CHAPTER IV

RESULTS AND DISCUSSION

1. Microemulsion formulation and phase diagram of microemulsion gel (MEG)

The microemulsion systems which were composed of isopropylmyristate (IPM), castor oil (CO) and soybean oil (SBO) as an oil phase, tween80 (T_{80}), cremophor EL (C_{EL}), cremophor RH (C_{RH}) as a surfactant, Lutrol F-68 (L_{68}), Brij 72 (B_{72}), Brij 721s (B_{721S}), Brij 35 (B_{35}), glycerin (G), propylene glycol (PG), butanol (B) and cetyl alcohol (C) as a co-surfactant were investigated. The formability of microemulsion gel (MEG) in each system is shown in Table 8. It could be seen that B_{72} and C as co-surfactant in the investigated formulations could not form MEG at all. Similarly, system with glycerin as co-surfactant could not form MEG at any surfactant:co-surfactant ratio as shown in Table 8 in system 14, 15, 17, 20. The results also showed that IPM as oil phase had more possibility to form MEG than CO and SBO. The pseudo-ternary phase diagrams of the microemulsion systems are presented in Figure 5-17. These figures illustrated the phase diagram which composed of IPM, CO and SBO as an oil phase using non-ionic surfactant and water phase that aforementioned. The shaded area of the phase diagram represented the area where microemulsion existed as semi-solid, viscous and transparent gel.

MEG area could be found in specific type and ratio of surfactant and co-surfactant used. Furthermore, the area was unique and had narrow range that was limited by a certain type and ratio of the component in each formulation as shown in each pseudo-ternary phase diagram in Figure 5 to 17. In this study, formation of MEG from various oil, surfactant and co-surfactant was conducted by spontaneous formation except for the systems in which surfactant and co-surfactant were in solid state such as system using L_{68}, B_{35}, B_{72}, B_{721S} and C as cosurfactant. Melting method at 60°C was used in order to melt the solid composition before mixing together with the other components of each system.

For the MEG systems that contained the same main composition; oil phase (IPM), surfactant (T_{80}) and water phase with co-surfactant, the system that composed of T_{80}: L_{68} at the same ratio of surfactant: co-surfactant as (2:1) and (1:1) but different water phase which was water or mixture of water and PG (4:1) as shown in Figure 5, 7, 6 and 8 revealed that system contained PG as co-solvent could form less MEG area than system without PG in water phase. These might be explained by the effects of surfactant: cosurfactant mixing ratio even the opposing effects of surfactant and the cosurfactant. Theoretically, cosurfactants increased the size of polar head group of surfactant and influenced the curvature of surfactant film to form microemulsion. However, increasing or presence 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 microemulsion was decreased with the increasing amount of cosurfactant. On the other hand, the results of surfactant was 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.

Figure 9 and 10. illustrates the existence of MEG area form by system with and without cosurfactant in the system as shown in system 5 and 6 from Table 8. The results showed that system with C_{EL} as cosurfactant in this system could form MEG in a narrow area than system without C_{EL} as cosurfactant. In addition, the system that composed of B_{721S} and B_{35} as co-surfactant could form MEG in a specific minimum ratio of oil: surfactant at 8:2, 7:3, 6:4, 5:5 and 4:6 and when percentage of water was less than 20% in B_{721S} as co-surfactant system as shown in Figure 11. When using B_{35} as co-surfactant, the oil: surfactant ratio which MEG area could be obtained were specific at oil:surfactant ratio were 3:7, 2:8 and 1:9 with percentage of water less than 15% as shown in phase diagram in Figure 12. Pseudo-ternary phase diagram showed that area of MEG which formed by B_{35} as co-surfactant was less than B_{721S} as co-surfactant. Furthermore, MEG area of B_{721S} and B_{35} as co-surfactant were also less than other co-surfactant system containing the same main composition of IPM: T_{80}: W system. In contrast, the system that composed of B_{72} and C as co-surfactant system could not form MEG in any ratio as shown in Table 8. This result is undoubtedly due to the high melting point of long saturated hydrophobe hindering the formation of any surfactant aggregates. Cetyl alcohol had 16 carbon atom whereas Brij 72 had 18 carbon atom which had high melting point about 45°C and 43°C respectively. In particular the relative lengths of hydrophobic chains of surfactant and oil are extremely important in determining whether the small or larger molecular volume oils are solubilized to the greater extent (Warisnoicharoen et al, 2000).

Comparison of MEG areas from various surfactant systems that contained the same composition of water phase and oil phase as shown in Figure 13 and 14 revealed that the largest MEG area was obtained from the system composed of IPM : C_{EL} : W+PG(4:1). This area decreased when the surfactant was changed from C_{EL} to C_{RH}. Furthermore, this area also decreased and could form MEG in specific ratio of surfactant when oil phase was altered from IPM to CO at the same composition of surfactant and water phase according to Figure 13 and 16. Similarly, for the system of IPM: T_{80}: W and SBO: T_{80}: W that had the same surfactant and water phase, IPM could form the larger MEG area than SBO as shown in Figure 9 and 17.

For MEG containing CO as an oil phase, the existence of MEG could not be obtained from most surfactants, co-surfactants at any investigated ratios. The formability of MEG which CO was an oil phase in this study could be obtained only by CO: C_{EL}: W:PG (4:1) system at specific oil: surfactant ratio were 5:5, 4:6 and 3:7 as shown in Figure 16. This could be the high molecular weight and high density of castor oil than others. Molecular weight of CO was more than SBO and IPM (CO = 939.5, SBO = 881 and IPM= 270.5). Therefore, it could be solubilized less than smaller molecular volume oil (Malcomson et al, 1993). According to MEG area, butanol as co-solvent system could not form MEG at any ratio but could form microemulsion as transparent liquid or fluid in large area. These could be explained by the low molecular weight with short chain alcohol of butanol which had influenced on the formation of microemulsion by both interfacial and bulk effects. Their amphiphilic nature, short hydrophobic chain (4 carbon atom; $CH_3CH_2CH_2CH_2OH$) and terminal hydroxyl group, enable them to interact with surfactant monolayers at the interfacial and influence the curvature of the interfacial energy.

The formation and structure of LC and MEG phase depended on the characteristic of the amphiphilic compound, the other component in the system and the ratio of the components. The type of surfactant and co-surfactant as well as the their relative concentrations was seen to have a pronounced effect on the region of the existence of MEG.

According to the maximum amount of oil solubilized by various system studies as shown in Table 8, it was seen that MEG regions for system IPM : C_{EL} : W: PG (4:1) and IPM : C_{RH} : W: PG (4:1) had the highest maximum amount of oil solubilization as 82% and 80% w/w, respectively. In system containing B_{721S} and B_{35} as co-surfactant, the maximum amount of oil solubilization was obtained as 80% and 30% w/w, respectively. The maximum amount of oil solubilization system composed of IPM : T_{80} : L_{68} : W:PG (T_{80}:L_{68} = 2:1 and 1:1) was 70% w/w. Similarly with the maximum amount of oil solubilization of system IPM : T_{80} : L_{68} : W(T_{80}:L_{68} = 2:1 and 1:1) that were 70% and 72% whereas the lowest maximum amount of oil solubilization was 40% in system 13 which butanol as co-surfactant. The results indicated that the maximum amount of oil solubilization was depended on the molecular volume of oil (Malcomson et al, 1993). The oil with low molecular weight could be solubilized to a greater extent than that with the higher molecular weight as shown that the maximum amount of oil solubilization of IPM was more than SBO and CO that was ranked from low to high molecular weight (IPM<SBO<CO). Molecular weight of CO, SBO and IPM were 939.5, 881 and 270.5, respectively (Kibbe, 2000).

Transparent gels containing both water and oil or microemulsion gel (MEG) or transparent oil-water gel (TOW gel) as proposed by Provost and Kinget (1988) could be described as viscous semisolid systems that consist mainly of water, oil and surfactant They have jelly-like consistency and transparent, clear and homogeneous, optically isotropic and thermodynamically stable. A characteristic "ring" or resonance occurs when a container full of TOW gel is tapped or gently bounced. The high viscosity and stiffness gel may be the cubic phase, hexagonal phase or reverse hexagonal phase of LC or MEG system that limits its potential use as the delivery system by itself. However, the ability of the less viscous lamellar phase gel to form cubic phase or reverse hexagonal phase gel upon absorbing more water has resulted in novel drug delivery opportunities in term of routes of administration and applications (Hatefi and Amsden, 2002).

The oil phase used in this study were IPM, CO and SBO; they also consumed as edible oil regarded as an essentailly non-toxic and non-irritant material. CO is high melting point oil used in oral and topical pharmaceutical formulation and stable at temperature up to 150 °C. In topical formulation, CO was used to provide stiffness to cream and emulsions. In oral formulation, it used to prepared sustained release tablets and capsule. IPM and SBO are widely used in parenteral, oral, intramuscular as drug vehicle or as component of emulsion (Kibbe, 2000). Moreover, effect of amount of oil were reported that the molecular volume of oil appeared to be an important to determine phase behavior. Warisnoicharoen et al (2000) also studied the formation of o/w microemulsion solubilized by non-ionic surfactant and various molecular weight oil. The results suggested that within the same series of oil, the low molecular volume oil was solubilized to a greater extent than large molecular volume oil.

The surfactant and cosurfactant used in this study were non-ionic surfactant which was widely used in application in pharmaceutical, cosmetic, food product, oral, parenteral, intravenous fat emulsion and topical pharmaceutical formulations. Interest in using non-ionic surfactants is due to their low irritation and high chemical stability, non-toxic, non-irritant material, compatible with other surfactant, retain this utility over the broad range of pH value from 3 to 10. Non-ionic surfactants of polyoxyethylene class are generally used in the formation of ME. Therefore, T_{80}, C_{EL}, C_{RH}, L_{68}, Brij and glycerin were selected to produce MEG in this study. These surfactant and co-surfactant also stable such as C_{EL} and C_{RH} can be sterilized by autoclaving for 20 minutest at 121°C (Kibbe, 2000). Furthermore, the amount of oil in microemulsion was increased with the increasing ratio of surfactant: cosurfactant. Similar result was obtained from composition of mineral oil, water using Brij 96 as surfactant and glycerin, propylene glycol as cosurfactant (Kale and Allen, 1989).

