CHAPTER V

DISCUSSION AND CONCLUSIONS

1. Formulation of microemulsion and phase diagram

Non-ionic surfactants find wide application in pharmaceutical and cosmetic products. Interest in using non-ionic surfactants is increasing due to their low irritation and high chemical stability. They are compatible with other surfactants and retain this utility over the broad range of pH values from pH 3 to 10. Non-ionic surfactants of polyoxyethylene class are generally used in the formation of microemulsions. Therefore, tween 20 and tween 80 were selected to produce microemulsions in this study. Furthermore, they can be used in parenteral dosage form (Kibbe, 2000; Nema et al., 1997; Powell et al., 1998).

In their simplest form, microemulsions are small droplets of one liquid dispersed throughout another by the presence of a fairly large concentration of a suitable combination of surfactants. An essential requirement for their formation and stability is the attainment of a very low interfacial tension between oil and water. Since microemulsions have a very large interface between oil and water because of the small droplet size, they can only be thermodynamically stable if the interfacial tension is low. Thus, the role of the surfactants in the system is to reduce the interfacial tension between oil and water (typically about 50 mN/m to this low level). In general, it is not possible to achieve the required interfacial tension with the use of a single surfactant (Attwood, 1994). The cosurfactant is added to further lower the interfacial tension between oil and water phase, fluidize the hydrocarbon region of the interfacial film, and influence film curvature. Thus most appropriate cosurfactant is typically an alcohol of short to medium chain length (C_3-C_8) which can diffuse rapidly between the bulk of oil and water phases and the interface (Swarbrick and Boyland, 1994). Unfortunately, short to medium chain length alcohols use as cosurfactants limit the potential in the microemulsion due to their toxic and irritant

properties. Furthermore, the evaporation of alcohol can destabilize the system (Attwood, 1994).

Many reports claimed that the hydrophilic molecule such as glycerin and sorbitol could form microemulsion (Attwood et al.,1992; ale and Allen, 1989). Hence, glycerin, propylene glycol, and polyethylene glycol 400 were selected as cosurfactant in this study. In addition, they are low price, commercially available and parenterally acceptable compounds. Soybean oil was selected because it was widely used intramuscularly as a drug vehicle, or as a component of emulsion used in parenteral nutrition regimens (Kibbe, 2000; Nema et al., 1997; Powell et al., 1998).

In this study, microemulsions were prepared by titration method. This technique was convenient and allowed a large number of compositions to be examined within a short period of time. However, the microemulsions should be equilibrated at room temperature before any physicochemical characterization studies.

Effect of surfactants and cosurfactants

Theoretically, the size of microemulsions is in the range of 10-140 nm (Swarbrick and Boyland, 1994). It is very small droplet thus the curvature of the interphase must be high to form microemulsions. From the results, microemulsion could not be produced when using tween 20 as surfactant while tween 80 could produce microemulsions. Moreover, only systems with glycerin and polyethylene glycol 400 as cosurfactant were found to form microemulsion. While systems with propylene glycol as cosurfactant could not form microemulsion. The chemical structure of each compound shown in Appendix A. The chemical structures of tween 20 and tween 80 are similar but the hydrophobic chain of tween 20 is lauric acid (C12) whereas tween 80 is oleic acid (C18) (Kibbe, 2000). Thus, hydrophobic chain of tween 20 is much shorter than that of tween 80.

Tween 80 was water-soluble non-ionic surfactant. It was adsorbed at the interface of between hydrophobic oil phase and water. It was noteworthy that hydrophilic cosurfactants molecules would influence the polar head group area of surfactant by altering the size of head groups of surfactant. It would penetrate into the

hydrophilic region or polyoxyethylene chain of surfactant and form hydrogen bond. Therefore the molecular geometry of surfactant was changed. The hydrophilic part of surfactant was expanded and consequently bulkier than hydrophobic part. Furthermore, it increased the curvature and fluidity of surfactant film.

The chemical structure of glycerin or propane-1,2,3-triol has hydroxyl groups at all carbon in molecule. Possible structure of surfactant film formations in tween 80:glycerin systems as shown in Figure 107. It would preferably penetrate in the polyoxyethylene chain of tween 80 and formed hydrogen bond in this area. One molecule of glycerin or more than that might penetrate between molecule of tween 80. Therefore, hydrophilic part of tween 80 was bulkier than hydrophobic part. It would expand and influence the film curvature. The interface curves of surfactant film toward water and would envelop the soybean oil.

