CHAPTER IV

RESULTS AND DISCUSSION

1. The preparation of micelle systems

A series of surfactants (Tween[®] 20, Tween[®] 80, Solutol[®] HS15 and Cremophor[®] EL) and cosurfactants (glycerol, propylene glycol, Palsgaard[®] 0093 and Imwitor[®] 308) were used to investigate the ability to form micellar solution. The weight ratios of surfactant to cosurfactant at 4:1, 2:1 and 1:1 at various concentrations from 10% to 90% w/w were studied. The systems, which were clear solution at every concentration investigated, were classified as micelle solutions. The result was shown that the mixtures consisted of Palsgaard[®] 0093 as cosurfactant were milky emulsion and highly viscous and failed to form micellar solution with all surfactants investigated. It was suggested that Palsgaard[®] 0093 was long chain monoglyceride and had highly melting points about 67^0C, at which room temperature it was white powder. The mixtures using Imwitor[®] 308 and all surfactants at 2:1 and 1:1 weight ratios of surfactant to cosurfactant were turbid except for formulation containing Tween[®] 20 to Imwitor[®] 308 and Tween[®] 80 to Imwitor[®] 308 weight ratio of 2:1 as well. The systems using glycerol and propylene glycol as cosurfactant were readily formed

micellar solution. The systems listed in Table 4 were able to produce micellar solution and selected for preparing oil-in-water (O/W) microemulsions. The appearance of this investigation is shown in Appendix B.

Table 4. The formulations producing micellar solution

Surfactant	Cosurfactant	Weight Ratio of surfactant: cosurfactant
Tween® 20	-	-
	Glycerol	4:1
		2:1
		1:1
	Propylene glycol	4:1
		2:1
		1:1
	Imwitor® 308	4:1
		2:1
Tween® 80	-	-
	Glycerol	4:1
		2:1
		1:1
	Propylene glycol	4:1
		2:1
		1:1
	Imwitor® 308	4:1
		2:1
Solutol® HS15	-	-
	Glycerol	4:1
		2:1
		1:1
	Propylene glycol	4:1
		2:1
		1:1

Table 4. The formulations producing micellar solution (continued)

Surfactant	Cosurfactant	Weight ratio of surfactant: cosurfactant
Solutol® HS15	Imwitor® 308	4:1
Cremophor® EL	-	-
	Glycerol	4:1
		2:1
		1:1
	Propylene glycol	4:1
		2:1
		1:1
	Imwitor® 308	4:1

2. Study of phase behavior of microemulsions

The long chain triglyceride, soybean oil, was attempted to use as an oil phase to form microemulsion. However, no soybean oil could be incorporated into all micellar systems to form microemulsion. The mixture became an emulsion. In contrast, the medium chain, isopropyl myristrate (IPM), showed an ability to be solubilized in the micellar systems investigated. This was suggested that the difference in lipophilic chain length of oil effected the ability of oil penetration into surfactant interfacial film. The same result was found for alkane (C_nH_{2n+2}) oils in that the greater oil penetration of alkane with decreased the alkyl chain length of oil (Monduzzi et al, 1997). Such

soybean oil was too large molecule to easily penetrate into surfactant film compared to isopropyl myristate, which was medium chain length oil.

The area of microemulsion existences using nonionic surfactant (as previously described) and cosurfactant (glycerol, propylene glycol or Imwitor® 308) at 4:1, 2:1 and 1:1 weight ratios of surfactant to cosurfactant, isopropyl myristate (IPM) and distilled water, after one month storage, were obtained (Figures 12-24). The percentages of maximum oil solubilization and microemulsion region are shown in Table 5. The phase diagrams of microemulsion observed after preparation for 24 hrs and one week are shown in Appendix B. The areas of microemulsion existence with the time of determination, 24 hrs, 1 week and 1 month were similar. However,there was slightly difference observed in systems using Tween® 80 and Solutol® HS15 as surfactant with some cosurfactants.

Figure12 shows the phase diagrams of microemulsions containing nonionic surfactant with the absence of cosurfactant. The result was emphasized the ability of surfactants (Tween® 20, Tween® 80, Solutol® HS15 and Cremophor® EL) to form microemulsion. The system containing Tween® 20, IPM and water produced the largest microemulsion area (16%) compared to other surfactants. There was no gel formation observed when using Tween® 20 as surfactant. The difference in microemulsion area of phase behavior in Figure 12 could relate to the difference in

Table 5. The percent of area of microemulsion existence, isotropic area, gel area and maximum oil solubilization

of T 20, T 80, Sol and Cre with various cosurfactant

Surfactant	Cosurfactant	Weight ratio of surfactant:cosurfactant	Isotropic area (%)	Isotropic gel area (%)	Microemulsion area (%)	Maximum oil solubilization (%)
T20	none	none	16	0	16	20
	Gly	4:1	13	0	13	20
		2:1	7	0	7	10
		1:1	5	0	5	20
	PG	4:1	13	0	13	20
		2:1	6	0	6	10
		1:1	3	0	3	5
	Im	4:1	22	0	22	20
		2:1	36	0	36	30
		1:1	ND	ND	ND	ND
T 80	none	none	6	2	4	10
	Gly	4:1	4	1	3	30
		2:1	10	1	9	30
		1:1	3	0	3	20
	PG	4:1	23	1	22	40

ND = not determineed; microemulsion not found

Table 5. The percent of area of microemulsion existence, isotropic area, gel area and maximum oil solubilization

of T 20, T 80, Sol and Cre with various cosurfactant (continued)

Surfactant	Cosurfactant	Weight ratio of surfactant:cosurfactant	Isotropic area (%)	Isotropic gel area (%)	Microemulsion area (%)	Maximum oil solubilization (%)
T 80	PG	2:1	18	0	18	20
		1:1	9	0	9	20
	Im	4:1	23	0	23	50
		2:1	33	0	33	60
		1:1	ND	ND	ND	ND
Sol	none	none	10	1	9	50
	Gly	4:1	6	0	6	60
		2:1	2	1	1	5
		1:1	0	0	0	0
	PG	4:1	24	0	24	40
		2:1	23	0	23	30
		1:1	17	0	17	30
	Im	4:1	34	0	34	30
		2:1	ND	ND	ND	ND
		1:1	ND	ND	ND	ND

ND = not determineed; microemulsion not found

Table 5. The percent of area of microemulsion existence, isotropic area, gel area and maximum oil solubilization

of T20, T 80, Sol and Cre with various cosurfactant (continued)

Surfactant	Cosurfactant	Weight ratio of surfactant:cosurfactant	Isotropic area (%)	Isotropic gel area (%)	Microemulsion area (%)	Maximum oil solubilization (%)
Cre	none	none	14	2	12	20
	Gly	4:1	15	1	14	60
		2:1	5	2	3	5
		1:1	3	0	3	40
	PG	4:1	23	4	19	30
		2:1	17	2	15	20
		1:1	15	0	15	20
	Im	4:1	38	0	38	50
		2:1	ND	ND	ND	ND
		1:1	ND	ND	ND	ND

ND = not determineed; microemulsion not found
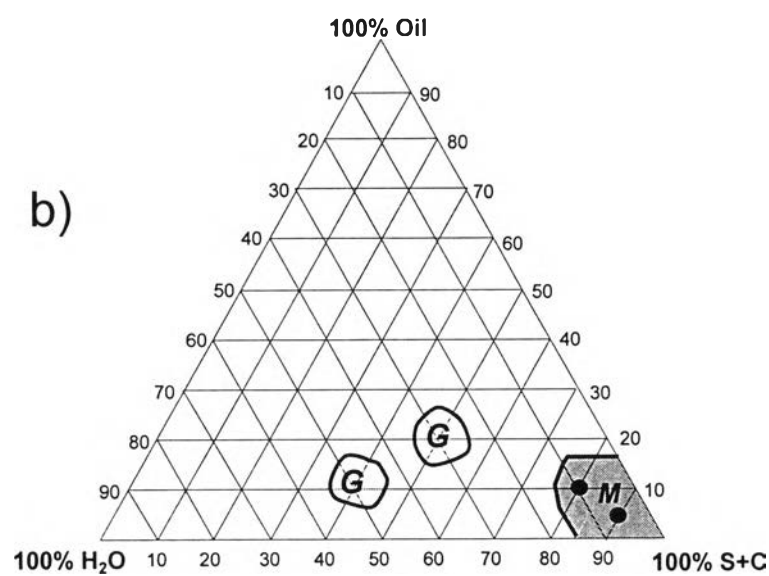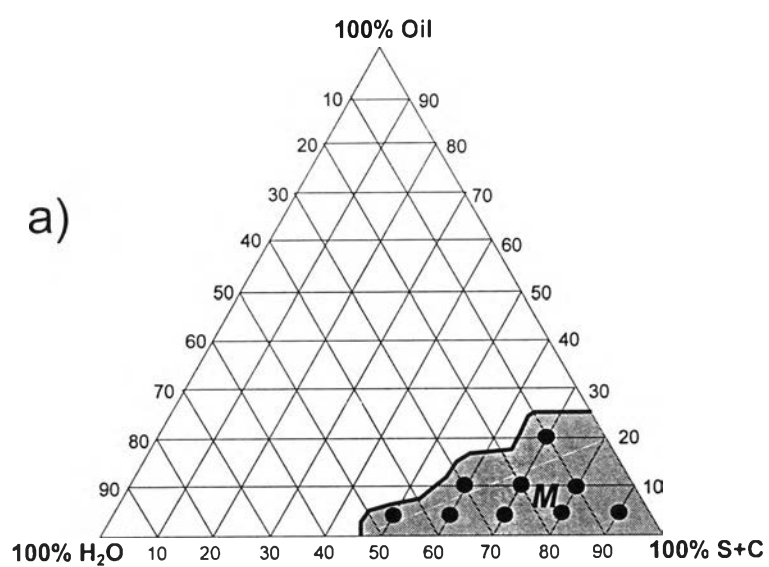
Figure 12 . Pseudo-ternary phase diagram of surfactant / IPM / water microemulsion

a) IPM / T20 / water   b) IPM / T80 / water

G = Gel,   M = Microemulsion

● Represented the clear solution
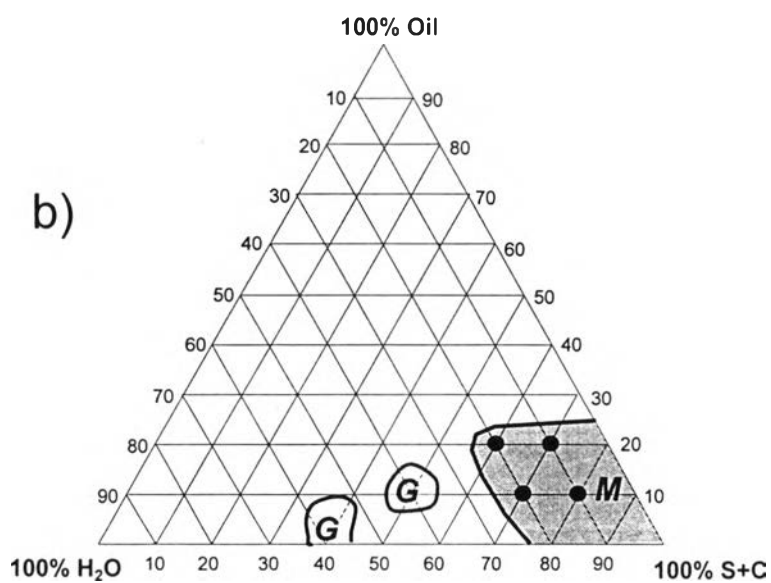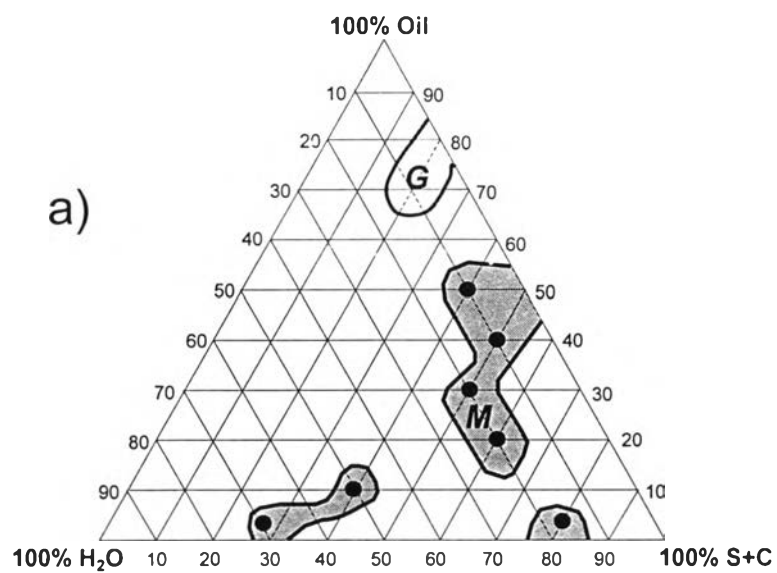
Figure 12 . Pseudo-ternary phase diagram of surfactant / IPM / water microemulsion

(continued)

a) IPM / Sol/ water   b) IPM / Cre/ water

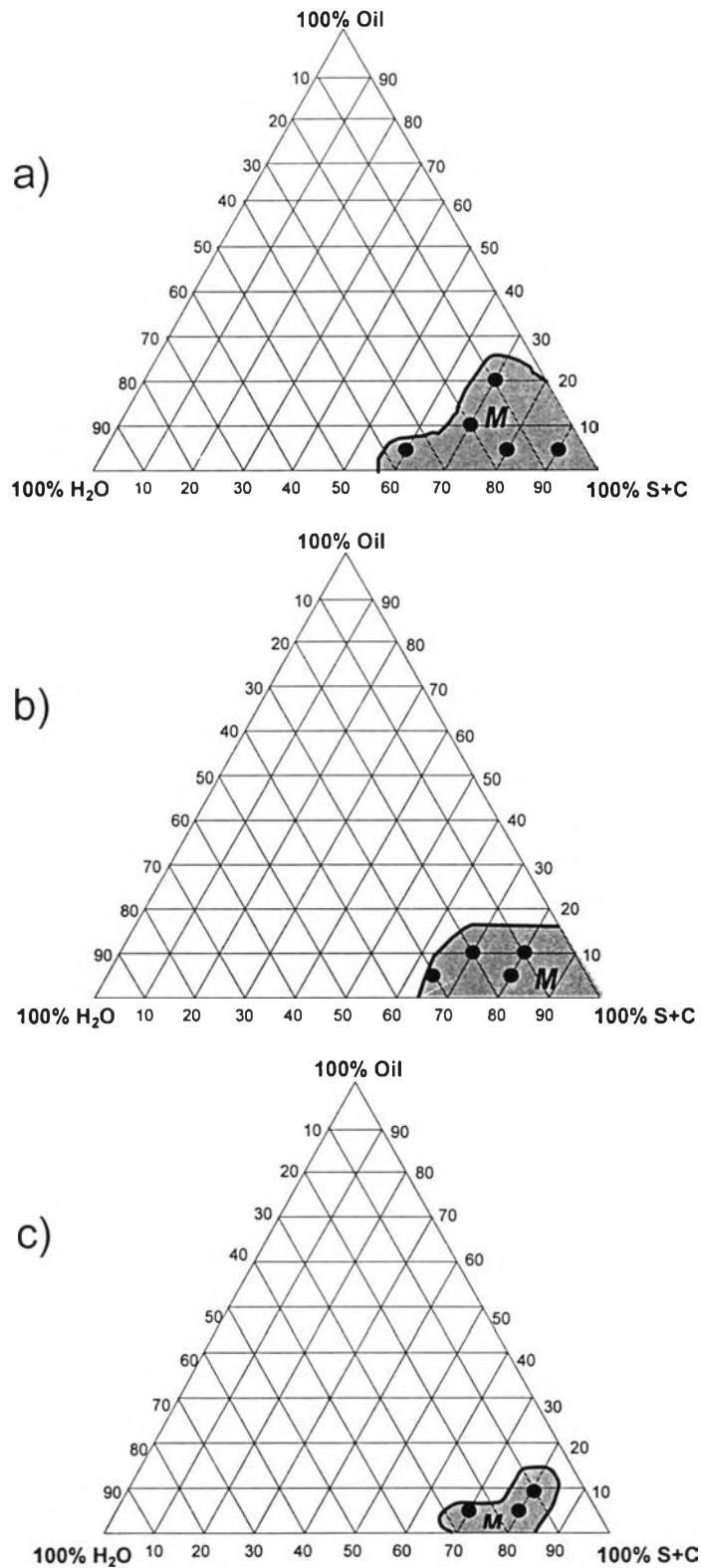G = Gel,   M = Microemulsion

● Represented the clear solution

Figure 13.   Pseudo-ternary phase diagram of IPM /T20 / Gly / water

system at 4:1(a), 2:1(b) and 1:1(c) weight ratio of surfactant to cosurfactant

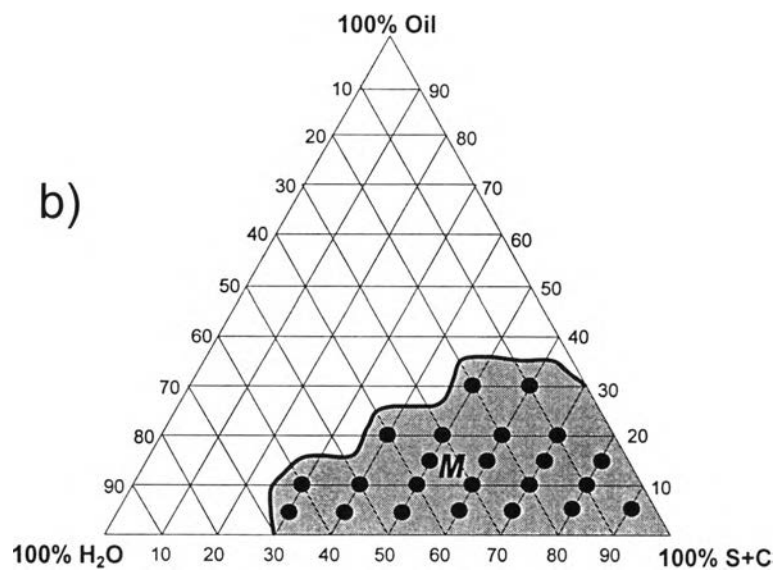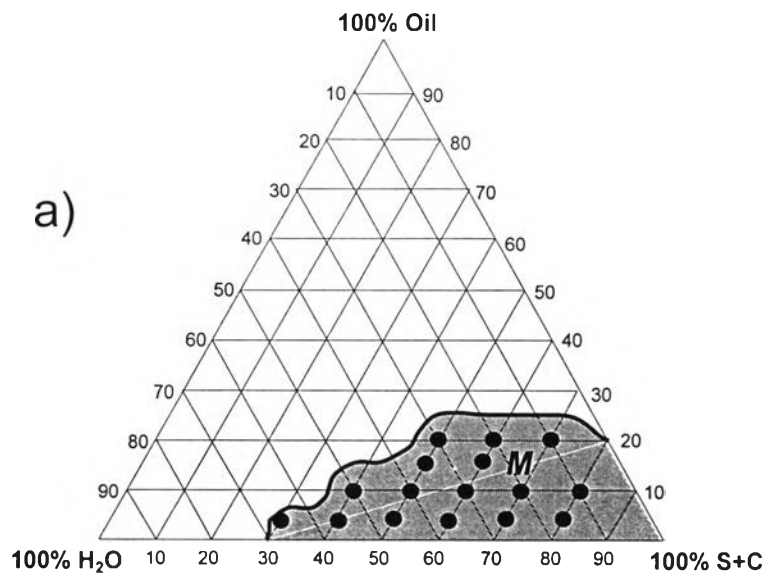G = Gel,   M = Microemulsion