Another interesting application of the in situ formed cubic phase gel was for periodontal delivery of antibiotics for prevention and treatment of infections. The larmellar phase can be injected into periodontal pocket where it would transform into a stiff, cubic phase or reverse hexagonal phase gel and release the antibiotic locally preventing infection. In this study, the less viscous larmellar phase gel was recorded to be the beginning point to form MEG until viscous stiffness cubic phase gel was detected as the end point which was confirmed by cross-polarizing microscope. These mean that if this formulation contact with the amount of gingival fluid in periodontal pocket, less viscous lamellar gel phase may be transform to viscous stiffness MEG within periodontal pocket that could be controlled drug release and could receive local action which may reduce adverse drug reaction (Norling et al, 1992).

Table 8 The formability of the investigated MEG systems.

System	Oil	Surfactant (E1)	Cosurfactant (E2)	Water phase	Ratio E1:E2	Formability*	maximum oil solubilized (%w/w)
1	IPM	T_{80}	L_{68}	W: PG (4:1)	2:1	+	70%
2	IPM	T_{80}	L_{68}	W: PG (4:1)	1:1	+	70%
3	IPM	T_{80}	L_{68}	W	2:1	+	70%
4	IPM	T_{80}	L_{68}	W	1:1	+	72%
5	IPM	T_{80}	-	W	-	+	71%
6	IPM	T_{80}	C_{EL}	W	1:1	+	60%
7	IPM	T_{80}	B_{72}	W	1:1	–	–
8	IPM	T_{80}	B_{721S}	W	1:1	+	80%
9	IPM	T_{80}	B_{35}	W	1:1	+	30%
10	IPM	T_{80}	C	W	1:1	–	–
11	IPM	C_{EL}	-	W: PG (4:1)	-	+	82%
12	IPM	C_{RH}	-	W: PG (4:1)	-	+	80%
13	IPM	C_{RH}	B	W	1:1	+	40%
14	CO	T_{80}	G	W	2:1	–	–
15	CO	T_{80}	G	W	1:1	–	–
16	CO	T_{80}	-	W: PG (4:1)	-	–	–
17	CO	C_{EL}	G	W	1:1	–	–
18	CO	C_{EL}	-	W: PG (4:1)	-	+	50%
19	SBO	T_{80}	-	W	-	+	60%
20	SBO	T_{80}	G	W	1:1	–	–

+ = The system could form microemulsion gel

- = The system could not form microemulsion gel

* = The results from weight ratio of oil :surfactant 9:1 to 1:9 and the characteristic of microemulsion were semi-solid, viscous, stiffy and transparent gel which confirmed by polarized light microscopic and TEM.

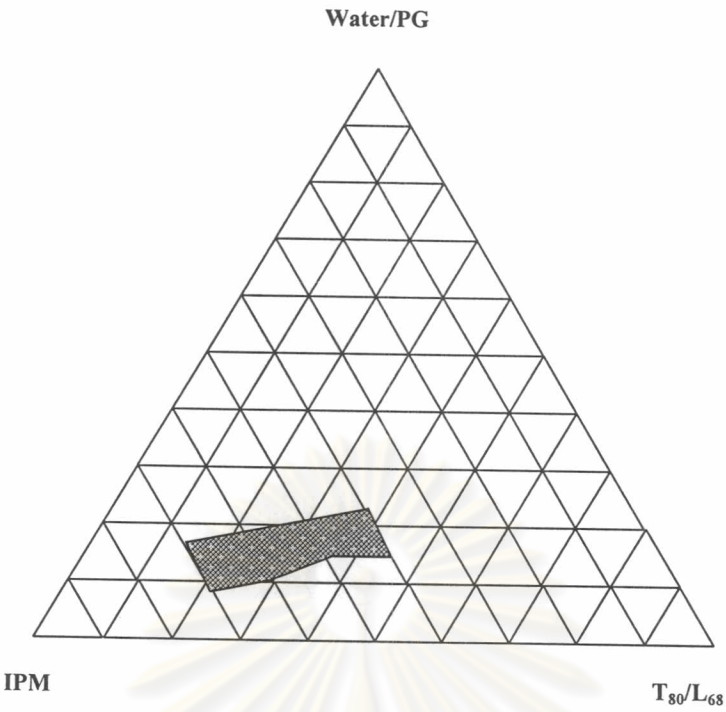


Figure 5 Pseudo-ternary phase diagram from the system of isopropyl myristate (IPM) : tween 80 (T_{80}) : Lutrol F-68 (L_{68}) ratio of (T_{80}:L_{68}) = (2:1) and co-solvent of water:propylene glycol (4:1). The shaded area represents the microemulsion gel and liquid crystal zone.

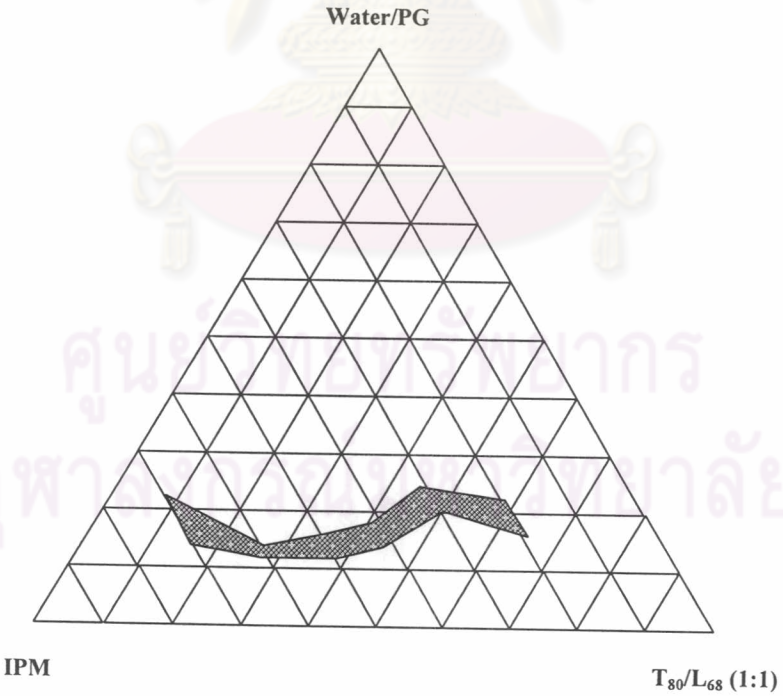


Figure 6 Pseudo-ternary phase diagram from the system of isopropyl myristate (IPM) : tween 80 (T_{80}) : Lutrol F-68 (L_{68}) ratio of (T_{80}:L_{68}) = (1:1) and co-solvent of water:propylene glycol (4:1). The shaded area represents the microemulsion gel and liquid crystal zone.

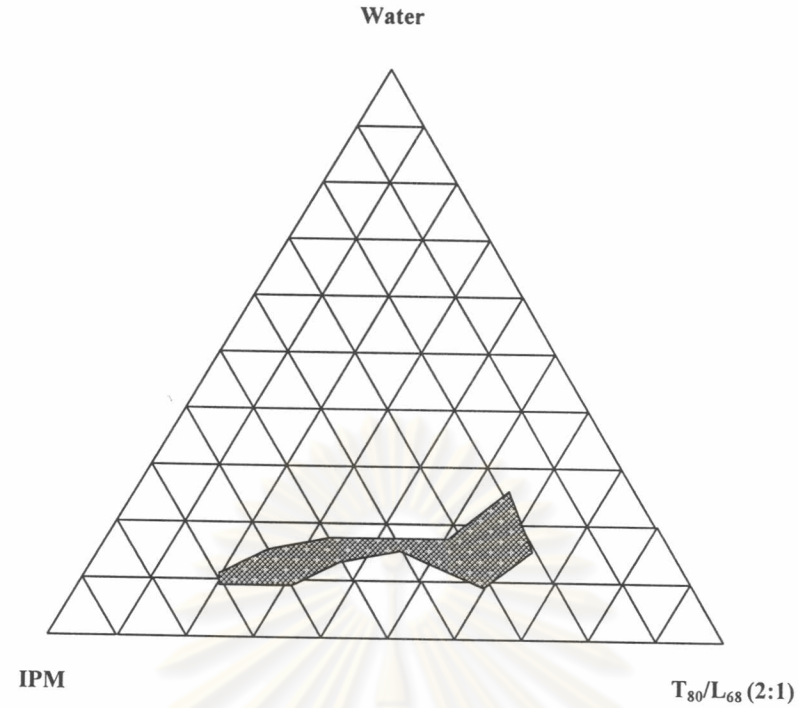


Figure 7 Pseudo-ternary phase diagram from the system of isopropyl myristate (IPM) : tween 80 (T_{80}) : Lutrol F-68 (L_{68}) at the ratio of (T_{80}:L_{68}) = (2:1) and purified water (W). The shaded area represents the microemulsion gel and liquid crystal zone.