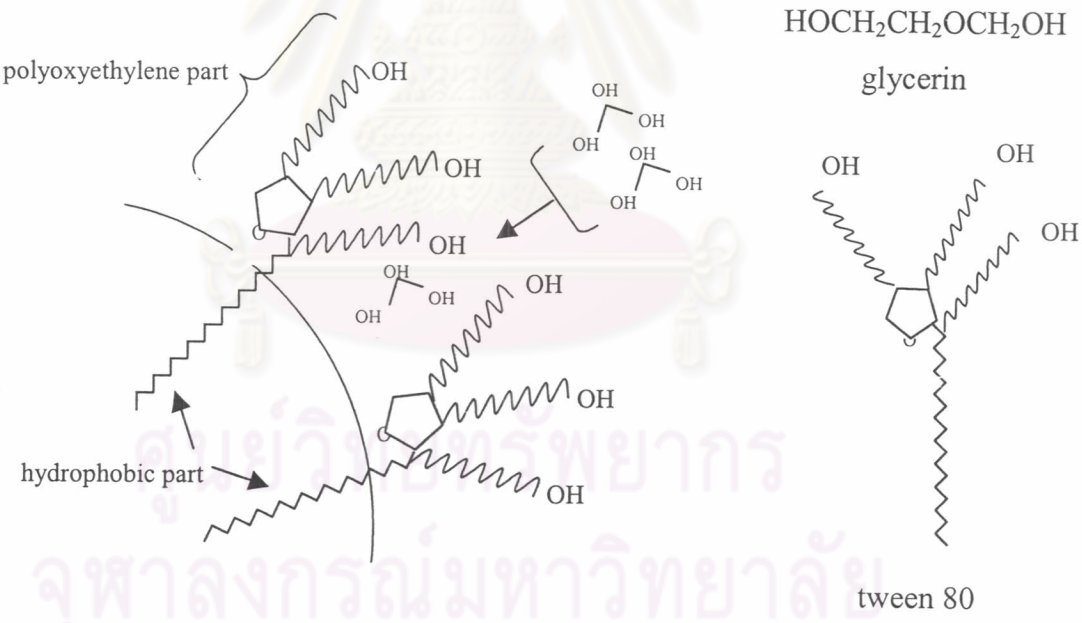

Figure 107 Possible structure of surfactant film formation in tween 80:glycerin microemulsion systems.

Similar to glycerin, polyethylene glycol 400 has hydroxyl groups at the both ends of molecule. Thus, hydrogen bonding was formed between hydroxyl groups of

polyethylene glycol 400 and polyoxyethylene chain of surfactant as revealed in Figure 108. Thus, the curvature of surfactant film increased. Because of this effect, water-soluble hydrophilic materials were used to aid microemulsion formation. The same tendency was reported by Attwood et al. (1992) in the study of microemulsions prepared from isopropyl myristate (IPM), sorbitol, water and between tween 80, 60 or 40 systems.

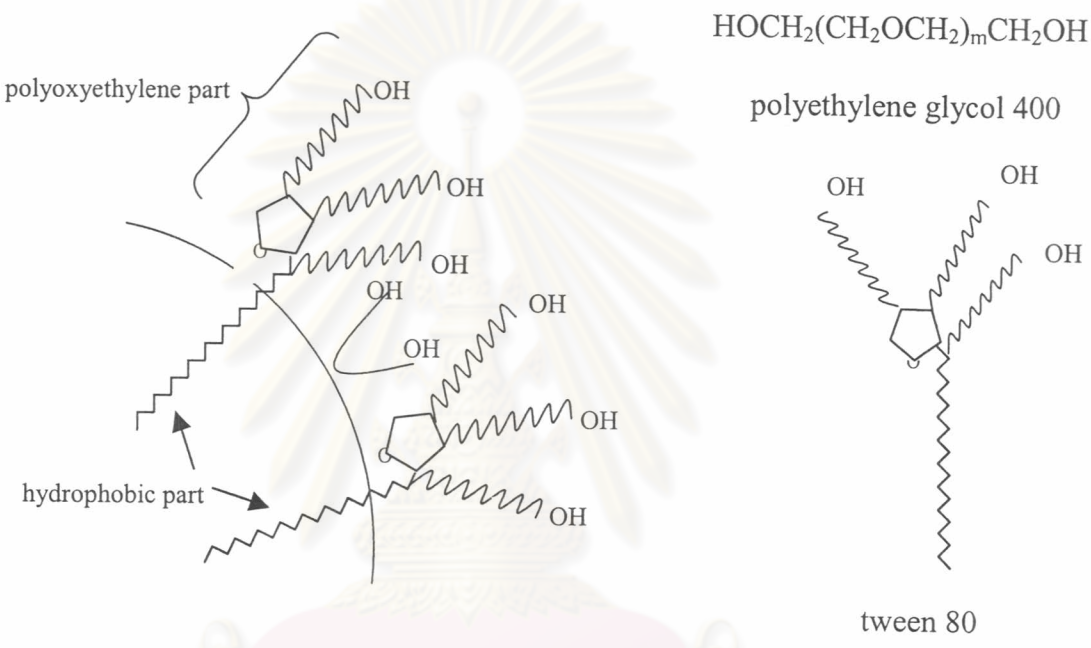

Figure 108 Possible structure of surfactant film formation in tween 80:polyethylene glycol 400 microemulsion systems.

Glycerin as cosurfactant could form microemulsion at all ratios while polyethylene glycol 400 as cosurfactant could form microemulsion at ratio 1:0.7 and 1:0.5. Glycerin was a small molecule with molecular weight of 92.09 (Kibbe, 2000). It could easily penetrate into polyoxyethylene chain of surfactant film. While the chemical structure of polyethylene glycol 400 was long chain polyol. It could penetrate into polyoxyethylene chain of surfactant film more difficult than glycerin. It would show less entrapment in polar area head group of surfactant than glycerin. In addition, the mole of surfactant and cosurfactants in each ratio of surfactant:cosurfactant as exhibited in Table 16. It indicated that at the same ratio of surfactant:cosurfactant, the mole of glycerin more than the mole of polyethylene

glycol 400. Therefore, the molecule of glycerin could penetrate between the molecule of tween 80 more than the molecule of polyethylene glycol 400. However, some molecule of glycerin and polyethylene glycol 400 might dissolve in aqueous phase and did not form microemulsions.