● Represented the clear solution
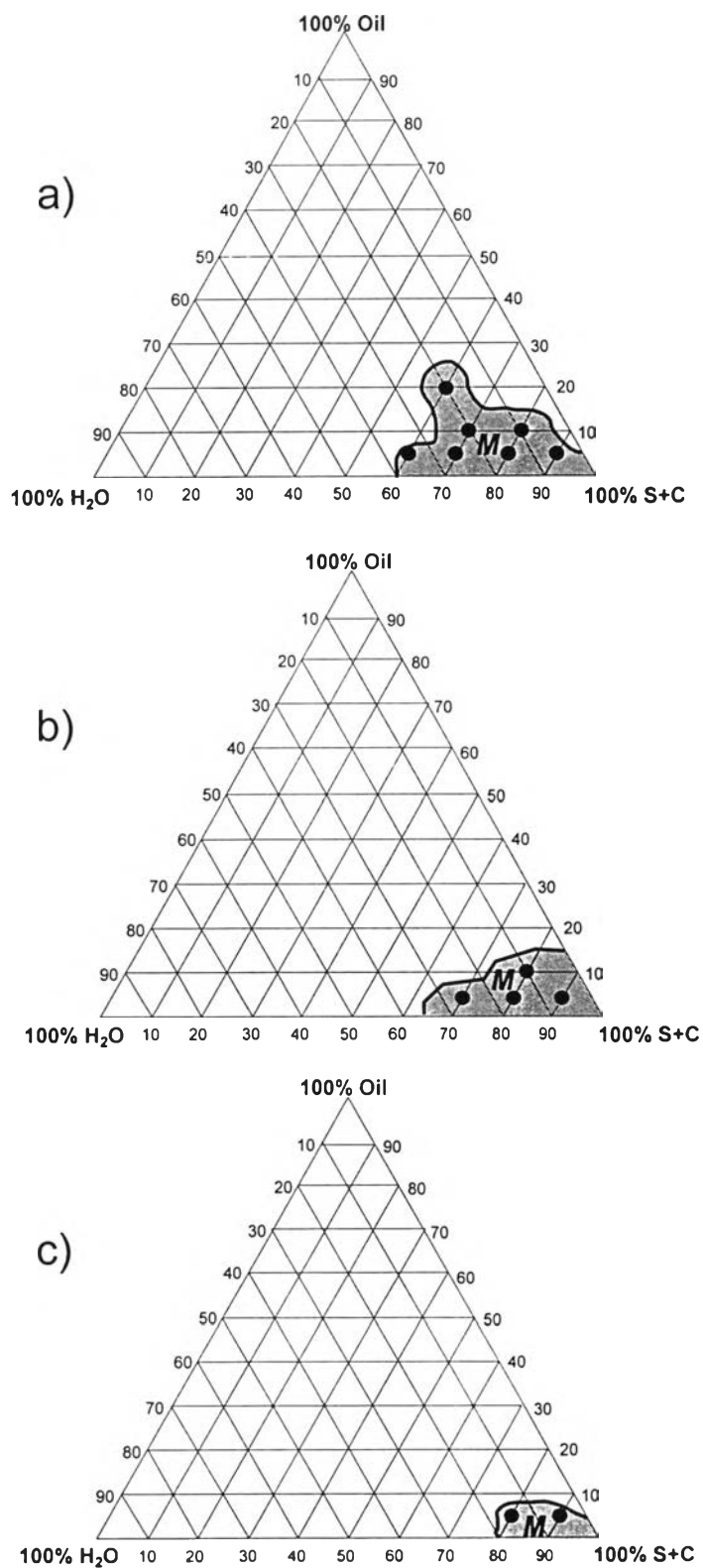
Figure 14. Pseudo-ternary phase diagram of IPM / T20 / PG / water

system at 4:1(a), 2:1(b) and 1:1(c) weight ratio of surfactant to cosurfactant

G = Gel,   M = Microemulsion

● Represented the clear solution

Figure 15. Pseudo-ternary phase diagram of IPM / T20 / Im/ water system at 4:1(a) and 2:1(b) weight ratio of surfactant to cosurfactant

G = Gel,   M = Microemulsion
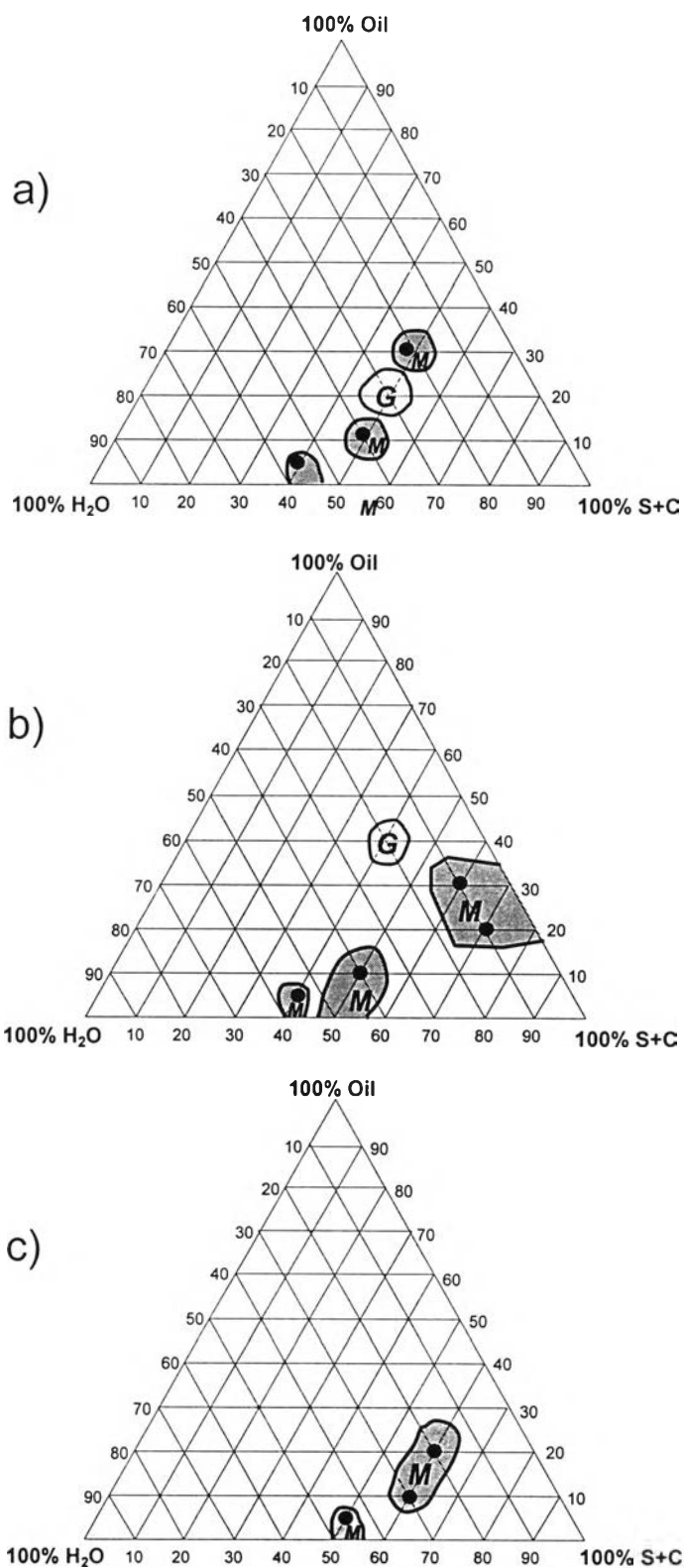
● <u>Represented the clear solution</u>

Figure 16. Pseudo-ternary phase diagram of IPM / T80 / Gly / water

system at 4:1(a), 2:1(b) and 1:1(c) weight ratio of surfactant to cosurfactant

G = Gel,   M = Microemulsion
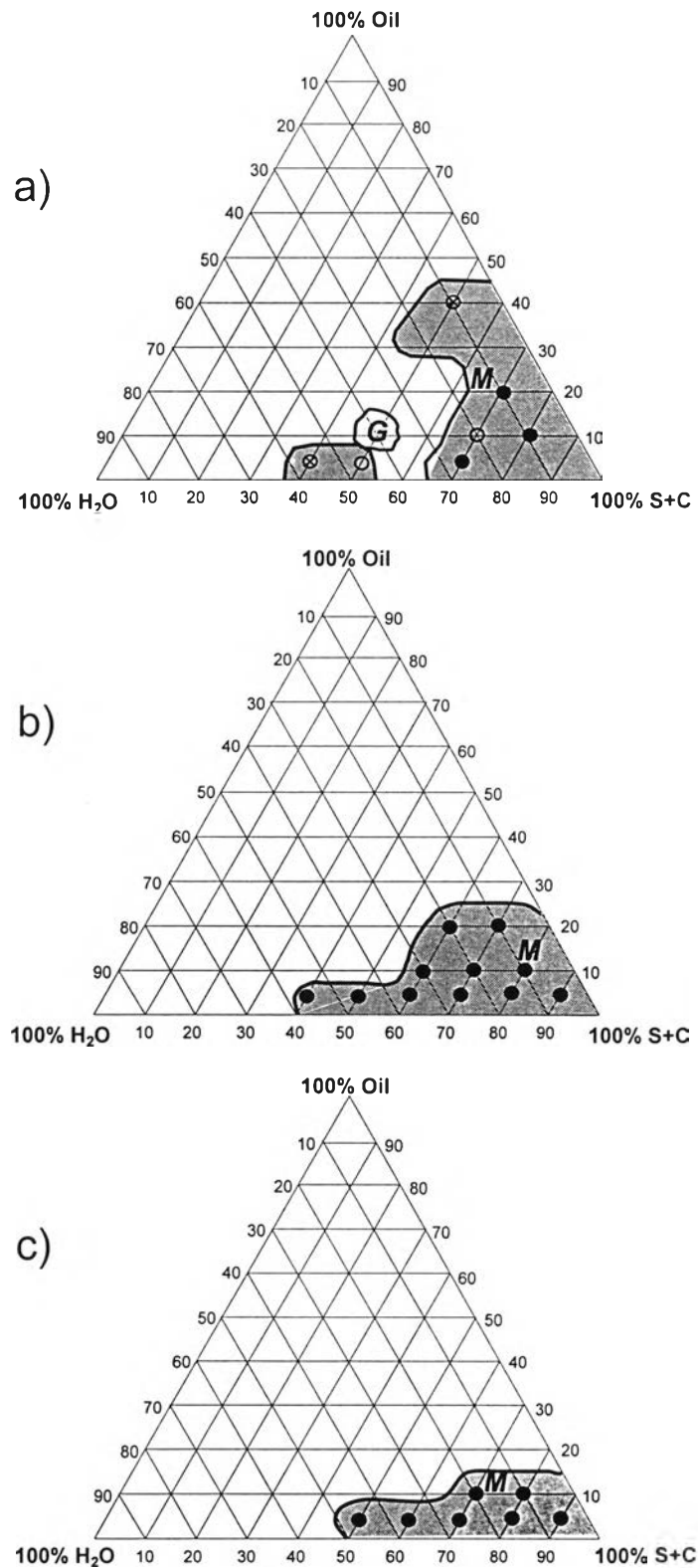
● Represented the clear solution

Figure 17. Pseudo-ternary phase diagram of IPM / T80 / PG / water

system at 4:1(a), 2:1(b) and 1:1(c) weight ratio of surfactant to cosurfactant

G = Gel,   M = Microemulsion
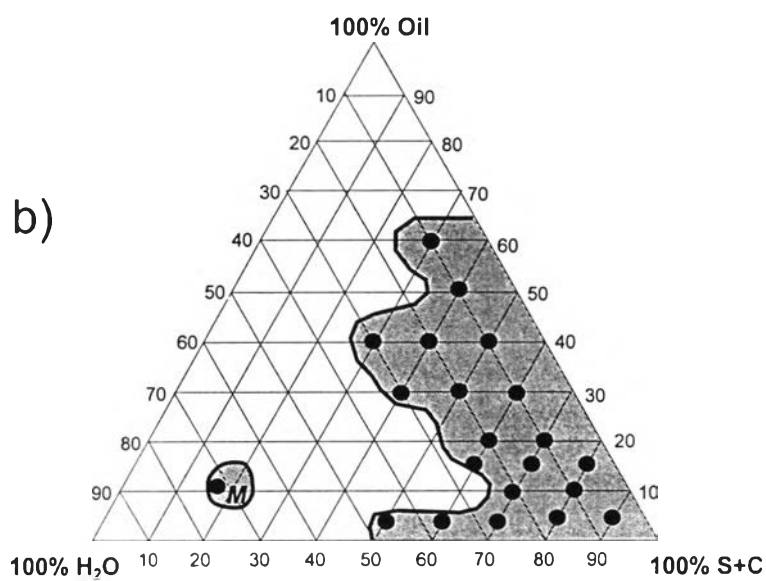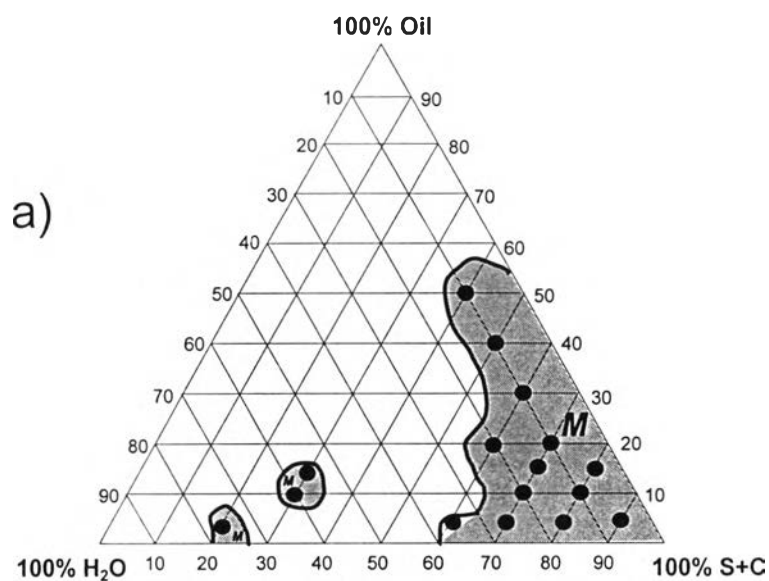
● Represented the clear solution

Figure 18. Pseudo-ternary phase diagram of IPM / T80 / Im / water

system at 4:1(a) and 2:1(b) weight ratio of surfactant to cosurfactant

G = Gel,   M = Microemulsion
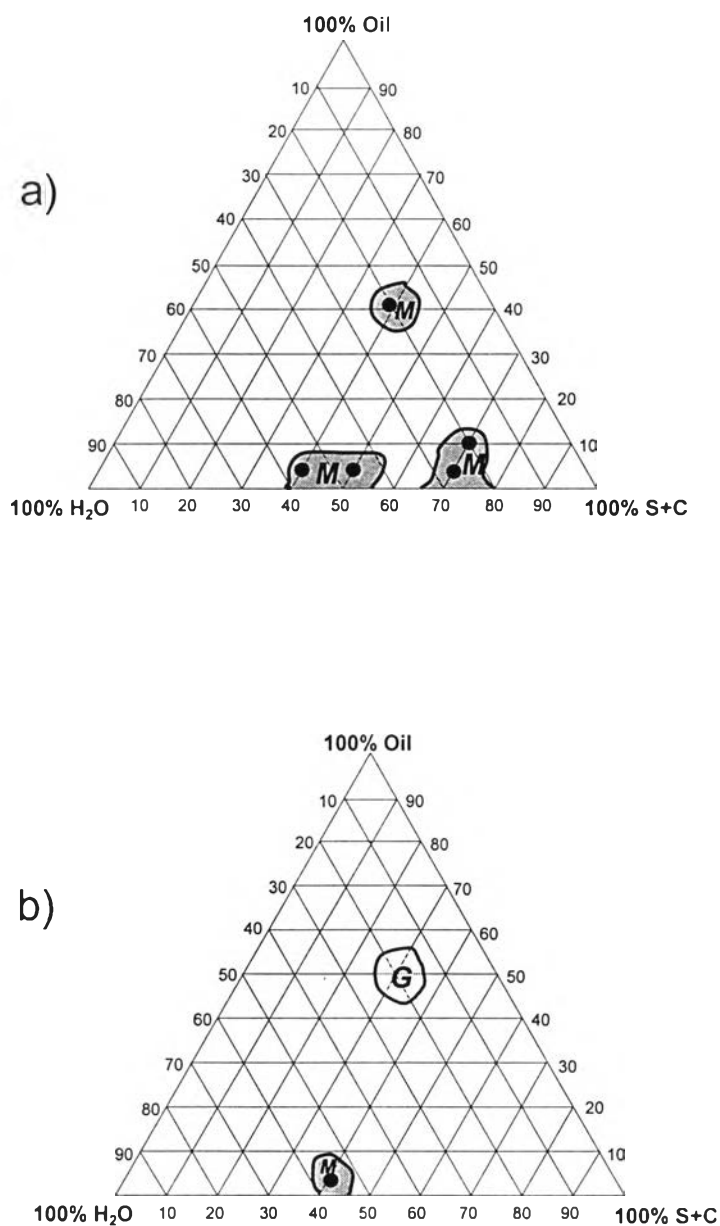
● Represented the clear solution

Figure 19.   Pseudo-ternary phase diagram of IPM / Sol/ Gly/ water

system at 4:1(a),and   2:1(b)weight ratio of surfactant to cosurfactant

G = Gel,   M = Microemulsion
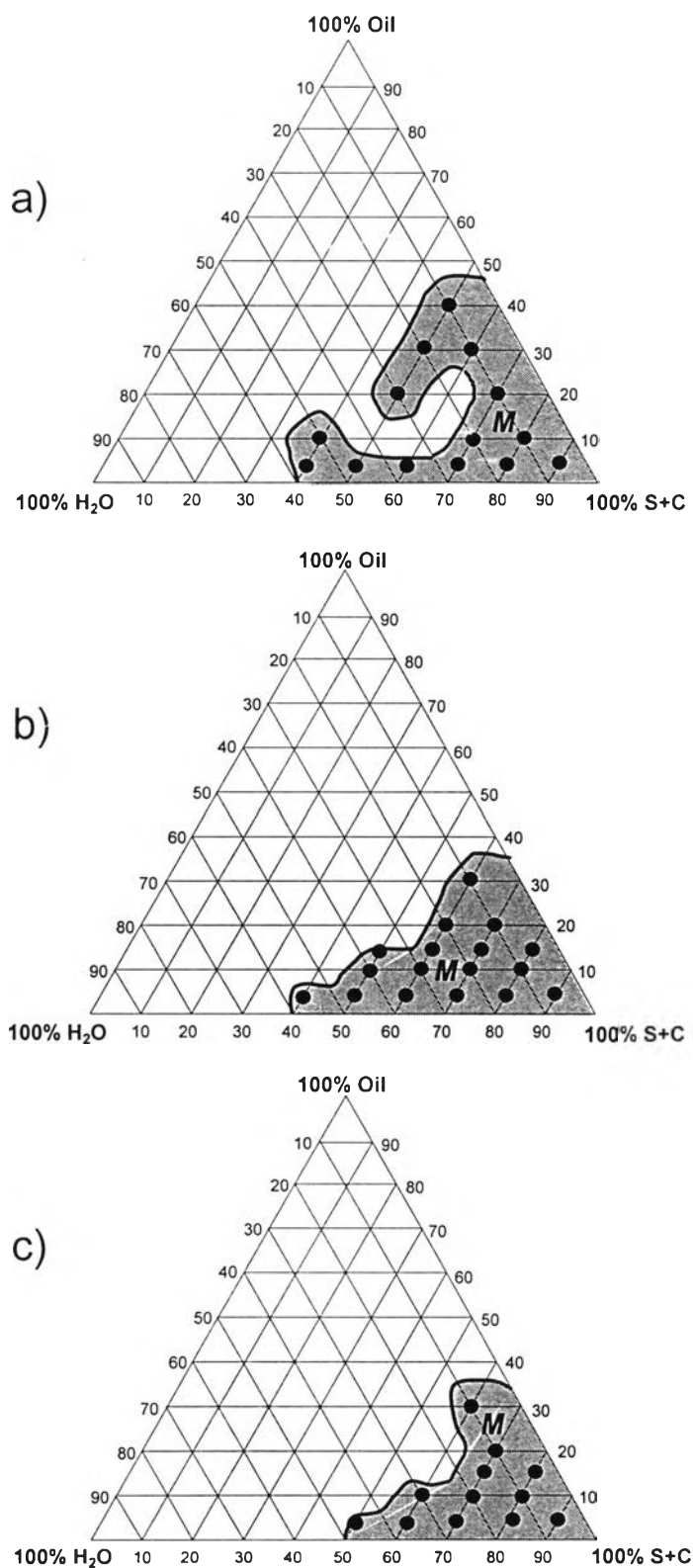
● Represented the clear solution

Figure 20. Pseudo-ternary phase diagram of IPM / Sol/ PG / water

system at 4:1(a), 2:1(b) and 1:1(c) weight ratio of surfactant to cosurfactant

G = Gel,   M = Microemulsion

● Represented the clear solution

Figure 21. Pseudo-ternary phase diagram of IPM /Sol/ Im/water

system at 4:1 weight ratio of surfactant to cosurfactant

G = Gel,   M = Microemulsion

● Represented the clear solution

Figure 22. Pseudo-ternary phase diagram of IPM /Cre/Gly / water system at 4:1(a), 2:1(b) and 1:1(c) weight ratio of surfactant to cosurfactant

