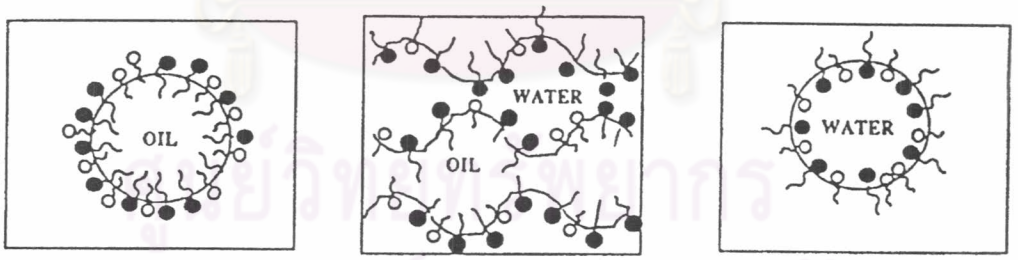
CHAPTER II

LITERATURE REVIEW

Introduction and Definition

Microemulsions are thermodynamically stable, transparent (or translucent), homogeneous, single optically isotropic, spontaneous formation and size between 10-140 nm. Generally, microemulsion are systems consisting of water, oil and surfactant (s), dispersions of oil and water stabilized by an interfacial film of surfactant molecules. The surfactant may be pure, a mixture, or combined with other additives. The systems containing low concentrations of dispersed phase be composed of droplets of either oil dispersed in water (o/w) or water dispersed in oil (w/o) while in systems containing comparable amount of oil and water, equilibrium bicontinuous structures in which the oil and the water domains interpenetrate in a more complicated manner are formed (Lawrence and Rees, 2000; Malmsten, 1999; Swarbrick and Boylan, 1994).



O/W Microemulsion Bicontinuous W/O Microemulsion

Figure 1 Oil-in water (o/w), bicontinuous, and water-in-oil (w/o) microemulsion structures. The filled larger circles ∿● represent the surfactant, and the smaller empty circles ∿○ the cosurfactant (from Swarbrick and Boyland, 1994)

Microemulsions are distinctly different from emulsions in that the formers are thermodynamically stable one-phase systems whereas the latter are kinetically stabilized dispersions. Thus, microemulsions require no work for their formation and once formed they are "infinitely" stable. Emulsions, on the other hand, require work for their formation and display a kinetically controlled instability and size between 1-10 μm. This fundamental difference between emulsions and microemulsions are not always appreciated within the pharmaceutical literature (Attwood, 1994; Malmsten, 1999).

Theory of Microemulsion Formulation

Historically, three approaches have been used to explain microemulsion formation and stability. These are: (I) interfacial or mixed film theories, (II) solubilization theories, and (III) thermodynamic treatments (Lawrence and Rees, 2000; Paul and Moulik, 1997; Swarbrick and Boyland, 1994).

I. Mixed-film theories

The spontaneous formation of microemulsion droplets is considered to be due to the formation a complex film at the oil-water interface by the surfactant and cosurfactant. This causes a reduction in oil-water interfacial tension to very low values (from close to zero to negative). The mixed interfacial film in equilibrium with both oil and water is considered to be liquid and duplex in nature (i.e., showing different properties at the oil and water sides) with a two-dimensional spreading pressure. A negative interfacial tension results, and energy is available to increase the interfacial area, effectively reducing droplet sizes.

A major drawback to Schulman's concept was the high value of the spreading pressure necessary to give the transient negative interfacial tension. Prince (1977) later postulated that the negative interfacial tension could be a result of the depression of the oil/water interfacial tension without the film present, rather than the unrealistically high initial pressure in the original model. The alcohol cosurfactant

partitions between the oil phase and the interface, with the fraction in the oil phase able to significantly depress the oil/water interfacial tension without the film presents from its normal value of approximately 50 mNm^{-1} to a new value of around 15 mNm^{-1}.

The molecular structures of the oil, surfactant, cosurfactant and the concentrations of each influence the type of microemulsion. Since it is generally easier to expand the oil side of an interface (by penetration of the oil or cosurfactant into the hydrocarbon chain area) than the water side, it is easier to form w/o rather o/w microemulsions. A short-to-medium chain length cosurfactant ensures that the film is flexible enough to readily deform around the droplets.

For the dynamic role of the cosurfactant in microemulsion formation was considered by Gerbacia who observed that the interfacial tension can be temporarily reduced due to the diffusion of cosurfactant through the interface to form mixed duplex film. The dispersion process involves a transient reduction of interfacial tension to near zero or negative values at which the interface expands to form fine dispersed droplets enough to bring the interfacial tension positive again, when the second process, that of stabilization, is initiated by the interfacial film of alcohol and surfactant. The stability of the o/w systems is considered to be controlled by the interfacial charge. If the diffuse double layer at the interface is compressed by high concentrations of counterions, water-in-oil microemulsions are formed (Paul and Moulik, 1997).

II. Solubilization theories

In the solubilization concept introduced by Gillberg and Shinoda, microemulsions are considered as swollen micellar systems, i.e. with oil and water solubilized in normal or reverse micelles. This stemmed from the studies of three and four component phase diagrams on the one hand and the solubilization studies of water and hydrocarbons by non-ionic surfactants on the other (Paul and Moulik, 1997).

