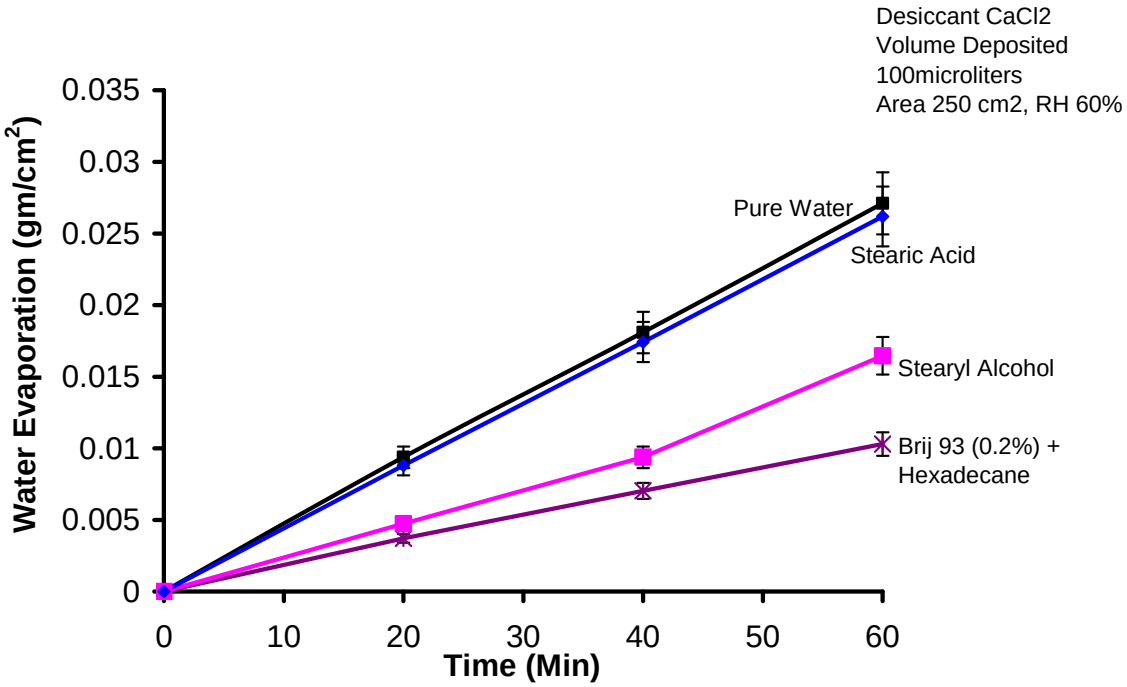


Figure 4-3. Comparison of water evaporation reduction by duplex film and monolayers.

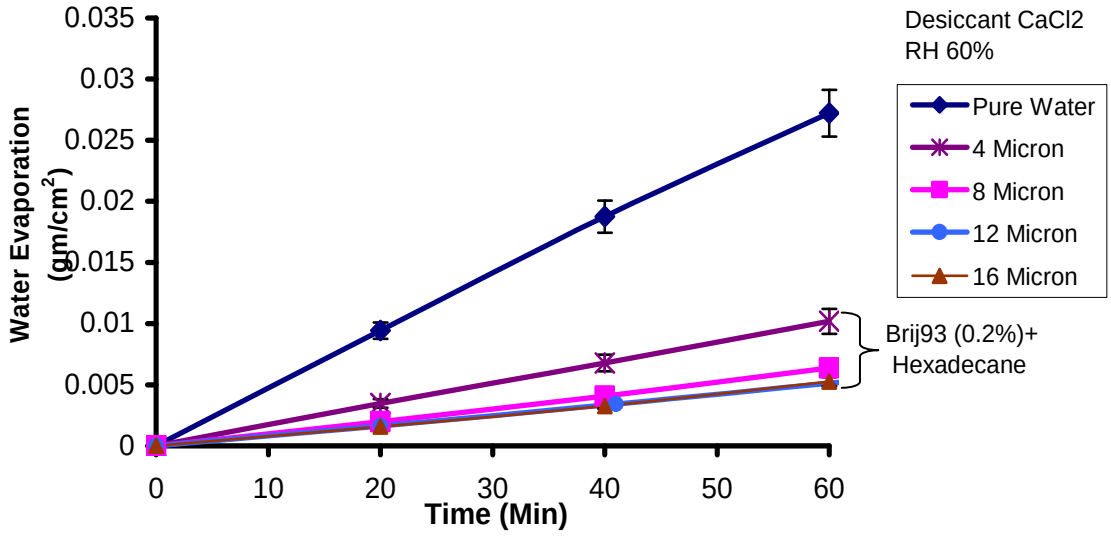


Figure 4-4. Effect of film thickness on evaporation.

