CHAPTER II

LITERATURE REVIEW

1. Definition and terminology of microemulsions

Microemulsions was introduced by Hoar and Schulman in 1943. They have reported the spontaneous formula of a transparent or translucent solution upon mixing of oil, water and an ionic surfactant combined with a cosurfactant (i.e., a medium chain length alcohol). At that time, Hoar and Schulman referred to this new type of colloidal dispersion, consisting of two immiscible phases, as an oleophatic hydromicelle. The other workers such as Bowcott and Schulmm introduced transparent emulsion at later stages of their studies. Subsequently the microemulsion is defined as a system of water, oil, amphiphile normally with a cosurfactant, which is a single optically isotropic and thermodynamically stable liquid solution (Danielson and Lindman, 1981).

The composition of microemulsions and macroemulsions are the same; however, their characteristics and appearance are different (Table 1).

Table 1. Characteristics of microemulsion and macromulsion (From Friberg and Kayali, 1991)

Feature	**Macroemulsion**	**Microemulsion**
Appearance	Turbid, milky	Transparent
Droplet (μm)	0.15 — 100	0.0015 — 0.15
Formation	Mechanical or Chemical Energy added	Spontaneous
Thermodynamic stability	No	Yes
Autoxidation of liquid	High	Low
Viscosity	High	Low

2. Theories of microemulsion formation

Microemulsion formation and the thermodynamically stability of microemulsion system have been described for three main theories (Tenjarla, 1999). The first theory is the mixed film theory. The second theory is solubilization theory, and the last is thermodynamic theory. However, there is no one approach alone

covering all aspects of microemulsion structure and stability (Lawrence and Rees, 2000; Paul and Moulik, 1997;Swarbrick and Boylan, 1994)

2.1. Mixed-film theory

This theory is emphasized in interfacial film and ultra low interfacial tensions.

In this theory, the interfacial film of surfactant is considered as a complex film or a duplex film having different properties on the water side and the oil side, where interfacial tension (γ_T) is,

$$\gamma_T = (\gamma_{o/w})_a - \pi \qquad (1)$$

where $(\gamma_{o/w})_a$ is the o/w interfacial tension in presence of alcohol as a cosurfactant, and π is the spreading pressure of the mixed film. π is attached by the presence of amphiphiles and penetration of oil into the hydrocarbon part of the interfacial surfactant film. With the expansion of the interface, π increases and γ_T tends to zero favoring dispersion of one phase in the other.

As described above, the flat duplex film may have the difference in tension gradient (surface pressure) on both oil and water sides, respectively as π_o and π_w, respectively.

The reduction of this tension gradient by equalizing the two surface pressures and tensions is the driving force for the film curvature (Figure 1). Both sides of the interface expand spontaneously with penetration of oil and cosurfactant until the two pressures become equal, corresponding to π_o andπ_w as the pressure on oil and water sides, respectively, after curvature. The side with the higher tension would be concave and would envelop the liquid on that side, making it the internal phase. The pressure gradients, and hence the type of microemulsion, are influenced by the molecular structures of the oil, surfactant, and cosurfactant and the concentrations of each. Since it is generally easier to expand the oil side of an interface, $\pi_o > \pi_w$, (by penetrations of the oil or cosurfactant into the hydrocarbon chain area) than the waterside, it is easier to form w/o rather than o/w microemulsions (Paul and Moulik, 1997; Swarbrick and Boylan, 1994).

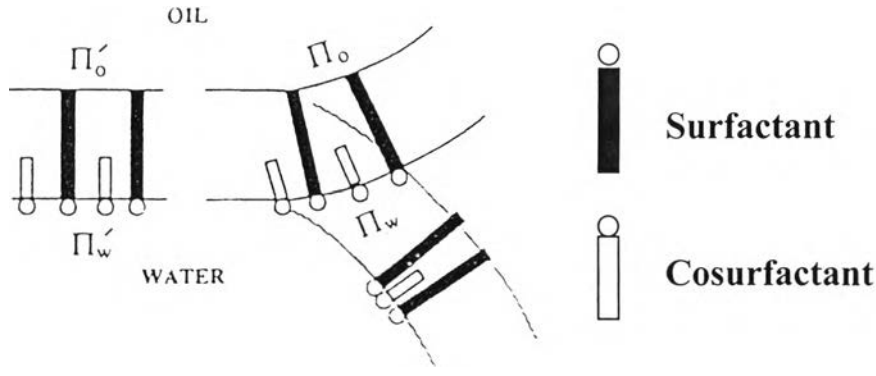


Figure 1. The mechanism of curvature of a duplex film of microemulsion (From Swarbrick and Boylan, 1994)

In additon to the duplex film theory, the flexible surface model regrarding the curvature of the surfactant film is considered (Garti et al., 2001). The flexible surface model describes the properties of surfactant film separating the water and oil parts. One parameter for this film is spontaneous mean curvature H_0, which is defined as, $H_0 = \frac{1}{2}(c_1 + c_2)$, where c_1 and c_2 are the principle curvatures of the surfactant film. H_0 expresses the natural tendency of monolayer to bend away from a flat geometry and it is, by definition, positive if it tends to enclose the oil and negative if it tends to include the water. H_0 depends on the nature of the surfactant and also on the composition of aqueous and oil phases it separates. For example, H_0 is positive for surfactants with a large head group and a small nonpolar group and decreases with an increase in the number and size of the alkyl chain of the nonpolar group. Decreasing the polarity of polar phase by adding cosurfactant and any penetration by nonpolar phase in the

hydrocarbon tail of the surfactant decrease H_0. For bicontinuous microemulsion, H_0 is close to zero.

Another parameter in flexible film model is elasticity of the surfactant film. Generally, an elastic or flexible surfactant film favors the formation of a microemulsion, whereas a lamellar phase is formed with a more rigid or stiff film. The elasticity of the film depends on the molecular structure of the surfactant and can be reduced by adding cosurfactant. Moreover, the elasticity is also affected by any penetration by the nonpolar phase in the hydrocarbon part of surfactant (Corswant, Thoren and Engström, 1998).