III. Thermodynamic theories

The free energy of microemulsion formation can be considered to depend on the extent to which surfactant lowers, the surface tension of oil-water interface and the change in entropy of the system such that

$$\triangle G_f = \gamma \triangle A - T \triangle s \quad (1)$$

where $\triangle G_f$ is the free energy formation, γ is the surface tension of oil-water interface, $\triangle A$ is the change interfacial area on microemulsification, $\triangle s$ is the change in entropy of the system which is effectively the dispersion entropy, and T is the temperature. It should be noted that when a microemulsion is formed the change in $\triangle A$ is very large, due to the large number of very small droplets formed. Originally workers proposed that in order for a microemulsion to be formed a (transient) negative value of γ was required, it is now recognized that while value of γ is at all times, it is very small (of the order of fraction of mN/m), and is offset by the entropic component. The dominant favourable entropic contribution is the very large dispersion entropy arising from the mixing of one phase in the other in the form of large numbers of small droplets. However, there are also expected to be favourable entropic contributions arising from other dynamic processes such as surfactant diffusion in the interfacial layer and monomer-micelle surfactant exchange. Thus a negative free energy of formation is achieved when large reductions in surface tension are accompanied by significant favourable entropic change. In such case, microemulsion is spontaneous and the resulting dispersion is thermodynamically stable (Lawrence and Rees, 2000).

Phase Diagram

When oil, water and surfactants are mixed, microemulsions are only one of a number of association structure (including ordinary emulsions, micellar, and mesomorphic phases of various constructions such as lamellar, cubic and various gels and oily dispersions) that can form, depending on the chemical nature and

concentration of each of components, as well as the prevailing temperature and pressure. A useful approach to illustrate the complex series of interactions that occur when different ratios of components are mixed is by the construction of a phase diagram.

A regular tetrahedron composed of four equilateral triangles can be used to plot the composition of four-component systems, with the pure components represented by each corner of four-component systems, with the pure components represented by each corner of the tetrahedron and the edges represented by each corner of the tetrahedron and the edges representing binary mixtures. Quaternary diagrams, however, are time-consuming to prepare and often difficult to interpret. In practice it is therefore more usual to investigate planar sections of the tetrahedron by plotting a two-dimensional triangular diagram (pseudoternary phase diagram) by either keeping the composition of one component fixed and varying the other three, or by using a constant ratio of two components, generally the surfactant and cosurfactant or cosolvent. Figure 2 represents schematically the pseudoternary phase diagram at constant surfactant-to-cosurfactant ratio. Microemulsions can also exist in equilibrium with excess water, excess oil, or both.

Transition between the different types and between the single-phase microemulsions can be readily by a change in temperature (non-ionic surfactant), a change in salinity (i.e., ionic strength), or by modifying the surfactant-to-cosurfactant ratio (Lawrence and Rees, 2000; Swarbrick and Boyland, 1994).

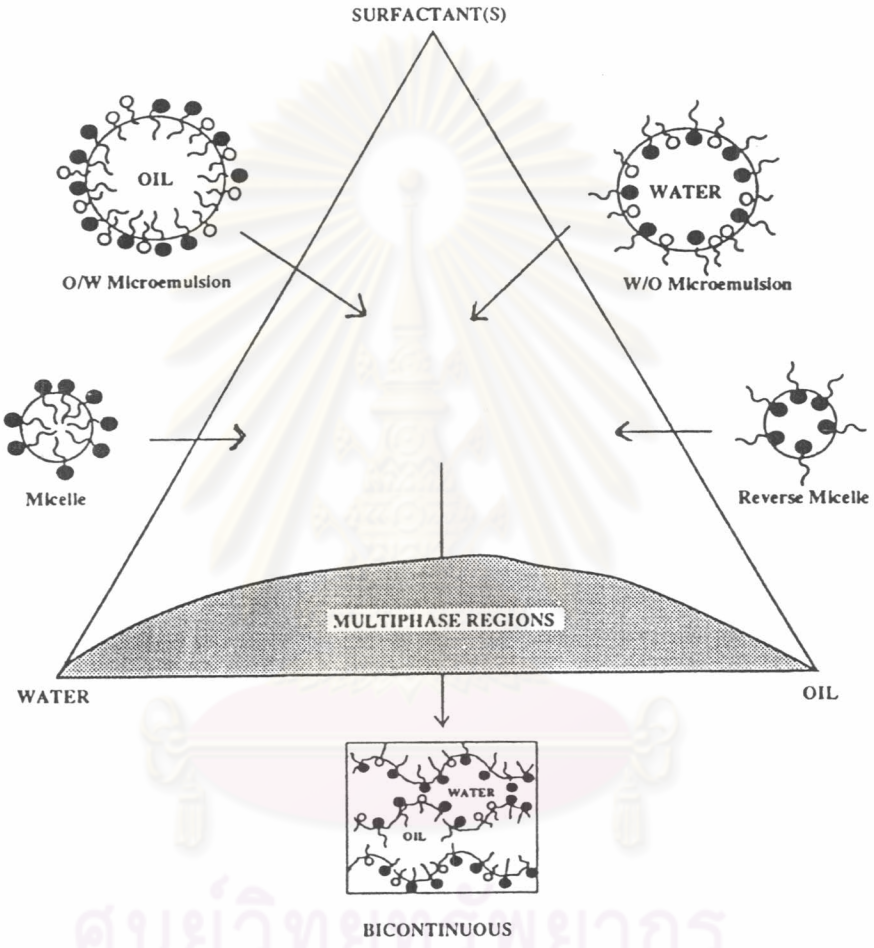


Figure 2 Triangular phase diagram showing micellar, microemulsion, and liquid crystalline regions (from Swarbrick and Boyland, 1994).