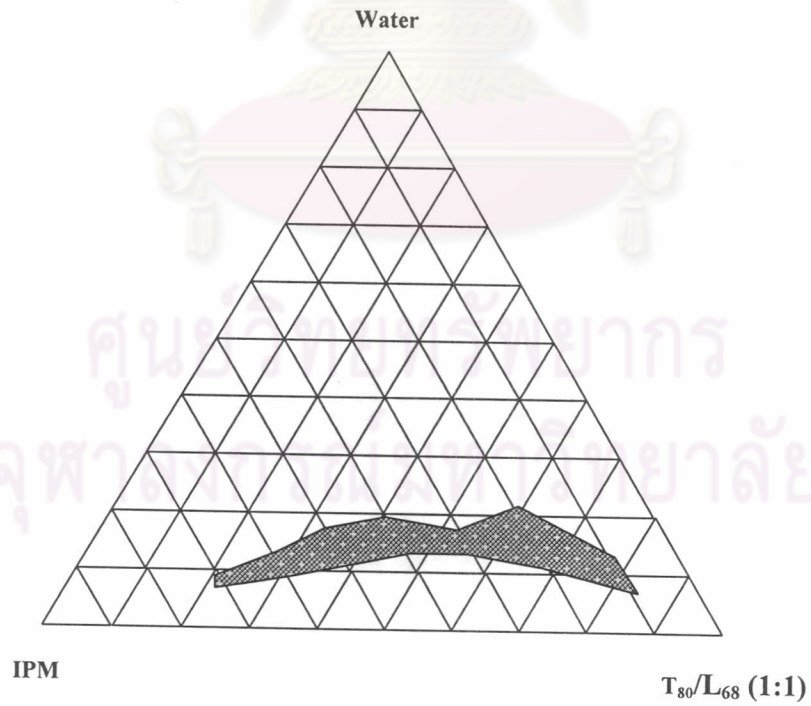


Figure 8 Pseudo-ternary phase diagram from the system of isopropyl myristate (IPM) : tween 80 (T_{80}) : Lutrol F-68 (L_{68}) at the ratio of (T_{80}:L_{68}) = (1:1) and purified water (W). The shaded area represents the microemulsion gel and liquid crystal zone.

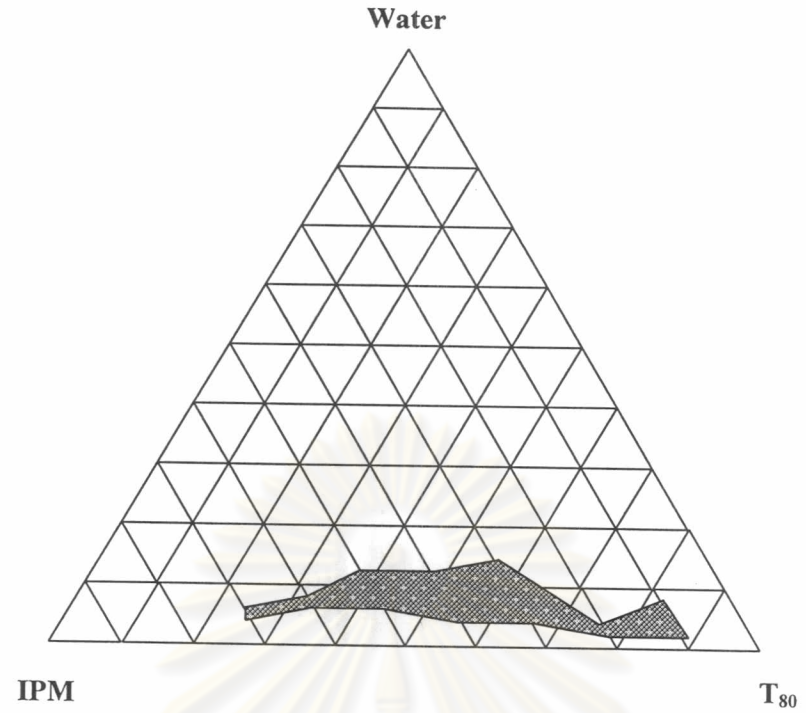


Figure 9 Pseudo-ternary phase diagram from the system of isopropyl myristate (IPM) : tween 80 (T_{80}) and purified water (W). The shaded area represents the microemulsion gel and liquid crystal zone.

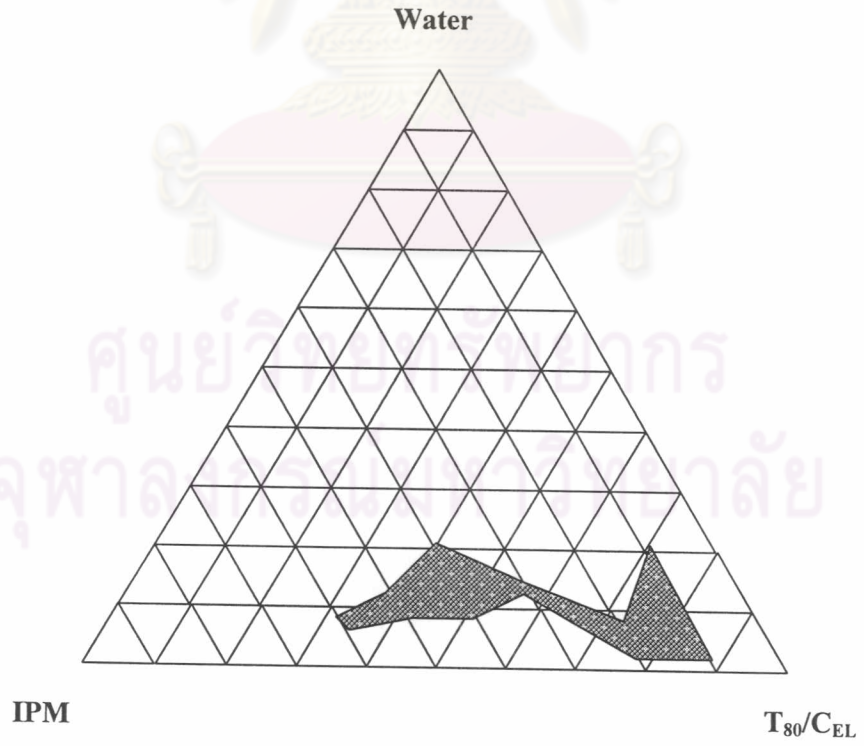


Figure 10 Pseudo-ternary phase diagram from the system of isopropyl myristate (IPM) : tween 80 (T_{80}) : cremophor EL (C_{EL}) at the ratio of (T_{80}:C_{EL}) = (1:1) and purified water (W). The shaded area represents the microemulsion gel and liquid crystal zone.

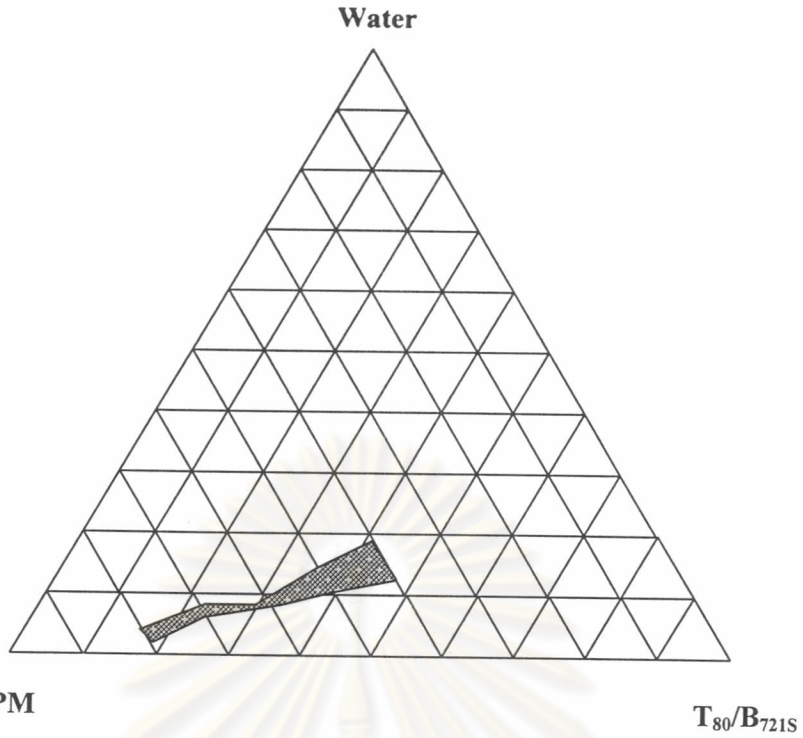


Figure 11 Pseudo-ternary phase diagram from the system of isopropyl myristate (IPM) : tween 80 (T_{80}) : Brij 721S (B_{721S}) at the ratio of (T_{80} : B_{721S}) = (1:1) and purified water (W). The shaded area represents the microemulsion gel and liquid crystal zone.

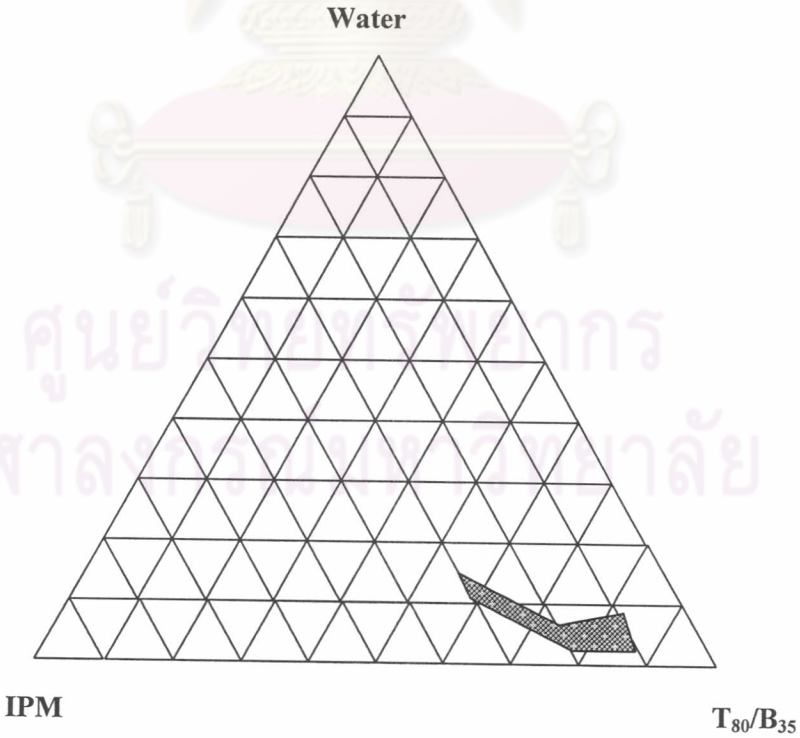


Figure 12 Pseudo-ternary phase diagram from the system of isopropyl myristate (IPM) : tween 80 (T_{80}) : Brij 35 (B_{35}) at the ratio of (T_{80} : B_{35}) = (1:1) and purified water (W). The shaded area represents the microemulsion gel and liquid crystal zone.