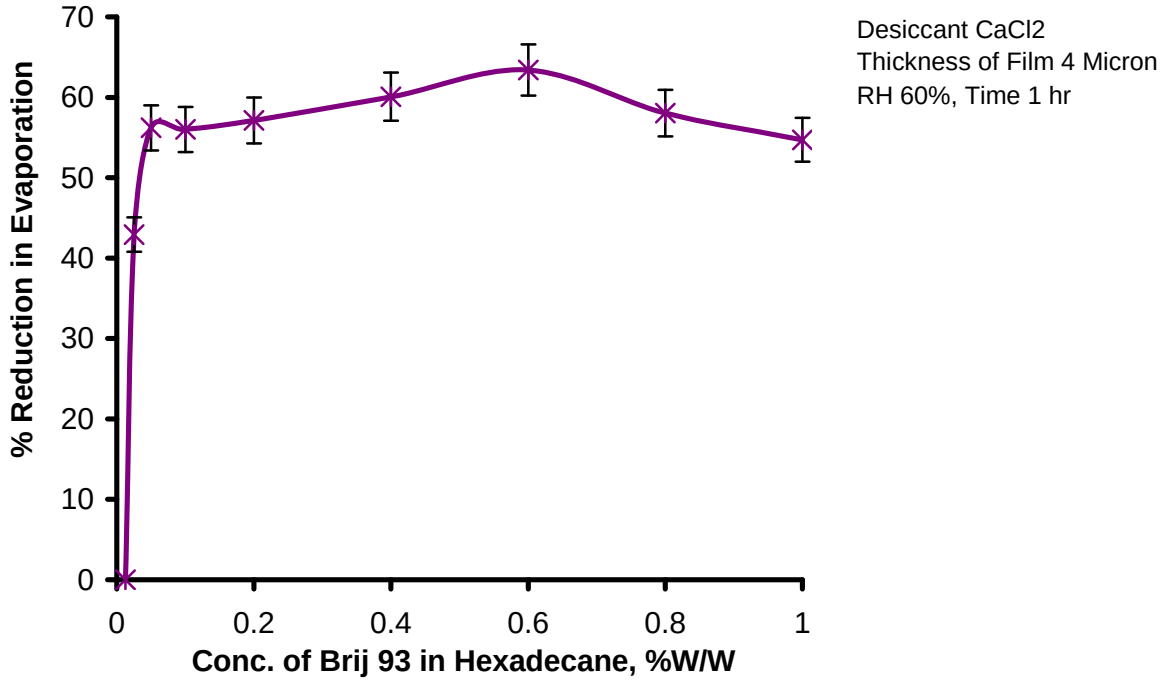


Figure 4-5. Effect of concentration of Brij 93 in hexadecane on evaporation.