Table 16 The mole of surfactant and cosurfactants in each ratio of microemulsion system.

Ratio of surfactant: cosurfactant	Mole of surfactant and cosurfactants		
	Tween 80	Glycerin	Polyethylene glycol 400
1:1.5	0.0223	0.4761	-
1:1	0.0279	0.3967	-
1:0.7	0.0319	0.3174	0.0731
1:0.5	0.0361	0.2569	0.0592

Propylene glycol or 1,2-propanediol as cosurfactant could not form microemulsions although its chemical structure was similar to glycerin. The chemical structure of propylene glycol revealed less hydrophilic than that of glycerin. It has only two hydroxyl groups at the position one and two of carbon atom. The hydroxyl groups of propylene glycol could form hydrogen bond with polyoxyethylene part of surfactant film whereas carbon atom at third position could penetrate into hydrophobic part of surfactant film as shown in Figure 109. Consequently, propylene glycol affected the polar head group of surfactant much less than glycerin. In addition, the penetration into hydrophobic part of surfactant film was small. Since the bond distance between carbon atom was short (1.54 A°) (Lien, 2000). Thus, the bending of surfactant film was not enough to form microemulsion at all ratios.

Furthermore, the systems composed of tween 20 as surfactant could not form microemulsion. As expected, hydrophobic chain of tween 20 was short. When cosurfactant penetrated into surfactant film, the bending of surfactant film was not enough to form microemulsion. While hydrophobic chain of tween 80 was longer.

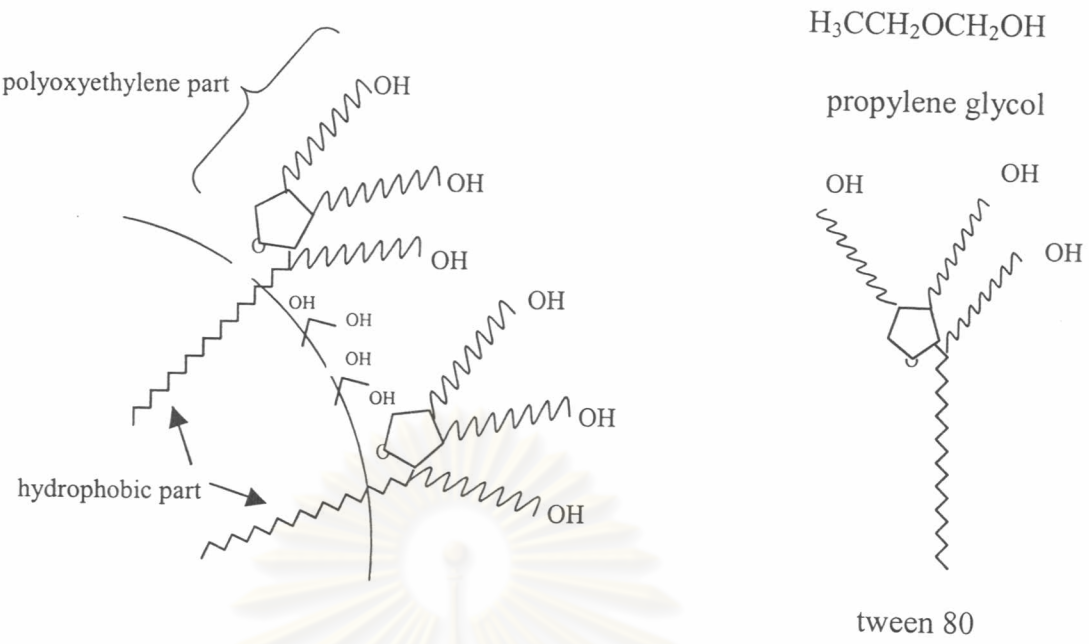

Figure 109 Possible structure of surfactant film formation in tween 80:propylene glycol systems.

Effect of surfactant:cosurfactant mixing ratio

Pseudo-ternary phase diagram of tween 80:glycerin and tween 80:polyethylene glycol 400 systems revealed that the amount of oil in microemulsions was increased with the increasing ratio of surfactant:cosurfactant. It indicated that the maximum amount of oil incorporated in microemulsion could be increased with the increasing of the ratio of surfactant:cosurfactant. A similar result was obtained from composition of mineral oil, water solution using Brij 96 as surfactant and glycerin, ethylene glycol, and propylene glycol as cosurfactants (Kale and Allen, 1989). The effect of surfactant:cosurfactant mixing ratio may be explained by the opposing effects of the surfactant and the cosurfactant. As aforementioned, cosurfactants increased the size of polar head group of surfactant and influenced the curvature of surfactant film to form microemulsions. However, increasing the amount of cosurfactant decreased the amount of surfactant in the systems or decreased the surfactant:cosurfactant ratio. Hence, the amount of surfactant in the systems may not be enough to form microemulsion. Consequently, the area of microemulsions was decreased with the increasing the amount of cosurfactant. In contrast, the results of

surfactant were opposite. Higher amount of surfactant in the system or increasing the ratio of surfactant:cosurfactant would result in enough surfactant to form microemulsion. The area of microemulsion would subsequently increase.