Choice of components

Although there are no strict rules for choosing the appropriate microemulsion components, there are a number of general guidelines base on empirical observes. A crucial step lies in the choice of surfactant and cosurfactant for the particular oil. The surfactant chosen must

- lower interfacial tension to a very small value to aid dispersion processes during the preparation of the microemulsion

- provide a flexible film that can readily deform round small droplets

- have the appropriate hydrophic-lipophilic character to provide the correct curvature at the interfacial region for the desired microemulsion type, o/w or w/o type.

These conditions have been achieved in several ways, for example, by using a combination of an anionic or cationic surfactant of high HLB with a cosurfactant of lower HLB, a single chained non-ionic surfactant of the polyethylene glycol alkyl ether type at appropriate temperature (Swarbrick and Boyland, 1994).

Many of the surfactants and oils that are regarded as acceptable are food grade materials or have a history of use in the pharmaceutical arena for example as parenteral emulsion dosage forms (Duro et. al., 1999). Non-ionic surfactants can be useful alternatives to naturally occurring surfactants, and polyoxyethylene sorbitan *n*-acyl esters (Tweens), for example, have been reported to have minimal toxicity. Although there are some restrictions, the use of polyoxy ethylene sorbitan monooteate (Tween 80) and polyoxyethylene sorbitan monolaurate (Tween 20) appear acceptable for oral or parenteral use (Kibbe, 2000). Furthermore, the insensitivity of non-ionic microemulsions to pH and electrolyte concentration relatively to their ionic counterparts represented an added benefit. There are consequently a large number of formulation studies involving non-ionic surfactants as microemulsion excipients

(Attwood, Mallon et al., 1992; Kale and Allen, 1989; Malcomson and Lawrence, 1993; Malcomson et. al., 1998).

Numerous triglyceride oils have been investigated in the search for stable, non-toxic oil for use in parenteral dosage form. A wide variety of natural oils, including cottonseed, soybean, safflower, sesame, cod liver, linseed, coconut, corn, peanut, olive, coca butter, and butter oil have been studied. Medium chain triglycerides (MCTs) are being used more frequently in combination with long chain triglycerides (LCTs). These fatty acids contain between 6 and 12 carbon atoms. MCTs are reported to be 100 times more soluble in water than are LCTs. Thus, they may have an increased ability to solubilize liposoluble drugs (Floyd and Jain, 1996).

Soybean and safflower are the only oils that have continued long-term commercial acceptability in parenteral dosage form and can be found in several products. The composition and characteristics of some commercial parenteral dosage form are summarized in Table 1 (Floyd and Jain, 1996).

In many cases, a requirement for cosurfactant causes difficulty in the formulation of acceptable microemulsions because the majority of studies have chosen to employ medium chain length alcohols as the cosurfactant of choice. Unfortunately, there are significant toxicity and irritancy issued with these materials, which preclude their use in pharmaceutical formulations. Alternatives to medium chain alcohols have been evaluated such as short chain amines (Wormuth and Kaler, 1987) and alkanoic acids (Aboofazeli et al., 1994) however these cosurfactants behave in much the same way as the alcohol and toxicity remains and issue. Polyhydric alcohols such as sorbitol and sucrose have also been used as additives to facilitate microemulsification (Attwood, Mallon et al., 1992).

Table 1 Composition of Intravenous Fat Emulsions (in grams).

	Intralipid[a]	Liposyn[b]	Liposyn II[b]	Lipomul[c] Infonutrol[d]	Lipofundin[e]	Lipofundin S[e]	Trivé 1000[f]	Nutrafundin[e]
Soybean oil	100,200	-	50,100	-	-	100,200	38	38
Safflower oil	-	100,200	50,100	-	-	-	-	-
Cottonseed oil	-	-	-	150	100	-	-	-
Egg phospholipids	12	12	12	-	-	-	-	-
Soybean phosphatides	-	-	-	12	7.5	7.5, 15	7.0	3.8
Glyercol	22.5	25	25	-	-	-	-	-
Glucose	-	-	-	40	-	-	-	-
Xylitol	-	-	-	-	-	50	-	100
Sorbitol	-	-	-	-	50	-	100	-
Pluronic F-68	-	-	-	3	-	-	-	-
DL-a-tocopherol	-	-	-	-	0.585	-	0.4	-
Maleic acid	-	-	-	-	-	-	10	-
Amino acid mixture	-	-	-	-	-	-	60	60
Water for injection to	1000	1000	1000	1000	1000	1000	1000	1000

[a]Clintec, U.S. Note: Similar formulas are marketed by Vitrum, Sweden; Travenol, U.S.; alpha Therapeutic, U.S.; Green Cross, Japan; and Daigo, Japan.

[b]Abbott, U.S.

[c]Upjohn, U.S. (withdrawn from market).

[d]Astra-Hewlett, Sweden (withdrawn from market).

[e]Braun, West Germany.

[f]Egic, France.