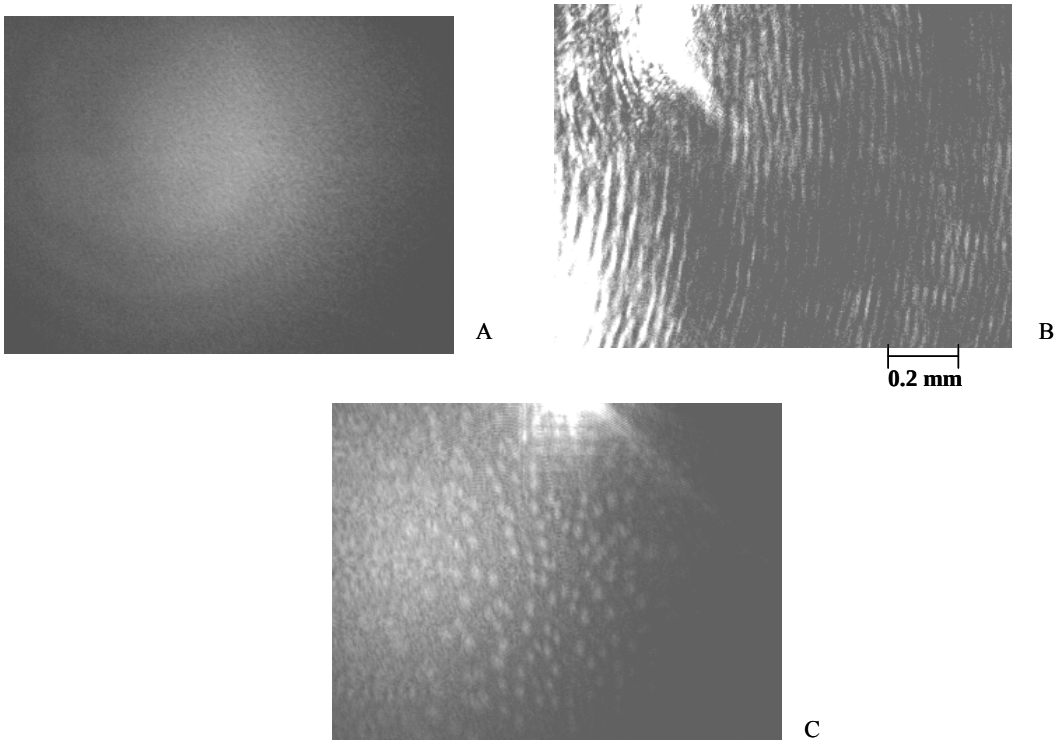


Figure 4-6. Brewster Angle Microscopy images of duplex film of hexadecane and Brij-93. A) 0.2 wt.% Brij-93 in hexadecane. B) 0.5 wt.% Brij-93 in hexadecane. C) 0.8 wt.% Brij-93 in hexadecane.

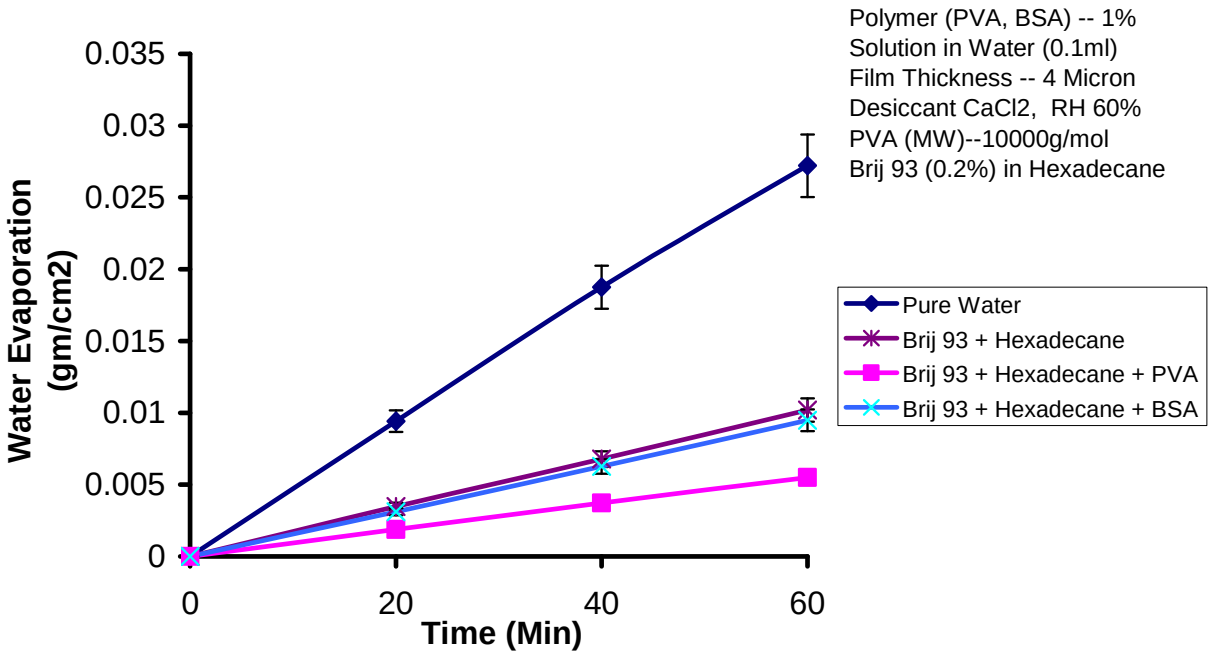


Figure 4-7. Effect of various polymers on evaporation.