Effect of amount of oil

In pseudo-ternary phase diagrams, the microemulsions were formed at low oil content. The maximum oil solubilized was only 21% w/w. This was possibly due to the large molecular volume of soy bean oil (1563 $A^{\circ 3}$) (Malcomson et al., 1998). Thus, it could be solubilized less than oil of smaller molecular volume oils. Warisnoicharoen et al. (2000) studied the formation of o/w microemulsions solubilized by the non-ionic surfactant such as polyoxyethylene-10-dodecyl ether and containing two groups of pharmaceutically acceptable oils that is triglyceride oils and ethyl esters. The triglyceride oils were tributryrin, Miglyol 812, and soybean oil. While ethyl esters were ethyl butyrate, ethyl caprylate, and ethyl oleate. The result suggested that within the same series of oil, the oil with low molecular volume was solubilized to a greater extent than that with the large molecular volume. Moreover, Aboofazeli et al. (1995) reported that the molecular volume of oil appeared to be more important in determining phase behavior than its polarity.

2. Determining of microemulsion type

Microemulsion type is another important consideration for pharmaceutical applications. In general, the presence of o/w microemulsion droplets is likely to be a feature in microemulsions where the volume fraction of oil is low. Conversely, w/o droplets are likely when the volume fraction of water is low. For systems where the amounts of water and oil are equal, a bicontinuous microemulsions may result (Lawrence and Rees, 2000). In addition, it is generally accepted that low HLB (3-6) surfactants are favored for the formation of w/o microemulsions whereas surfactants with high HLB (8-18) are preferred for the formation of o/w microemulsion systems (Attwood, 1994). There are many methods to determine the microemulsion type. A single method may yield incorrect results. In stead, the type of microemulsion should

always be confirmed by means of a second method. Therefore, dilution and dye solubility tests were used to determine the type of microemulsion in this study since it provided a convenient and useful tool for the investigation of microemulsion type (Constantinides, 1995; Ho et al., 1996). Tween 80 is a non-ionic surfactant. Its has HLB value is about 15. All preparations contained small amount of oil. Moreover, the results from dilution and dye solubility tests showed that all preparations examined could be diluted with water and stained by water soluble dye, tartrazine. Thus, the microemulsions were certainly o/w microemulsions.

On the other hand, the HLB also determines the type of microemulsion through its influence on molecular packing and film curvature. The o/w structures are favored if the effective polar part is more bulky than hydrophobic part (CPP<1) and the interface curves spontaneous toward water (positive curvature). While the w/o structures are formed when the interface curves in opposite direction (CPP>1, negative curvature). At zero curvature, when the HLB is balanced (CPP~1), either bicontinuous or lamellar structure may form according to rigidity of the film (Swarbrick and Boyland, 1994). In this experiment, microemulsions could form by using glycerin and polyethylene glycol 400 as cosurfactant. They are hydrophilic molecules that affect the polar head group of surfactant. The polar part of tween 80 was more bulky than hydrophobic part. The head group area of surfactant (a) increased thereby the critical packing parameter (CPP) decreased. Thus, the interface curves toward the water phase. Consequently, o/w microemulsions were formed and dilution and dye solubility tests confirmed this result. Furthermore, non-birefringent property is an important consideration to designate as microemulsion. All preparations examined under polarized light were non-birefringent as expected. It was confirmed that they were microemulsion.

3. Drug free microemulsions

The physical appearance of all microemulsions before autoclaving was observed to be clear, transparent and one-phase that was characteristic of microemulsion system (Attwood, 1994; Malmsten, 1999; Swarbrick and Boyland,

1994). But after autoclaving, the microemulsions containing glycerin as cosurfactant showed phase separations. The upper phase was yellow solution while the lower phase was clear, colorless solution. Soybean oil and tween 80 are yellow solutions while glycerin is clear, colorless solution (Kibbe, 2000). Thus, the upper phase was likely to be a mixture of tween 80 and soybean oil whereas the lower phase was a mixture of glycerin and water. Upon sterilization with high temperature and stress condition, the high temperature produced high kinetic energy. This might affect or destroy the layer of surfactant film. Hence soybean oil was separated from surfactant and cosurfactant. The hydrogen bonding between hydroxyl groups of glycerin and polyoxyethylene chains of tween 80 was reported to have bond energy of approximately 6 kcal/mole (Martin, 1993). It was possibly broken down by high temperature and stress condition during sterilization therefore glycerin was separated from surfactant film. Furthermore, glycerin was small molecule thus it could easily be separated from surfactant film. Consequently, phase separation was obtained after autoclaving. However, the preparations were recovered to one-phase microemulsions by gently shaking because microemulsions were spontaneous formation, which was the outstanding feature of microemulsion.

In contrast, microemulsion containing polyethylene glycol 400 as cosurfactant showed good appearance after autoclaving. It was expected that polyethylene glycol 400 was long chain molecule. Therefore, it was more difficult to separate from surfactant film than glycerin and produced stronger surfactant film than glycerin.