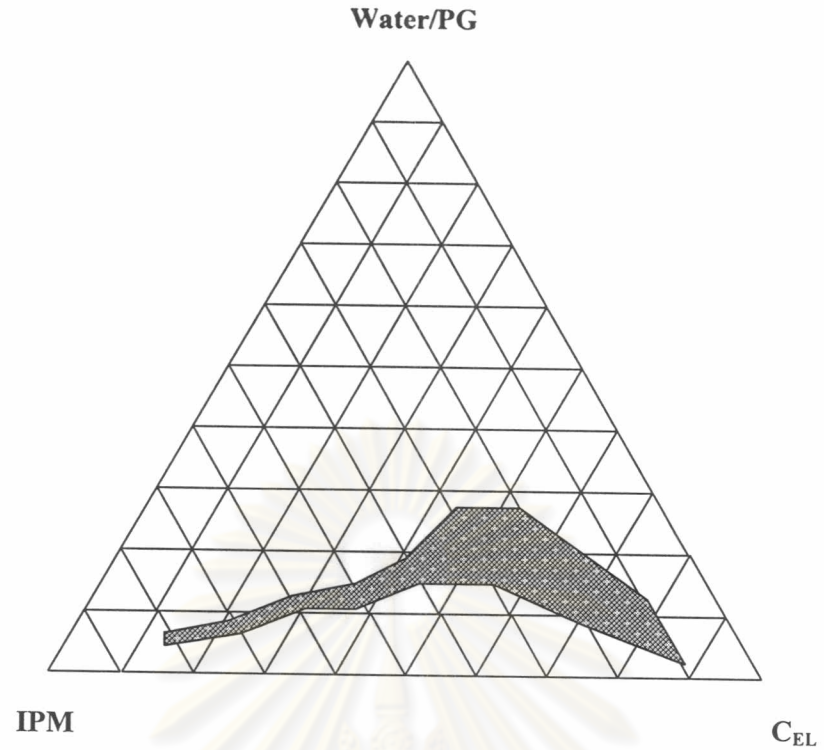


Figure 13 Pseudo-ternary phase diagram from the system of isopropyl myristate (IPM) : cremophor EL (C_{EL}) and co-solvent of water : propylene glycol (4:1). The shaded area represents the microemulsion gel and liquid crystal zone.

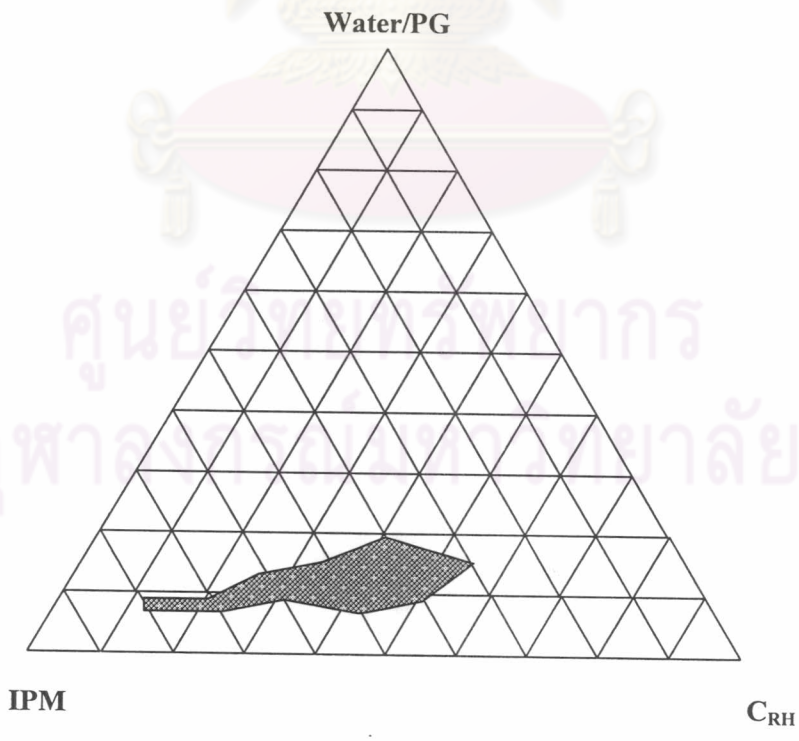


Figure 14 Pseudo-ternary phase diagram from the system of isopropyl myristate (IPM) : cremophor RH40 (C_{RH}) and co-solvent of water : propylene glycol (4:1). The shaded area represents the microemulsion gel and liquid crystal zone.

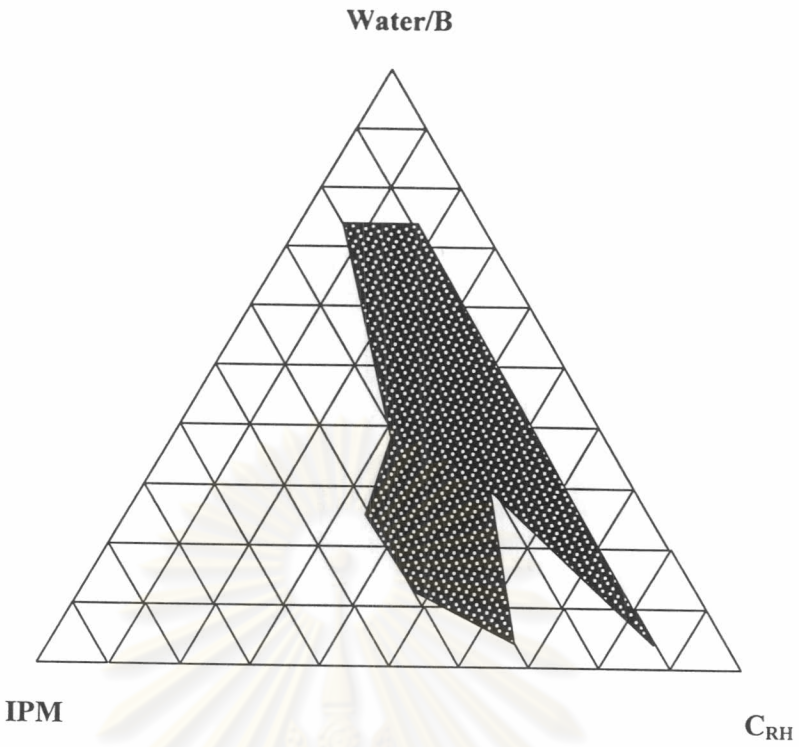


Figure 15 Pseudo-ternary phase diagram from the system of isopropyl myristate (IPM) : cremophor RH40 (C_{RH}) and co-solvent of water : butanol (4:1). The shaded area represents the liquid microemulsion area.

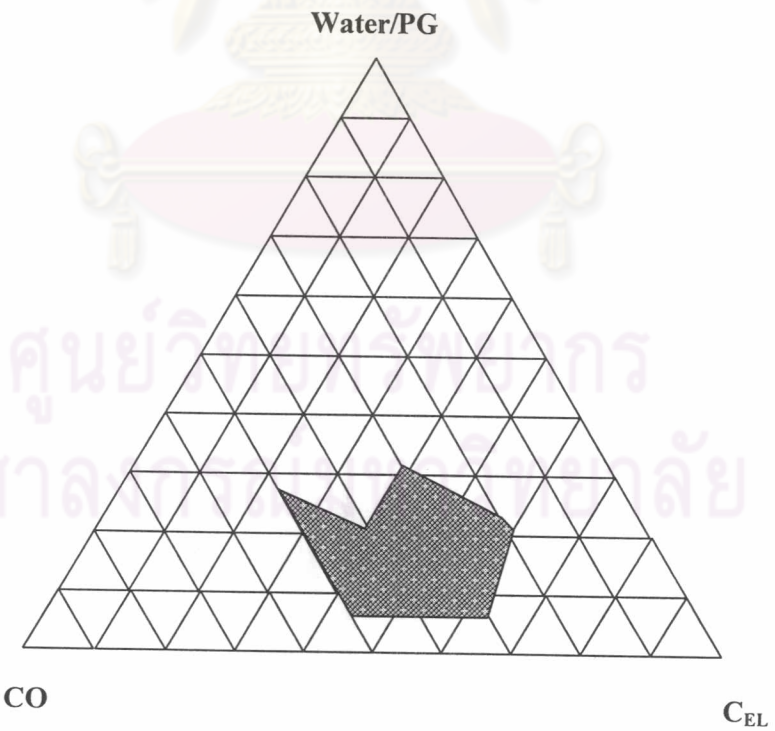


Figure 16 Pseudo-ternary phase diagram from the system of castor oil (CO): cremophor EL (C_{EL}) and co-solvent of water : propylene glycol (4:1). The shaded area represents the microemulsion gel and liquid crystal zone.