(table from Floyd and Jain, 1996)

The role of surfactant

The surfactants used to stabilize the single-phase microemulsion systems may be: (I) non-ionic, (II) zwitterionic, (III) cationic, or (IV) anionic surfactants. Combinations of these, particularly ionic and non-ionic, can be very effective at increasing the extent of the microemulsion region. Examples of non-ionics include polyoxyethylene surfactants such as Brij 35 ($C_{12}E_{23}$) or a sugar esters such as sorbitan monooleate (Span 80). Phospholipids are a notable example of zwitterionic surfactant and exhibit excellent biocompatibility. Quaternary ammonium alkyl salts form one of the best known classes of cationic surfactants, with hexadecyltrimethyl ammonium bromide (CTAB) and the twin-tailed surfactant didodeccyl ammonium bromide (DDAB) amongst the most well known. The most widely studied anionic surfactant is probably sodium bis-2-ethylhexylsulphosuccinate which is twin-tailed and is a particularly effective stabilizer of w/o microemulsions (Lawrence and Rees, 2000).

Attempts have been made to rationalize surfactant behavior in terms of the hydrophile-lipophile balance (HLB), as well as the critical packing parameter (CPP). Both are fairly empirical but can be a useful guide to surfactant selection. The HLB takes into account the relative contribution of hydrophilic and hydrophobic fragments of the surfactant molecule. It is generally accepted that low HLB (3-6) surfactants are favoured for the formation of w/o microemulsions whereas surfactants with high HLB (8-18) are preferred for the formation of o/w microemulsion systems.

In contrast, the CPP relates the ability of surfactants to form particular aggregates to the geometry of molecule itself. The CPP can be calculated using the following equation:

$$CPP = v / a.l \qquad (2)$$

where v is the partial molar volume of the hydrophobic portion of the surfactant, a is the optimal head group area and l is the length of the surfactant tail. The latter parameter is often expressed as l_c, that is the critical length of the hydrophobic chain, generally assumed to be 70-80% of its fully extended length. The effect of changing

CPP is illustrated in Figure 3 but put simply, cone-shaped surfactants will pack at curved interfaces whereas surfactants whose geometry can be represented by truncated cones or rectangular blocks prefer to form worm-like micelles or lamellar structure (Attwood, 1994; Lawrence and Rees, 2000).

Of course, changes in microemulsion composition will modify the microenvironment of the surfactant, which will lead to changes in the apparent CPP of the surfactant. For example, increases in ionic strength would be expected to result in a decrease in the effective head group area of ionic surfactants as the double layer shrinks and screening of the head groups allow closer approach. The presence of hydrophilic molecules such as glycerol and sorbitol in the aqueous phase will also influence optimal head group area by altering the solubility of the head group in the aqueous phase. Because of these effects, water-soluble hydrophilic materials have been used as to aid microemulsion formation (Attwood, Malion et al., 1992).

In most cases, single-chain surfactants alone are unable to reduce the oil/water interfacial tension sufficiently to enable a microemulsion to form. Medium chain of alcohols which are commonly added as cosurfactants have the effect of further reducing the interfacial tension, whilst increasing the fluidity of the interface thereby increasing the entropy of the system. Medium chain length alcohols also increase the mobility of the hydrocarbon tail and also allow greater penetration of the oil into this region. Furthermore, any alcohol present may also influence the solubility properties of the aqueous and oil phases due to its partitioning between these phases. But medium chain length alcohols limits the potential use of the microemulsion due to their toxic and irritant properties and the evaporation of alcohol can destabilize the system (Attwood, 1994; Swarbrick and Boyland, 1994).

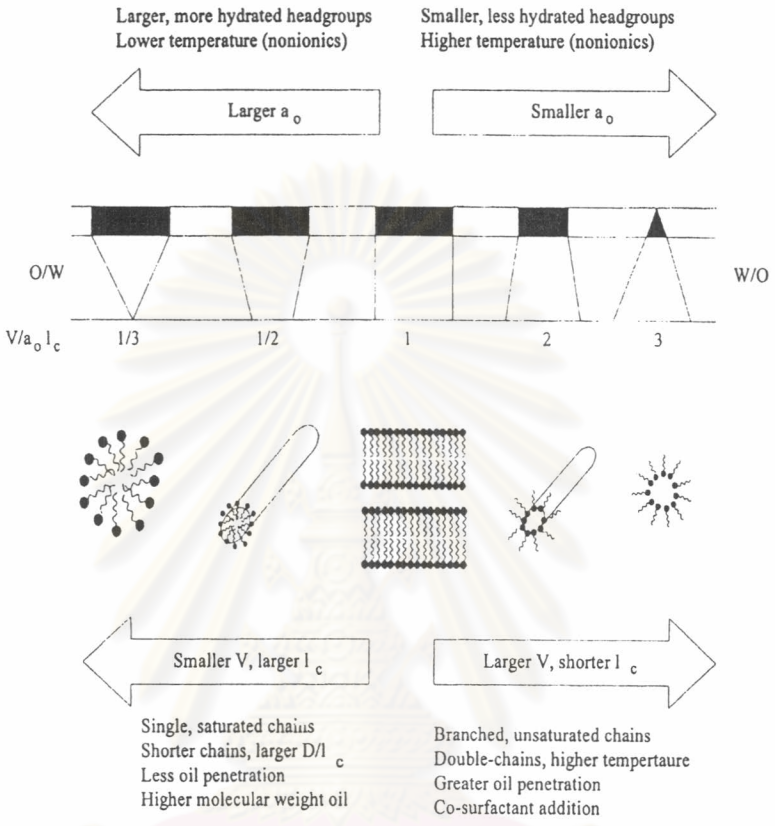


Figure 3 Effect of molecular moieties and solution conditions on the CPP of a surfactant and the resulting range of possible surfactant aggregates in water or aqueous solution (from Lawrence and Rees, 2000).