The refractive index of a substance is the ratio of the velocity of light in air to the velocity of light in the substance. It is valuable for the identification of substances and the detection of impurities (USP24/NF19, 2000). In this experiment, the refractive index of all microemulsions was in the range 1.43 to 1.44. Comparison of the refractive indices of microemulsions and their compositions in Appendix C found that the refractive index of microemulsions was not related to the compositions. Soybean oil, glycerin and polyethylene glycol 400 have the refractive indices about 1.47 except water for injection which is about 1.33. This was due to the external phase. It was a mixture of water for injection, tween 80, and glycerin or polyethylene glycol 400. Furthermore, the refractive index decreased when the amount of water in formulation increased. A similar result was obtained from Kale and Allen (1989) that

the refractive index of the microemulsion systems was found to decrease upon dilution with water. Consequently, it was ascertained that the external phase of microemulsions was water. This was in agreement with the aforementioned that the microemulsions are o/w type.

The pH of emulsion is generally adjusted to 7-8 to allow physiological compatibility and maintain physical integrity, minimizing fatty acid ester hydrolysis of medium chain and long chain triglycerides. The main degradation pathway of fat emulsion leads to the formation of fatty acids, which gradually reduce the pH of the emulsion (Benita and Levy, 1993; Levy and Benita; 1989). In this experiment, the pH of microemulsions significant decreased ($p<0.05$) after sterilization from the range of 6.95-7.19 to 6.77-7.09 in tween 80:glycerin systems and 6.55-6.91 to 6.07-6.41 in tween 80:polyethylene glycol 400 systems. The study of Yalin et al. (1997) also reported that the pH of intravenous lorazepam emulsion was decreased after autoclave sterilization. This result explained that autoclave sterilization caused some hydrolytic breakdown, resulting in the liberation of free fatty acids with a consequent reduction in pH of the microemulsions (Kibbe, 2000; Levy and Benita, 1991; Yalin et al., 1997).

It is well known that the viscosity of emulsion depends on the composition of the system. There is a linear relationship between emulsion viscosity and the viscosity of the continuous phase. And the greater volume of the internal phase, the greater is apparent viscosity. In addition, the viscosity of emulsions increased upon aging (Lachman et al., 1986). In this study, the viscosities of tween 80, glycerin, and polyethylene glycol 400 were 420.51, 812.74, and 87.54 cps, respectively. Comparison within the tween 80:glycerin system at the ratio of 1:1.5 showed that viscosity of Formulation T1 G1.5 O6 was lower than those of Formulations T1 G1.5 O8 and T1 G1.5 O4. It was possible that the amount of surfactant and cosurfactant in Formulation T1 G1.5 O6 were lower than the other formulations. Furthermore, the amount of water in this formulation was 25 % w/w, which was higher than other formulations. The water would reduce the viscosity of the formulation. Thus, the lowest viscosity was obtained in formulation T1 G1.5 O6.

Formulation T1 G1.5 O4 had higher amount of surfactant and cosurfactant than Formulation T1 G1.5 O8 and equal amount of water but its viscosity was lower than Formulation T1 G1.5 O8. This was attributed to the effect of internal phase. The amount of internal phase had an effect the viscosity of formulation. The greater volume of the internal phase or oil phase, the greater apparent viscosity was obtained (Lachman et al., 1986). Thus, Formulation T1 G1.5 O4, which had amount of oil two folds less than Formulation T1 G1.5 O8 exhibited lower viscosity.

Similar results were obtained in tween 80:glycerin system at the ratio of 1:1. Formulation T1 G1 O6 had less amount of surfactant and cosurfactant than Formulations T1 G1 O8 and T1 G1 O4 but the amount of water was higher. Thus, Formulation T1 G1 O6 produced the lowest viscosity. Formulation T1 G1 O4 had more amount of surfactants than Formulation T1 G1 O8 but viscosity of Formulation T1 G1 O4 was lower than Formulation T1 G1 O8. These results explained similar to the results of tween 80:glycerine system at the ratio of 1:1.5.

Among microemulsions in tween 80:polyethylene glycol 400 systems, the highest viscosity was obtained from formulations containing 8 % w/w oil. It decreased when the amount of oil in formulations decreased to 6 % w/w and 4 % w/w. It was concluded that the viscosity of formulation increased when increasing the volume of the internal phase.

Particle size of microemulsion was a prominent property normally used to characterize microemulsion. From TEM photomicrographs, the particle shape of microemulsion was mostly spherical. Particle size distribution was quite wide. The particle size of all formulations was in the size range of about 50-100 nm. This small particle was designated as microemulsion. Comparison of the mean particle diameter between before and after autoclaving showed that after autoclaving the mean particle diameter was significantly increased ($p<0.05$). Formulations in tween 80:glycerin systems showed two phase separations after autoclaving but recovered to one phase by gentle shaking. Accordingly, the new layer of surfactants film of microemulsions was reformed thereby the mean particle diameter of microemulsions was altered. Similarly, the mean particle diameter of formulations in tween 80:polyethylene glyclo400 systems showed an increased after autoclaving. Coalescence of some

particle might occur during autoclaving thereby the mean particle diameter increased after steam sterilization.