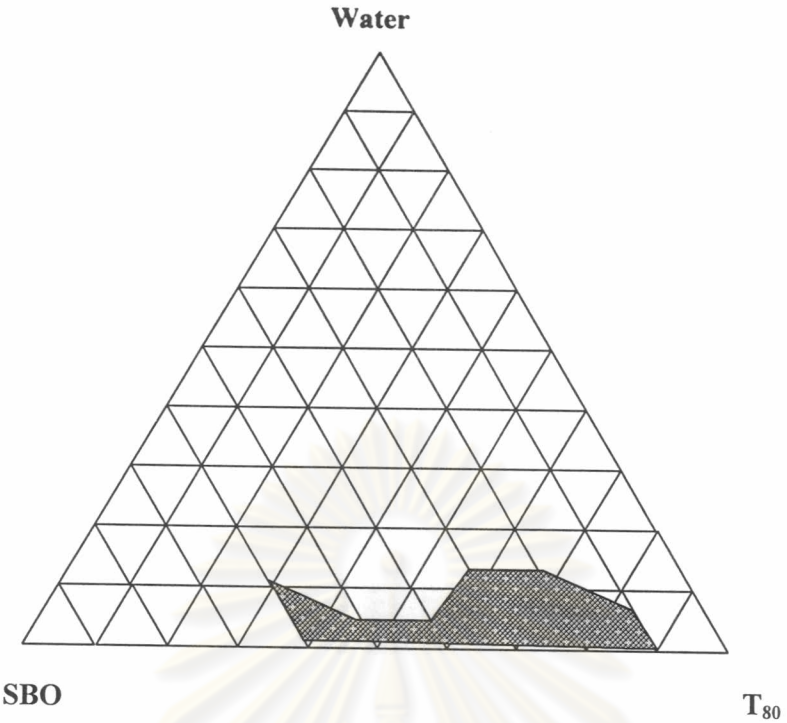


Figure 17 Pseudo-ternary phase diagram from the system of soybean oil (SBO): tween 80 (T_{80}) and purified water (W). The shaded area represents the microemulsion gel and liquid crystal zone.

2. Preparation and physicochemical characterization of microemulsion gel base

2.1 Physical appearance

The physical appearance and microscopic pattern of MEG base and MEG containing metronidazole by visual inspection and polarized light microscopy technique are listed in Table 9. From the results, the characteristic of the obtained MEG and liquid crystal systems may be classified into 4 characters according to Table 9; 1= transparent, viscoelastic lamellar gel, 2=transparent, liquid-viscous gel, 3= transparent, rigid, highly viscous gel (ringing gel), 4= phase separation. Moreover, the microscopic pattern were classified into 4 groups; A=black background, B= birefringent, lamellar phase, C=birefringent, hexagonal phase and D=birefringent, butterfly pattern.

Crystal or precipitation of drug also observed under cross-polarizer. Crystal or precipitation were not found in most system except in formulation 1/1 to 1/5 and 2/5, 2/6, 4/4 and 6/1 that precipitation and crystallization of metronidazole occurred. This might be seen that crystal or precipitation of metronidazole could be found in the occurrence of phase separation system as shown in Table 9. For non-separation system, precipitation or crystallization was also found in formulation 1/1, 1/2 ,1/4 and 6/1 that. In some MEG base of 2/5 and 2/6 system phase separation occurred after 7 days storage while MEG containing metronidazole at the concentration of 1.5% w/w showed phase separation in system 1/3, 1/5, 2/5, 2/6, 3/1, 3/6 and 4/4 as shown in Table 9 but the preparation were recovered to one phase system by gently shaking for a few seconds. These might be explained by the solubility of drug in MEG system. The excess of drug solubility could be result to precipitation. On the other hand, the ability of such oil:surfactant: cosurfactant system to solubilize the molecular of drug were limited or not appropriated to solubilize the drug in formulation.

The visual inspection of all systems revealed that the color of the product was depended on the main composition of gel such as oil, surfactant and co-surfactant. The MEG system from soybean oil had yellowish color while system from IPM and CO were colorless, clear transparent gel. The system that had T_{80} and C_{EL} as surfactant were of yellowish color whereas L_{68}, C_{RH} and B_{35} as surfactant and co-surfactant gave colorless, clearly transparent product. In addition, higher amount of surfactant and co-surfactant in formulation caused the color of the product similar to the color of surfactant or co-surfactant. In contrast, system composed of C_{EL} or T_{80} as surfactant, the appearance of final product occurred as pale yellow, viscous formula like the main component. Furthermore, the type of oil used in system also effect the color of formulation. According to this study, SBO, CO and IPM were used and the color of the last two oil are colorless whereas SBO are appear yellow. This could be effect to the appearance or character of the obtained MEG.

The appearance of each formulation depended on the characteristic of component used in formulation including surfactant and cosurfactant. Furthermore amount of such component also affect the characteristic or appearance of final product. System that composed of highly viscous surfactant such as C_{RH} or L_{68} would have the final appearance had viscous like the nature of them. C_{RH} occurs as a white semi-solid paste that made the final formulations were clearly transparent and viscous

gel. Their viscosity are ranged between 650-850 cps whereas L_{68} are practically odorless and tasteless which brought about colorless gel (Kibbe, 2000).

In addition, the distinguish of formulation 7/1 that occurred a "ringing gel" which was an excellent in all characteristic; physical stability, appearance, viscosity. The appearance which had seen might be occurrence in a various type of oil: surfactant: cosurfactant mixture. Transparent oil in water gel (TOW) and micellar solution, both microscopically isotropic, presented a marked difference in viscosity behavior.

These TOW gels can be described as semi-solid systems that consist mainly of water, oil and emulsifying agent. They have jelly-like consistency and are transparent, clear and homogeneous, optically isotropic and thermodynamically stable. A characteristic 'ring" or resonance occurs when a container full of TOW gel is tapped or gently bounced. A survey of literature Provost (1988) indicated that many terms are used interexchangeably to designate these gels, transparent emulsion gel, clear resonance gel, cream gel, viscous isotropic phase and ringing gel.

The MEG base and MEG containing metronidazole that were clear, transparent gel with birefringent property and had specific characteristic such as changing from liquid to semi-solid, rigid, stiffing or jelly-like transparent gel when contacted with certain amount of water were selected to further investigation.

Table 9 Appearance and microscopic pattern of MEG base after 7-day storage for equillibrium.

Composition of formula	System	Ratio of oil:surfactant	Physical appearance		Microscopic pattern		crystal
			Base	with MTZ	Base	with MTZ	
IPM : T_{80} : W	1/1	5:5 (water 10%)	1	1	B	B	found
	1/2	4:6 (water 8%)	2	2	A	A	found
	1/3	4:6 (water 10%)	1	4	B	B	found
	1/4	3:7 (water 8%)	2	2	A	A	found
	1/5	3:7 (water 10%)	1	4	B	B	found
	1/6	1:9 (water 7%)	2	2	A	A	no
CO : C_{EL} : W: PG (4:1)	2/1	2:8 (water 23%)	2	2	A	A	no
	2/2	2:8 (water 20%)	2	2	A	A	no
	2/3	3:7 (water 10%)	2	2	A	A	no
	2/4	3:7 (water 25%)	2	2	A	A	no
	2/5	5:5 (water 13%)	4	4	A	A	found
	2/6	5:5 (water 20%)	4	4	A	A	found
IPM : T_{80} : L_{68} : W (T_{80}:L_{68} = 2:1)	3/1	3 : 4.67 : 2.33 (water 15%)	1	4	C	C	no
	3/2	3 : 4.67 : 2.33 (water 20%)	1	1	C	C	no
	3/3	3 : 4.67 : 2.33 (water 25%)	1	1	C	C	no
	3/4	2 : 5.33 : 2.67 (water 20%)	1	1	C	C	no
	3/5	2 : 5.33 : 2.67 (water 25%)	3	3	C	C	no
	3/6	2 : 5.33 : 2.67 (water 17%)	1	4	C	C	no
IPM : C_{EL} : W: PG (4:1)	4/1	1:9 (water 10%)	2	2	A	A	no
	4/2	2:8 (water 25%)	1	1	D	D	no
	4/3	3:7 (water 25%)	3	3	B	B	no
	4/4	3:7 (water 20%)	1	4	B	B	found
IPM : C_{RH}: W: PG (4:1)	5/1	3:7 (water 14.52%)	1	1	D	D	no
	5/2	4:6 (water 20%)	3	3	B	B	no
	5/3	4:6 (water 15%)	1	1	B	B	no
	5/4	5:5 (water 15%)	3	3	D	D	no
	5/5	5:5 (water 15%)	1	1	B	B	no

Table 9 (Continue)

Composition of formula	System	Ratio of oil:surfactant	Physical appearance		Microscopic pattern		crystal
			Base	with MTZ	Base	with MTZ	
IPM : T_{80} : C_{EL} : W	6/1	3 : 3.5 : 3.5 (water 15%)	1	1	D	D	found
	6/2	2 : 4 : 4 (water 9%)	2	2	A	A	no
	6/3	1 : 4.5 : 4.5 (water 15%)	2	2	B	B	no
	6/4	1 : 4.5 : 4.5 (water 20%)	1	1	D	D	no
IPM : T_{80} : B_{35} : W	7/1	3 : 3.5 : 3.5 (water 15%)	3	3	B+D	B+D	no
SBO : T_{80} : W	8/1	1:9 (water 7%)	2	2	A	A	no

Note; Physical appearance : 1 = transparent, viscoelastic lamellar gel
2 = transparent, liquid- viscous gel
3 = transparent, rigid, highly viscous gel (ringing gel)
4 = phase separation

Microscopic pattern ; A = black background
B = birefringent, lamellar phase
C = birefringent, hexagonal phase
D = birefringent, butterfly pattern

2.2 Preparation of microemulsion gel (MEG) containing metronidazole

The saturation solubility of drug in MEG and LC was performed by varyingconcentrations of metronidazole incorporate into MEG base of each system from 0.50, 0.75, 1.00, 1.50, 2.00, 2.50 to 5.00 %w/w. The saturation solubility of metronidazole in each MEG base and LC systems was determined by visual observation and polarized light microscopy. The selected formula that possessed viscous-stiffing, transparent and stable gel bases were selected to incorporate metronidazole. Then the formulas were observed for 1 week, the highest drug concentration at which drug crystals were not found was considered to represent the saturation solubility of metronidazole in that system. The non-syneresis, non-separated, non-precipitated and stable formulations were selected. Figure 18, shows the crystal precipitate under cross-polarizing microscope. The occurrence of precipitation or crystal found in over loading metronidazole MEG system might be confirmed by the birefringent property of crystal under cross-polarizing microscope. The phase transformation of MEG with metronidazole could not observed in this study.