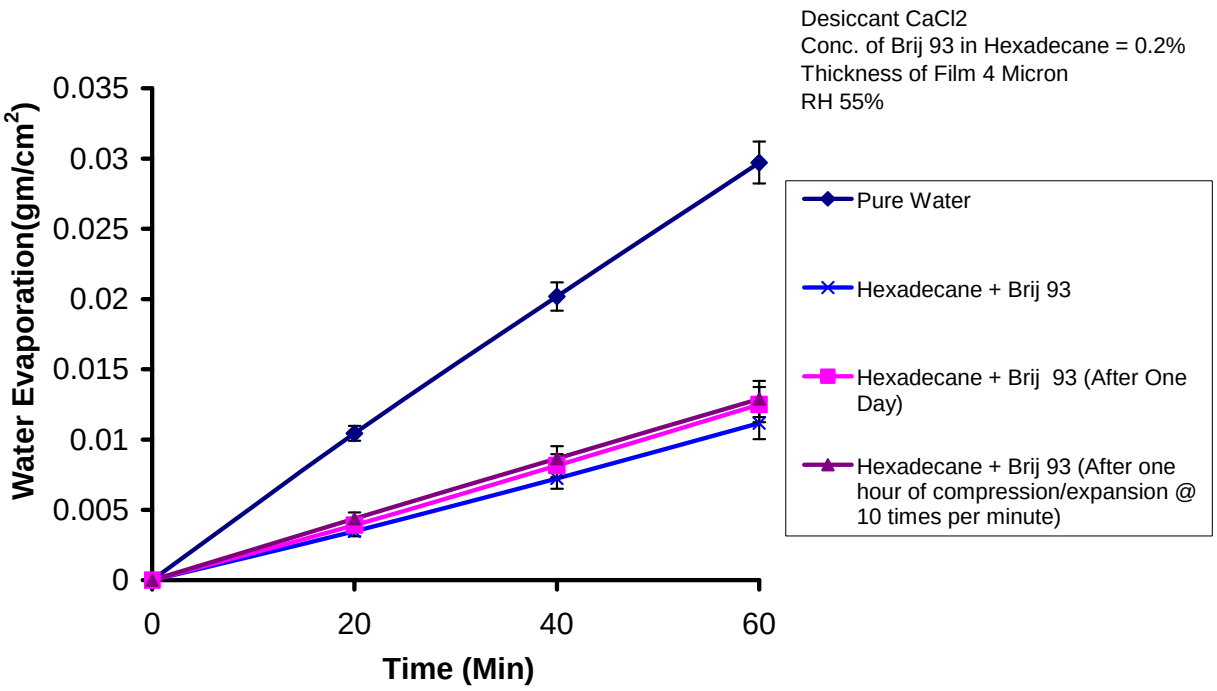


Figure 4-8. Effect of aging and compression/expansion of film on evaporation.