Comparison of tween 80:glycerin systems both before and after autoclaving at equal amount of oil, the mean particle diameter of microemulsions at the ratio of surfactant:cosurfactant 1:1 was smaller than that at ratio 1:1.5. Similarly, tween 80:polyethylene glycol 400 systems, the mean particle diameter of microemulsions at the ratio of surfactant:cosurfactant 1:0.5 was smaller than ratio 1:0.7. These results indicated that the mean particle diameter decreased with the increasing of the surfactant:cosurfactant ratio. This result was in accordance with the report that the addition of surfactant to the microemulsion systems decreased the amount of cosurfactant at the interfacial film. The interfacial film was thereby condensed and stable thus smaller particle was obtained. While the addition of cosurfactant caused the film to expand due to amount of cosurfactant that penetrated into the interfacial film increased therefore larger particle was obtained. Similar results were obtained from Attwood et al. (1992), Goa et al. (1998), and Kale and Allen (1989). Comparison between microemulsions containing polyethylene glycol 400 and glycerin as cosurfactant showed that the particle size of microemulsion containing polyethylene glycol 400 as cosurfactant at the ratio of 1:0.7 and 1:0.5 was smaller than microemulsion containing glycerin as cosurfactant at the ratio of 1:1.5 and 1:1. These results agreed with the mean particle diameter decreased with the increasing of the surfactant:cosurfactant ratio. Moreover, microemulsion using polyethylene glycol 400 as cosurfactant produced smaller particle than those using glycerin as cosurfactant. It was possible that molecule of polyethylene glycol 400 which penetrated between surfactant molecules increased the bending of surfactant film more than molecule of glycerin.

4. Drug loaded microemulsions

The obtained microemulsion systems contained soybean oil, tween 80 as surfactant, glycerin, polyethylene glycol 400 as cosurfactants could solubilize diazepam up to 10 mg/ml. This was an about 200 fold increased compared to its

solubility in water (0.05 mg/ml) (Macdonald et al., 1972). Commercially, the concentration of diazepam in hydroalcoholic preparation is 5 mg/ml. A total volume of 2 ml is usually injected either intramuscular or intravenous as a single dose. Diazepam loaded microemulsion could contain more drug.

The physical appearance of drug loaded microemulsions was similar to drug free microemulsions both before and after autoclaving. No precipitation of drug could be seen in all formulations. The content of diazepam in all formulations was in the range of 97.54 to 105.89% of labeled amount after autoclaving. The pH of microemulsion containing diazepam before autoclaving was mostly increased. This was due to the pK_a of diazepam (pK_a = 3.3) (Lund, 1994). The drug was a weak base. Some amount of drug could solubilize in surfactants and slightly soluble in water. And these surfactants were neutral molecules that could solubilize in water. Therefore diazepam which solubilized in these surfactants increased the pH of microemulsions. However, it had minute influence to the pH of microemulsions. After autoclaving, pH of all formulations was significantly decreased ($p<0.05$). The results were similar to drug free microemulsions as aforementioned.

The viscosity of microemulsions increased when diazepam was incorporated. As expected, addition of diazepam would increase the concentration of disperse phase due to its solubility in soybean oil. Therefore, the viscosity of formulations increased. Furthermore, when the amount of diazepam increased from 5 mg/ml to 10 mg/ml, the viscosity was markedly increased in all formulations as illustrated in Figure 110. The mean slope of formulations was 18.42. Consequently, the viscosity of microemulsion containing diazepam 10 mg/ml was higher than microemulsion containing diazepam 5 mg/ml.

The shape of microemulsions remained sphere. But wider distribution was obtained. The particle size of microemulsions containing diazepam of tween 80:glycerin systems showed no statistically significant difference ($p>0.05$). While in tween 80:polyethylene glycol 400 systems showed significant decreased ($p<0.05$). The following two possibilities of the decreasing of the particle size could be considered. First, a certain portion of undissolved drug could act as an emulsifying agent by the deposition of drug at the interface of microemulsions. Due to the

chemical structure of the drug contained polar group of carbonyl (C=O) in the molecule as seen in Figure 4. The polar group was oriented with the polar group at the interface of microemulsion amongst the polar head group of surfactants and the hydrophobic group buried inside the hydrophobic chains of surfactant as mentioned by Park and Kim, 1999. Therefore, drug increased the curvature of surfactant film. Consequently, the drug-loaded microemulsions had particle size smaller than drug-free microemulsions.