The MEG containing metronidazole systems were studied for their loading capacity and physical stability. The results of this experiment are shown in Table 10. The MEG from IPM : C_{RH}: W: PG (4:1) and IPM : T_{80} : B_{35} : W system could hold a maximum of 2.5 and 5%w/w of metronidazole. Maximal saturation concentration of metronidazole in IPM : T_{80} : W, CO : C_{EL} : W: PG (4:1), IPM : T_{80} : L_{68} : W (T_{80}:L_{68} = 2:1), IPM : C_{EL} : W: PG (4:1), IPM : T_{80} : C_{EL} : W and SBO : T_{80} : W systems were 1.5% w/w of metronidazole. The results showed that the solubility of metronidazole in liquid crystal systems was more than the solubility of metronidazole in water (0.1%w/w). Metronidazole is an amphiphile molecule. It is only slightly soluble in both water and lipid phases, and thus the drug molecule may align between the surfactant molecules at the interface of liquid crystalline systems that was expected to increase its solubility by being incorporated into the structure of liquid crystals. Another location of drug are proposed by Makai et al (2003) revealed that in the systems which had lamellar phase structure, the incorporated drug might be partly built between the lamellar space and partly located at the given polarity part of the amphiphillic surfactant molecules.

According to the incorporation of drug in MEG system, as in surfactant and block copolymer system, it is important to realize that the presence of a drug in a microemulsion may affect its stability and structure. For example, if the drug is soluble in water and charged, it behaves largely as a salt, and might therefore have a significant effect on the properties on microemulsions formed by ionic surfactants, whereas those formed by nonionic surfactants are less sensitive to the presence of the drug. For water –insoluble but oil-soluble drugs, on the other hand, the drug behaves essentially as an oil, thereby causing a progression of structures toward oil or more curved toward water for both ionic and nonionic and surfactants. At higher concentration of drug, phase separation may occur if the surfactant system is not balanced as shown in Table 10 in formulation 2/5 and 2/6 which phase separation occurred. For surface-active drugs, finally, the effect of the drug is determined by a delicate balance between different interactions. Nevertheless, addition of surface active drugs can be expected to have significant effect on the microemulsion stability and structure (Esposito et al, 2003).

Most of the loading drug were completely dissolved in all formulation at the concentration from 0.5% w/w until 1.5% w/w. At three days after preparation, the solubility of metronidazole was decreased in the MEG systems containing more than 1.5% w/w of metronidazole and the crystal were observed. It is indicated that the excess amount of drug that existed in the interface of the oil-surfactant mixture was released to the aqueous phase and grew the crystal or precipitation in the course of time. This results similar to the preparation flubiprofen-loaded microemulsion for parenteral delivery from ethyl oleate and tween 20 system (Park and Kim, 1999). The amount of drug which could be incorporated into microemulsions depended on both the microemulsion structure and the properties of the drug. As previously discussed, unless the microemulsion was completely balanced. An o/w microemulsion can only incorporate a certain fraction of oil or hydrophobic drug and a w/o microemulsion only a certain amount of water or a polar drug without phase separation (Esposito et al, 1996).

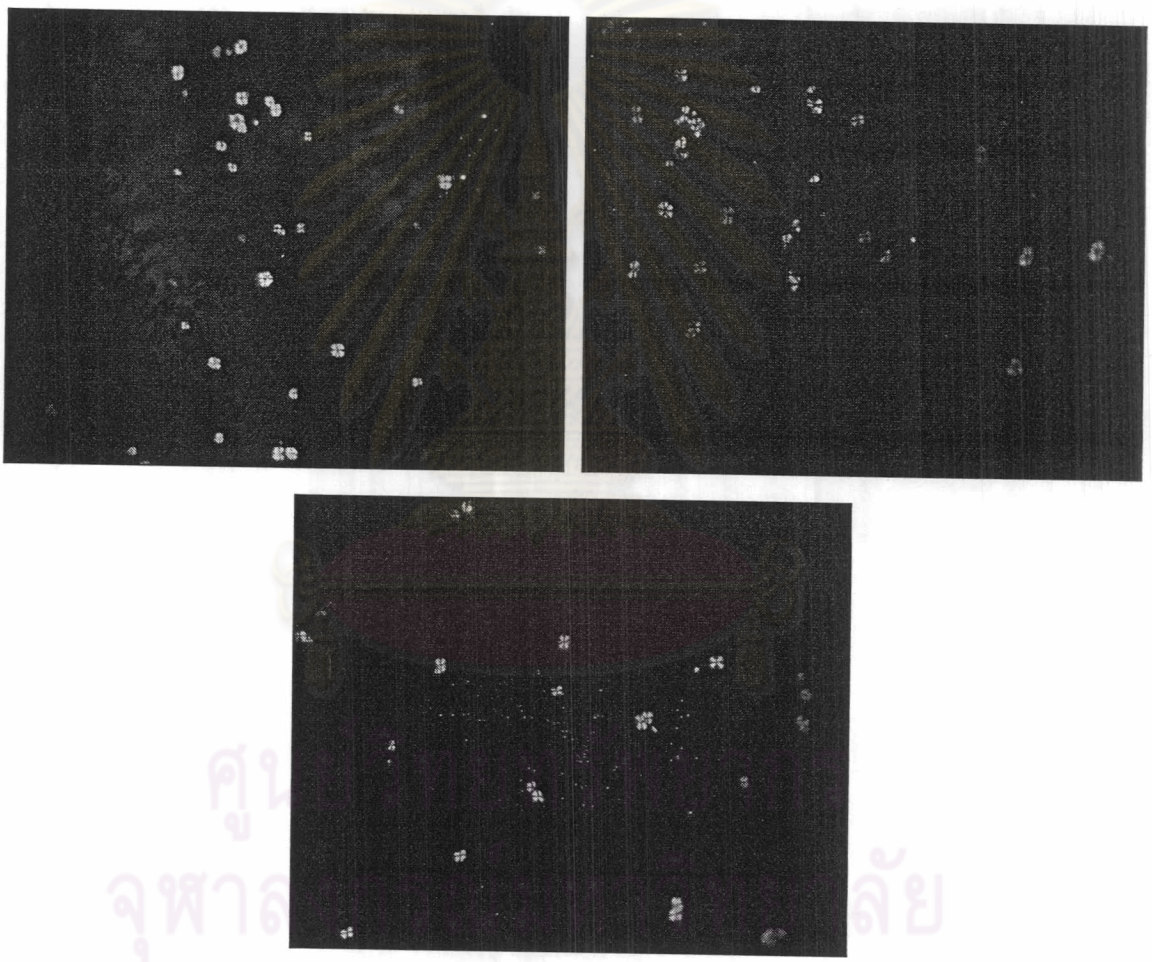


Figure 18 The photomicrographs from cross-polarizing microscope showing crystals and precipitate of metronidazole that occurred after three days of incorporation of metronidazole over maximum loading capacity.

Some system such as IPM : C_{RH}: W: PG (4:1) and IPM : T_{80} : B_{35} : W could hold maximum loading at 2.5 % w/w and 5% w/w, respectively without any precipitation or crystallization was observed as shown in Table 10. These might be the appropriated system for this drug loaded to from aggregation with hydrophobic part of surfactant molecule (Djordjevic et al, 2004).

Table 10 Loading capacity of metronidazole microemulsion gel.

Composition of formula	System	Ratio of oil:surfactant	Drug loading (%w/w)						
			0.50	0.75	1.00	1.50	2.00	2.50	5.00
IPM : T_{80} : W	1/1	5:5 (water 10%)	-	-	-	-	+	++	+++
	1/2	4:6 (water 8%)	-	-	-	-	+	++	+++
	1/3	4:6 (water 10%)	-	-	-	-	+	++	+++
	1/4	3:7 (water 8%)	-	-	-	-	+	++	+++
	1/5	3:7 (water 10%)	-	-	-	-	+	++	+++
	1/6	1:9 (water 7%)	-	-	-	-	+	++	+++
CO : C_{EL} : W: PG (4:1)	2/1	2:8 (water 23%)	-	-	-	-	+	++	+++
	2/2	2:8 (water 20%)	-	-	-	-	+	++	+++
	2/3	3:7 (water 10%)	-	-	-	-	+	++	+++
	2/4	3:7 (water 25%)	-	-	-	-	+	++	+++
	2/5	5:5 (water 13%)	-	-	-	*	+	++	+++
	2/6	5:5 (water 20%)	-	-	-	*	+	++	+++
IPM : T_{80} : L_{68} : W (T_{80}:L_{68} = 2:1)	3/1	3 : 4.67 : 2.33 (water 15%)	-	-	-	-	+	++	+++
	3/2	3 : 4.67 : 2.33 (water 20%)	-	-	-	-	+	++	+++
	3/3	3 : 4.67 : 2.33 (water 25%)	-	-	-	-	+	++	+++
	3/4	2 : 5.33 : 2.67 (water 20%)	-	-	-	-	+	++	+++
	3/5	2 : 5.33 : 2.67 (water 25%)	-	-	-	-	+	++	+++
	3/6	2 : 5.33 : 2.67 (water 17%)	-	-	-	-	+	++	+++

Table 10 Continue.