2.2 Solubilization theory

Solubilization concept was introduced by the group of Friberg and Venable (1983). The microemulsions are considered as a swollen micellar system such oil and water solubilization in normal (oil-swollen, o/w) or reverse (water-swollen, w/o) micelles. The relationship between reverse micelles and microemulsions was illustrated by Swarbrick and Boylan (1994) with the aid of phase diagrams. The inverse micellar region of the ternary system of water, pentanol, and sodium dodecyl sulfate (SDS) is shown as the base triangle in Figure 2. The region is composed of water solubilized in

reverse micelles of SDS in pentanol. The addition of up to 50% p-xylene gave rise to transparent w/o microemulsion regions containing a maximum of 28% water with 16% pentanol and 6% surfactant. The quaternary phase diagram constructed on addition of the hydrocarbon clearly shows the relationship of these areas to isotropic inverse micellar phase.

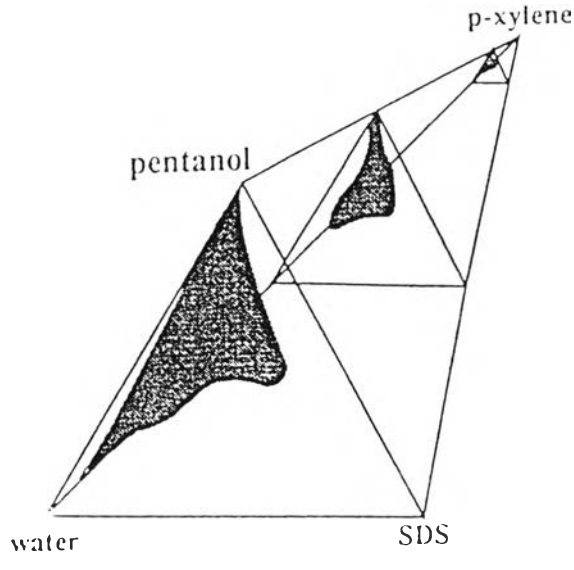


Figure 2. Phase diagram illustrating that the w/o microemulsion region containing p-xylene is a direct continuation of the inverse micellar solution of the three structure-forming elements in the base triangle, water, surfactant (SDS) and cosurfactant (pentanol) (From Swarbrick and Boylan, 1994)

2.3 Thermodynamic theory

The theory focuses on the factors that can effect on microemulsion formation. The thermodynamic equation is that,

$$\Delta G_f = \gamma \Delta A - T\Delta S \qquad (2)$$

where ΔG_f is the free energy of formation, γ is the surface tension of the oil-water interface, ΔA is the change in interfacial area of microemulsification, ΔS is the change in entropy of the system which is effectively the dispersion entropy, and T is the temperature (Swarbrick and Boylan, 1994).

The surfactant and cosurfactant are able to reduce the oil-water interfacial tension closely to zero, while the very large dispersion entropy arises from the mixing of one phase in the others. If microemulsion are formed, the change in interfacial area is very large by increasing the number of small droplets. It is noted that microemulsion can be formed when there is a large reduction in interfacial tension to a very small value, 10^{-2}-10^{-3} mN/m, attended by entropic change, thus, a free energy of formation is negative. For this reason, microemulsification is spontaneous and the dispersion is thermodynamically stable. Nevertheless, the thermodynamic of microemulsion is well understood as the interplay between a small interfacial free energy and small entropy of mixing. However, because of the being small in droplet

size, other effects, such as the influence of curvature on the interfacial film and the fluctuation, become important (Kegel, Overbeek and Lakkerkerker, 1999).

3. Structures and phase behavior of microemulsion

The microemulsions are generally formed in three systems, such as oil-in-water (o/w) microemulsions, which water is the continuous phase; water-in-oil (w/o) microemulsions, which oil is the continuous phase and bicontinuous microemulsions, which, normally, water volume is equal to oil volume. The formation depends on the nature and concentration of oil, surfactant and cosurfactant (Swarbrick and Boylan, 1994) (Figure 3)

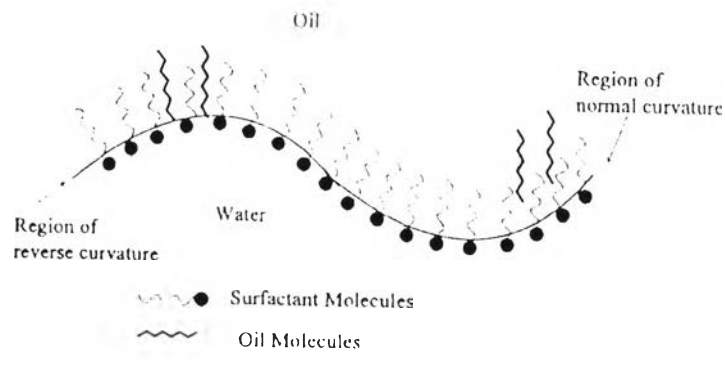


Figure 3. The interfacial surfactant monolayer of bicontinuous microemulsion (From Lawrence and Rees, 2000)

In general, the phase behavior is used to determine the structure boundaries of the mixture of oil, water and surfactant. The mixture may display isotropic solution, emulsion or liquid crystals. The phase diagram of microemulsions comprisely oil, water, surfactant and cosurfactant is investigated by using ternary phase diagram in which each apex of the diagram is 100% of that particular component. Most commonly, pseudo-ternary phase diagrams are used to describe the system consisting of three or more components and a corner of diagram perhaps represents a binary mixture of components. Apex of surfactant can consist of a mixture of surfactant and cosurfactant at a specific ratio (Figure 4).