G = Gel,　M = Microemulsion

● <u>Represented the clear solution</u>

Figure 23. Pseudo-ternary phase diagram of IPM/Cre/PG/water

system at 4:1(a), 2:1(b) and 1:1(c) weight ratio of surfactant to cosurfactant

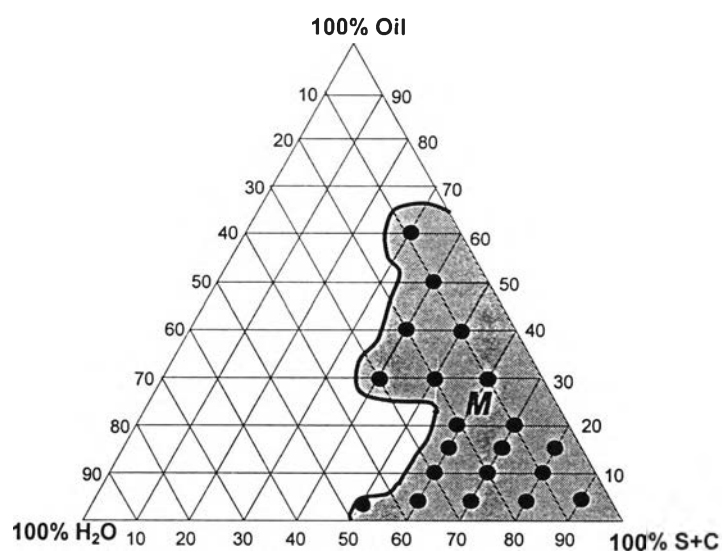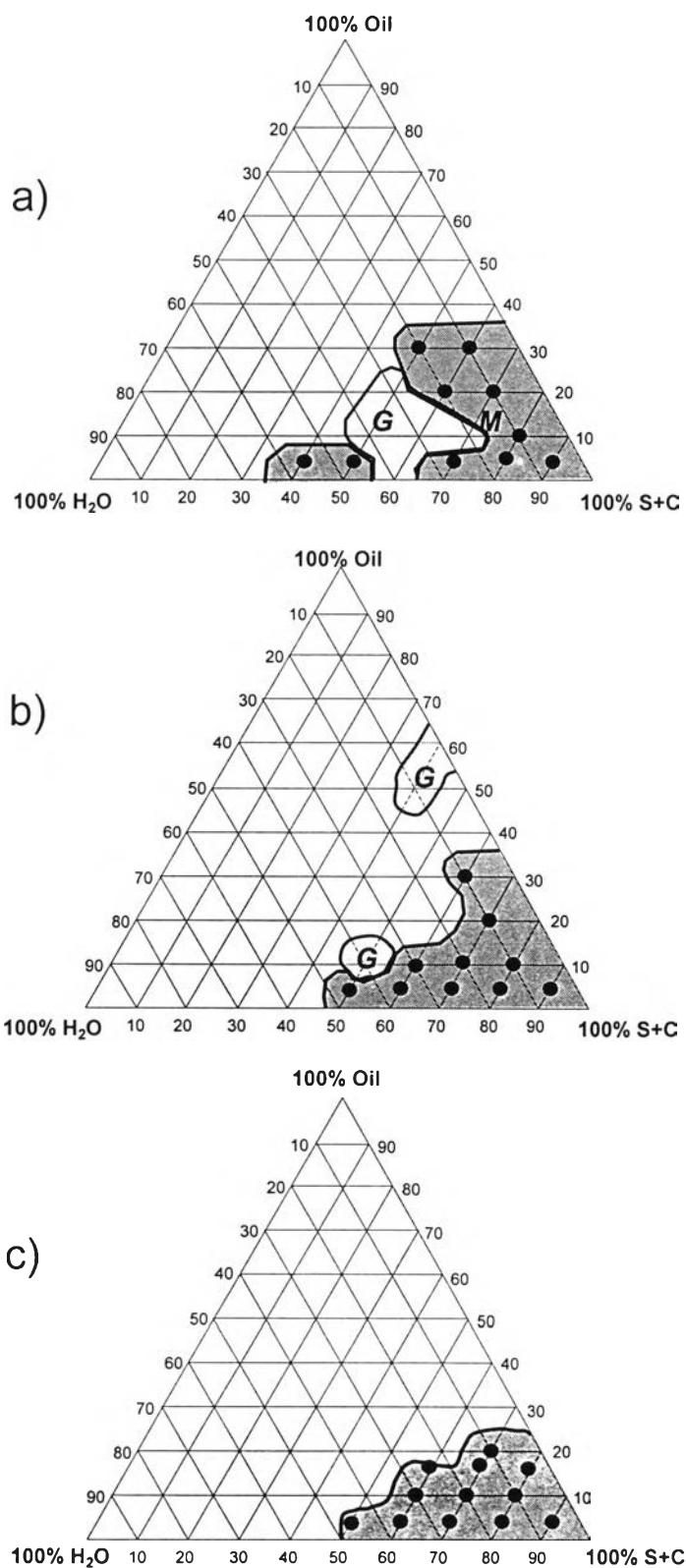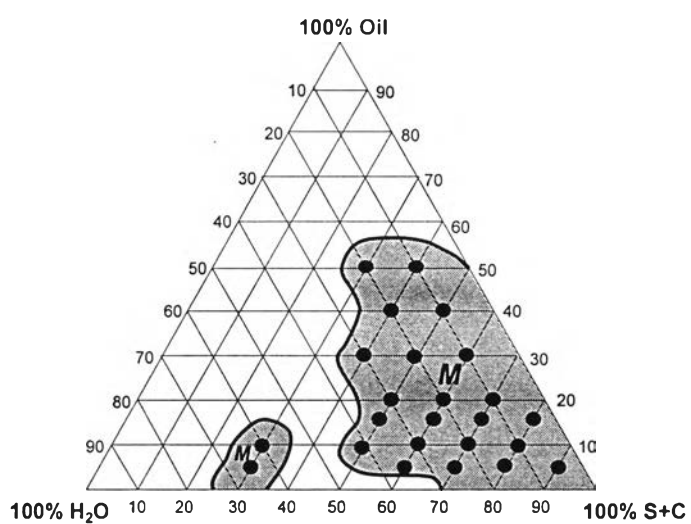G = Gel,   M = Microemulsion

● Represented the clear solution

Figure 24. Pseudo-ternary phase diagram of IPM / Cre/ Im/ water

system at 4:1 weight ratio of surfactant to cosurfactant

G = Gel,   M = Microemulsion

● __Represented the clear solution__

structure and HLB value of surfactant. Tween® 20 and Tween® 80 are polyethylene sorbitan fatty acid esters, which HLB are 16.7 and 15 respectively. Conversely, Solutol® HS15 and Cremophor® EL are polyethylene glycol derivatives with HLB in between 12-14. The HLB value describes the relative polarity of the surfactant and may indicate the extent of favorable for surfactant migrating into the o/w interface. It seemed that all surfactants could migrate into the interface to reduce interfacial tension to lower level to form microemulsion. In present study, the microemulsion areas of Solutol® HS15 (9%) and Cremophor® EL (12%) microemulsion systems were slightly different. While the area of Tween® 20 microemulsion (16%) were four times higher than that of Tween® 80 microemulsion (4%) even they had the similar HLBs. The result could suggest that HLB might not be a suitable guideline for determination of microemulsion formation. In addition, CPP of surfactant might be another influencing factor to be considered in terms of its effect on the curvature and fluidity of interface. The CPP basically depends upon the relative size of hydrophilic and the hydrophobic group. However, the CPP could be changed with several factors such as oil penetration, temperature. So, it was not well mentioned to use the CPP alone to study the formation of microemulsion (Warisnoicharoen, Lansley and Lawrence, 2000). Moreover, the oil which is smaller than hydrophobic chain of the surfactant is possible to be solubilized; however, this rule was not true in some systems.

Figures 13-15 show the pseudo ternary phase diagrams of system containing

Tween® 20, IPM, water and cosurfactant; namely, glycerol (Figure 13), propylene

glycol (Figure 14) or Imwitor® 308 (Figure 15) at 4:1, 2:1 and 1:1 weight ratio of

surfactant (s) to cosurfactant (c). A cosurfactant molecule was highly incorporated into

surfactant film leading to a decrease in moduli and an increase in the volume of the

amphiphilic unit of the interfacial film. Some cosurfactant; such as ethanol, can

increase the HLB of surfactant by decreasing the hydrophilicity of aqueous phase

promoting the formation of microemulsion of surfactant with lower HLB (Lawrence,

1996). So, the use of cosurfactant was in favor of microemulsion formation (Garti et al.,

2001). In systems using glycerol or propylene glycol and Tween® 20, microemulsion

areas decreased from 13% to 5% and 13% to 3%, respectively, when the ratio of

surfactant to cosurfactant decreased from 4:1 to 1:1 (Table 5). In other words, it could

be that an increase in the cosurfactant volume had no effect on increasing the area of

the microemulsion. It was surprising that upon addition of glycerol or propylene glycol

in microemulsion using Tween® 20, area of microemulsion existence was lower than

the corresponding microemulsion without any cosurfactant. In contrast, when Imwitor®

308 was added, the dramatically higher areas of microemulsion existence were obtained

compared to microemulsion formed using the other cosurfactants. Moreover, when the

weight ratio of Tween[®] 20 to Imwitor[®] 308 decreased, the area of microemulsion existence increased approximately 14 % (from 22 % for 4:1 up to 36 % for 2:1).

Compared among the cosurfactants studied, system containing [®] 20 exhibited highest area of microemulsion and oil solubilization when using Imwitor[®] 308. Using glycerol as cosurfactant, the microemulsion area observed at 2:1 and 1:1 weight ratio of Tween[®] 20 to glycerol was higher than the same systems using propylene glycol. This could be explained by the difference in structure of cosurfactant. Glycerol and propylene glycol are polyhydric alcohol; however, glycerol has three of hydroxyl groups in molecule, while propylene glycol has two hydroxyl groups. The hydroxyl group is hydrophilic and likes to dissolve in water so the glycerol prefers to locate in aqueous phase than at the interface resulting in less ability to reduce the interfacial tension and effectively change the CPP. The result was similar to the study of Aboofazeli et al. (1994) in that the differences in the phase diagram were produced by the distribution of alcoholic cosurfactant between the three domains, namely, the aqueous phase, oil phase and interfacial film. Another point was reported that branched chain alcohol had a higher propensity to be located in aqueous phase, so large amounts were necessary in order to lower the interfacial tension.

The number of cosurfactant molecules located at the interface isreduce as the weight ratio increases. For Tween[®] 20 microemulsion, the lower weight ratio of

surfactant to glycerol or propylene glycol increased the microemulsion existence showing that a limited amount of these hydrophilic cosurfactant was required to form microemulsion with hydrophilic surfactant.

In system containing Tween® 80 and glycerol, area of microemulsion existence at 2:1 weight ratio of Tween® 80 to glycerol was higher than system without cosurfactant added and system with higher amount of glycerol was increased. However, at 4:1 and 1:1 weight ratios of surfactant to cosurfactant, the microemulsion areas were not seemed to be much increased (Figure 16). However, the isotropic gel region was 1 % lower in the presence of glycerol because the cosurfactant could penetrate into surfactant interfacial film and then destroyed gel structure (El-Nokaly, Hiler and McGrady, 1991). The areas of microemulsion existence of systems containing Tween® 80 and propylene glycol were the same as the corresponding systems using Tween® 20 in that an increase in microemulsion region was found with increasing concentration of propylene glycol (Figure 17). All of the microemulsions containing propylene glycol exhibiting the higher area of microemulsion compared to the system without cosurfactant added. Again, propylene glycol might preferably located at the interface showing the more potential of cosurfactant than glycerol. It seemed that an addition of cosurfactant investigated was greatly reduce gel structure in systems containing

glycerol and gel structure did not appear in system using propylene glycol or Imwitor® 308 as cosurfactant.

An addition of Imwitor® 308 in system containing Tween® 20 or Tween® 80 had an higher effect on increasing microemulsion area and the level of oil incorporation. The area of microemulsion existence of IPM/ Tween® 20 (or Tween® 80)/ Imwitor® 308 microemulsion were higher upon decreasing the ratio of Imwitor® 308 from 4:1 to 2:1. However, there were some differences in microemulsion containing Tween® 20 and Tween® 80 in that for Tween® 80/ Imwitor® 308 microemulsion at ratios of 4:1 and 2:1, the microemulsion area increased toward the oil corner when the ratio was decreased (Figure 18). For Tween® 20/Imwitor® 308 /IPM system, the microemulsion area increased toward to water corner when the ratio of surfactant to cosurfactant decreased (Figure 15).

Figures 19-21 show the ternary phase behavior of Solutol® HS 15 with various cosurfactants. It was found that glycerol had not effect on increasing microemulsion area especially when weight ratio of Solutol® HS 15: glycerol decreased. Conversely, the areas of microemulsion containing propylene glycol and Imwitor® 308 were higher than system without cosurfactant (Table 5). The microemulsion areas of IPM/Solutol® HS 15/propylene glycol/water formulation (Figure 20, Table 5) at various weight ratios of Solutol® HS 15 to propylene glycol were slightly different. However, the

microemulsion area seemed to be decreased when concentration of propylene glycol was increased. When Imwitor[®] 308 was used at 4:1 weight ratio of surfactant to cosurfactant, the microemulsion area increased toward the oil axis (Figure 21).

In Figure 22 and Table 5, when glycerol was added in IPM/Cremophor[®] EL/ glycerol/ water microemulsion at 4:1 weight ratio of Cremophor[®] EL to glycerol, microemulsion area increased about 2% compared with microemulsion containing Cremophor[®] EL without cosurfactant added, and the area of microemulsion existence decreased with decreasing the ratio of surfactant and cosurfactant. For the system using propylene glycol at the Cremophor[®] EL to propylene glycol ratios of 4:1, 2:1 and 1:1, the higher ratio of Cremophor[®] EL to propylene glycol allowed the higher microemulsion area. Surprisingly, the gel formed was increased in system using 4:1 weight ratio of Cremophor[®] EL to propylene glycol compared to systems without cosurfactant (Figure 23). Compared between all surfactants used, Imwitor[®] 308 was the cosurfactant that could increase area of microemulsion existence to the greater extent. However, only 4:1 weight ratio of Cremophor[®] EL to Imwitor[®] 308, microemulsion could be formed (Figure 24).

It could be generally concluded that propylene glycol and Imwitor[®] 308 were cosurfactant that were capable of increasing microemulsion area in system containing of IPM, water and surfactant, namely, Tween[®] 20, Tween[®] 80, Solutol[®] HS 15 and

Cremophor® EL. Such an increase was dependent upon type of surfactant, cosurfactant and weight ratio of surfactant to cosurfactant in system. It could be explained by the action and arrangement of surfactant and cosurfactant used. However, the maximum level of oil solubilization seemed not to be increased with a higher microemulsion area in system using cosurfactant. The system which the oil solubilization extremely increased with increasing microemulsion area were those containing propylene glycol with Tween® 80 and Imwitor® 308 with Tween® 20, Tween® 80 and Cremophor® EL.

The pH 7.4 phosphate buffered saline (PBS) was used to replace distilled water for investigating the change in the area of microemulsion existence in order to use the microemulsion in PBS in hemolysis study. The systems studied were 4:1 and 2:1 weight ratios of Tween® 20(and Tween® 80) to Imwitor® 308 and 4:1 weight ratio of Solutol® HS 15(and Cremophor® EL) to Imwitor® 308. The areas of microemulsion of all systems in PBS were rarely difference to the same systems in water. It was concluded that the addition of electrolyte, which was known to reduce head group hydration of ionic surfactant had no obvious effect on the microemulsion formation using nonionic surfactant investigated here. The nonionic surfactant, however, is more sensitive to a change in temperature. Conversely, the ionic microemulsion have no sensitivity to thermal change but is highly sensitive to electrolytes (Lawrence and Rees, 2000).

In addition, the requirement of heating process in microemulsion was investigated. Microemulsion were prepared as previously deseribed in Chapter 3 but no heating process was used. The Tween® 20/ Imwitor® 308 microemulsion at 2:1 weight ratio of Tween® 20 to Imwitor® 308 with 5%-15% oil solubilization could be prepared without heating at any amount of sample used. For Cremophor® EL system with the same condition as Tween® 20, the microemulsion could be formed, however, when the total amount of mixture was increased to more than 2 g, the system was required the heat for reducing the preparation time. When Tween® 80 and Solutol® HS 15 were replaced Tween® 20, the mixture were turbid and highly viscous, so the heating process was an advantage. It seemed that Tween® 20 and Imwitor® 308 mixture was favored to easily form microemulsion than the other surfactants, which might require a longer period of time to spontaneously form a microemulsion.

From the phase behavior study, it was found that Tween® 20 was more favorite to form microemulsion especially when it was matched with Imwitor® 308. In addition, the area of microemulsion containing Tween® 20 with Imwitor® 308 tended to present o/w microemulsion as the microemulsion area was toward to the water corner whereas the microemulsion area prepared using the other surfactants tended to expand to oil axis and at high concentration of surfactant mixture. The result was suggested that a difference in surfactant structure had an effect on microemulsion formation.

Comparing polyoxyethylene sorbitan surfactant, Tween[R] 20 and Tween[R] 80 which have the same polar head group, polyethylene sorbitan, with different alkyl chain length. Tween[R] 20 had shorter alkyl chain than Tween[R] 80. Comparing between Solutol[R] HS15 and Cremophor[R] EL, both of them have polyethylene glycol as hydrophilic part, however, Cremophor[R] EL has ethoxylated glycol as hydrophilic part as well. Cremophor[R] EL has higher polar part and longer chain length than Solutol[R] HS15. It was suggested that a difference in chain length and head group of surfactant had effect on CPP value that results in the difference in CPP of surfactants. However, the CPP can change by penetration of oil molecule, thereby, the surfactant chain will increase the effective hydrocarbon chain volume of surfactant molecules and thus, increase the CPP. Consequently, IPM is relatively large molecule then the possibility of some penetration into the surfactant chains should increase CPP (Aboofazeli and Lawrence, 1993). In the present study, the incorporation of IPM into Tween[R] 80, Solutol[R] HS15 and Cremophor[R] EL might lead to an increase in CPP. If the CPP is higher than 1, the aggregations tended to form w/o microemulsion.

In this study, Imwitor[R] 308 presented the highest ability to increase microemulsion formation with all surfactants. It was that Imwitor[R] 308 is a surfactant in nature then it could penetrate into interfacial film higher than the other cosurfactants.

For this reason, the areas of microemulsion using any surfactants were higher when adding Imwitor®308 in system.

3. The determination of the type of microemulsion

Microemulsion formed in the previous study was determined for the type of microemulsion using the measurement of electrical conductivity and the dye solubilization method. The conductivity values of microemulsion were normally in range of 10-140μ S/cm (Appendix D). It was found that the values of conductivity in all microemulsion systems increased with increasing the weight fraction of water, ϕ_w, which is weight ratio of water to total amount of microemulsion. It was suggested that systems consisting of water at a ϕ_w lower than 0.15, the structure of the system was water in oil microemulsion. (Alany et al., 2001).

The type of microemulsions was tested by dye solubilization method using water soluble dye (methyl orange) and oil soluble dye (sudan III). The results were found that in the systems which ϕ_w > 0.15 methyl orange were highly dissolved and expanded through the continuos phase while sudan III was not dissolved easily. The high solubilization of methyl orange and the conductivity $\geq$ 20 μS/cm of system might indicate that microemulsion were o/w type. In contrast, system having

conductivity $\leq$ 10 μ S/cm, and the sudan III was greatly dissolved, the systems were considered to be w/o microemulsion. Unfortunately, the sensitivity of the conductivity meter was in the range of 10-9990 μ S/cm. Hence, system having conductivity lower than 10 μ S/cm could not be accurately detected and was reported as 10 μ S/cm.

However, the exception was noted for some systems which were found that the conductivity was 20, while the sudan III was more dissolved. An example was the system including the system containing 60% concentration of 2:1 weight ratio of Tween® 80 to Imwitor® 308 with 0.2-0.3 for weight fraction of oil (ϕ_o). Hence, in these cases, there was some uncertainty to classify the type of microemulsion to be w/o microemulsion.

It was noted that, in some systems containing high weight fraction of surfactant mixture (ϕ_s), (0.8-0.9), it was difficult to investigate which dye were more dissolved as at high surfactant concentration the microemulsion was very viscous which interfered with the visually observation of dye test. These systems were the systems containing 80% w/w of 4:1 Tween® 80 to propylene glycol and 10% w/w of IPM as well as 80% w/w of 4:1 Tween® 80 to Imwitor® 308 and 5% w/w of IPM microemulsion. Surprisingly, the IPM/ Cremophor® EL/ Imwitor® 308/ water microemulsion at 4:1 ratio and ϕ_s was 0.6 having conductivity of 20 μS/cm and both of the dyes could dissolve equally in the system. It was possibly classified those systems to be

bicontinouos type. However, it was needed to be investigated by other techniques before the conclusion was made.

From the results, it was concluded that all microemulsions consisting of Tween® 20 were provided the o/w type while other systems were presented both of o/w and w/o types. The result was suggested that the type of microemulsion depended on the type of surfactant and cosurfactant, the ratio of surfactant to cosurfactant as well as the concentration of each component.