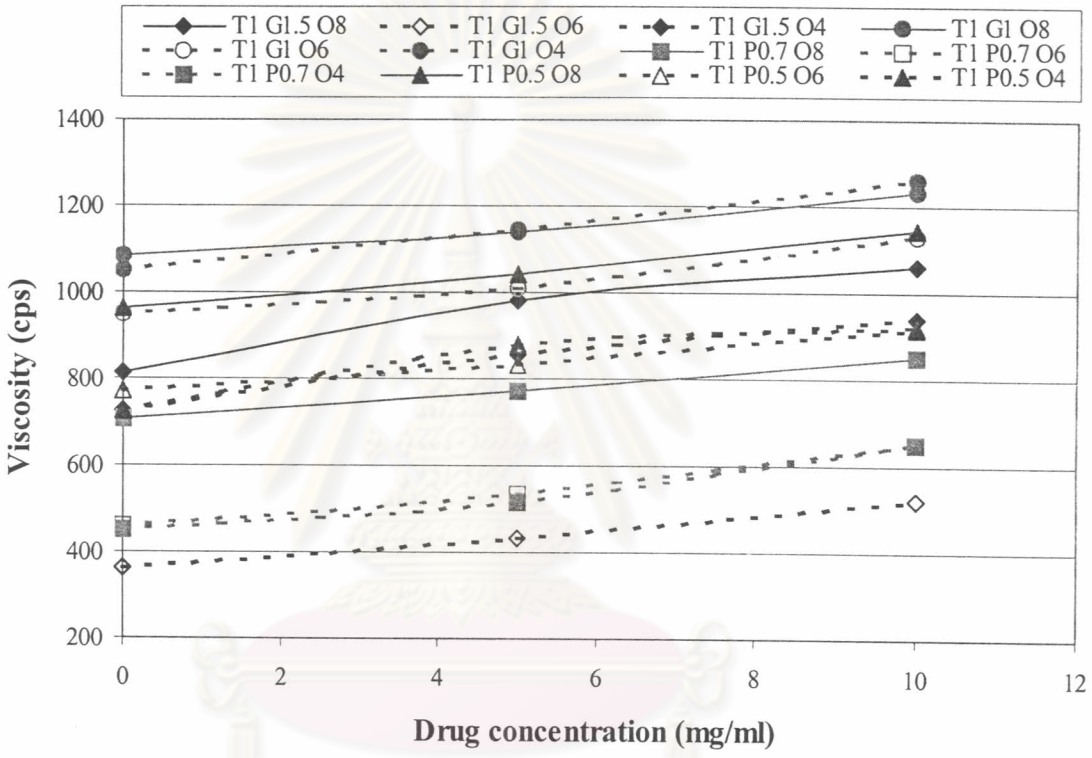

Figure 110 The viscosity of microemulsions.

In *in vitro* drug diffusion study, the mixture of 80% v/v of phosphate buffer pH 7.4 and 20% v/v of propylene glycol was used as diffusion medium. Due to high lipophilic drug (log P = 2.7) (Lund, 1994), the solubility of diazepam in the aqueous phase was very low. Large volumes were needed for the receptor phase. And this may cause difficulties in detection. The addition of organic solvents or surface active agents definitely improved the solubility of such drug in receptor phase (Benita and Levy, 1993; Washington, 1990).

The results of the diffusion studies indicated that the *in vitro* drug diffusion from microemulsions was lower than that from commercial product. The commercial product, diazepam injection (10 mg / 2 ml) was hydroalcoholic solution. The drug could directly diffuse through the dialysis membrane. The amount of drug diffusion at first 4 hours was high. After the fourth hour, the amount of drug diffusion gradually decreased. It was attributed to the decrease in the concentration of drug in donor part. Furthermore, increasing volume in donor compartment was observed during experiment due to the water went backward from the receptor compartment into donor compartment. Consequently, concentration gradient in donor part was decreased.

The drug diffusion from microemulsions was sustained more than 48 hours. The different amount of drug diffused from solution and from microemulsions could be attributed to the partition of the drug between the dispersed oily droplets and the continuous phase of the microemulsions. Two main processes governed the diffusion of drug from microemulsions. The transferring of drug from the disperse phase to the continuous phase and the diffusion of drug from the continuous phase through the membrane to the diffusion medium. Only the drug dissolved in the external aqueous phase was able to diffuse through the membrane. Thus, the diffusion of the drug through the membrane would be initially governed by the drug concentration in external aqueous phase of microemulsions (Trotta et al., 1989).

The *in vitro* drug diffusion study of each surfactant:cosurfactant systems showed that the amount of drug diffusion decreased when the amount of oil in formulation increased. This was attributed to the retention capacity of the dispersed oily droplets, the larger amount of which was able to sustained the drug release over longer period of time (Friedman and Benita, 1987). In this way a reservoir of the drug was produced and sustained release effect achieved as the drug continuously transfer from the oil droplets to continuous phase to replace drug diffusion from microemulsion. Furthermore, in each surfactant:cosurfactant systems the amount of drug diffusion from microemulsions containing diazepam at concentration of 10 mg/ml was higher than from microemulsions containing 5 mg/ml of diazepam at the equal amount of oil due to higher concentration gradient. Therefore, microemulsions containing high drug concentration could diffuse through dialysis membrane into

receptor compartment faster than microemulsions containing low drug concentration. However, the diffusion pattern of microemulsions both high and low drug concentration showed no statistically significant difference ($p>0.05$).

The viscosity and particle size of microemulsions may influence the amount of drug diffusion. Generally, low viscosity and small particle size would increase the amount of drug diffusion. However, the formulations containing drug 10 mg/ml have higher viscosity but the amount of drug diffusion was higher than the formulations containing lower amount of diazepam. It was concluded that concentration gradient had more prominent effect to the amount of drug diffusion than viscosity of microemulsions. Moreover, the formulations containing diazepam 10 mg/ml had large particle size and higher the amount of drug diffusion than the formulations containing diazepam 5 mg/ml. As expected, the particle sizes of the same formulation containing lower and higher concentration of drug were slightly changed in the range of 1-7 nm. But amount of drug in formulations was 2-fold difference. Thus, the amount of drug in formulations influenced to the amount of drug diffusion more than particle size in this experiment.

The dissolution kinetic of drug from microemulsions in each surfactant:cosurfactant system was mostly fitted with Weibull model and cube root model. Pharmaceuticals systems following the Weibull model indicated that there was no fundamental kinetic adequately to characterize the diffusion kinetic properties of the drug. In addition, there was no single parameter related with the intrinsic diffusion rate of drug (Costa and Lobo, 2001). However, the diffusion kinetic of drug from microemulsion was also fitted with the cube root model. This model normally applies to pharmaceutical dosage form such as tablet. When the model was used, it was assumed that the release rate was limited by the drug particles dissolution rate not by the diffusion (Costa and Lobo, 2001). To apply this model to microemulsions, it assumed that internal oil droplets of microemulsion were drug particles. Thus, cube root model was appropriate to explain the diffusion kinetic of drug in this experiment because the amount of drug diffusion from microemulsions depended on drug diffusion from internal oily phase to external aqueous phase.