When the individual phase diagrams are formed, a viscous of gel phase with isotropic and nonisotropic region may appear. The type of equilibriums of phase behavior can be modified by a change in temperature (nonionic surfactant), a change in salinity (ionic surfactant) or a change in surfactant and cosurfactant weight ratio. These effects on phase diagrams could be explained by the Winsor classification (Figure 5) as follow:

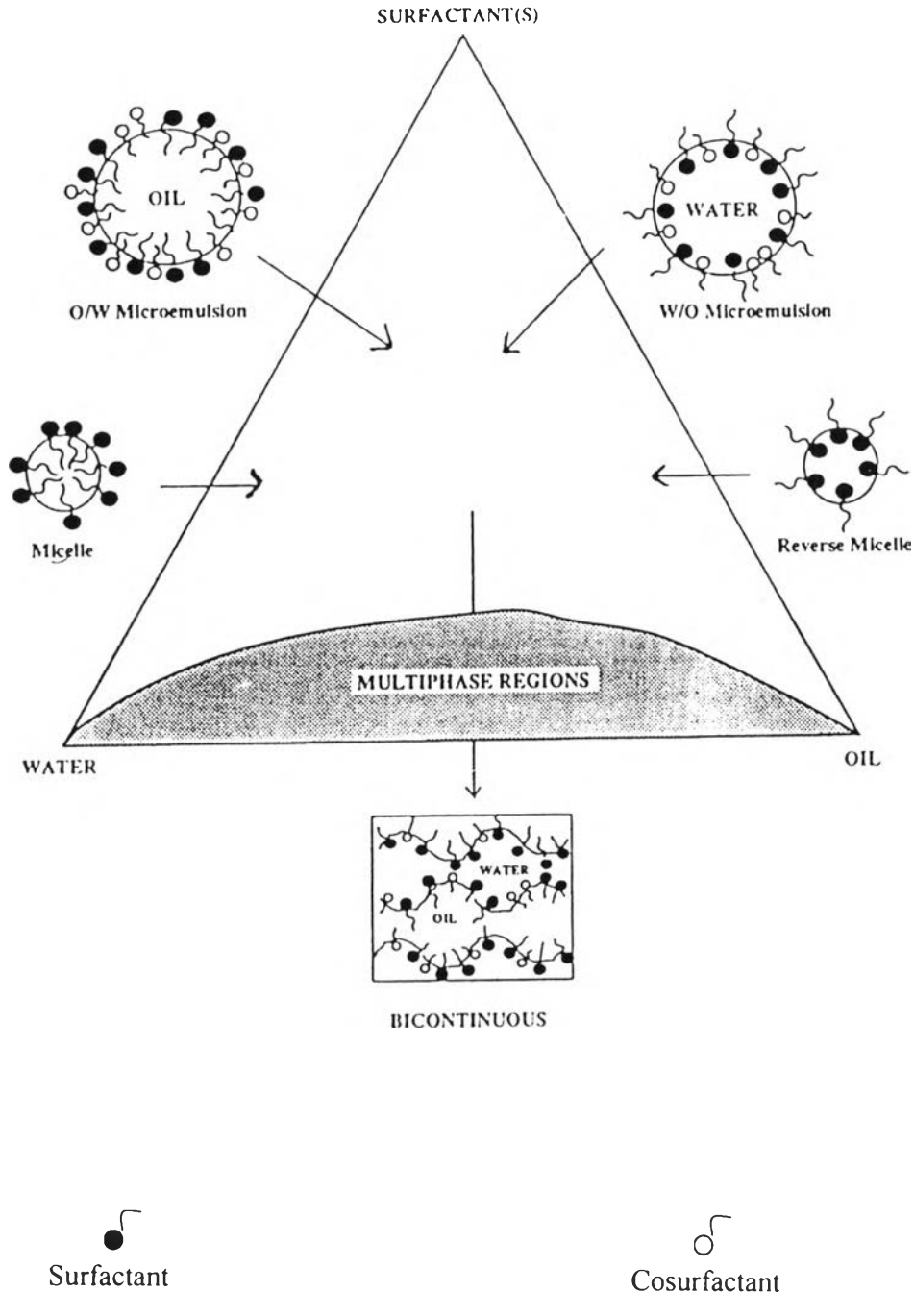


Figure 4. Pseudo-ternary phase diagram of an oil/surfactants/water system with emphasis on microemulsion and emulsion (From Swarbrick and Boylan, 1994)

Winsor I. At low temperature for the non-ionic surfactant and at low electrolyte concentration for the ionic surfactant, the surfactant is preferentially soluble in the water leading to the formation of oil-in-water (o/w) microemulsions. No surfactant aggregates exist in the excess oil phase, only a small concentration of surfactant monomer.

Winsor III. As temperature is raised for the non-ionic surfactant or electrolyte concentration increased for the ionic, eventually the system separates into three phases. The middle phase contains oil, water and the majority of the surfactant and is referred to as a middle-phase microemulsion. No surfactant aggregates now exist in either the aqueous or the oil phase.

Winsor II. With further heating or electrolyte addition, the system becomes two phases with excess water but now the surfactant is mainly in the oil phase stabilizing a water-in-oil (w/o) microemulsion; no surfactant aggregates are left in the aqueous phase, merely a small concentration of monomer with which the microemulsion is in equilibrium (Clint, 1992).

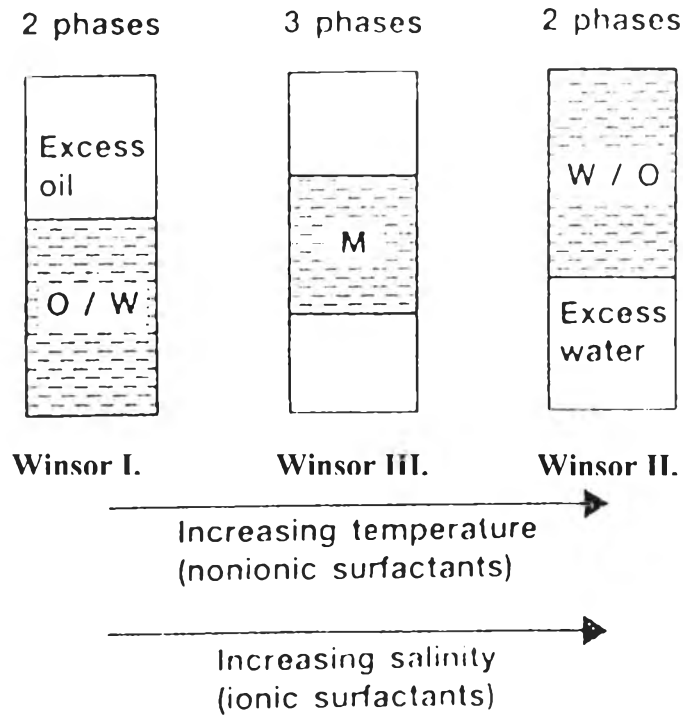


Figure 5. Sequence of microemulsions following a change in temperature and salinity. Most of the surfactant is in (The shade area represents microemulsion phase). In the three-phase system the microemulsion contains both oil and water (From Clint, 1992)