4. Dilutability of microemulsion

The ability of microemulsion to be diluted was investigated for the stability of microemulsion after dilution because dilute microemulsion was prepared for particle size and size distribution and hemolysis studies. In addition, a cosurfactant likes to partitions to aqueous phase so this was influenced to o/w interface and cause to destroy the microemulsion droplet (Lawrence and Rees, 2000) leading to unstable turbid solution. Figures 25-37 show the ability of microemulsion when diluted with distilled
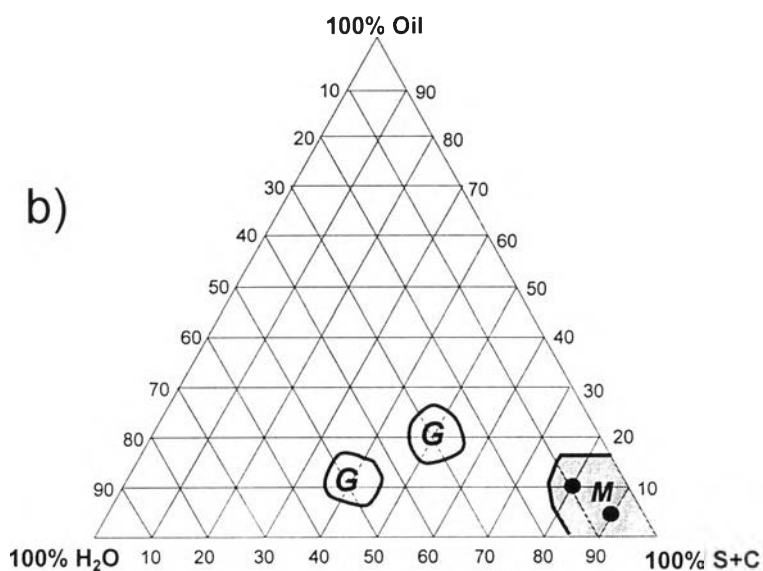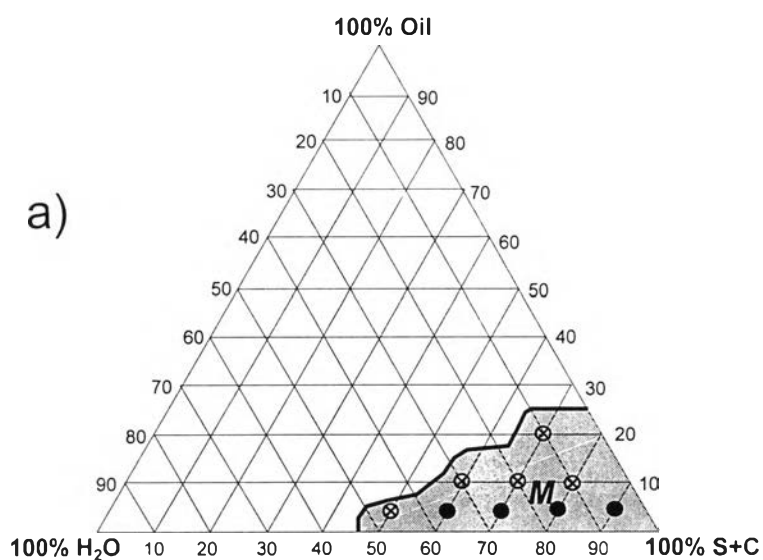
Figure 25. Pseudo-ternary phase diagram of surfactant / IPM / water microemulsion

       upon dilution

a) IPM / T20 / water   **b) IPM / T80 / water**

G = Gel,   M = Microemulsion

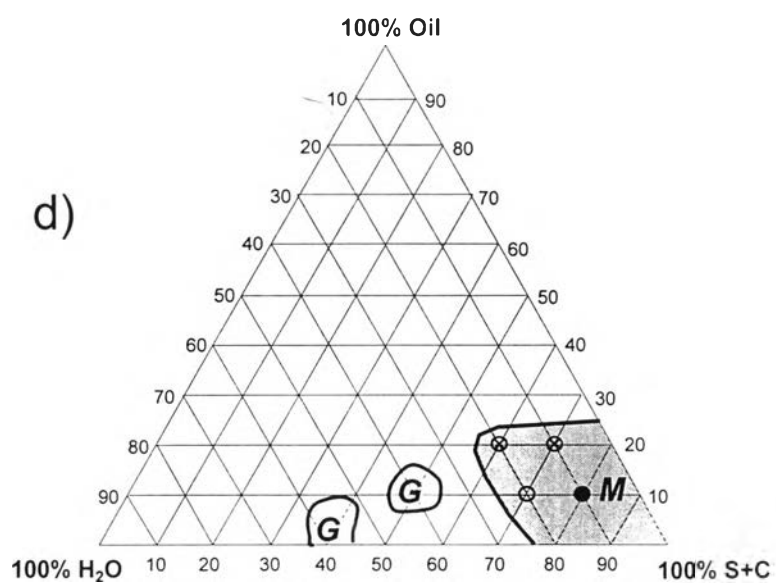● **Represented the clear solution**   ⊗ **Represented the turbid solution (unstable)**
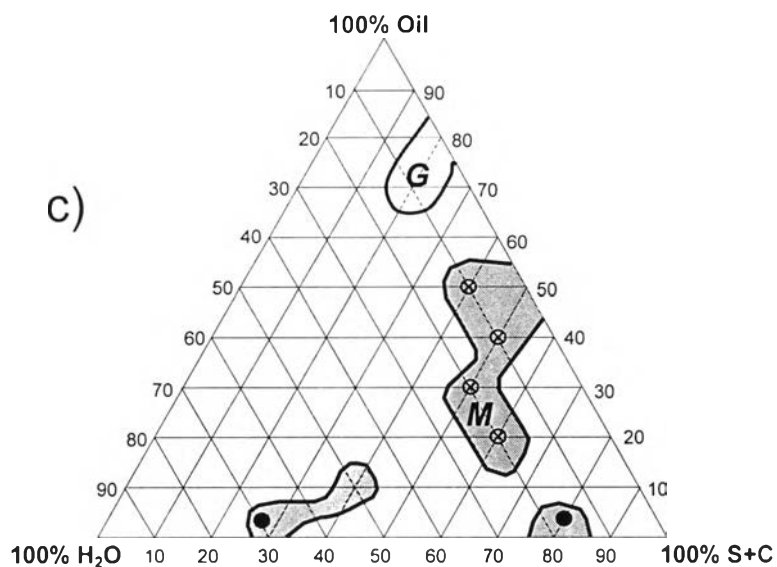
Figure 25. Pseudo-ternary phase diagram of surfactant / IPM / water microemulsion

upon dilution(continued)

c) IPM / Sol / water   d) IPM / Cre / water

G = Gel,   M = Microemulsion

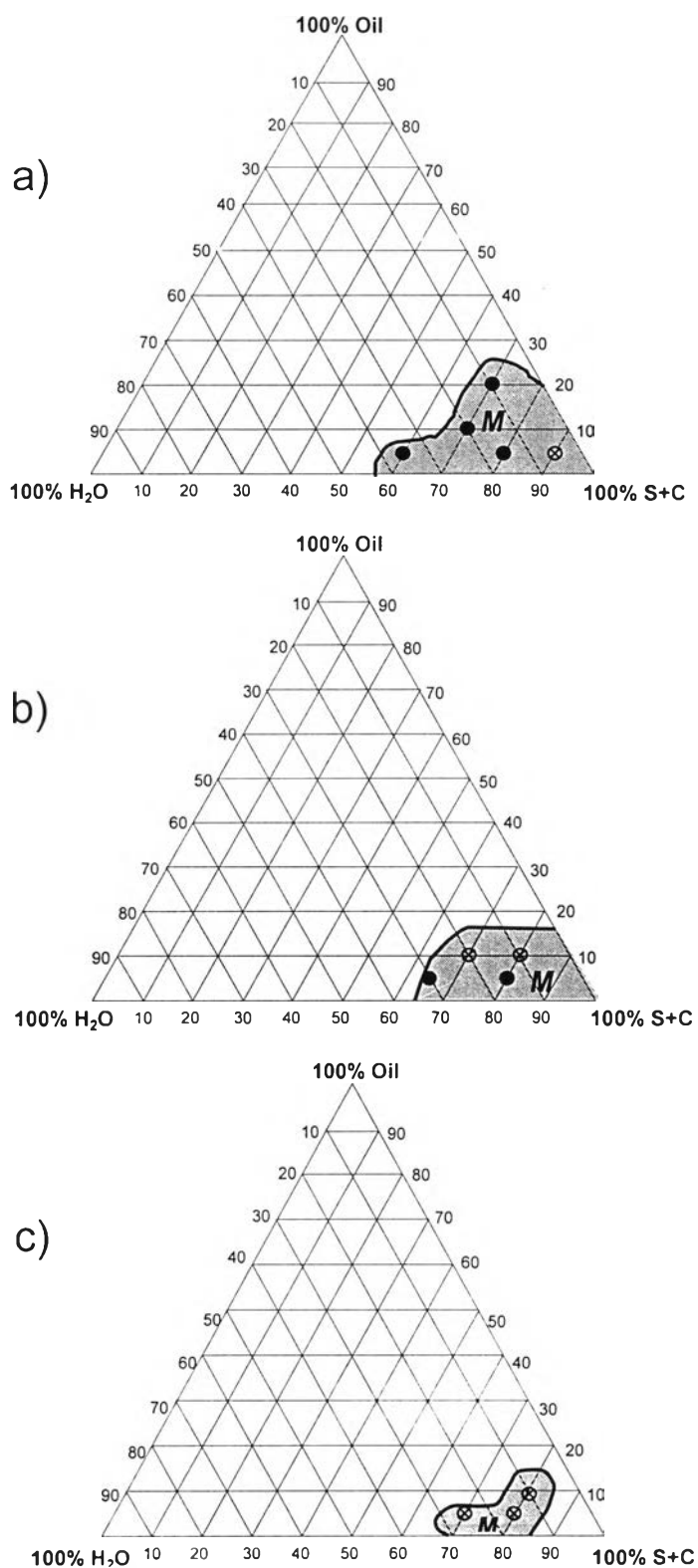● Represented the clear solution   ⊗ Represented the turbid solution(unstable)

Figure 26. Pseudo-ternary phase diagram of IPM / T20 / Gly / water

system at 4:1(a), 2:1(b) and 1:1(c) weight ratio of surfactant and cosurfactant upon dilution

G = Gel,   M = Microemulsion

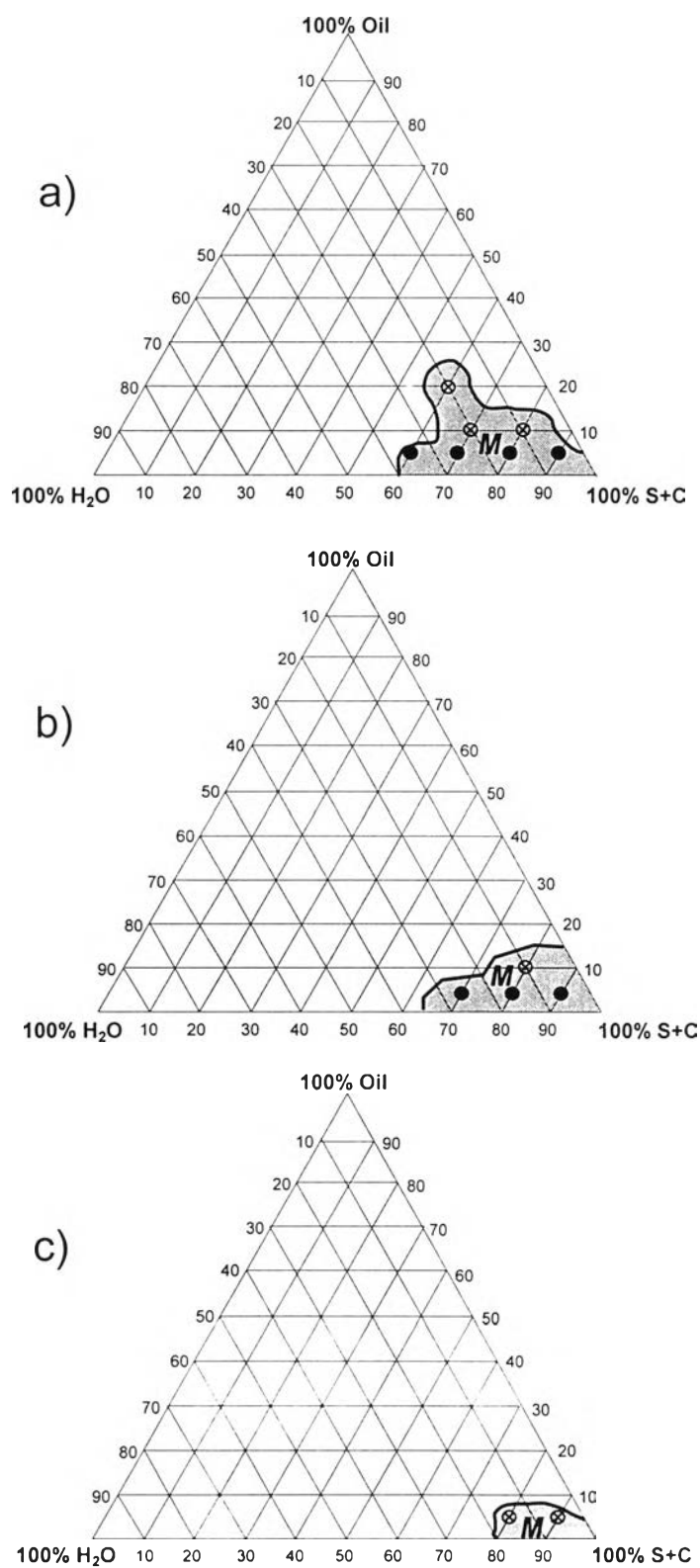● Represented the clear solution   ⊗ Represented the turbid solution(unstable)

Figure 27. Pseudo-ternary phase diagram of IPM / T20 / PG / water

system at 4:1(a), 2:1(b) and 1:1(c) weight ratio of surfactant to cosurfactant upon dilution

G = Gel,   M = Microemulsion

● <u>Represented the clear solution</u>   ⊗ <u>Represented the turbid solution(unstable)</u>
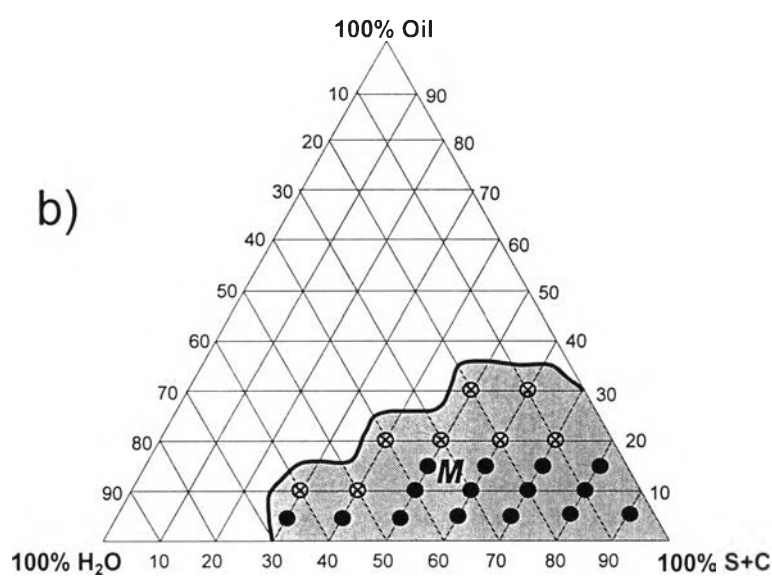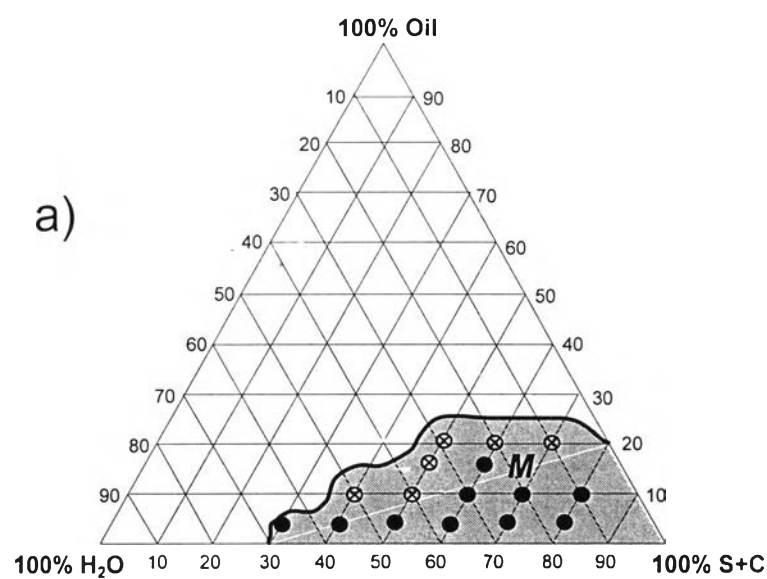
Figure 28. Pseudo-ternary phase diagram of IPM / T20 / Im/ water

system at 4:1(a) and 2:1(b) weight ratio of surfactant and cosurfactant opon dilution

G = Gel,   M = Microemulsion

● Represented the clear solution   ⊗ Represented the turbid solution(unstable)

Figure 29. Pseudo-ternary phase diagram of IPM / T80 / Gly / water

system at 4:1(a), 2:1(b) and 1:1(c) weight ratio of surfactant to cosurfactant upon dilution

G = Gel,   M = Microemulsion

● <u>Represented the clear solution</u>   ⊗ <u>Represented the turbid solution(unstable)</u>

Figure 30.   Pseudo-ternary phase diagram of IPM / T80 / PG / water

system at 4:1(a), 2:1(b) and 1:1(c) weight ratio of surfactant to cosurfactant upon dilution

G = Gel,   M = Microemulsion

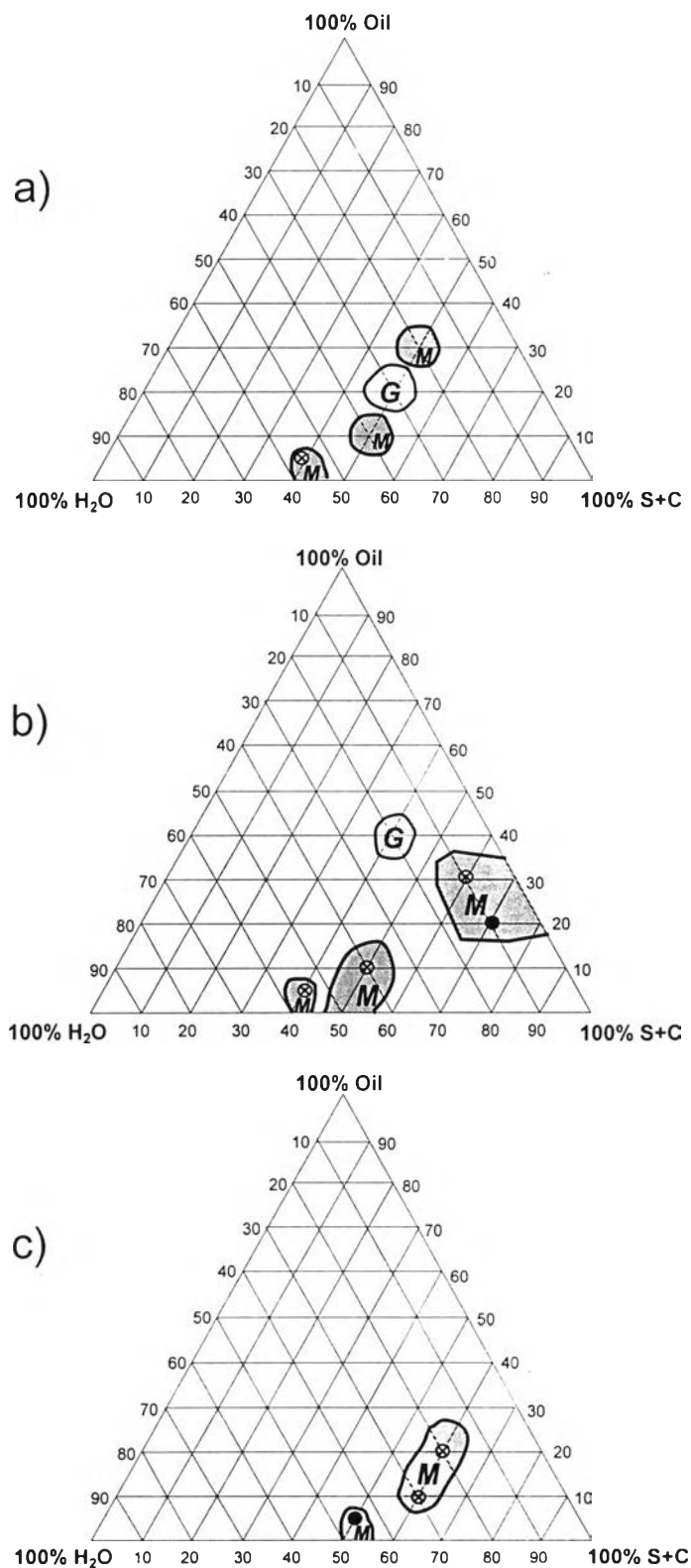● Represented the clear solution   ⊗ Represented the turbid solution(unstable)