Preparation of microemulsions

Since microemulsions are thermodynamically stable, they can be prepared simply by blending oil, water, surfactant and cosurfactant with mild agitation. No significant energy contribution is required.

The usual method of preparing microemulsion is to dissolve the surfactant in the oil and then add the water to solution of oil and surfactant with gentle shaking. The microemulsion rapidly becomes first translucent and then optically clear after a few seconds. Microemulsion can be sterilized by filtration, as the mean diameter of the droplets is below 0.22 μm (Swarbrick and Boyland, 1994).

The order of mixing the components is generally considered not to be critical since microemulsions form spontaneously. However, Rosano and co-workers, (1988) demonstrated that, although microemulsion is a spontaneous process, the driving forces are small and the time taken for these systems to reach an equilibrium interfacial tension can be long. Large transitory fluctuations in interfacial tension can occur during the microemulsion mixing process, as the components arrange themselves in such a way that the resulting interfacial and bulk microstructures lead to an overall minimum in the free energy. The time to establish equilibrium is influenced by the order of mixing. This is established more slowly if the cosurfactant is injected into the oil phase, as its greater solubility in this phase hinders its diffusion into the aqueous phase.

Evaluation of microemulsion for parenteral administration

1. Syringeability

Syringeability describes the ability of emulsion to pass easily through a hypodermic needle or transfer from the vial prior to injection. It includes characteristics such as the ease of withdrawal, clogging and foaming tendencies, and accuracy of dose measurements. Increasing the viscosity, density and particle size

hinders the syringeability of emulsion. A suitable test is to ensure that the entire emulsion passes through a 25-gauge needle of internal diameter 0.3 mm.

2. Injectability

Injectability refers to the performance of emulsion during injection and includes factors such as pressure or force required to injection, evenness of flow, aspiration qualities, and freedom from clogging. The syringeability and injectability are closely related to the viscosity and particle characteristics of the emulsion.

3. Clogging

Clogging or blockages of syringe needles while administering an emulsion may occur because of a single large particle or an aggregation that block the lumen of the needle or because of a bridging effect of the particles. It is advisable to avoid particles greater than one-third of the internal diameter of the needle to prevent clogging. Clogging, if observed at or near the needle end, is usually caused by restrictions to flow from the emulsion and may involve combination of factors such as vehicle, particle size, shape and distribution, viscosity, and flow characteristics.

4. Drainage

Drainage refers to the ability of the emulsions to break cleanly away from the inner walls of the primary container-closure system and is another characteristic of a well-formulated parenteral emulsion. Silicone coating of containers, vials, and plugs with dimethicone can improve the drainage of slightly overflocculated systems as well as of good emulsions.

5. pH

The pH of the emulsion is usually adjusted to approximately 8.0 prior to sterilization. This is preferred because the pH of the emulsion falls on autoclaving, and also as a function of time during storage, as the result of glyceride and

phosphatide hydrolysis liberating free fatty acids (FFA). The rate of FAA production is minimal if the pH of the emulsion is between 6 and 7 after sterilization (Floyd and Jain, 1996). This parameter affects to physical and chemical stability. The pH meter is generally instrument to be used for determining the pH of preparation.

6. Particle size

Particle size of lipid globules has a direct effect on both toxicity and stability. Particle greater than 4 to 6 μm are known to increase the incidence of emboli and blood changes. For intravenous injections, particle should be less than 1 μm in diameter. For subcutaneous or intramuscular injections, the particle should preferably be less than 250 μm in diameter. Larger particle sizes can be used for oral formulations. The microemulsion has particle sizes in the range 10-140 nm. Hence, the microemulsion has the advantage of very small disperse phase diameter, which may impart thermodynamic stability.

7. Osmotic pressure

Osmotic pressure is a colligative property and therefore can be related to the relative molecular mass of the colloidal material. This property is important because it affects directly to the cells, especially red blood cells. The osmotic property is determined by the gradient of some colligative properties, such as freezing point, boiling point, or pressure vapour. Osmomat® O30-D is instrument to determine the osmotic pressure using freezing point depression method.

8. Viscosity

Viscosity describes the resistance to flow with applied stress for a particular system; a more viscous system requires greater force or stress to make it flow at the same rate as a less viscous system. This parameter is directly used to describe the syringeability and injectability of emulsion for parenteral administration. Many techniques could be used to measure this parameter. Capillary viscometers and the falling ball viscometers are simple instruments for measuring viscosity but only for Newtonian liquids. Rotational viscometers including to coaxial cylinder sensor

systems (cup-and-bob viscometers) and cone-and-plate sensor systems are instruments may be used with both Newtonian and non-Newtonian liquids (Martin, 1993).

9. In vitro drug release

During the last decade there has been a considerable increase in interest in the use of disperse systems as drug carriers. Dissolution characteristic of drugs from these dosage forms is important factor for absorption and bioavailability. Several studies have shown that the dissolution rate is a rate-limiting step for absorption and bioavailability of drugs administered in formulations (Banakar, 1992). Several types of apparatus have been proposed and implemented in assessing the dissolution rate of microdisperse systems. One of the common problems associated with most methods is retention of the dissolving material within the confines of the dissolution chamber. Thus, no method is suggested to be the best for determining the dissolution characteristics of microdisperse systems.