Temperature has an influence on the solubilization of nonionic microemulsion refers to phase inversion temperature (PIT), at which temperature the microemulsion structure inverts. The temperature influences on area of surfactant head group. At low temperature, nonionic surfactants are hydrophilic and form normal o/w microemulsion. At higher temperature, the hydration of the head group of surfactant is reduced, allowing microemulsion droplet to come together. Consequently, the droplets are in

contact then the interfacial surfactant films can re-align forming droplets of w/o microemulsion. For ionic surfactant, the phase inversion is strongly influenced by pH and electrolyte. The temperature is also influenced on the packing ratio, P, because the area of surfactant head group is changed with changing the temperature. This effect depends on the nature of oil and surfactant. Thus, it is important to avoid any complication due to the presence of a PIT, the intended temperature of use should be 30^0C below the PIT (Lawrence, 1999)(Figures 6-7)

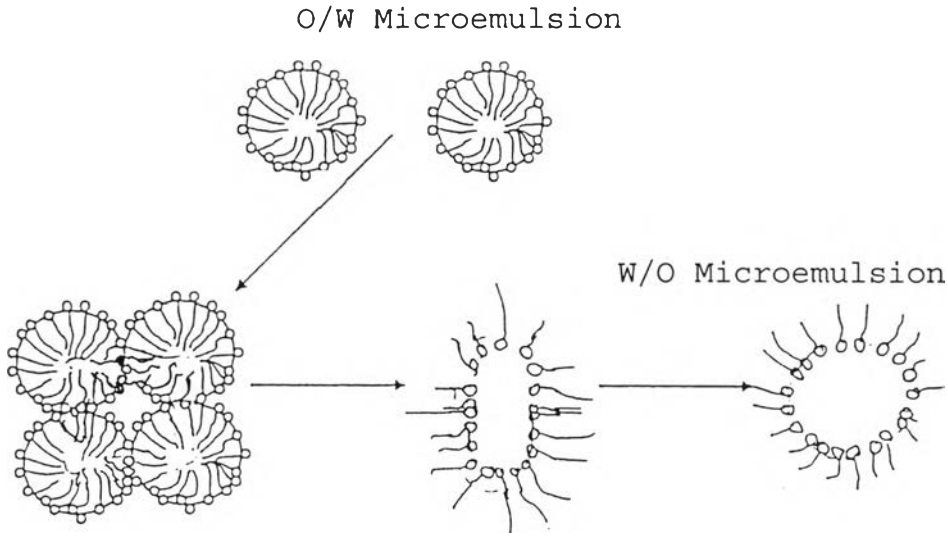


Figure 6. The scheme of phase inversion from normal to reverse microemulsion upon adding charged molecules or increasing temperature (Lawrance, 1999)

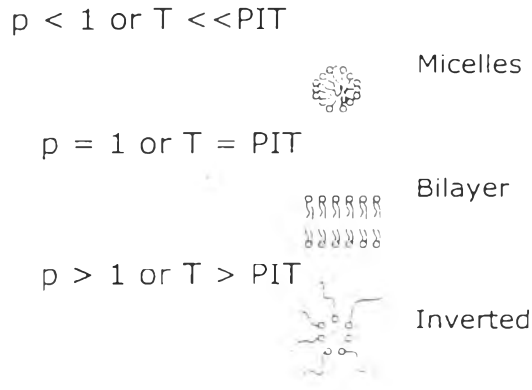


Figure 7. The relation of phase inversion temperature (PIT) or the packing ratio (P) and structure of surfactant aggregate in solution (Lawrance, 1999)

4.Characterization of microemulsion

4.1 Particle size and size distribution

4.1.1 Light scattering

Scattering methods have been widely applied in the study of microemulsions. These include small-angle X-ray scattering (SAXS), small-angle neutron scattering (SANS), and static as well as dynamic light scattering. The lower

limit of size that can be measured with these techniques is about 2 nm. The upper limit is about 100 nm for SANS and SAXS and a few micrometers for light scattering. These methods are very valuable for obtaining quantitative information on the size, shape, and dynamics of the components (Solans, Pons and Kunieda, 1997).

Static light scattering technique has also been widely used to determine microemulsion droplet size and shape. In the experiment, the intensity of scattered light is generally measured at various angles and for different concentrations of microemulsion droplets. For dynamic light scattering, also referred to as photon correlation spectroscopy (PCS), is used to analyze the fluctuation in the intensity of scattering by the droplets due to Brownian motion. The hydrodynamic radius of the particles can be determined from the diffusion coefficient of the scattering centers when the interparticle interaction becomes zero. Although dynamic light scattering measurement is relatively easy and fast, extrapolation of results to infinite dilution is not possible in most microemulsion systems and hydrodynamic radius of the particle obtained should be corrected because of interparticle interaction.

There is major difficulty in study of microemulsions with use of scattering techniques. Dilution of sample in order to reduce interparticle interaction may not appropriate for some systems because it can destroy the structure and the composition of pseudo-phases. Nevertheless, successful determinations have been

achieved by using a dilution technique that maintains the identity of droplets and extrapolating the result obtained at infinite dilution to gain the size, shape, etc., or by measurement at very low concentrations of equal or less than 1 % w/w of surfactant (Solans, Pons and Kunieda, 1997).