Figure 31. Pseudo-ternary phase diagram of IPM / T80 / Im / water

system at 4:1(a) and 2:1(b) weight ratio of surfactant to cosurfactant upon dilution

G = Gel, M = Microemulsion

● Represented the clear solution   ⊗ Represented the turbid solution(unstable)
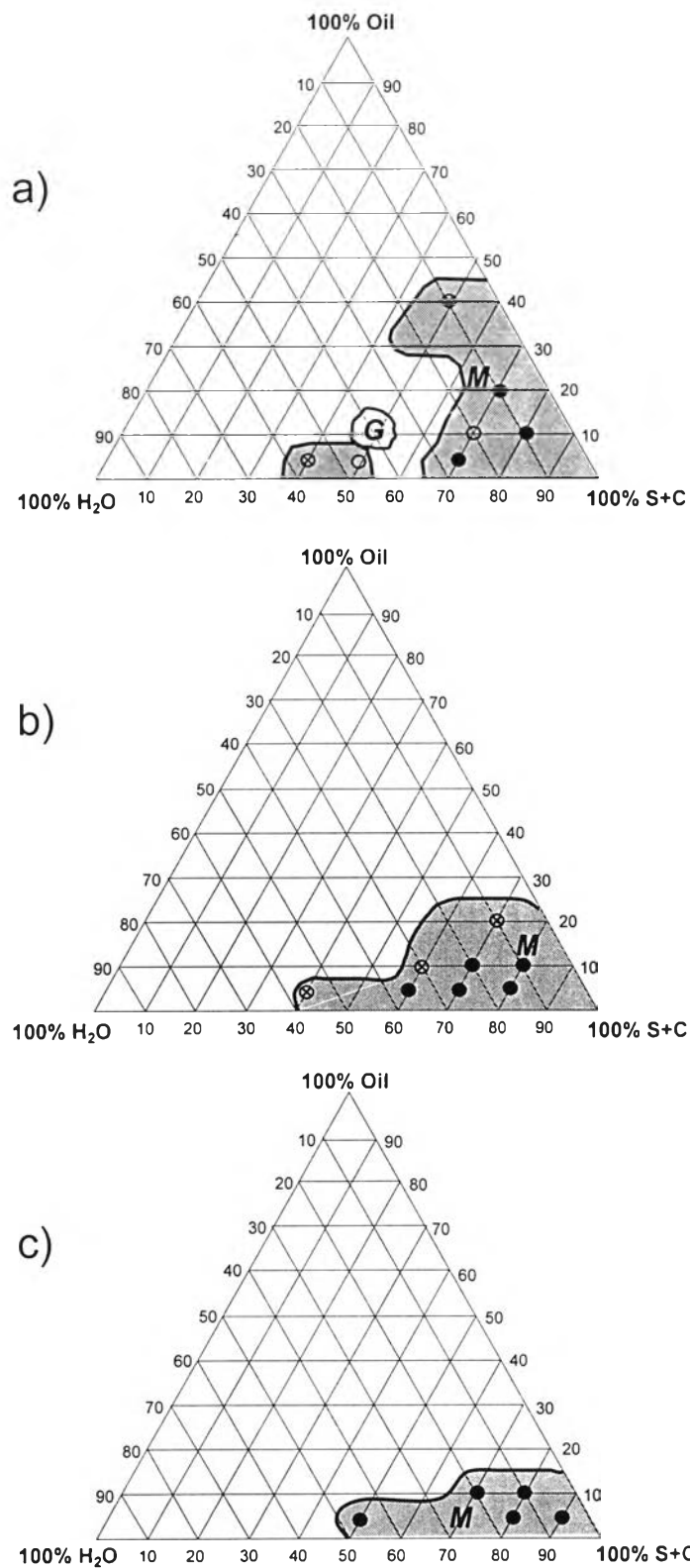
Figure 32. Pseudo-ternary phase diagram of IPM / Sol/ Gly/ water

system at 4:1(a) and 2:1(b) weight ratio of surfactant to cosurfactant upon dilution

G = Gel, M = Microemulsion

● Represented the clear solution   ⊗ Represented the turbid solution(unstable)

Figure 33.   Pseudo-ternary phase diagram of IPM / Sol / PG/ water

system at 4:1(a), 2:1(b) and 1:1(c) weight ratio of surfactant to cosurfactant upon dilution

G = Gel,   M = Microemulsion

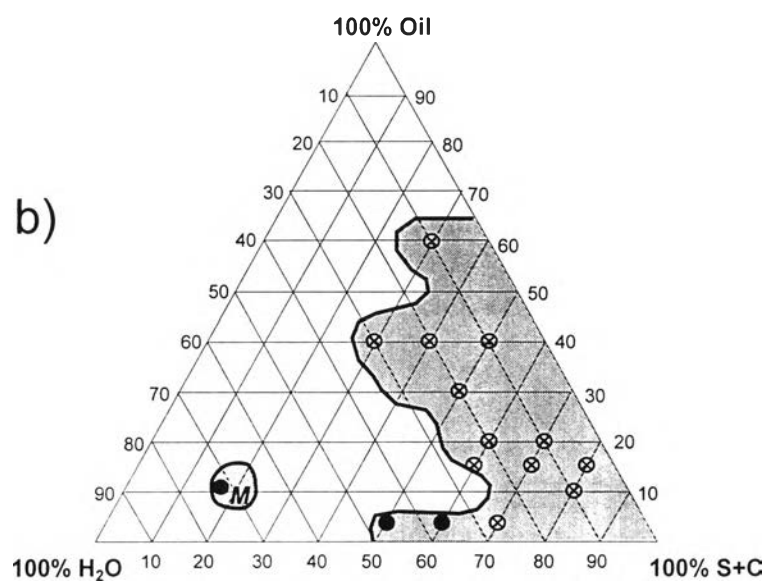● Represented the clear solution   ⊗ Represented the turbid solution(unstable)
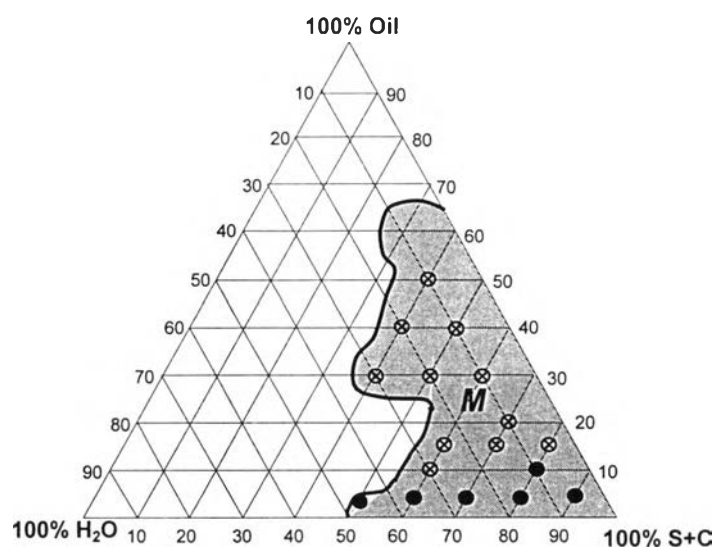
Figure 34. Pseudo-ternary phase diagram of IPM /Sol/ Im/water

system at 4:1 weight ratio of surfactant to cosurfactant opon dilution

G = Gel, M = Microemulsion

● <u>Represented the clear solution</u> ⊗ <u>Represented the turbid solution(unstable)</u>
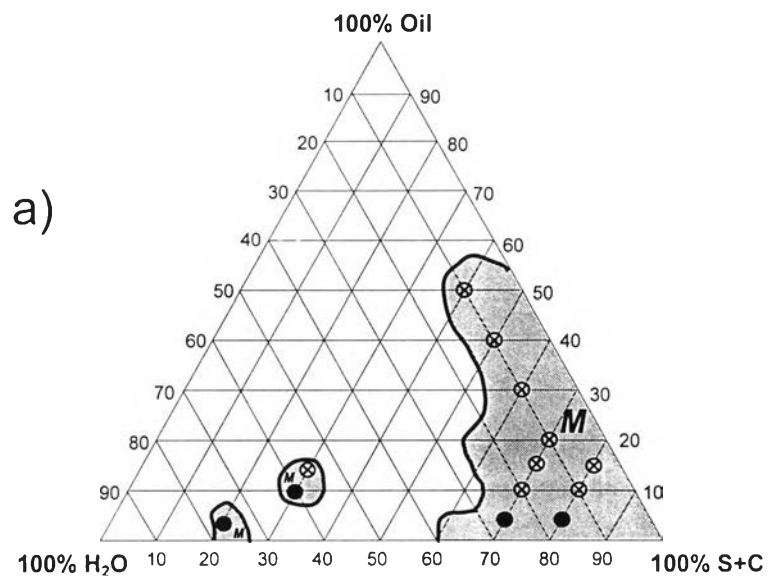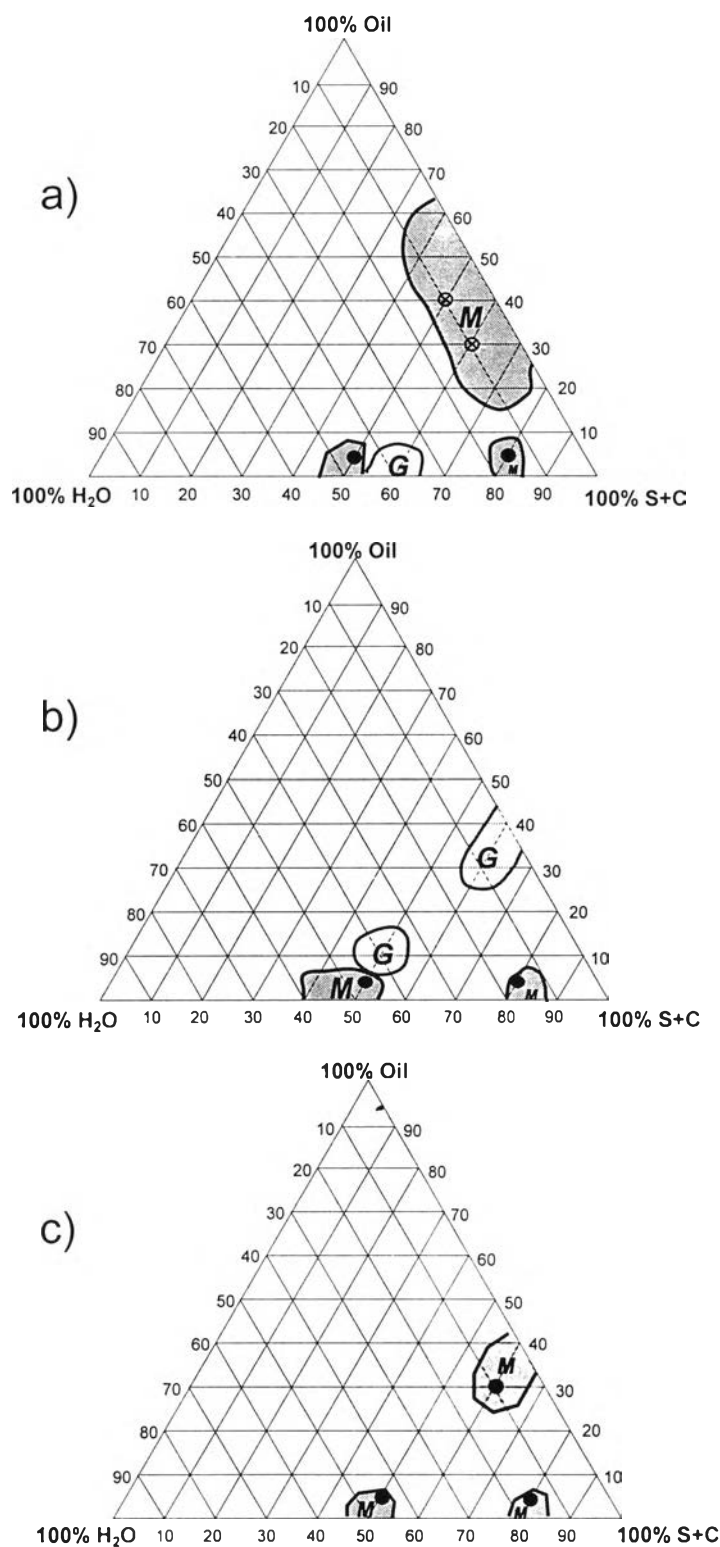
Figure 35. Pseudo-ternary phase diagram of IPM /Cre/ Gly / water

system at 4:1(a), 2:1(b) and 1:1(c) weight ratio of surfactant to cosurfactant upon dilution

G = Gel,   M = Microemulsion

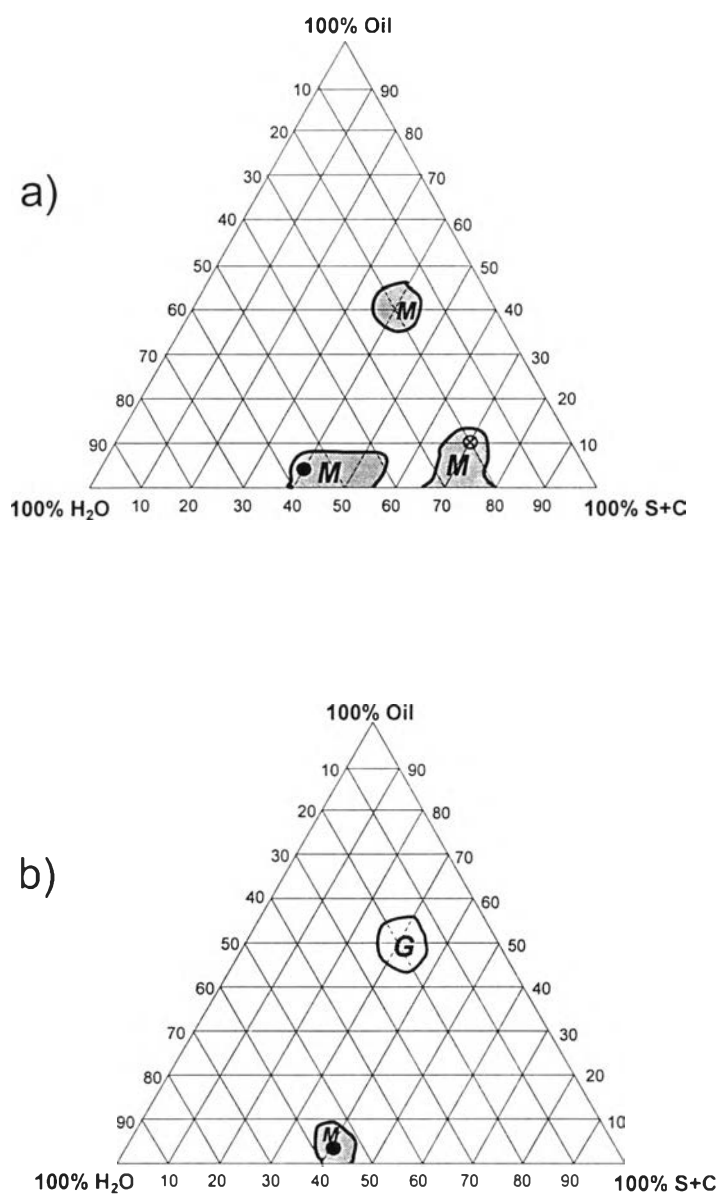● Represented the clear solution   ⊗ Represented the turbid solution(unstable)

Figure 36. Pseudo-ternary phase diagram of IPM/Cre/PG/water

system at 4:1(a), 2:1(b) and 1:1(c) weight ratio of surfactant to cosurfactant upon dilution

G = Gel,   M = Microemulsion

● **Represented the clear solution**   ⊗ **Represented the turbid solution(unstable)**
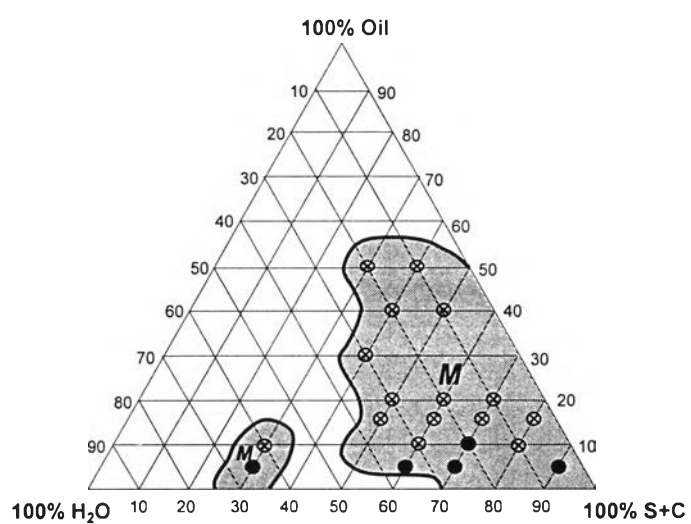
Figure 37. Pseudo-ternary phase diagram of IPM / Cre / Im/ water

system at 4:1 weight ratio of surfactant to cosurfactant upon dilution

G = Gel,   M = Microemulsion

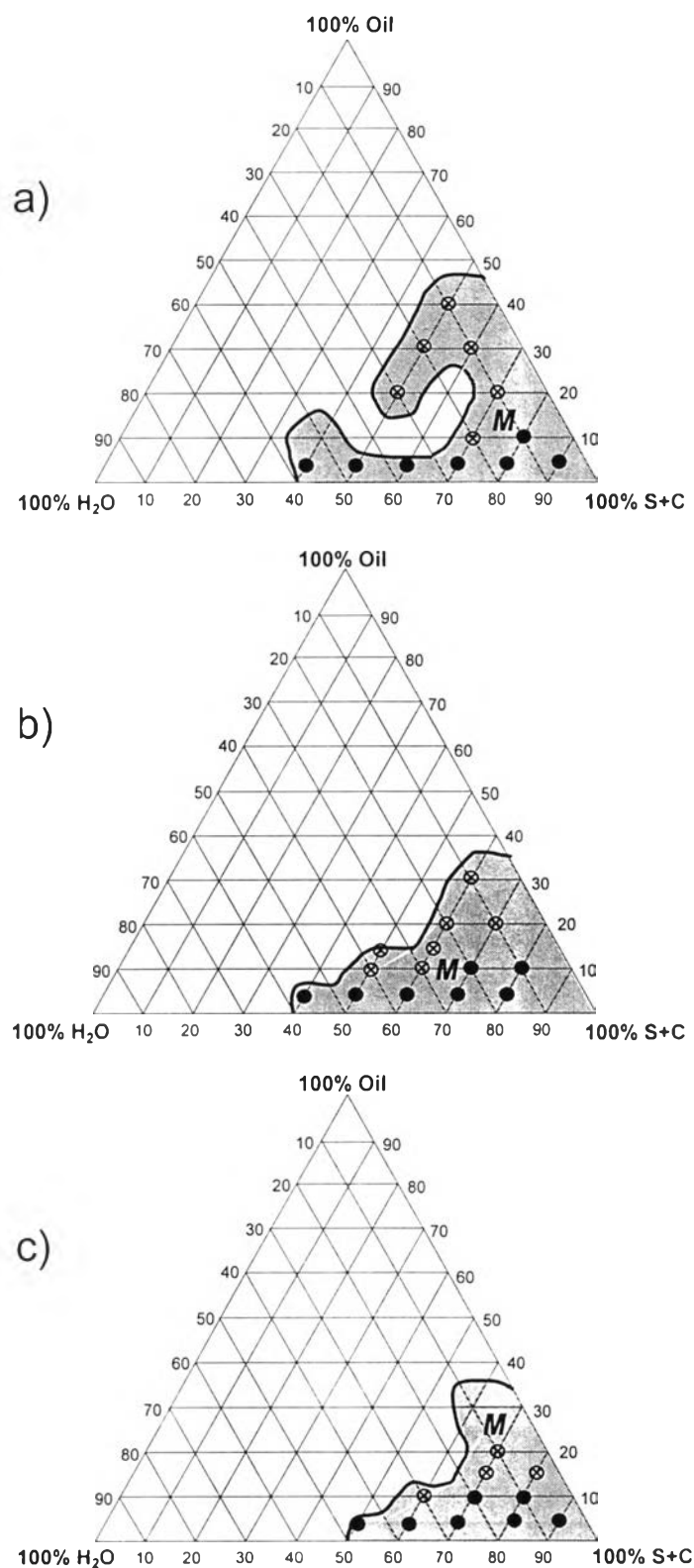● Represented the clear solution   ⊗ Represented the turbid solution(unstable)
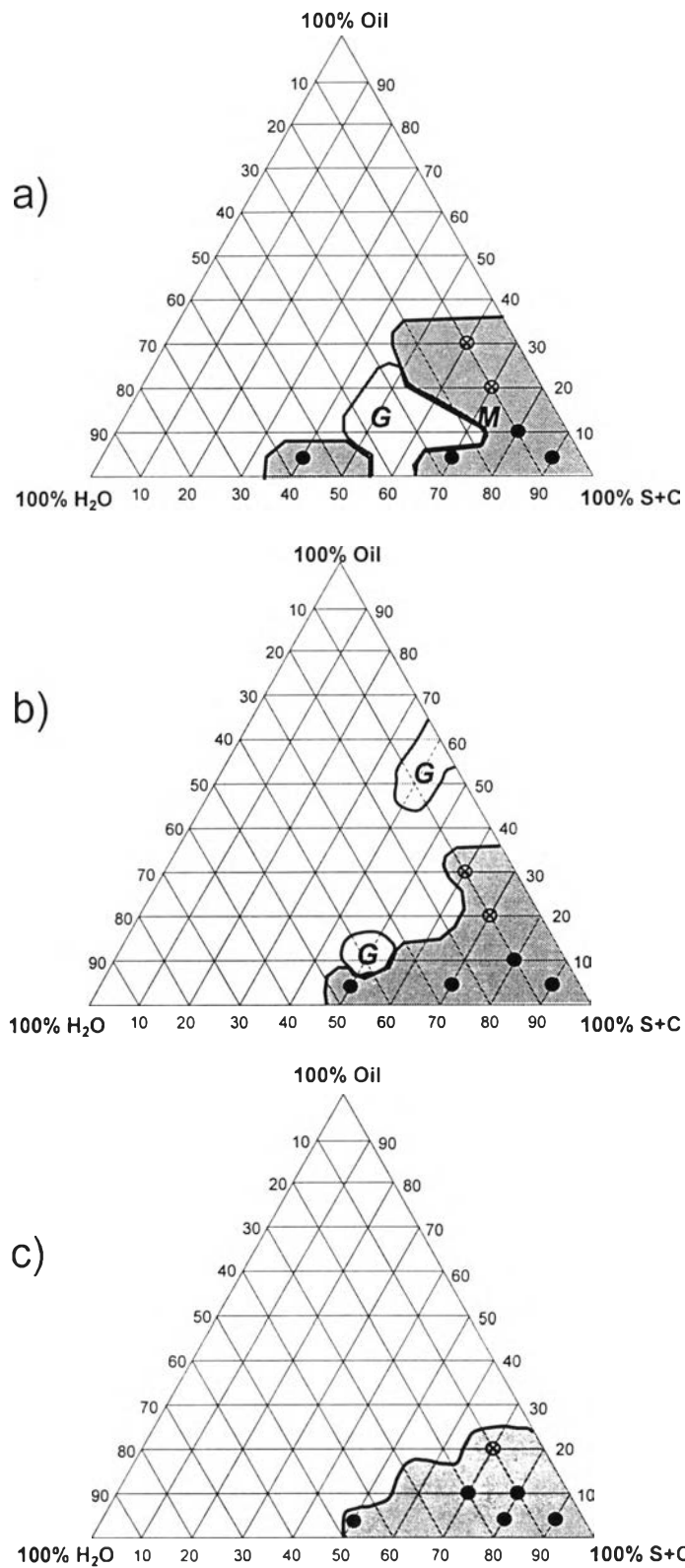
water. It was seemed that the system being microemulsion area toward to water axis such as systems containing Tween® 20 and Imwitor®308 were more stable than the microemulsion having area toward to oil axis such as the system consisting of Imwitor®308 and Solutol®HS15, Cremophor ®EL and Tween® 80. The result related to the type of microemulsion, as o/w microemulsion was more stable than w/o microemulsion when diluted with water. The results were in the same trend as the type of microemulsions in that most of Tween® 20 microemulsions were o/w type that could theoretically stable upon dilution.