Composition of formula	System	Ratio of oil:surfactant	Drug loading (%w/w)						
			0.50	0.75	1.00	1.50	2.00	2.50	5.00
IPM : C_{EL} : W: PG (4:1)	4/1	1:9 (water 10%)	-	-	-	-	+	++	+++
	4/2	2:8 (water 25%)	-	-	-	-	+	++	+++
	4/3	3:7 (water 25%)	-	-	-	-	+	++	+++
	4/4	3:7 (water 20%)	-	-	-	-	+	++	+++
IPM : C_{RH}: W: PG (4:1)	5/1	3:7 (water 14.52%)	-	-	-	-	-	-	+
	5/2	4:6 (water 20%)	-	-	-	-	-	-	+
	5/3	4:6 (water 15%)	-	-	-	-	-	-	+
	5/4	5:5 (water 15%)	-	-	-	-	-	-	+
	5/5	5:5 (water 15%)	-	-	-	-	-	-	+
IPM : T_{80} : C_{EL} : W	6/1	3 : 3.5 : 3.5 (water 15%)	-	-	-	-	+	++	+++
	6/2	2 : 4 : 4 (water 9%)	-	-	-	-	+	++	+++
	6/3	1 : 4.5 : 4.5 (water 15%)	-	-	-	-	+	++	+++
	6/4	1 : 4.5 : 4.5 (water 20%)	-	-	-	-	+	++	+++
IPM : T_{80} : B_{35} : W	7/1	3 : 3.5 : 3.5 (water 15%)	-	-	-	-	-	-	-
SBO : T_{80} : W	8/1	1:9 (water 7%)	-	-	-	-	+	++	+++

- = clear, no crystal precipitation +, ++, +++ = precipitation and crystal found * = phase separation

2.3 Determination of MEG type and conductivity

MEG type is another important consideration for pharmaceutical applications. A single method may yield misinterpret results. Instead, the type of MEG should always be confirmed by mean of other methods. In this study, the dilution test and dye solubility test were employed since it provided a convenient and useful tool for the investigation of MEG type (Constantinides, 1995). Furthermore, conductivity test was used to confirm the determination of MEG type in each system.

Table 11 summarizes the results of the tests for MEG type and conductivity of MEG base and MEG containing 1.5%w/w metronidazole determined by three methods. From the dilution test on MEG without metronidazole, the MEG became slightly turbid when water was added and mixed for a few minutes. After thirty minutes standing at room temperature all preparations were clear transparent gel and phase separation was not shown except formulation 1/1-1/6, 2/3, 2/5, 6/1, 6/2 and 8/1 in which the opposite results were obtained. In contrast, most formulations were turbid after adding IPM into system and phase separation occurred after standing at room temperature for thirty minutes except for corresponding formulations aforementioned. The upper phase was slightly turbid solution whereas the lower phase was more turbid than the upper phase. For the MEG containing 1.5%w/w metronidazole, the results from dilution test were similar to MEG without metronidazole. Thus, the results indicated that the external phase of most MEG formulation was hydrophilic phase except for the formulation 1/1-1/6, 2/3, 2/5, 6/1, 6/2 and 8/1 that external phase was lipophilic phase which could be classified as water in oil system.

From the dye solubility test of drug free MEG, when tartrazine was added it was easily miscible with MEG. Most systems exhibited clear, homogeneuos yellow solution except for formulation 1/1-1/6, 2/3, 2/5, 6/1,6/2 and 8/1 in which coagulation of tartrazine solution were observed. In contrast, when oil soluble dye (D&C red no.17) was added the dye was not miscible with MEG and the system became turbid. For the MEG containing 1.5%w/w metronidazole, the results from dye solubility test were not different from MEG without metronidazole.

Furthermore, the conductivity testing of both drug free and drug loaded MEG revealed that the conductivity of formulation 1/1-1/6, 2/3, 2/5, 6/1,6/2 and 8/1 were lower than the others which correspondingly confirmed with the dilution and dye solubility test. Therefore, the results from dilution test, dye solubility test and conductivity test indicated that most formulation of MEG with and without drug were o/w MEG except formulation 1/1-1/6, 2/3, 2/5, 6/1, 6/2 and 8/1 which were w/o MEG. In addition, the trend of conductivity value of MEG containing 1.5%w/w metronidazole were more than those without metronidazole except formulation 5/3, 5/4 and 5/5 which drug loaded MEG had lower conductivity than MEG base as shown in Figure 20.

This might be due to the changing in phase behavior of such formulations after drug-loading. The drug molecule might adsorbed the water from system in order to solubilize its molecule. Therefore, the lower of water in continuous phase was obtained which resulted in the lower in electrical conductivity value. Another aspect

revealed that the solubilized macromolecules can even induce temperature percolation followed by a phase separation (Esposito et al, 2003). Moreover, it was also observed from complex behavior of the higher electrical conductivity with increasing percentage of water content in formulation. A ternary system containing water, toluene and triton X-100 (40: 40: 20% w/w) had shown a very drastic change in electrical conductivity where a marked fluctuation was expected as a sign of dynamics occurring at the boundary (Hyde, 1996).

The percolation behavior in microemulsion has been exploited to explore the structural property of microemulsion. The other study of conductivity along with flow and electrical birefringent methods for isotropic phase of AOT/water/dodecane system had reported for a constant oil: water ratio and increasing water content, the microemulsion showed a transition in the conductivity from high to low conductant state (Gao et al, 1998). They have also shown that the charge transport mechanisms between the clustered nanodroplets is different for ionic and non-ionic surfactant (Malcomson et al, 1993).

The thermodynamics of clustering of microdroplets during percolation has also studied by Paul and Moulik (1997). The phenomenon of percolation of conductance has been known to occur in binary inclusions containing conductor and insulator. It has been found that after a threshold volume fraction of spherical particles of the conductors the conductance increases sharply and then levels off. This phenomenon is known as 'static percolation' and has been critically studied and analyzed.The phenomenon of conductance percolation in colloidal solution, i.e. w/o microemulsion, is equally interesting if not more so. The droplets containing surfactant ions come to a threshold distance wherein transfer of charge between them occurs efficiently; they are physicochemically dynamic and can approach their neighbours by diffusion to transfer charge. This is how 'dynamic percolation' arises after a threshold volume fraction at a constant thermal condition or temperature. The phenomenon can be augmented by the increase of temperature at a fixed droplet concentration. The w/o microemulsions formed with non-ionic amphiphiles containing soluble salts in the aqueous core can exhibit conductance percolation as well.

The mechanism of percolation might be favour of transfer by the hopping of surfactant from one droplet to another and the other by the mechanism of fusion, interfacial layer opening and ion-transfer as shown in Figure 19 which (A) Hopping mechanism by ion hop in the direction indicated by the curl head (B). Ion transport by fusion and fission by ion in the droplets (Moulik and Paul, 1998).

In general, the presence of o/w MEG droplets is likely to be a feature in MEG 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 structure may results (Lawrence and Rees, 2000). In addition, it is generally accepted that low HLB (3-6) surfactants are favored for the formation of w/o MEG whereas surfactants with high HLB (8-18) are preferred for the formation of o/w MEG system (Attwood, 1994).

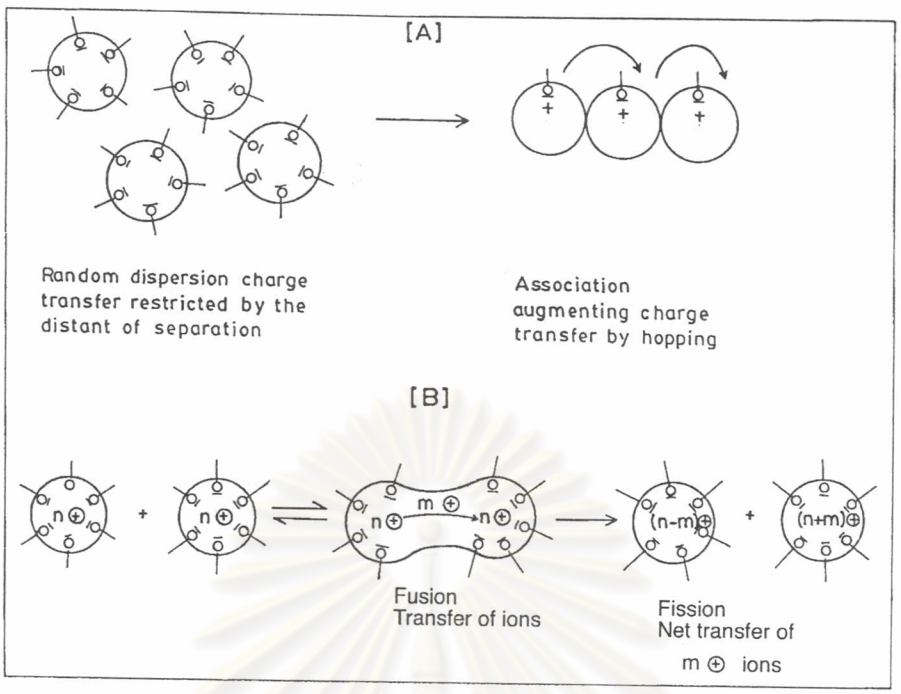


Figure 19 A schematic representation of the models of charge transfer (from Moulik and Paul, 1998).