Membrane diffusion technique is the most popular method for studying the dissolution characteristic of microdisperse systems (Levy and Benita, 1990; Lostritto et al., 1987; Saarinen-Savolainen et al., 1997; Washington, 1990). In this approach the carrier disperse phase, suspended in a small volume of continuous phase, is separated from a large bulk of sink phase by a dialysis membrane which is permeable to the drug. The sample and sink are well stirred. The drug diffuses out of the carrier and through the membrane to the sink, wherein it is periodically assayed. Thus, the accumulation of the drug in the sink is controlled by the consecutive rate processed of (non-sink) partition and diffusion of the drug across the membrane. By this method, the *in vitro* membrane permeation systems for transdermal can be applied to separate the sample compartment and receptor compartment by dialysis membrane (Banakar, 1992; Huang, 1987).

The measurement of release profiles requires good sink condition, implying that release must occur into a large volume of sink medium. It has been recommended as a rule of thumb that the drug concentration in the sink phase in dissolution experiments must be kept below 10% of saturation (Washington, 1990).

This poses a problem since the drug must be assayed in the sink medium, and as the sink volume is increased the concentration of drug being measured the concentration of drug being measured decreases. Especially for insoluble drugs, a method of assay is required which is very sensitive to less quantity of drug in solution.

Microemulsion characterization

Microemulsions have been evaluated using a wide range of different techniques over the years, but a complementarity of methods is generally required in order to fully characterize these systems. At the macroscopic level viscosity, conductivity and dielectric methods provide useful information. Viscosity measurements for example can indicate the presence of rod-like or worm-like reverse micelles, and conductivity measurements provide a means of determining whether a microemulsion is oil-continuous or water-continuous as well as providing a means of monitoring percolation or phase inversion phenomena. Dielectric measurements are a powerful means of probing both the structural and dynamic features of microemulsion systems.

The isotropic nature of microemulsions and their optical clarity make their study by spectroscopic techniques straightforward, particularly in comparison to conventional macroemulsions. Pulsed field gradient NMR for example has been used extensively to measure self-diffusion coefficients of the various components and yield information on the mobility and microenvironment. Scattering methods have also been invaluable in elucidating microemulsion structure and methods employed include dynamic and static light scattering, small-angle X-ray scattering (SAXS). Freeze-fracture electron microscopy has also been used to study microemulsion structure, however extremely rapid cooling of the sample is required in order to maintain structure and minimize the possibility of artifacts (Lawrence and Rees, 2000; Paul and Moulik, 1997; Swarbrick and Boyland, 1994).

Furthermore, dye solubility tests and dilution tests were used to determine the type of microemulsion. The former method involved the addition of a water- or oil-

soluble dye to the microemulsion. Thus, intense staining of the external phase after addition of a water-soluble dye indicated an o/w microemulsion. The addition of an oil-soluble dye to the same microemulsion would result in the staining of the droplets of the internal phase. The dilution test involved observation of the microemulsion following its dilution with oil or water to see whether separation had been effected. If water was easily dispersed in the continuous phase, the microemulsion was termed o/w microemulsion. If oil was dispersible in the external phase, the microemulsion was termed w/o microemulsion (Ho et al., 1996). Transmission Electron Microscopy (TEM) has also been used to study shape and size of microemulsion (Amsetem and Friedman, 1998).

Advantage of the use of microemulsions as carriers of drugs

Microemulsions have generated considerable interest over the years as potential drug delivery systems (Gasco, 1997; Lawrence and Rees, 2000; Swarbrick and Boyland, 1994). Their thermodynamic stability allows self-emulsification of the system, whose properties are not dependent on the process followed; the temperature range over which the phase does not separate can be rather wide.

Microemulsions act as supersolvents of drugs (including drugs that are relatively insoluble in both aqueous and hydrophobic solvents), probably a consequence of the presence of the surfactant and the cosurfactant.

The dispersed phase, lipophilic or hydrophilic (oil in water or water in oil microemulsion, respectively), can behave as a potential reservoir of lipophilic or hydrophilic drug, respectively. The drug will be partitioning between dispersed and continuous phase, and when the system comes into contact with a semipermeable membrane, with skin or mucous membrane, the drug can be transported through the barrier. Drug release with pseudo-zero-order kinetic can be obtained (Gasco, Carlotti et al., 1988), depending on the volume of the dispersed phase, the partition of the drug among interphase and continuous and dispersed phase, and the transport rate of the drug.

Microemulsions can be sterilized by filtration, as mean diameter of droplets is below 0.22 μm and the microemulsions formed can be used as sustained release formulations. The optical transparency and low viscosity of microemulsions ensure that they are good appearance and easy to handle and pack. Moreover, the technology required to prepare microemulsions is simple, because their thermodynamic stability means that no significant energy contribution is required.

The use of microemulsions as delivery systems can improve the efficacy of a drug, allowing the total dose to be reduced and thus minimizing side effects.

The attraction of o/w microemulsion systems lies in their ability to incorporate hydrophobic drugs into the apolar oil phease thereby enhancing their solubility. For example, o/w microemulsions have been used to solubilize steroidal drugs such as prednisolone, hydrocortisene, betamethasone, testosterone and its esters and progesterone (Malcomson, Satra, Kantaria et al., 1998; Malcomson and Lawrence, 1993). Interestingly, it has been noted that hydrophobic drugs need to have a significant solubility in the dispersed oil phase for the o/w microemulsion system to offer a marked benefit over the micellar system alone (Malcomson and Lawrence, 1993). However the oil in which the drug is most soluble do not necessarily from microemulsions with the highest drug solubilization capacity (Malcomson et al., 1998).