4.1.2 Nuclear magnetic resonance

This technique has been used to study the structure and dynamic of microemulsions. Self-diffusion measurement using different tracer techniques, in common the radioactive label, supplies information on mobility of the compounds as the self-diffusion coefficient. The limits of this technique are time consuming and the use of labeled molecules in multicomponents systems such as microemulsion is not practical (Lindman, Olsson and Soderman, 1999).

However, the Fourier transform pulsed field gradient spin-echo NMR (FT PGSE NMR) allows simultaneous and rapid determination of self-diffusion coefficients (in the range of $10^{-9} m^2 s^{-1}$). In this technique the replacement of nuclear spin in controlled magnetic field gradient is monitored, and the contributions of different components are resolved by Fourier transformation of the NMR signal (spin echo). The PGSE experiment consists of two equal and rectangular gradient pulses of magnitude

(g) and length (δ), sandwiched on either side of 180° rf pulse in simple Hahn echo experiment. For molecules undergoing free diffusion characterized by diffusion coefficient of magnitude (D), the echo attenuation due to diffusion (I) is given by

$$I(\Delta,\delta,g) = I_0 \exp [-\gamma 2g2\delta2(\Delta-\delta/3)D] \quad (3)$$

where Δ represents the distance between the leading edges of the two gradient pulses, γ is the magnetogyric ratio of the monitored spin, and I_0 denotes the echo intensity in the absence of any field gradient. By varying either g, while at the same time keeping the distance between the two rf pulses constant, δ, or Δ, D is obtained by fitting this equation to the observed intensities (Figure 8).

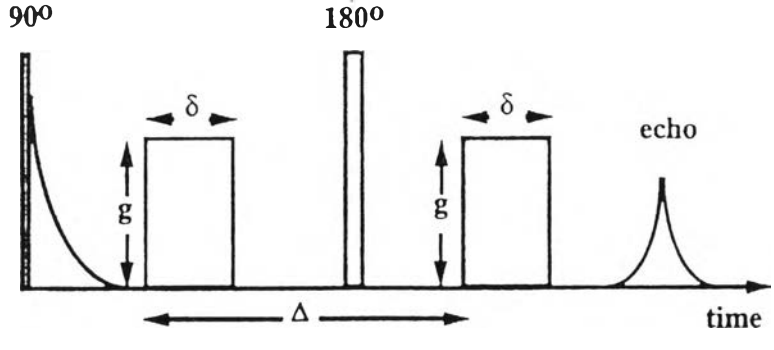


Figure 8. A schematic of the Fourier transform pulsed filed gradient spin-echo NMR method (From Lindman, Olsson and Soderman, 1999)

In w/o microemulsion, water diffusion is slow and corresponds to that of the droplets (of the order of $10^{-11} m^2 s^{-1}$), oil diffusion is high (of the order $10^{-9} m^2 s^{-1}$), and diffusion of surfactant molecules, located at the interface, is of the same order as that of the droplets. In contrast, o/w microemulsions the diffusion coefficients of water are higher than that oil. In bicontinuous microemulsions the diffusion coefficients of water and oil are both high (of the order of $10^{-9} m^2 s^{-1}$) and the diffusion coefficient of the surfactant has been found to be intermediate between the value of nonassociated surfactant molecules and the value for a droplet-type structure (of the order of $10^{-10} m^2 s^{1}$) (Lindman, Olsson and Soderman, 1999; Solans, Pons and Kunieda, 1997).

4.1.3 Electron microscopy

Most of electron microscopic techniques have been attempted for the characterization of microemulsions. Because of the high liability of the samples and the danger of artifacts, electron microscopy should be considered as a misleading technique in microemulsion studies. However, images showing clear evidence of the microscopy, a well-established method in the biological field, has been successfully applied to microemulsions. Moreover, the careful control of the temperature of sample

before freezing and ultrarapid cooling followed by fracture and replication of fracture face yield image of the microstructure of these systems (Solans, Pons and Kunieda, 1997).

Transmission electron microscopy-freeze fracture electron microscopy (FFEM) is used to determine the particle size of microemulsion. In this technique, the microemulsion must be cooled very rapidly to prevent phase separation or crystallization. Rapid cooling can be attained with propane jetting, liquid cryogen or spray freezing. A very rapid cooling rate is achieved to freeze a drop of microemulsion in less than 20 millisecond. The frozen sample is fractured, visibility enhanced by depositing a platinum-carbon layer, and viewed under a transmission electron microscopy (TEM). Direct imaging of the microemulsions in the frozen hydrated state (using a cryostage in TEM) can be conducted with the help of glass-forming microemulsions, which do not break during cooling, nor do they separate or crystallize (Tenjarla, 1999).

4.2 Interfacial tension of microemulsions

The technique is used to gain information about the formation and the properties of microemulsion. The ultra low interfacial tension of microemulsion can be

measured with the spinning-drop apparatus and surface laser-light scattering. The measured interfacial tension corresponds to that at the planar interface between the microemulsion and the excess oil (Winsor I) or water phase (Winsor II). For systems that have a three-phase region (Winsor III), interfacial tension can be measured between the middle-phase microemulsion and both oil and water excess phases. The interfacial tensions are derived from the measurement of the shape of a drop of the low-density phase, rotating in a cylindrical capillary filled with the high-density phase (Clint, 1992).

4.3 The conductivity of microemulsions

Electrical conductivity is widely used to determine the nature of the external phase and to detect phase inversion phenomena. The distinction between o/w and w/o microemulsion is quite straightforward in that o/w and w/o systems having high conductivity and low conductivity, respectively. In microemulsion, this behavior is more complex. For example, a sharp increase in conductivity in w/o microemulsion was observed at low volume fractions of water (Solans, Pons and Kunieda, 1997).