5. The particle size of micelles and microemulsions

The particle size and polydispersity values of each microemulsion are shown in Table 6. Comparing the particle size of micelle using Imwitor®308 with various surfactants at the same total concentration (60 %w/w) and weight ratio of surfactant to cosurfactant (4:1), the particle sizes were smaller in an order of Cremophor ® EL < Solutol®HS15 < Tween® 80. The micelle and microemulsion were diluted to obtain the 1 % w/w concentration of surfactant and cosurfactant (as previously described in Chapter 3) before the particle size analysis.

Table 6. The particle size and size distribution of microemulsion using Imwitor®308 as cosurfactant with various surfactants at 2:1 or 4:1 weight ratio of surfactant to cosurfactant.

Surfactant/ Cosurfactant	Ratio*	Conc** (%w/w)	Oil (%w/w)	Size[a] ± SD (nm)	P[b] ± SD
Tween® 20/Imwitor®308	2:1	50	0	10.5 ± 0.1	0.146± 0.028
Tween® 20/Imwitor®308	2:1	50	1	10.3 ± 0.1	0.207 ±0.031
Tween® 20/Imwitor®308	2:1	50	3	10.6 ± 0.2	0.139 ±0.027
Tween® 20/Imwitor®308	2:1	50	5	11.1 ± 0.1	0.139± 0.023
Tween® 20/Imwitor®308	2:1	50	7	12.4 ± 0.1	0.098± 0.035
Tween® 20/Imwitor®308	2:1	50	10	12.7 ± 0.1	0.082± 0.017
Tween® 20/Imwitor®308	2:1	50	12	ND	ND
Tween® 20/Imwitor®308	2:1	50	15	ND	ND
Tween® 20/Imwitor®308	4:1	60	0	ND	ND
Tween® 20/Imwitor®308	4:1	60	5	10.2 ± 0.1	0.090± 0.044
Tween® 20/Imwitor®308	4:1	60	10	ND	ND
Tween® 20/Imwitor®308	4:1	60	15	ND	ND
Tween® 80/Imwitor®308	4:1	60	0	23.8 ± 1.3	0.267± 0.051
Solutol®HS15/Imwitor®308	4:1	60	0	12.4 ± 0.1	0.026± 0.021
Solutol®HS15/Imwitor®308	4:1	60	5	14.5 ± 0.0	0.027± 0.010
Cremophor®EL/Imwitor®308	4:1	60	0	11.5 ± 0.1	0.042± 0.027
Cremophor®EL/Imwitor®308	4:1	60	5	14.0 ± 0.0	0.044 ±0.005

* = weight ratio of surfactant to cosurfactant

** = concentration of surfactant and cosurfactant

[a] = particle size

[b] = polydispersity

ND= not determined

In microemulsion containing Tween® 20 at the ratio of 2:1, the droplet sizes grew up with increasing oil concentration. Conversely, the polydispersity of Tween® 20 systems decreased with increasing percent oil. The value of polydispersity of systems was around 0.1 which was lower than 1 suggesting that there were monodispersed (Langevin and Rouch, 1999). Hence, when more oil incorporated, the systems became less polydispersed. It was noticed that the polydispersity of Tween® 80 system was higher than the other systems.

The result was suggested that the different surfactants provided the different surfactant aggregate and the allowance of cosurfactant incorperation. As the PCS method directly measures the translation diffusion coefficient for microemulsion droplet that are noninteracting especially at diluted concentration (0.1-1.0 % w/w). Further, the droplet hydrodynamic diameter (or alternatively the correlation length) could then be calculated from the Stokes-Einstein equation under the assumption of noninteracting droplet (Tenjarla, 1999). The stability of microemulsion investigated upon dilution was decreased with increasing either weight ratio of surfactant to cosurfactant or the amount of oil in systems. The ability of microemulsion to be diluted was essential for measurement of the droplet size. Hence, the limitation of system to be measured by PCS was found in undiluted system. The particle size of microemulsion containing Tween® 20 and Imwitor® 308 depended on oil solubilization. In addition,

the way of cosurfactant incorperation might affect the value of CPP, v/a_0l_c, hence the

size of aggregate was changed (Aboofazeli and Lawrence, 1993).

6. The determination of phase inversion temperature (PIT)

The temperatures at which phase inverted are shown in Table 7. The PIT of

IPM/Tween$^®$ 20/ Imwitor$^®$308/ water microemulsions at 2:1 weight ratio of Tween$^®$

20 to Imwitor$^®$308 with 50 %w/w of total concentration surfactant and cosurfactant

were slightly different with various amounts of oil incorporation. The PIT tended to

increase with increasing concentration of oil. It was suggested that the PIT of

microemulsions depended on the amount and nature of the oil incorporation and the

nature of the surfactant (Lawrence and Rees, 2000). If the oil would locate at the core

of oil microemulsion, the PIT would found to be increase upon increasing more oil.

Moreover, it was suggested that the droplet size of microemulsion was increased upon

adding more oil locating at the droplet core (Warisnoichoreon, Lansley and Lawrence,

2000). Hence, IPM was likely to be solubilized in the oil core of Tween$^®$ 20

microemulsion. The finding was in agreement with particle size analysis in that the size

of droplets increased when adding more oil.

Table 7. Phase inversion temperature of IPM/ Tween$^{®}$ 20/ Imwitor$^{®}$308/ water microemulsion at 2:1 weight ratio of Tween$^{®}$ 20 to Imwitor$^{®}$308 and at 50% total concentration of surfactant mixture

Concentration of oil (% w/w)	N_1		N_2		T average PIT $\pm$ SD
	T_1	T_2	T_1	T_2	
0	67.3	65.8	65.7	64.1	65.7 $\pm$ 1.3
1	66.3	65.4	62.0	63.5	64.3 $\pm$ 1.9
3	67.4	69.2	65.2	64.6	66.6 $\pm$ 2.1
5	66.6	66.9	65.8	66.1	66.4 $\pm$ 0.5
7	69.6	70.3	68.2	68.6	69.2 $\pm$ 1.0
10	74.3	73.0	68.2	68.1	70.9 $\pm$ 3.2
12	72.3	71.2	68.2	68.1	70.0 $\pm$ 2.1
15	74.5	74.8	70.9	71.9	73.0 $\pm$ 1.9

N_1= First experiment

N_2= Second experiment

T_1= temperature at onset of turbidity

T_2 = temperature at onset of clearity

7. The viscosity of microemulsions

The effects of surfactant and cosurfactant on the viscosity of microemulsion were determined. The viscosity values of the formulations determined by Haake$^{®}$ viscometer are shown in Table 8.

For formulations using Tween$^{®}$ 20 as surfactant at the 2:1 weight ratio of surfactant to cosurfactant, the viscosity values of microemulsion containing Tween$^{®}$ 20 and Imwitor$^{®}$ 308 were not different when increasing concentration of oil. Moreover, when the shear rate was decreased from 200 s^{-1} to 150 s^{-1} and 100 s^{-1}, the viscosity values of Tween$^{®}$ 20/ Imwitor$^{®}$ 308/ IPM/ water with 15 % w/w oil were slightly decreased from 60.98 to 60.81 and 58.99, respectively. The result could be suggested that the micreomulsion showed the Newtonian flow which was in agreement with other studies (Gradzielski and Hoffmann, 1999). An addition of oil was found to decrease the viscosity of micellar solution of Tween$^{®}$ 20 and Imwitor$^{®}$ 308. The same results were found upon addition of oil to micelle of Solutol$^{®}$ HS15/ Imwitor$^{®}$ 308 and Cremophor$^{®}$ EL/ Imwitor$^{®}$ 308. While the weight ratio of surfactant to cosurfactant increased, from 2:1 to 4:1, the viscosity of microemulsion was about 2 times higher. However, the concentration of surfactant and cosurfactant was also increased from 50%

Table 8. The viscosity values of microemulsions containing Imwitor[®] 308 and various surfactants

Surfactant/ Cosurfactant	Weight ratio of Surfactant: Cosurfactant	Conc[*] (%w/w)	Oil (%w/w)	Viscosity±SD (mPa S)
Tween[®] 20	0	50	5	528.31 ±2.45
Tween[®] 20/ Imwitor[®]308	2:1	50	0	64.25 ± 9.17
Tween[®] 20/ Imwitor[®]308	2:1	50	1	58.67 ±0.29
Tween[®] 20/ Imwitor[®]308	2:1	50	3	59.06 ± 1.60
Tween[®] 20/ Imwitor[®]308	2:1	50	5	58.06 ± 1.17
Tween[®] 20/ Imwitor[®]308	2:1	50	7	57.97 ± 0.85
Tween[®] 20/ Imwitor[®]308	2:1	50	10	61.00 ± 1.18
Tween[®] 20/ Imwitor[®]308	2:1	50	12	59.38 ± 1.18
Tween[®] 20/ Imwitor[®]308	2:1	50	15	60.98 ± 1.46
Tween[®] 20/ Imwitor[®]308	4:1	60	5	136.77 ± 1.87
Tween[®] 20/ Imwitor[®]308	4:1	60	10	139.34 ± 1.29
Tween[®] 20/ Imwitor[®]308	4:1	60	15	125.06 ± 1.75
Tween[®] 80/ Imwitor[®]308	4:1	60	0	121.07 ± 1.48
Solutol[®] HS15/ Imwitor[®]308	4:1	60	0	196.73 ± 1.64
Solutol[®]HS15/ Imwitor[®]308	4:1	60	5	121.07 ± 1.48
Cremophor[®]EL/ Imwitor[®]308	4:1	60	0	399.61 ± 2.21
Cremophor[®]EL/ Imwitor[®]308	4:1	60	5	328.86 ± 2.29

[*] = concentration of surfactant and cosurfactant

to 60% w/w. This was consistent with the study of Ktistis (1990) who reported an increase in viscosity with increasing polysorbate (surfactant) and sorbitol (cosurfactant) and oil concentrations in microemulsion. Although, in the present study, the amount of oil solubilization had not much effect on the viscosity of microemulsion.

The viscosity of Tween[®] 20 without Imwitor[®] 308 was the highest (528 mPa s) than the formulation adding cosurfactant at the same oil concentration, 5 %w/w. The result was in agreement with the previous report, in that the system with cosurfactant had a lower viscosity compared with the corresponding cosurfactant free system (Alany et al., 2001).

Microemulsion containing either Cremophor[®] EL or Solutol[®] HS15 had the lower viscosity compare to the corresponding micellar solution. Compared among the micelles of all surfactant, the higher in viscosity were increased in the order of Tween[®] 20, Tween[®] 80, Solutol[®] HS15 and Cremophor[®] EL, respectively. It was possibly due to the structure and molecular weight of the surfactant.

8. The osmolality of microemulsion system.

The nonionic microemulsions were prepared in PBS. The osmolality behaviors of various microemulsion formulations are shown in Table 9. In Tween® 20 systems, the osmolality of Tween® 20 microemulsions using Imwitor® 308 was greatly lower than microemulsion without cosurfactant at the same percent of oil approximately 1,000 mOsm/kg. The higher oil solubilization, the osmolality values seemed to be increased. The same finding was found in microemulsion using Cremophor® EL in that micelles having the lower viscosity than corresponding microemulsion. For micellar solution of surfactant and Imwitor® 308, the osmolality values were varied from lower to higher when Tween® 80, Tween® 20, Cremophor® EL and Solutol® HS15, respectively were used. Some formulations could not be measured, as the values were much greater than the limitation of equipment. It was noted that an increase in oil concentration of microemulsion caused an increase in the concentration of the system. Thus, the osmolality of microemulsion was increased, as well.

Normally, the serum osmolality is approximately 285 mOsm/kg. The parenteral formulation should have the osmolality similar to the serum osmolality; however, some peripheral infusions have the osmolality exceeding the normal value approximately 700

Table 9. The osmolality of microemulsions

Surfactant/ Cosurfactant	Weight ratio of Surfactant: Cosurfactant	Conc* (%w/w)	Oil (%w/w)	Osmolality (mOsm/kg)
Tween® 20	0	50	5	2717±268
Tween® 20/ Imwitor® 308	2:1	50	0	1475±84
Tween® 20/ Imwitor® 308	2:1	50	1	1546±64
Tween® 20/ Imwitor® 308	2:1	50	3	1702±81
Tween® 20/ Imwitor® 308	2:1	50	5	1676±40
Tween® 20/ Imwitor® 308	2:1	50	7	1785±121
Tween® 20/ Imwitor® 308	2:1	50	10	1903±6
Tween® 20/ Imwitor® 308	2:1	50	12	2100±29
Tween® 20/ Imwitor® 308	2:1	50	15	2237±9
Tween® 20/ Imwitor® 308	4:1	60	5	ND
Tween® 20/ Imwitor® 308	4:1	60	10	ND
Tween® 20/ Imwitor® 308	4:1	60	15	ND
Tween® 80/ Imwitor® 308	4:1	60	0	1235±30
Solutol® HS15/ Imwitor® 308	4:1	60	0	3344±69
Solutol® HS15/ Imwitor® 308	4:1	60	5	ND
Cremophor® EL/ Imwitor® 308	4:1	60	0	2129±189
Cremophor® EL/ Imwitor® 308	4:1	60	5	2664±115

* = concentration of surfactant and cosurfactant

ND = not determined

to 800 mOsm/kg (Siegel, 1990). From the result, the microemulsion investigated may

be used as parenteral infusion.

9. The pH of microemulsions

The pH values of the preparations are shown in Table 10. PBS (pH 7.4) was

used as aqueous phase in all formulations. In microemulsion containing Tween[®] 20

and Imwitor[®] 308 at 2:1 and 4:1 weight ratios of surfactant to cosurfactant, the pH

values slowly increased with increasing concentration of oil. From the result, the

concentration of oil in formulation had slightly effect on the pH value. Similarly, the

compositions in microemulsion, surfactant and cosurfactant, had gradually effect on the

pH value. However, the ranges of pH values of all microemulsions were 6-7 that closed

to neutral pH, the pH ranges from 3 to 10.5 upon intravenous administration. The blood

itself is an excellent buffer and it can dilute and distribute the solution throughout the

circulatory system very rapidly (Lund, 1994).

Table 10. The pH values of microemulsions

Surfactant/ Cosurfactant	Weight ratio of Surfactant: Cosurfactant	Conc* (%w/w)	Oil (%w/w)	pH
Tween® 20	0	50	5	7.05
Tween® 20/ Imwitor®308	2:1	50	0	6.35
Tween® 20/ Imwitor®308	2:1	50	1	6.25
Tween® 20/ Imwitor®308	2:1	50	3	6.35
Tween® 20/ Imwitor®308	2:1	50	5	6.36
Tween® 20/ Imwitor®308	2:1	50	7	6.38
Tween® 20/ Imwitor®308	2:1	50	10	6.40
Tween® 20/ Imwitor®308	2:1	50	12	6.44
Tween® 20/ Imwitor®308	2:1	50	15	6.41
Tween® 20/ Imwitor®308	4:1	60	5	6.55
Tween® 20/ Imwitor®308	4:1	60	10	6.61
Tween® 20/ Imwitor®308	4:1	60	15	6.71
Tween® 80/ Imwitor®308	4:1	60	0	6.45
Solutol®HS15/ Imwitor®308	4:1	60	0	7.04
Solutol®HS15/ Imwitor®308	4:1	60	5	7.03
Cremophor®EL/ Imwitor®308	4:1	60	0	6.73
Cremophor®EL/ Imwitor®308	4:1	60	5	6.74

* = concentration of surfactant and cosurfactant

10. Hemolysis study of microemulsion

Formulations presenting high o/w microemulsion area, high maximum oil solubilization and ability to be diluted were chosen for hemolysis study. The systems included Tween® 20, Imwitor® 308, IPM microemulsion at 2:1 and 4:1 weight ratio of Tween® 20 to Imwitor® 308. The total concentration of 50% w/w was selected to study. Unfortunately, system at weight ratio of 4:1 could not be diluted if the oil concentration were more than 5% w/w.

These formulations were prepared by replacing distilled water with pH 7.4 phosphate buffered saline (PBS). Formulations were diluted with PBS to various concentrations of surfactant and cosurfactant of 0.005, 0.01, 0.05, 0.075, 0.1, 0.25, 0.50 and 1.00 % w/v, which were the concentrations above the critical micelle concentration (cmc) of Tween® 80 that had the lowest cmc , for hemolysis study. However, the study was extended to investigate into the effect of other systems using different surfactants. Thus, the formulations containing Tween® 80, Solutol® HS15 and Cremophor® EL as surfactant at 4:1 weight ratio and at 60% w/w total concentration of surfactant and cosurfactant were tested for hemolytic activity. The corresponding micellar systems

using the same surfactants in the absence and presence of Imwitor® 308 were also

studies. Some microemulsions investigated, appeared turbid upon dilution (Table 11)

were not used for hemolysis study to compare any toxicity.

Generally, the hemolysis test *in vitro* served as a screening method for toxicity

of lytic agent contained in intravenous formulation. The comparison of the hemolytic

activity of micelles and microemulsions are shown in Figures 38-42 (Table f1 – f6 in

Appendix F). Each point in the graph represented the mean value from 9 experiments.

The hemolytic action of IPM/ Tween® 20/ Imwitor® 308/ PBS microemulsions

are shown in Figure 39. The percent hemolysis of all formulations at the concentration

of surfactant and cosurfactant from 0.005% to 0.075% w/v slowly increased upon

increasing concentration. At the concentration of 0.075% to 0.1% w/v microemulsion

containing 5 % w/w oil showed higher percent hemolysis than the formulation

containing 10% w/w oil and 15% w/w oil, respectively. However, when the

concentration above 0.1% w/w, the percent hemolysis increased to closely 100 %

hemolysis with increasing oil concentration in formulation.