Solubilization of antifungal drugs (clotrimazole, ciclopirox olamine and econazole nitrate) in ternary water/non-ionic surfactant/oil systems was studied. The solubilization of these antifungal drugs ranged from practically insoluble to slightly soluble in water as well as in most of the oils used in pharmaceutical formulations. The maximum drug solubilization values were obtained with water/polysorbate 80/oil systems. The results show that it is possible solubilize 1% w/w of antifungal agent in suitable topical microemulsions with a water content higher than 50% w/w (Garcia-Celmar et al., 1994).

For labile drugs such as peptides, generally have little or no activity when delivered orally and are highly susceptible to proteolysis in the gastrointestinal tract. Parenteral drug administration especially for chronic condition is not well accepted by

patients and can lead to issues with compliance. Consequently, the oral delivery of labile drugs is the focus of growing attention, particularly as many of the new therapeutic agents in development are hydrophilic drugs such as peptides or oligonucleotides. Hydrophilic drugs of this kind can be successfully incorporated into the dispersed aqueous phase of w/o microemulsion droplets where they are afforded some protection from enzymatic degradation when administered orally (Sarciaux et al., 1995). In addition, the presence of surfactant and in some cases cosurfactant, for example medium chain diglycerides in many cases serves to increase membrane permeability thereby increasing drug uptake (Constantinides, 1995; Constantinides, Scalart, Lancester et al., 1994).

Applications of microemulsions in the pharmaceutical field

Many studies have been performed *in vitro* and in vivo and various components have been examines. Biocompatibility, which is obviously the first requirement, limits the choice of the components for application in the pharmaceutical field to a limited number of oils, surfactants, and cosurfactant.

1. Oral Delivery

The formulation of w/o microemulsions for use as self-microemulsifying drug delivery systems (SMEDDS) has been investigated using blends of low and high HLB surfactants, which were commercially available and pharmaceutical acceptable, typically sorbitan esters and Tween 80. The oil phase comprised long or medium chain length glyceride (Constantinides and Scalart, 1997). In a related study, an optimized o/w microemulsion formulation for the delivery of cyclosporin A prepared using Cremophor EL® as surfactant, Transcutol® as a cosurfactant and Captex 355® as the oil phase has been reported.

Bioavailability enhancements of 3.5 and 1.25 were observed relative to the Sandimmune® and Sandimmune Neoral® formulation (Gao et al., 1998). The formulation and performance of SMEDDS containing the anti-malarial halofantrine

has also been reported. Six-to eight-fold improvements in bioavailability were observed relative to tablet formulations (Khoo et al., 1998).

2. Transdermal Delivery

The transdermal delivery of the hydrophilic drug diphenhydramine hydrochloride from a w/o microemulsion into excised human has been investigated. The formulation was based on combination of tween 80 and span 80 with isopropyl myristate (IPM). However two additional formulations were tested containing cholesterol and oleic acid, respectively. Cholesterol increased drug penetration whereas oleic acid had no measurable effect, but the authors clearly demonstrated that penetration characteristics could be modulated by compositional selection (Schmalfuß et al., 1997).

An interesting application of gelatin microemulsion-based organogels (MBGs) which exploits the presence of surfactant-stabilized conducting aqueous channels has been their use in the iontophoretic transdermal delivery of a model hydrophilic drug. The MBGs were prepared using a variety of pharmaceutically acceptable surfactants and oils including tween 80 and IPM (Kantaria et al., 1999). Novel sorbitan monostearate organogels have also been prepared from vegetable oils and IPM. Prepared at elevated temperatures and then cooled, the surfactant self-assembles into inverse vesicles and then rod-shaped tubules. The organogels are opaque and thermoreversible, and may have been suggested as novel delivery vehicles for drugs and antigens (Murdan et al., 1999).

3. Ocular Delivery

The development and characterization of o/w microemulsions designed for ocular use has recently been reported (Hasse and Keipert, 1997). The microemulsions containing pilocarpine were formulated using lecithin, propylene glycol and PEG 200 as cosurfactants, and IPM as the oil phase. The formulations were low viscosity fluids with a refractive index lending themselves to ophthamological application. The test microemulsions were non-irritant in rabbit eyes or hen egg membrane. A prolonged pharmacological effect was observed in vivo compared to the drug

administered as a simple aqueous solution. This may have been related to increased bioavailability or enhanced retention or both. However, prolonged release was not observed *in vitro* using a cellulose membrane as permeability barrier.

4. Parenteral Delivery

O/W microemulsions are used mainly as carriers of lipophilic drugs in order to administer parenterally lipophilic substance that is not soluble in water. They can be administered intravenously, intramuscularly, or subcutaneously.

The preparation and evaluation of flurbiprofen-loaded o/w microemulsion in one of few recent reports of delivery systems designed for parenteral use. The systems of interest were prepared using ethyl oleate as the oil phase and tween 20 as surfactant, but there was no significant difference in the pharmacokinetics after administration in rats (Park and Kim, 1999).