4.4 The viscosity of microemulsions

There are several reports to study the viscosity of microemulsion for determining the hydration and the interaction of dispersed droplets. The viscosity equation is

$$\eta_{rel} = \exp^{a\varphi / (1-K\varphi)} \qquad (4)$$

where η_{rel} is the relative viscosity proposed based on the volume fraction of the dispersed phase, *a* is a constant with theoretical value (typically in range of 1-10), K is the hydrodynamic interaction coefficient (typically in range 1.3-2) and φ is the volume fraction of the dispersed phase (Gradzielski and Hoffman, 1999). K is dependent on the hydrodynamic interaction between the droplets and is inversely proportion to the droplet size. An increase in *a* and a decrease in K were observed with decreasing surfactant and cosurfactant mass ratio. The increase in *a* results from the greater hydrodynamic volume of the droplets (Tenjarla, 1999). Microemulsions are usually shown Newtonian behavior, which the viscosity is constant and independent on the applied shear stress or equivalently shear rate.

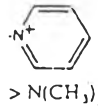
5. Excipient selection

Microemulsions usually involve three or four components such as oil phase, aqueous phase, surfactant and cosurfactant. Thus choices of surfactant and cosurfactant are important to matching with the particular oil.

5.1 Surfactant

Surfactant or surface-active agents are molecules adsorbed at an interface reducing the interfacial tension. The surfactants are the amphiphilic molecules having hydrophilic and hydrophobic parts. The hydrophilic region is used to classify the classes of surfactant (Table 2).

Table 2. Summary of main classes of surfactant and its application (From Clint, 1992)

Class	Head group	Main applications
Anionic	$-CO_2^- Na^+$	Soaps
	$-SO_3^- Na^+$	Synthetic detergents
	$-O-SO_3^- Na^+$	Detergents, personal care products
	$-O-PO_3^- Na^+$	Corrosion inhibitors, emulsifiers
	$-(OCH_2CH_2)_n-O-SO_3^- Na^+$	Liquid detergents, toiletries, emulsifiers
Cationic	$-N(CH_3)_3^+ Cl^-$	Bitumen emulsions
		Bactericides, antistatic agents
	$>N(CH_3)_2^+ Cl^-$	Fabric and hair conditioners
Zwitterionic	$-N^+(CH_3)_2-CH_2-CO_2^-$	Shampoos, cosmetics
	$-N^+(CH_3)_2-CH_2-SO_3^-$	
Semi-polar	$-(CH_3)_2N \rightarrow O$	Foam enhancers
Non-ionic	$-(OCH_2CH_2)_nOH$	Detergents, emulsifiers

When oil is mixed with water and surfactant, if an aqueous solution of a surfactant is drought into contact with a hydrocarbon oil phase, then an individual surfactant molecule, during its random diffusion around the system. has the opportunity to pass into the oil phase. It finds an environment, in which the hydrophobic tail prefers to be, but it takes with it a polar head group, which would have preferred to remain in the aqueous phase. For a system at equilibrium, the relative concentrations of molecules in each phase will depend on the balance between the hydrophobic and hydrophilic groups. The surfactant molecules tend to orient themselves to be substantially parallel each other with the polar head group remaining in the water as well as the hydrophobic tail in the oil (Clint, 1992).

Solution properties of surfactant are dependent on its concentration (Figure 9) and is attributable to the self-association of the amphiphile into micelles. The concentration at which the change of slope occurs is called the critical micelle concentration (cmc) (Florence and Attwood, 1988).

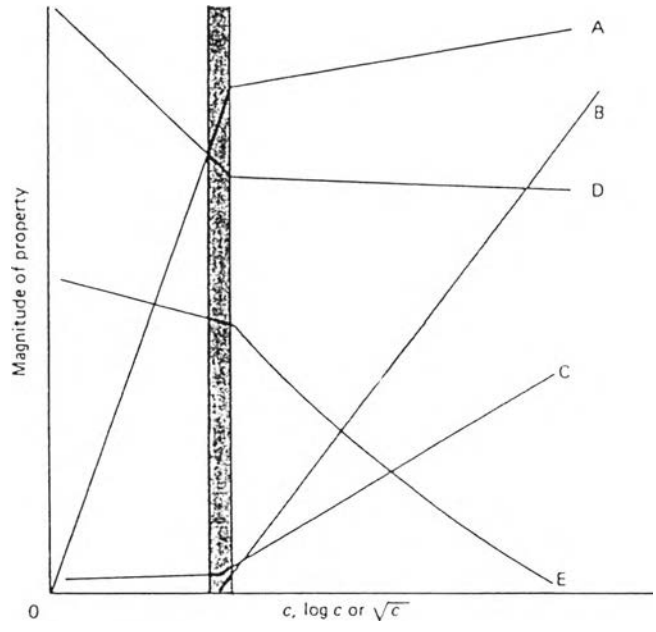


Figure 9. Solution properties of an ionic surfactant as a function of concentration,*c*. A, osmotic pressure (against *c*);B, solubility of a water-insoluble solubilisate (against *c*); C, intensity of light scattered by the solution (against *c*); D, surface tension (against log *c*); E, molar conductivity (against $\sqrt{c}$) (From Florence and Attwood, 1988)

In case of microemulsions, the surfactants chosen must reduce the oil and water interfacial tension for a very small value, provide a flexible film and give the correct curvature at the interfacial region (Swarbrick and Boylan, 1994). There are two rational guides to surfactant selection namely the hydrophile-lipophile balance (HLB) and the critical packing parameter (CPP) (Lawrence and Rees, 2000).

5.1.1 Hydrophile-lipophile balance (HLB)

The HLB value relates to the balance between hydrophilic and lipophilic of surface active agents and is a numerical scale based on the balance between these two opposing values . The higher HLB number is found in more hydrophilic part of surfactant, while the high lipophilic part has low HLB value. Thus the surfactant behavior can be classified by HLB number, as shown in Table 3

Table 3. Behavior in water and HLB of surfactant (Clint, 1992)

Behavior in water	HLB range
No dispersibility	1-4
Poor dispersibility	3-6
Milky dispersion after vigorous agitation	6-8
Stable milky dispersion, upper end translucent	8-10
Translucent or clear	10-13
Clear solution	13+

It is commonly concluded that low HLB (3-6) surfactants are preferred to form w/o microemulsions, where as surfactants with high HLB (8-18) are commonly used to prepare o/w microemulsions (Lawrence and Rees, 2000).