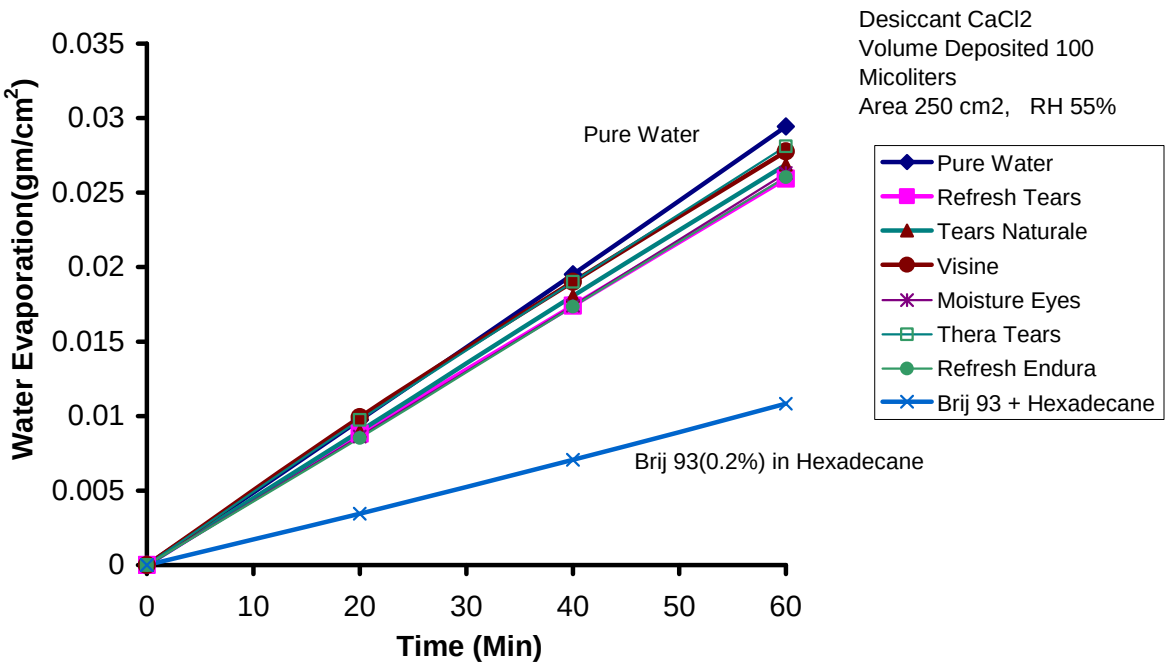


Figure 4-9. Effect of films of various commercial products on water evaporation.

CHAPTER 5
SUMMARY AND RECOMMENDATIONS FOR FUTURE WORK

5.1 Propofol Microemulsions for Anesthesia

5.1.1 Summary

Microemulsions offer a potential alternative carrier system for drug delivery because of their high solubilization capacity, transparency, thermodynamic stability, ease of preparation, and high diffusion and absorption rates. A number of factors must be considered when using microemulsions as drug delivery vehicle. First, the appropriate type of oil and surfactant must be used in order to dissolve and protect the drug. The surfactant can be non-ionic, or anionic (in combination of non-ionic). The type of drug dissolved and conditions of the target site will dictate the type of surfactant used to carry the drug. Second, the concentration of surfactant largely influences the carrier system, because this surfactant concentration will ultimately determine drug delivery effectiveness. Last, the conditions of the target site are critical to the effectiveness of a surfactant delivery system. Parameters such as temperature, pH will influence the solubility of a drug inside the microemulsion system.

Although o/w microemulsions are promising solvent systems for drug delivery, compared to other drug delivery methods, their potential has not been reached. There are several barriers that need to be overcome before this technology can be used in practice. The main challenge is the control of drug diffusion and partitioning between the dispersed and continuous phases present. Another factor influencing the use of microemulsions is the biological tolerance of the constituents of microemulsions, particularly the surfactants and cosurfactants, because they can cause disruptions in biological membranes. A high surfactant concentration in the body over a long period of time may disturb some bodily processes. Also, cosurfactants like medium chain length alcohols are skin or eye irritants, so their use in topical drug delivery systems is limited.

Nonionic surfactants such as ethoxylated alkyl chain ethers and sobitan easters, as well as nonionic block copolymers are generally less toxic than ionic surfactants. Also, many nonionic surfactants have an advantage over charged surfactants, as they can form microemulsions even without cosurfactants.

The need for a suitable alternative formulation is evidenced by past and ongoing research into other types of propofol delivery systems. In this thesis, we have demonstrated that microemulsion methods represent another suitable technology to deliver propofol intravenously with anesthetic parameters similar to the commercially available macroemulsions. Fewer drugs have been delivered using an intravenous route. Unlike other oil-in-water microemulsions wherein the active drug is dissolved in an oil excipient, in the present study propofol acted in two different, complementary roles in the present investigation. That is, the need for an excipients oil for propofol dispersal was obviated by the fact that propofol is itself an oil at room and physiological temperatures. Therefore, propofol could serve not only as the active pharmacodynamic agent, but also could exist as the physical platform for the preparation of these microemulsions. By doing so, the need for additional excipients oils (e.g., soybean or castor bean oil) is eliminated along with the potential for these excipients to nourish bacteria. The kinetics of general anesthesia with the propofol microemulsion was favorable compared to the commercially available propofol macroemulsion. Whereas the time for general anesthesia (defined by the protocol as loss of leg withdrawal to a pinch) during macroemulsion injection was 122-126 s, the latency periods during microemulsion treatment were 166-203 s. This short delay is favorable when compared to pro-drug technologies that rely on metabolism, a phenomenon that may vary significantly within any patient population.[147] The differences in induction times between experimental groups reported herein is caused by differential release of