The percent hemolysis of micelle using 4:1 weight ratio of Tween® 80 to

Imwitor® 308 at concentration of 60%w/w was lower than 1% at concentration of

0.005% to 0.1% w/v (Figure 40).

Table 11. The appearance of dilute micelles and microemulsions in PBS

Surfactant	Cosurfactant	Weight Ratio of surfactant: cosurfactant	Conc (%w/w)	Oil (%w/w)	Appearance
Tween® 20	-	-	50	-	Clear
Tween® 20	Imwitor® 308	4:1	50	5	Clear
Tween® 20	Imwitor® 308	4:1	50	10	Turbid
Tween® 20	Imwitor® 308	4:1	50	15	Turbid
Tween® 20	Imwitor® 308	2:1	50	5	Clear
Tween® 20	Imwitor® 308	2:1	50	10	Clear
Tween® 20	Imwitor® 308	2:1	50	15	Clear
Tween® 80	-	-	60	-	Clear
Tween® 80	Imwitor® 308	4:1	60	-	Clear
Tween® 80	Imwitor® 308	4:1	60	5	Turbid
Tween® 80	Imwitor® 308	4:1	60	10	Turbid
Tween® 80	Imwitor® 308	4:1	60	15	Turbid
Solutol® HS15	-	-	60	-	Clear
Solutol® HS15	Imwitor® 308	4:1	60	-	Clear
Solutol® HS15	Imwitor® 308	4:1	60	5	Clear
Cremophor® EL	-	-	60	-	Clear
Cremophor® EL	Imwitor® 308	4:1	60	-	Clear
Cremophor® EL	Imwitor® 308	4:1	60	5	Clear

However, the percent hemolysis slightly increased up to 90% hemolysis at the concentration of 1% w/v. It was seen that the percent hemolysis of Tween® 80 micelle without cosurfactant added was lower than that added Imwitor® 308 especially when increasing the concentration of surfactant. It was noted that the percent hemolysis increased when concentration of surfactant mixture increased.

The sigmoidal relationships between (− log) concentration of surfactant and cosurfactant and percent hemolysis were observed in Tween® 20 formulations in the micelle and microemulsion. The S-shaped curve was chosen to convert into straight line for calculate the IC_{50} (Concentration producing 50% hemolysis). The S-shaped curve was converted into straight line by re-scaling the y-axis by transformation into the unit known as probit. The concentrations used for the analysis were selected to produce responses (hemolysis) within 20% to 80% assuming the normal distribution of cumulative frequency of response (Bowman and Rand, 1980). The liner graphs from the regression and lysis of Tween® 20 systems were showed in Figures 43-46.

The calculated IC_{50} (and the 95% confidence limit) of the systems containing Tween® 20 and Imwitor® 308 at weight ratio of 2:1 with 0, 5, 10 and 15 % w/w oil were 0.06 (0.004-0.87) %w/v, 0.33 (0.19-0.59) %w/v, 0.33 % w/v and 0.29 (0.08-1.09) %w/v, respectively. The difference between IC_{50} of the systems were statistically tested using unpaired t-test. The values of IC_{50} of Tween® 20 and Imwitor® 308
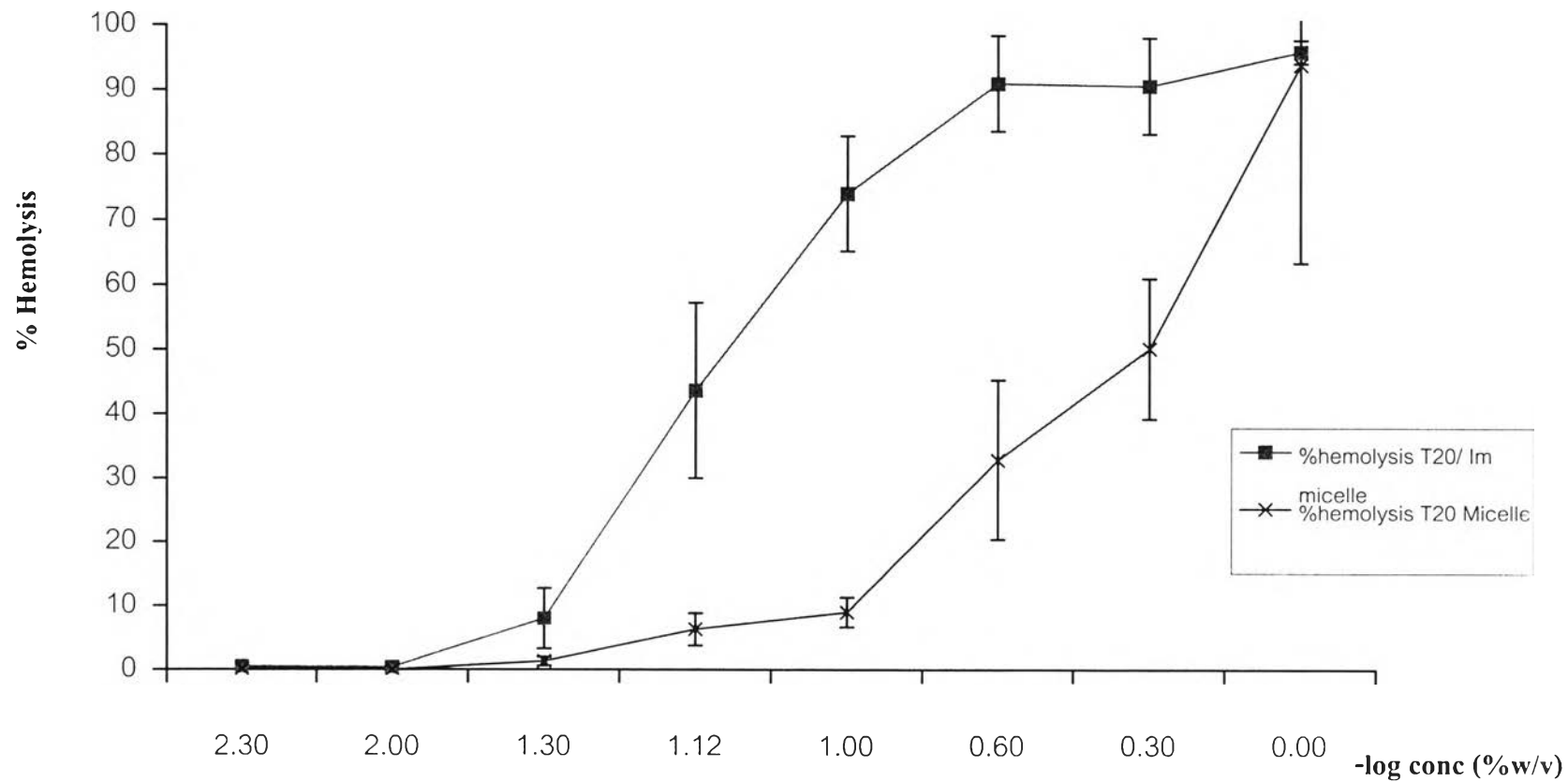
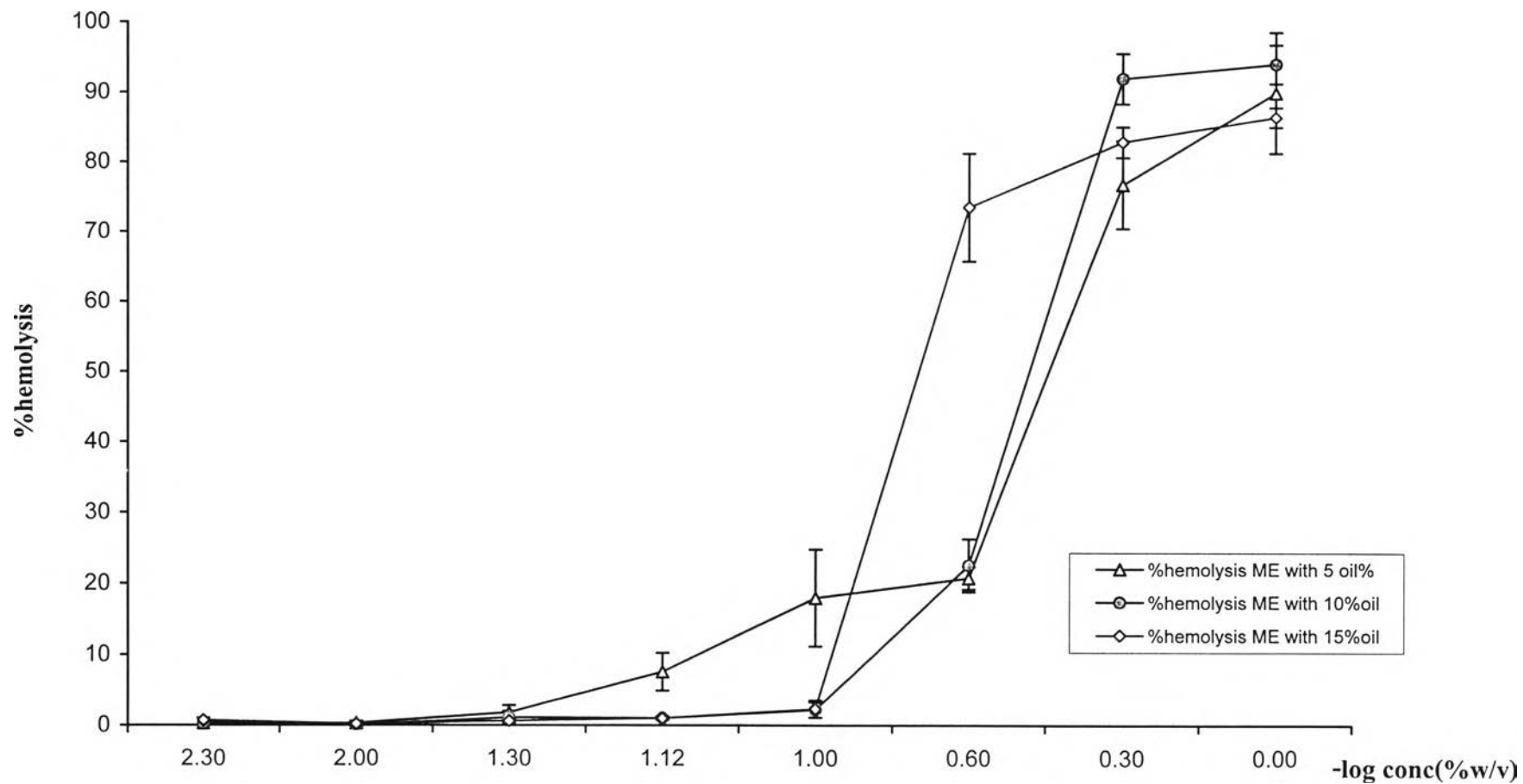**Figure 38. Hemolysis of T 20 micelle in PBS with /without Im (mean +/- SD)**

Figure 39. Hemolysis of T20/Im/IPM/PBS at 50% w/w concentration of surfactant mixture and 2:1 weight ratio of T20 to Im with 5, 10 and 15 %w/w oil (mean +/-SD)
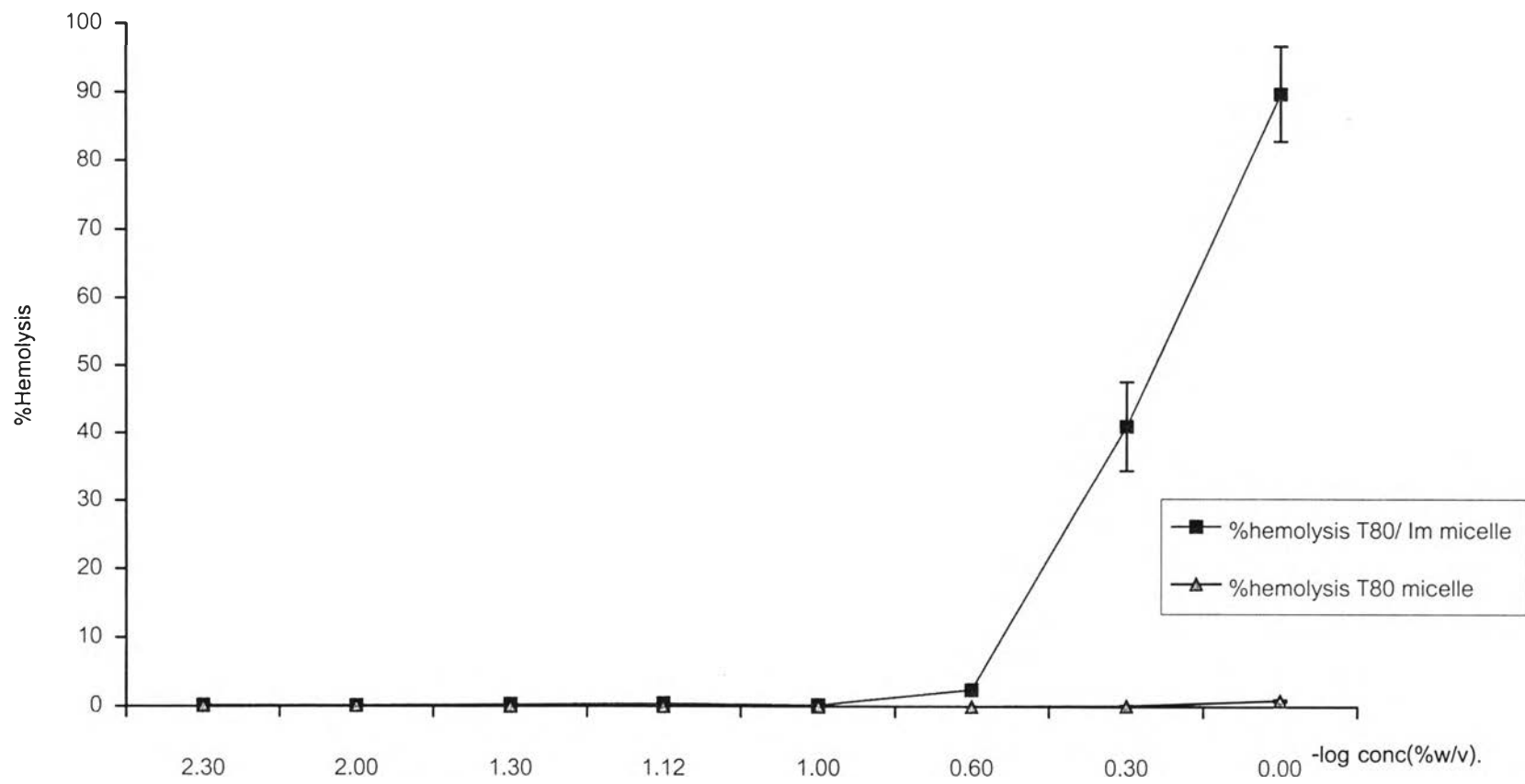
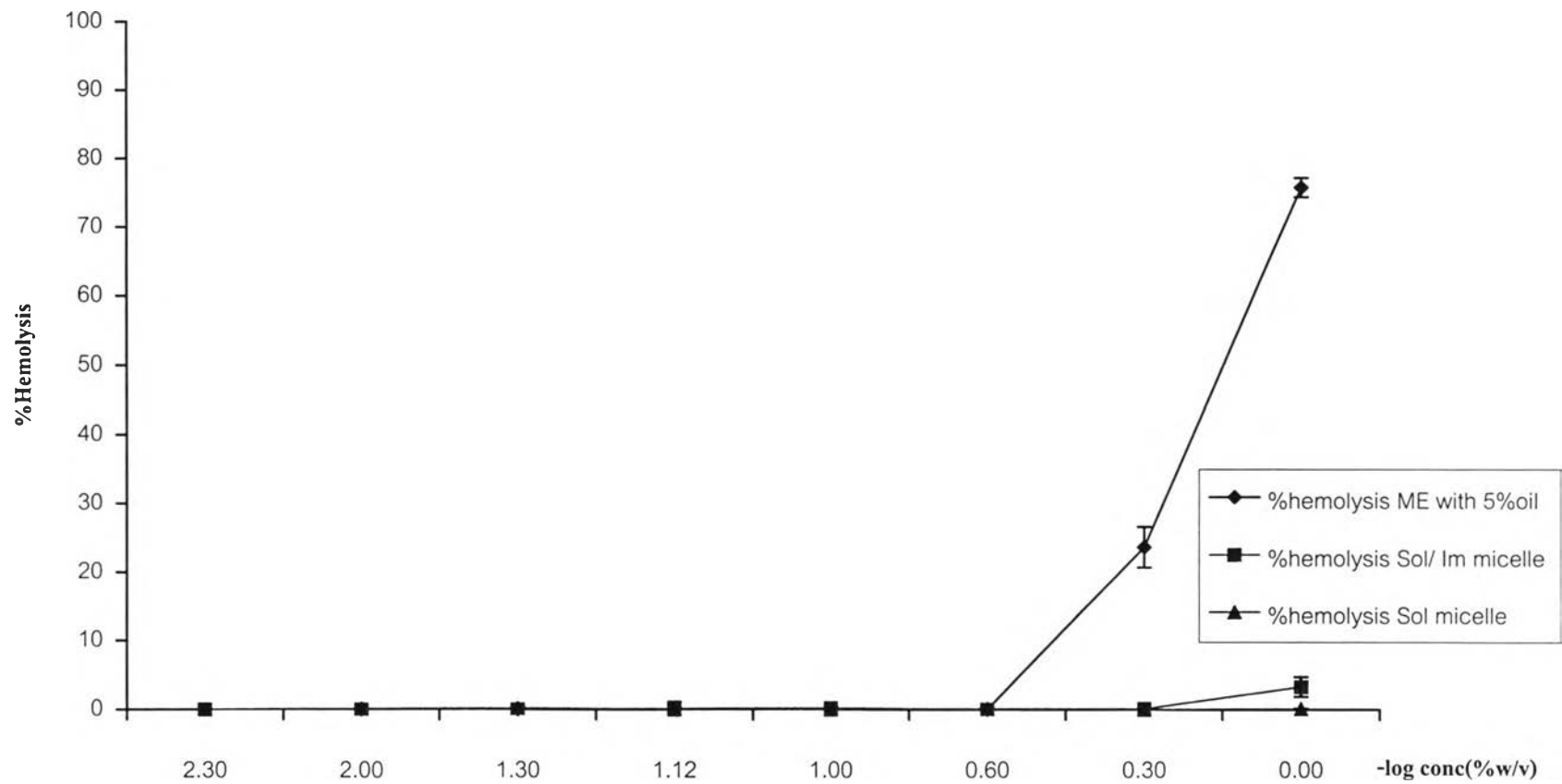**Figure 40. Hemolysis of T80 micelle in PBS with/without Im (mean+/-SD)**