5.1.2 The critical packing parameter (CPP)

This term has been described by Israelachvili, Mitchell and Ninham (1976). The CPP relates the ability of surfactants to form particular aggregates to the geometry of the molecule itself. The CPP equation is shown below;

$$CPP = \frac{v}{a_0 l_c} \qquad (5)$$

where v is the partial molar volume of the surfactant, a_0 is the cross-sectional area of surfactant head group and l_c is the critical length of the hydrophobic chain, generally assumed to be 70-80% of its fully extended length (Figure 10). The CPP is a measure of the preferred geometry adopted by the surfactant, and as a consequence is predictive of the type of aggregate that is likely to form. The factor and the effect changing CPP is shown in Figure 11. The 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. If the CPP is less than 1/3, o/w microemulsion are formed but when CPP is in between 1/3 and 1/2 then rod shaped aggregates are formed. As CPP increases beyond 1/2 towards 1, the flexible bilayer vesicle is present. At zero curvature, when the CPP equals to one,

bicontinuous or lamellar structures may be formed. For w/o microemulsion, the CPP is higher than 1. (Israelachvili, Mitchell and Ninham; 1976; Lawrence and Rees,2000; Porter,1993; Swarbrick and Boylan, 1994).

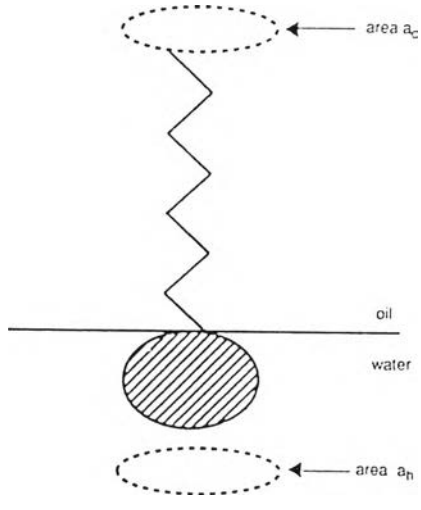


Figure 10. Schematic representation of a surfactant molecule with alkyl chain and head group (From Clint, 1992)

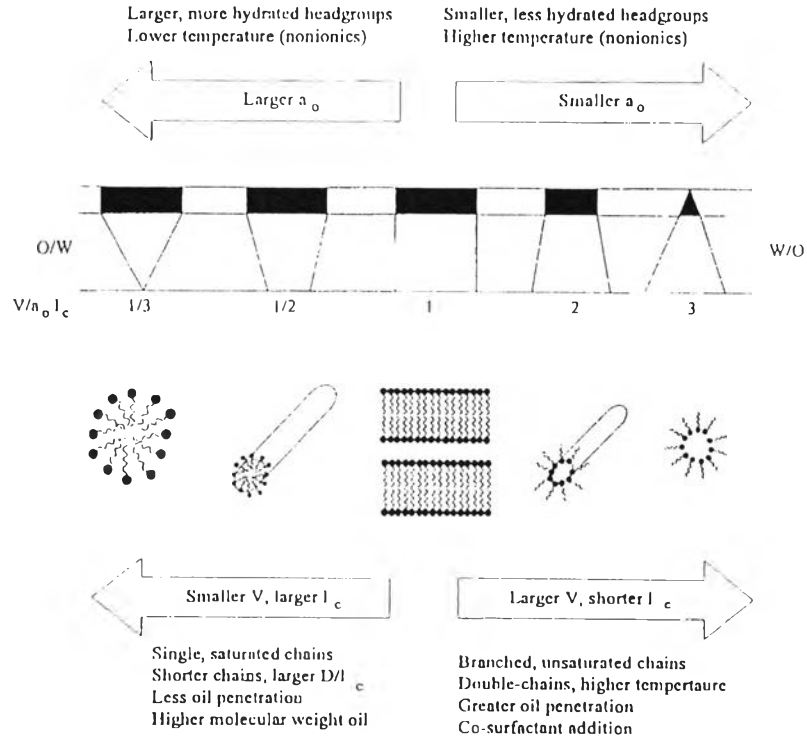


Figure 11. Factor, effecting the CPP of a surfactant and the possible surfactant aggregate (From Lawrence and Rees, 2000)

For toxicological point of view, an anionic surfactant (i.e. sodium alkylsulfate)greatly interacts with biological membranes and molecules of animal organs and highly irritates the animal skins. In addition, a cationic surfactant is a very harmful surfactant especially for animal cells such as quaternary ammonium salt showing anticholinergic activity and highly toxic ever in small dose. In addition, a nonionic surfactant, that is unionization in acidic or basic condition exhibits very low in toxicity so it can be used in pharmaceutical formulation. The examples of nonionic surfactant that have been reported to have less toxicity and suitable to be used in

parenteral preparations are polyoxyethylene sorbitan (Tween®), block copolymers of polyoxyethylene polyoxypropylene (poloxamer series), polyoxyethylene castor oil derivatives (Cremophor® EL) and polyoxyethylene-660-(12)-hydroxystearate (Solutol® HS15). The various pharmacopoeias for parenteral administration already include these surfactants (Klang and Benita, 1998).

5.2 Cosurfactant

Most single chain surfactants are unable to reduce the oil/water interfacial tension to form microemulsions. Thus, the cosurfactant is requried to lower and increase the fluidity of the interfacial tension between oil and water phases. It also increases the mobility of the hydrocarbon tail and allows greater penetration of oil into surfactant film. However, a number of double chain surfactants such as sodium bis-2-ethylhexylsulphosuccinate (AOT) and didodecylammonium bromide (DDAB) are able to form microemulsion without the aid of cosurfactant.

The cosurfactant is generally a short to medium chain length alcohols (C_3-C_8) as well as medium chain fatty alcohol, acid or amide. The examples of cosurfactant in the pharmaceutical industry are butanol, glycerin, ethylene glycol and

propylene glycol, polyhydroxy compounds (Kale and Allen, 1989; Lawrence and Rees, 2000).