After stability testing under accelerated condition, the microemulsions of tween 80:glycerin systems showed phase separation but recovered to the one-phase microemulsion by gently shaking while the microemulsions of tween 80:polyethylene glycol 400 systems showed good appearance. The pH and refractive index before and after stability testing was not significantly different ($p>0.05$) and remained in the pH range of 6 to 8. This result indicated that degradation of oil component did not occur during stability testing. Particle shape of microemulsions after accelerated stability testing was spherical. In addition, the particle size was significant increased ($p<0.05$). Although particle size of microemulsions increased but they remained below 100 nm. Furthermore, particle size of microemulsions showed less influenced on the amount of drug diffusion than concentration of drug in formulations. Generally, pharmacopoeia accept preparation contained not less than 90.0 percent and not more than 110.0 percent of the labeled amount of drug (USP24/NF19, 2000). In this study, the content of diazepam remained within the range of pharmacopoeia acceptance after stability testing. This indicated that o/w microemulsion systems could protect drug from degradation. Furthermore, enhancing both solubility and stability of the drug was obtained.

Conclusions

To date microemulsions are shown to be able to protect labile drug, control drug release, increase drug solubility, high stability and simplicity of manufacture. Furthermore, it has proven possible to formulate preparations suitable for most routes of administration. In this study, microemulsions could be produced when using commercially available and parenteral acceptable compounds. Type and ratio of surfactant:cosurfactant influenced the formability and existing region of microemulsions. Dilution test and dye solubility test were simultaneously employed to determine their types. The systems composed of soybean oil, tween 80 as surfactant, glycerin or polyethylene glycol 400 as cosurfactant and water for injection could form o/w microemulsions. Glycerin as cosurfactant could form microemulsion better than polyethylene glycol 400. The surfactant:cosurfactant ratio influenced the area of microemulsion in pseudo-ternary phase diagram by the area of microemulsion

increased with increasing the ratio of surfactant:cosurfactant. Moreover, the molecular volume of soybean oil might influence to the area of microemulsions.

The physical appearance of drug free microemulsions before autoclaving showed good appearance. After autoclaving, microemulsions containing glycerin as cosurfactant showed phase separation but they were recovered to one-phase microemulsion by gently shaking while the microemulsions containing polyethylene glycol 400 as cosurfactant showed good appearance. The pH of microemulsions was statistically decreased ($p<0.05$) after autoclaving but it remained in the range 6-8 that allowed physiological compatibility. However, the viscosity of microemulsions was high. It was increased when increasing the amount of oil in formulations. The TEM photomicrographs showed spherical particles. The mean droplet diameter of microemulsion was in nanometer size range 50-100 nm. And the surfactant to cosurfactant ratio had influence on the mean droplet diameter of microemulsion. The droplet size decreased with increasing the surfactant to cosurfactant ratio. After autoclaving, the mean droplet diameter was statistically increased ($p<0.05$).

The solubility of diazepam in o/w microemulsion increased up to 10 mg/ml. This is 200 fold increased, compared with the diazepam solubility in water. The physical appearance of drug loaded microemulsions was similar to drug free microemulsions both before and after autoclaving. In addition there was no precipitation of drug in all formulations. The pH was mostly increased when the drug was incorporated. After autoclaving, the pH of all formulations was decreased. The viscosity of microemulsions with drug was also increased. Furthermore, when the amount of drug increased from 5 mg/ml to 10 mg/ml, the viscosity markedly increased. The particle shape of microemulsion containing drug remained sphere. The particle size showed no significant changed ($p>0.05$) in tween 80:glycerin systems while tween 80: polyethylene glycol 400 systems showed significant decreased ($p<0.05$) when compare to drug free microemulsions. In an *in vitro* evaluation, the amount of drug diffusion from microemulsions was lower than that from commercial product. The drug diffusion from microemulsions was sustained more than 48 hours. Each surfactant:cosurfactant systems showed that the amount of drug diffusion increased when the amount of oil in formulations decreased. In addition, the amount of drug release with high drug concentration in microemulsions

was higher than the low drug concentration but the release pattern was no significantly difference ($p > 0.05$). The drug diffusion kinetic was the best fitted with Weibull model. Whereas the cube root model had high correlation coefficiency of more than 0.98. The amount of drug diffusion from microemulsions depended on drug diffusion from internal oily phase to external aqueous phase. After accelerated stability testing, the microemulsion of tween 80:glycerin systems showed phase separation but recovered to one-phase microemulsion by gently shaking whereas the microemulsion of tween 80:polyethylene glycol 400 systems showed good appearance. Particle size of microemulsions significantly increased ($p<0.05$) but it remained below 100 nm. And the content of diazepam remained within the range of pharmacopoeia acceptable. Consequently, it was concluded that this microemulsion system could be used as a parenteral drug carrier for lipophilic drugs, provided that enhanced the solubility and stability of diazepam. Furthermore, the sustained release of drug was obtained.