Figure 41. Hemolysis of Sol micelle with/without Im and microemulsion at 4:1weight ratio of Sol to Im (mean +/- SD)
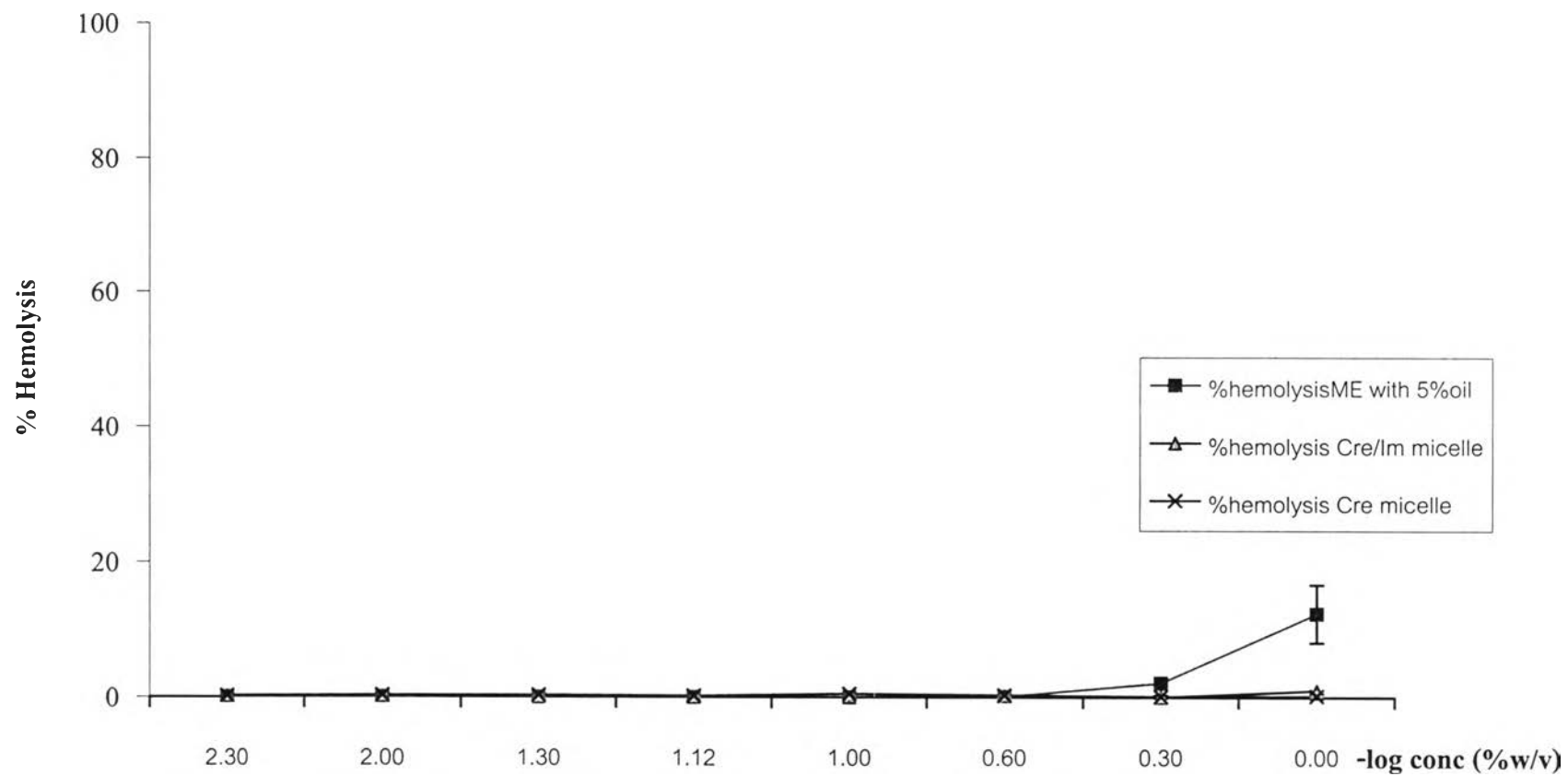
Figure 42. Hemolysis of Cre micelle with/ without Im and microemulsion at 4:1 weight ratio of Cre to Im (mean+/-SD)
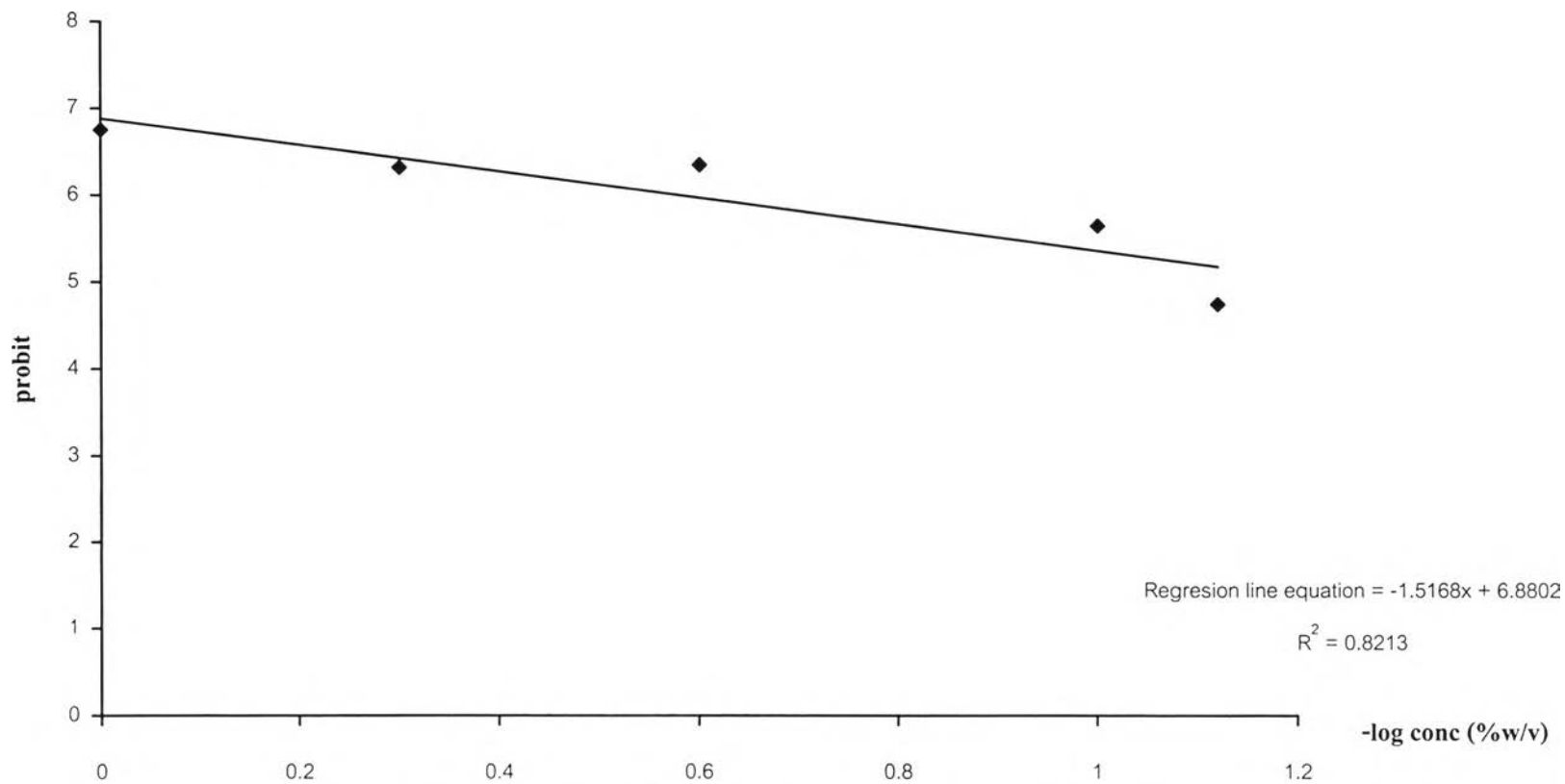
Figure 43. Relationship between concentration of micelle and probit of T20/Im/PBS at 50%w/w, 2:1 weight ratio
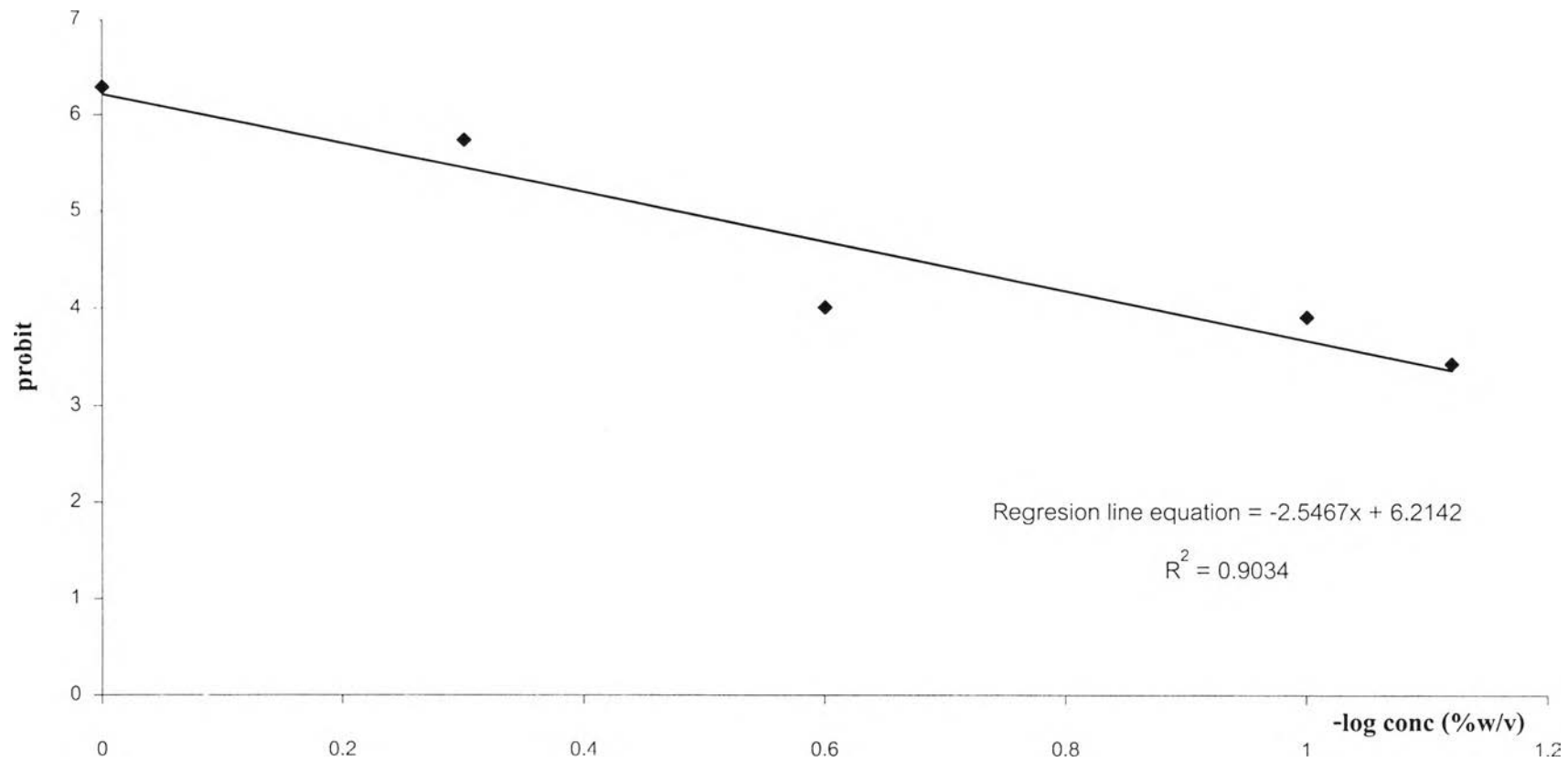
of surfactant to cosurfactant

Figure 44. Relationship between concentration of microemulsion and probit of T20/Im/PBS/ microemulsion at 50% w/w, 2:1 weight ratio of surfactant to cosurfactant with 5 % oil
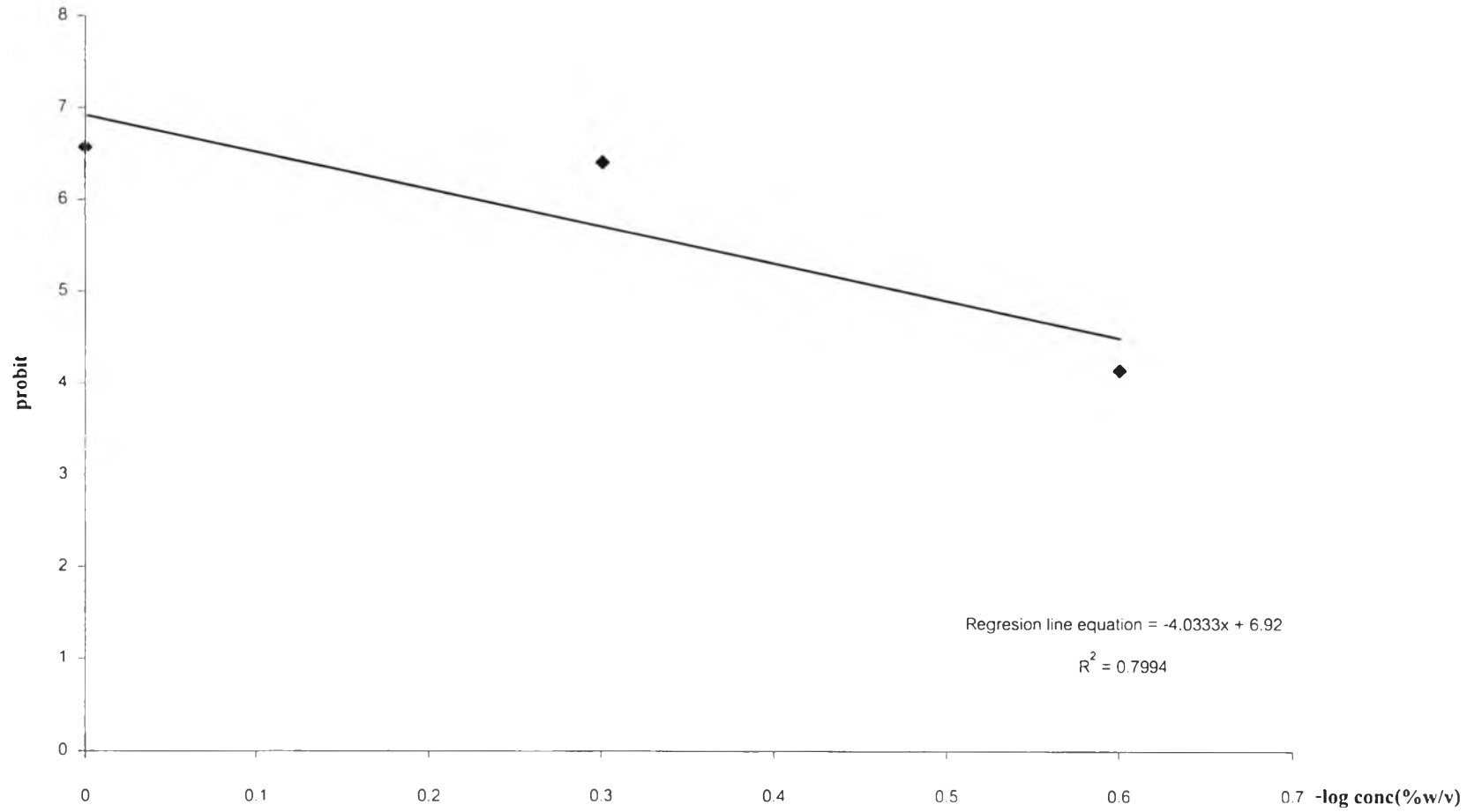
Figure 45. Relationship between concentration of microemulsion and probit of T 20/Im /PBS microemulsion at 50 %w/w, 2:1 weight ratio of surfactant to cosurfactant with 10% w/w oil
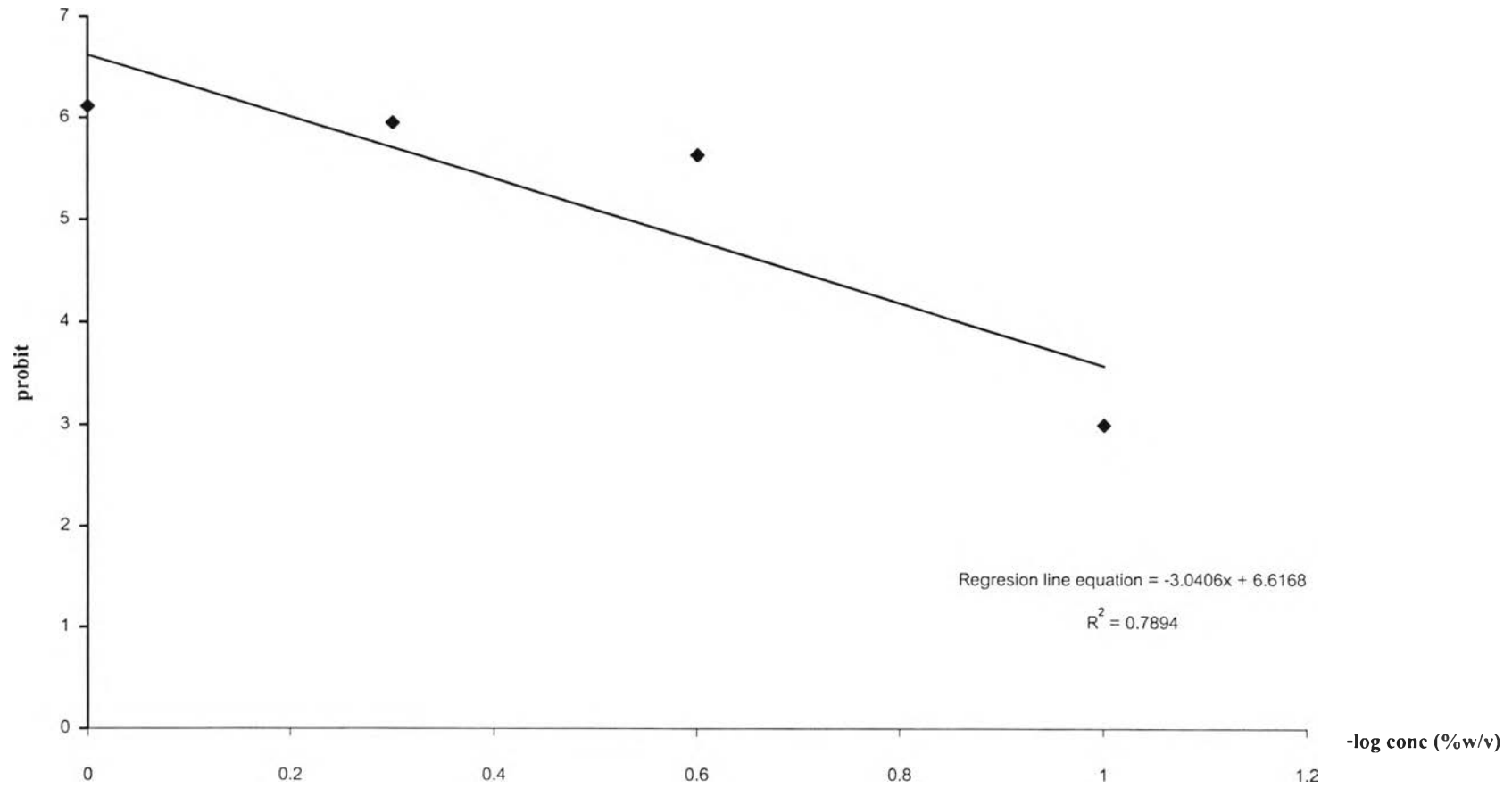
Figure 46. Relationship between concentration of microemulsion and probit of T 20/Im /PBS microemulsion at 50 %w/w, 2:1 weight ratio of surfactant to cosurfactant with 15% w/w oil

microemulsion containing 5, 10 and 15 % w/w oil were not significantly higher than the IC_{50} of the corresponding micelle (p $\geq$ 0.05). However, it was found that the percent hemolysis of microemulsion formulation was lower than the corresponding micelle containing Tween®20 and Imwitor®308 (Figures 38-39). It was suggested that the amount of surfactant and cosurfactant molecules self-assosiate to form microemulsion was larger than to form micelle thereby the free monomers of surfactant and cosurfactant in micelle solution were higher than microemulsion system leading to the erythrocyte hemolysis. The IC_{50} of Tween® 20 and Imwitor® 308 microemulsion with 5 % w/w oil was non-significantly higher than 15 % w/w oil (p $\geq$ 0.05); hence, increasing concentration of oil caused no effect on a decrease in toxicity.

Comparing between micelles containing Tween® 20 or Tween® 80 with cosurfactant, the Tween® 80 micelles were dramatically lower in toxicity than micelles containing Tween® 20. This result related to the previous study by Gould et al. (2000) in that a decrease in alkyl chain length of polyethylene alkyl ether surfactant (Brij) resulted in increasing hemolysis compared at the same surfactant concentration.

For system using Solutol® HS15 as surfactant, formulation consisting of IPM/ Solutol®HS15/Imwitor®308/PBS at 4:1 weight ratio and at 60 % w/w of Solutol® HS15 to Imwitor® 308 with 5 % w/w oil showed the toxicity to erythrocyte at

concentration of 0.5 % w/v. Again, the toxicity of Solutol® HS15 micelles with Imwitor® 308 added was lower than the corresponding microemulsion (Figure 41).

In formulations containing Cremophor® EL as surfactant, the hemolysis of the systems was in similar trend to systems containing Solutol® HS 15 in that the toxicity on human erythrocyte of Cremophor® EL microemulsion was higher than the corresponding micelle in the presence of Imwitor® 308 (Figure 42). The addition of oil at higher surfactant concentration presented an increase in percent of hemolysis.

The mechanism of hemolysis by surfactant may be described for three steps. Firstly, the surfactant molecule bind to erythrocyte membrane by lipophilicity. Secondly, the molecules are more incorporated into interior of lipid bilayer of cell membranes and result in disruption of bilayer structure as well as a change in cell shape and consequent small lesions. Thirdly, small solutes permeate through the lesions, which induce an osmotic change across the membrane. Thus, the lipid released from red blood cell membrane would correlate with the adsorption of the nonionic surfactant to the erythrocytes. As the alkyl chain length decreased, the amount of surfactant molecule adsorbed necessary to cause lysis was also decreased. This result meaned that the alkyl chain was important for membrane binding (Kondo and Tomizawa, 1968; Kobayashi, Onuki and Tachibana, 1999).

For this reason, it was suggested that the hemolysis of Tween® 20 micellar with/without cosurfactant formulation provided the highest percent hemolysis as its alkyl chain was shortest compared to the other surfactant solutions. Then, Tween® 20 molecules were easily adsorbed onto erythrocyte. However, The Tween® 20 microemulsions produced the large area of o/w microemulsion and high oil incorperation than the other formulations.

In addition, the Tween® 20 microemulsion studied might have higher in critical micelle concentration than the other, thus there were more surfactant free monomer interacting with erythrocyte. Nearly any hemolytic solution will become less hemolytic when diluted with normal saline or with blood (Yalkowsky, Krzyzaiak and Ward, 1998).