5.3 Oil

The majority of oils intended for pharmaceutical use are large and semi-polar molecules. The examples of oils used in the pharmaceutical formulation are reported below. Isopropyl palmitate (IPP) is mostly used in microemulsion gel. In parenteral formulation, medium chain length triglyceride such as Miglyol 812, fatty acid esters and isopropyl myristate (IPM) are widely used. Ethyl or methyl esters of lauric, myristic and oleic acid have been employed for microemulsion formulation. In addition, many alkyl ethylene glycol ethers such as ethylene glycol dibutyl ether is also used as the oil phase (Lawrence and Rees, 2000)

However, long alkyl chain length triglycerides such as soybean, rapeseed or sunflower oils are considered to hardly penetrate the interfacial film to form the optimum curvature (El-Nokaly, Hiler and McGrady, 1991).

6. Applications of microemulsion as drug delivery vehicle

Microemulsions have widely been studied because of their unique properties such as infinite stability, ease of manufacturing, clear solution, less viscosity and improved drug solubilization. In the early period, microemulsion formulations were used for floor polishing, cutting oil and wash solution. Since the mid-1980's, a lot of information about the significant advantage and the use of microemulsions have been reported in many fields such as cosmetic, photochemical, agriculture, beverages and specially in pharmaceutical fields. The studies about microemulsions as drug delivery vehicle are viewed as follows.

6.1. Oral delivery

The comparison of pharmacokinetic profiles of drug between Neoral®, cyclosporin A microemulsion, and Sandimmune®, cyclosporin A emulsion, was done in 140 volunteers (Chueh and Kahan, 1998). The pharmacokinetic profiles of volunteer using Neoral® were found to be significantly higher than Sandimmune®. In addition, there was microemulsion formulation consisting of cyclosporin A stabilized by Cremophor EL® as surfactant, Transcutol® as cosurfactant and Captex® as oil phase.

The bioavailability of cyclosporin A was reported to increase about 3.3 and 1.25 times for Sandimmune® and Neoral® respectively.

In the study of Kovarik et al.(1994) insulin containing o/w microemulsion was absorbed by ligated small intestinal segments approximately 2 times when compared with a solution of drug. The author concluded that the improved absorption resulted from a combination of smaller droplet size, type and digestibility of lipid phase of the formulation and the surfactant.

6.2 Parenteral delivery

The parenteral microemulsions comprised Miglyol 810n (MCT), soybean phosphatidylcholine (Epikuron 200), PEG 400, poly(ethylene glycol)(660)-12-hydroxystearate and ethanol were studied and the results from PGSE-NMR indicated that the microemulsion formed over a range of compositions was bicontinuous, even at high oil concentrations. After administration, the bicontinuous microemulsion formed o/w emulsions on dilution in the body. *In vitro* study showed that the resulting droplets were small, with mean radii typically in the range of 60-200 nm (Corswant, Thoren and Engström, 1998).

The intravenous administration of the microemulsion formulation above was performed by infusion into conscious rats over a 5 minutes period. It was found that the dose up to 0.5 ml/kg of microemulsion had no significant effect on acid-base balance, blood gases, plasma electrolytes, arterial blood pressure or heart (Corswant, Thoren and Engström, 1998).

In addition, the preparation and evaluation of flurbiprofen loaded oil-in-water microemulsion was investigated. The system of interest was prepared using ethyl oleate as oil phase and Tween® 20 as surfactant. The drug solubility was about eight times higher than in buffer solution (Park et al., 1999).

6.3 Topical delivery

The transdermal delivery of microemulsion of tetracaine hydrochloride was investigated for *in vivo* analgesic effect on rats and histopathology, irritation, and oxidative stress on mice. The microemulsion comprised aerosol-OT(AOT), water and isopropyl myristrate (IPM).The preliminary histopathology, irritations, and oxidative stress studies showed that microemulsion formulation was safe with a concentration of AOT : IPM up to 21:79 w/w. Moreover, microemulsion formulation showed the higher

analgesic responses of drug than in aqueous solution about eight times and the effect depended on a concentration of AOT in IPM (Changez and Varshney, 2000).

The study by Osborne, Ward and O'Neill. (1988) in the release of a hydrophilic drug from w/o microemulsion comprised octanol/ water/ dioctyl sodium sulfosuccinate (DSS) as topical vehicles was reported. This specific system was chosen because it can incorporate more than 70% water at ratio of DSS: octanol of 58:42. The water self-diffusion coefficient was determined by pulsed field gradient Fourier transform (PFGFT) NMR. The average self-diffusion values for water increased 10 folds when the dispersed phase water content increased from 15% to 58% by weight.

7. The determination of toxicity

A suitable, nonionic vehicle is required for administration of poorly soluble drug. There are several solubilizing techniques available, each with its own advantage and limitations. Microemulsions, because of their high solubilizing capability and thermodynamic stability, are attractive vehicles for parenteral administration. However, because of their potential toxicity, only a limited number of surfactant and cosurfactants can be used to prepare a pharmaceutical parenteral microemulsion (Tenjarla, 1999). The

determination of toxicity of microemulsion on erythrocyte is the one way for a clear understanding of the safety of microemulsion used for parenteral route.

In general, the hemolytic test *in vitro* serves as a screening method for the toxicity of lytic agents contained in intravenous formulation. For example, sodium oleate is often used in a commercial parenteral emulsions (Lipofundin® MCT, Abbolipid® and Schiwalipid®); however, only 0.005 %w/w of sodium oleate can lead to complete hemolysis after 30 minutes. Concentrations of sodium oleate from 0.03 to 0.05% w/w are generally used in commercial parenteral emulsion. It could be predicted that these preparations will also lead to complete hemolysis, as the sodium oleate concentrations are 10 times higher than the fine concentration inducing complete hemolysis in aqueous solutions of sodium oleate (Jumaa and Mü ller, 2000).

To determine the hemolysis, the free hemoglobin is used to assume the amount of erythrocyte hemolyis. When erythrocyte lysis occurs, the hemoglobin is released and can be detected using UV spectrophotometry at specific wavelength.