propofol from the individual droplets into the blood. That is, the different propofol nanoparticles have markedly different stabilities against dilution by blood based on the emulsifier structure and concentration selected for the formulation. In general, a microemulsion is thermodynamically stable at equilibrium. But, one can destabilize a microemulsion by significantly changing pressure, temperature, or chemical compositions. The last variable can be changed simply by diluting a microemulsion with saline or blood. It is well recognized that the formation of microemulsions requires an ultra low interfacial tension (e.g., ~10^{-3} mNewton/m) at the oil/water interface. Upon dilution with saline, the interfacial tension will increase substantially as the emulsifier molecules (i.e., both pluronic 68 and fatty acid salt molecules) desorb from the droplet surface. This event will markedly increase the interfacial tension at the droplet surface and ultimately destabilize the microemulsion, with release of the active pharmaceutical core (i.e., propofol). However, each emulsifier film around the microemulsion droplet has inherent molecular packing and, hence, stability. The extent of dilution required to destabilize a microemulsion represents its inherent stability. Thus, a microemulsion requiring greater dilution for destabilization indicates that its emulsifier film has a greater stability. C12 fatty acid salt microemulsions are the most stable as they require the greatest dilution to become a turbid macroemulsion. It is likely; however, that microemulsion destabilization is affected by more than just dilution in vivo as the surfactant can go to various other places in vivo dilution than in vitro. Further understanding and selectively modifying these destabilization rates by use adapting surfactant type and concentrations alludes to the possibility of controlling release times of active pharmaceuticals from nearly immediate (e.g., propofol) to longer times (e.g., chemotherapeutic or antifungal agents).

One benefit of slightly delayed propofol release due to these differential stabilities may be reduced pain on injection. That is, we hypothesize that the concentration of aqueous propofol in peripheral veins will be sufficiently low so that minimal-to-no pain will be experienced during induction. If the relaxation rate is sufficiently long to ensure nanoparticle integrity from the time of injection in a peripheral vein to the time they enter the central circulation (e.g., 10-15 s), then no pain should be caused during injection.

In dog study, we investigated the dose of propofol, micro- or macroemulsions, administered to dogs to induce anesthesia, along with possible effects on blood, the first tissue encountered by the formulations. The measured variables of anesthesia, vital signs, indices of blood cell populations or thrombosis, and plasma concentrations of propofol did not significantly differ between the groups of animals given either the propofol microemulsion or macroemulsion. In these studies, all animals experienced rapid anesthesia as assessed by loss of leg withdrawal with mean propofol doses of approximately 10 mg/kg for both the microemulsion and macroemulsion. This dose of propofol is greater than that previously reported in other studies investigating anesthetic induction in unpremedicated dogs using propofol formulated in a macroemulsion. In the end, the use of microemulsions as drug delivery vehicle is an exciting and attractive area of research, offering not only challenges to be overcome but also many potential extraordinary benefits.

5.1.2 Future Work

1. Development of non-ionic microemulsion systems: As already discussed ionic surfactants (even anionic) have very low acceptability limits in body as higher irritancy as well as cell response mechanisms. Whereas non-ionic systems can be used in high concentration without these side effects. So, non-ionic systems particularly Tween/PEG microemulsion system should be pursued for higher animal models for their efficacy for drug delivery.

2. Franz diffusion cell experiments for skin irritation: In Franz cell model, penetration studies are conducted to measure the rate and extent of penetration and absorption of

drug as well as surfactants into the skin (and various cell surfaces). From such studies, the optimal surfactant, co-surfactant combination can be selected and issues regarding penetration can be identified and resolved before costly clinical studies are embarked upon.

3. Pharmacokinetics and pharmacodynamics model for propofol system: Even though after 25 years of use of propofol we still don't know the exact mechanism of action of propofol. We need to develop a model for how the drug and surfactants included in the formulation affect the various systems in body. An analytical model of pharmacokinetics and pharmacodynamics is therefore required to minimize the animal experiments for efficacy studies.

4. Pharmacodynamics and pharmacokinetics in higher animal system like Pig: Once optimum system is defined in vitro, pharmacokinetics as well as pharmacodynamics studies needs to be done in bigger animal model like pigs. These studies will help us determine what various drug/surfactant does to the body as well as what body does to them.