The microemulsions comprised Miglyol 810N (MCT), soybean phosphatidylcholtine (Epicuron 200), PEG 400, poly (ethylene glycol) (660)-12-hydroxystearate and ethanol. PFG-NMR indicated that microemulsions formed over a range of compositions were bicontinuous, even at high oil concentration. After administration, the bicontinuous microemulsions form o/w microemulsions on dilution. *In vitro* studies showed the resulting droplets were small, with mean radii typically in the range 60-200 nm. Solubilization studies using felodipine (a calcium antagonist) and an antioxidant H290/58 in microemulsion system is ten times better that of both the PEG 400 vehicle and the soybean emulsion. The intravenous administration of the microemulsion formulations was performed by infusion into conscious rats over a 5-min period. Doses up to 0.5 ml/kg had no significant effect on acid-base balance, blood gases, plasma electrolytes, arterial blood pressure or heart rate (Corswant et al., 1998).

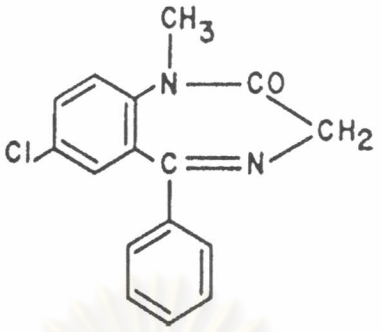
Lee et al. (1995) have been studied lipid microemulsions (LM) consisting of soybean oil and lecithin as a parenteral drug delivery system for site-specific delivery of non-water-soluble drugs. A major obstacle to targeting to non-reticuloendothelial system (RES) organs or maintaining high concentrations of LM in vasculature is their

rapid and extensive uptake by the RES in the liver and spleen. By replacing lecithin with hydrophilic poloxamer 338, it has been possible to avoid the normal deposition of LM in the liver and spleen (inverse targeting). Poloxamer 338-modified LM (PLM) containing ibuprofen octyl ester was intravenously administered to rats. Ibuprofen concentrations in the plasma and various organs were measured to elucidate the effect of inverse targeting to RES and targeting to other tissues in terms of the incorporated drug rather than the drug carrier. It was suggested that PLM could be exploited to direct lipophilic drugs in LM away from RES in the liver and spleen to other targeting tissues such as inflammatory tissues.

Physicochemical properties of diazepam.

Diazepam is a benzodiazepine with anticonvulsant, anxiolytic, sedative, muscle relaxant, and amnesic properties. It is used in the management of severe disability anxiety disorders and insomnia, in convulsions, particularly status epilepticus and febrile convulsions and in alcohol withdrawal. It is also used as a premedicant and sedative for surgical and other procedures, and for the relive of muscle spasm (Reynolds, 1996). Available dosage forms are tablet, syrup, emulsion, suppository and parenteral dosage form. The usual oral dosage for adults ranges between 2 to 10 mg, two to four times a day. For intramuscular or intravenous injection dosage for adults ranges between 2 to 15 mg that dosage repeated in three to four hours, if necessary. But no more than 30 mg should be given in an 8 hr period. Effective plasma levels vary from 0.2 to 0.5 μg/ml (Hanson, 1995).

The chemical name of diazepam is 7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benodiazepin-2-one. The chemical structure, molecular formula and molecular weight are show in Figure 4. The appearance of diazepam is a white or yellow crystalline powder, odorless or almost odorless. It has melting point in the range 131° to 135°C and pKa at 20°C is 3.3. Log of partition coefficient of diazepam between octanol and buffer pH 7.4 is 2.7 (Lund, 1994).

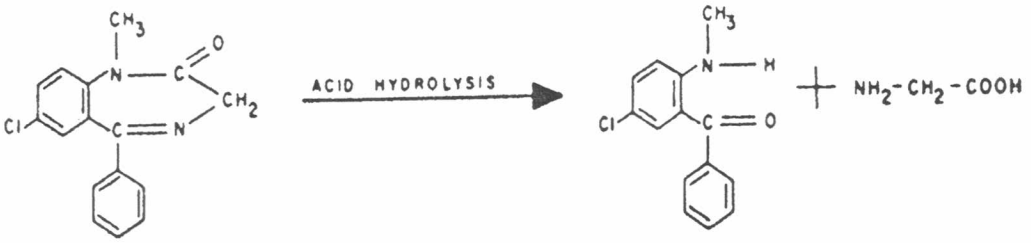


$C_{16}H_{13}ClN_2O$ Mol. Wt. = 284.75

Figure 4 The chemical structure, molecular formula and molecular weight of diazepam (from MacDonald et al., 1972).

Diazepam is very slightly soluble in water (0.05 mg/ml) and it is soluble 1 in 25 of ethanol, 1 in 8 of acetone, 1 in 39 of ether and 1 in 60 of propylene glycol and freely soluble in chloroform (1 in 2). The degradation of diazepam in aqueous solution by hydrolysis of 4,5-azomethine bond (ring opening) resulting in the formation of an intermediate which undergoes further hydrolysis to produce 2-methylamino-5-chlorobenzophenone and glycine derivative as shown in Figure 5. The reaction is reversible and pH dependent. Diazepam has maximum stability around pH 5. Its also appeared to be susceptible to photochemical decomposition (Hanson, 1995; Lund, 1994; MacDonald, 1972).

For parenteral dosage form, diazepam injection BP is a sterile solution of diazepam in water for injections or other suitable solvent. Injections containing 10 mg in 2 ml and 20 mg in 4 ml are usually available and pH in the range 6.2 to 7.0. While diazepam injection USP has pH in the range 6.2 to 6.9 (Lund, 1994).



Diazepam

2-methylamino-5-cholro- + Glycine benzophenone (MACB)

Figure 5 Hydrolysis of diazepam (from MacDonald et al., 1972).