5. Extension of this work on the water insoluble drugs: As new water insoluble drugs are being discovered, these microemulsion systems can be developed as a fingerprint of delivery system, based on solubility, stability, partition coefficient and various other process parameters. Few of the drugs for example can be rapamycin, geldanamycin, and various other water insoluble protein drugs.

6. Effect of various excipients on the performance of microemulsions: As we know pharmaceutical formulations consist of various excipients like antimicrobial agents, fillers, disintegrants, lubricants, glidants and binders. They are required for stability of drug and formulation, to stop bacterial growth, to ensure easy disintegration, disaggregation, and dissolution etc. So, it is necessary to study the effect over various excipients as mentioned above on the pharmaceutical performance of microemulsions made in a system containing all required excipients both in vivo and in vitro.

5.2 Reduction of Water Evaporation

5.2.1 Summary

Reduction of water evaporation directly touches many aspects of our lives. Knowledge, understanding of various methods to reduce water evaporation has revolutionary potential. A multimolecular (duplex) film of oil and surfactants is one of the approaches to reduce evaporation of water, can be a potential solution to this problem.

Mixture of Brij-93 and hexadecane forms a duplex film, which uniformly spreads on surface of water and retards evaporation of water. Increase in deposited volume of Brij-93 and

hexadecane reduces the evaporation rate and the maximum retardation in evaporation achieved was 80%. Corresponding changes in surface structure of duplex film due to increase in Brij-93 concentration were seen using Brewster Angle Microscope. Addition of polymer (Polyvinyl alcohol) was found to improve the retardation efficiency of the duplex film as compared to other polymeric additives.

5.2.2 Future Work

1. To find different kind of systems: Determine the different kind of surfactant and oil systems, which can spread on the water surface and form a duplex film. The work will include try using natural biodegradable long chain hydrophobic oils with different kind of superspreading surfactants. These surfactants decrease the interfacial tension at the oil/water interface to very low value and helps in spreading the duplex film.

2. Determine different kind of mixed surfactant monolayers, which reduce the rate of evaporation of water. In this case we want very compact interface so that through which water permeation rate is very slow, so we will try to form a very compact monolayer of surfactant. Especially mixed monolayers of Stearic acid, Oleic acid, Caster oil, Olive oil etc., with combination with long chain alcohols.

3. Effect of these films on permeation of gases: To study the rate of oxygen, carbon dioxide and other gases through these films, as continuous flow of oxygen/carbon dioxide is necessary for aquatic life and on the eye surface where oxygen is needed by top epithelium cell of the eye surface. So a continuous flow of oxygen, which is required by the epithelium cells, and carbon dioxide, which is excreted by the cells, is required for maintaining the normal vision.

4. Different kind of efficiency experiments on different systems of duplex film and mixed surfactant monolayers found in the first part, like surface structure behavior, film balance behavior, surface rheological properties to characterize these kinds of films. Various effects like lubrication efficacy, viscosity, and interaction with contact lenses of this film needs to be investigated. Following that, best surfactant and oil systems need to be identified which can directly spread on the surface and reduce water evaporation. These systems can be used directly to reduce evaporation of water or if incorporated in emulsion, it can be used in artificial tear solutions to reduce evaporation of water from tear film.

5. Identify the optimum systems weather monolayer or duplex film which reduces evaporation in vitro studies (film studies). Determine different properties of these films, and characterize these films. Determine mechanism by which these films spread and reduce the evaporation of water.

6. Incorporate the best duplex film/monolayer system as nanoemulsion in aqueous formulations and test its efficacy in efficiently reducing the evaporation rate.

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BIOGRAPHICAL SKETCH

Dushyant Shekhawat was born in Sardargarh, Rajasthan, India. He completed his high school from Kendriya Vidyalaya, Kota, Rajasthan. Later on, he joined the prestigious Indian Institute of Technology (IIT), Bombay from where he graduated with a bachelor's degree in chemical engineering in 2002. Here, at IIT, he was greatly inspired by his Professors (Dr. Jayesh Bellare, Dr. K. C. Khilar) and decided to pursue a Ph.D. degree in US universities. Of the several graduate school offers, he decided to join University of Florida owing to Dr. Dinesh O. Shah's fame in colloid and interface science area. In January 2003, Dushyant joined Professor Shah's group and completed his degree under his supervision in December of 2007.