There are many method to determine the MEG type. A single method may lead to incorrect results. In stead, the type of MEG should always be confirmed by at least two methods. Due to the high HLB of non-ionic surfactant used in this study; T_{80} (HLB=15), C_{EL} (HLB=13.5), C_{RH} (HLB=14-16), B_{35} (HLB=17), L_{68} (HLB=17), including that most formulation contained small amount of oil, thus, these MEG systems should be classified to be o/w type. However, some system using these surfactant exhibited low conductivity, separated when diluted with water which implied to be w/o MEG such as formulation 1/1-1/6, 2/3, 2/5, 6/1,6/2 and 8/1. These results might be explained by the low percentage of water presented in formulation 1/1-1/6 which were 10%, 8%, 10%, 8%, 10%, 7% and 10%, 13%, 15%, 9% and 7% in formulation 2/3, 2/5, 6/1, 6/2 and 8/1, respectively. Moreover, the results from dilution test, dye solubility test and conductivity test could be confirmed the w/o MEG type of these system (Swarbick and Boyland, 1994).

Similar results has been revealed by Djordjevic et al (2004). Percolation phenomenon was observed in conductivity and viscosity of system composed of water, IPM, Labrasol and Plurol that using caprylocaproyl macrogolglycerides based microemulsion drug delivery vehicles for an amphiphilic drug. Furthermore, it has been demonstrated that there is a strong correlation between specific structure and their electron conductive behavior. While the water volume fraction increase, the electrical conductivity slightly increased as well until the critical is reached when a sudden increase in conductivity is observed. The non-ionic amphiphiles also exhibited electroconductive behavior in spite of non-ionic type (Ho et al, 1996).

Table 11 The types and conductivity of MEG base and MEG containing 1.5%w/w metronidazole.

Composition of formula	System	Ratio of oil:surfactant (% water)	Dilution test		Dye solubility test		Conductivity		Type	
			water dilution	oil dilution	water soluble	oil soluble	MEG base	1.5%w/w MTZ	MEG base	1.5%w/w MTZ
IPM : T_{80} : W	1/1	5:5 (10%)	**	*	im	m	2.00	0.10	w/o	w/o
	1/2	4:6 (8%)	**	*	im	m	2.90	2.30	w/o	w/o
	1/3	4:6 (10%)	**	*	im	m	2.00	3.40	w/o	w/o
	1/4	3:7 (8%)	**	*	im	m	2.90	3.30	w/o	w/o
	1/5	3:7 (10%)	**	*	im	m	2.30	3.50	w/o	w/o
	1/6	1:9 (7%)	**	*	im	m	3.10	3.40	w/o	w/o
CO : C_{EL} : W: PG (4:1)	2/1	2:8 (23%)	*	**	m	im	11.10	16.70	**o/w**	**o/w**
	2/2	2:8 (20%)	*	**	m	im	11.90	12.20	**o/w**	**o/w**
	2/3	3:7 (10%)	**	*	im	m	3.80	3.80	w/o	w/o
	2/4	3:7 (25%)	*	**	m	im	21.30	23.20	**o/w**	**o/w**
	2/5	5:5 (13%)	**	*	im	m	1.70	4.60	w/o	w/o
	2/6	5:5 (20%)	*	**	m	im	15.80	14.30	**o/w**	**o/w**
IPM : T_{80} : L_{68} : W (T_{80}:L_{68} = 2:1)	3/1	3 : 4.67 : 2.33 (15%)	*	**	m	im	4.10	6.60	**o/w**	**o/w**
	3/2	3 : 4.67 : 2.33 (20%)	*	**	m	im	15.00	12.70	**o/w**	**o/w**
	3/3	3 : 4.67 : 2.33 (25%)	*	**	m	im	19.40	20.10	**o/w**	**o/w**
	3/4)	2 : 5.33 : 2.67 (20%)	*	**	m	im	6.40	6.00	**o/w**	**o/w**
	3/5	2 : 5.33 : 2.67 (25%)	*	**	m	im	10.70	9.80	**o/w**	**o/w**
	3/6	2 : 5.33 : 2.67 (17%)	*	**	m	im	4.30	8.00	**o/w**	**o/w**
IPM : C_{EL} : W: PG (4:1)	4/1	1:9 (10%)	*	**	m	im	4.80	5.20	**o/w**	**o/w**
	4/2	2:8 (25%)	*	**	m	im	14.70	15.80	**o/w**	**o/w**
	4/3	3:7 (25%)	*	**	m	im	12.20	13.80	**o/w**	**o/w**
	4/4	3:7 (20%)	*	**	m	im	8.60	11.00	**o/w**	**o/w**
IPM : C_{RH}: W: PG (4:1)	5/1	3:7 (14.52%)	*	**	m	im	9.00	14.30	**o/w**	**o/w**
	5/2	4:6 (20%)	*	**	m	im	20.70	14.60	**o/w**	**o/w**
	5/3	4:6 (15%)	*	**	m	im	14.80	13.20	**o/w**	**o/w**
	5/4	5:5 (15%)	*	**	m	im	12.70	11.00	**o/w**	**o/w**
	5/5	5:5 (15%)	*	**	m	im	15.20	11.00	**o/w**	**o/w**

Table 11 (Continue).

Composition of formula	System	Ratio of oil:surfactant (% water)	Dilution test		Dye solubility test		Conductivity		Type	
			water dilution	oil dilution	water soluble	oil soluble	MEG base	1.5%w/w MTZ	MEG base	1.5%w/w MTZ
IPM : T_{80} : C_{EL} : W	6/1	3 : 3.5 : 3.5 (water 15%)	**	*	im	m	3.30	3.00	w/o	w/o
	6/2	2 : 4 : 4 (water 9%)	**	*	im	m	4.10	4.30	w/o	w/o
	6/3	1 : 4.5 : 4.5 (water 15%)	*	**	m	im	10.90	11.90	**o/w**	**o/w**
	6/4	1 : 4.5 : 4.5 (water 20%)	*	**	m	im	12.00	12.10	**o/w**	**o/w**
IPM : T_{80} : B_{35} :W	7/1	3 : 3.5 : 3.5 (water 15%)	*	**	m	im	9.10	11.00	**o/w**	**o/w**
SBO : T_{80} : W	8/1	1:9 (water 7%)	**	*	im	m	3.40	3.80	w/o	w/o

water soluble dye = tartrazine

oil soluble dye = D&C red no.17

* = no separation
** = separation
m = miscible
im = immiscible

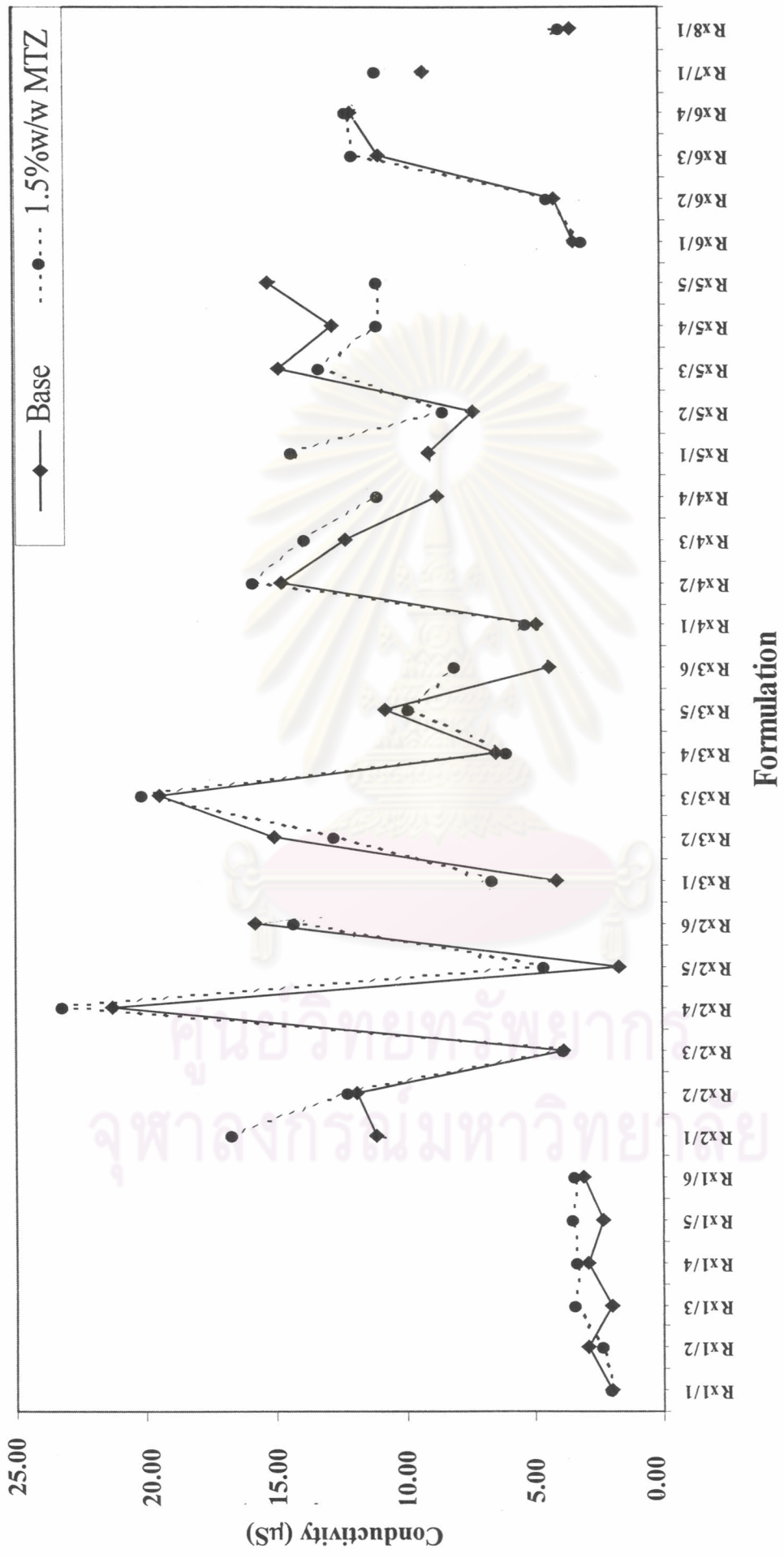


Figure 20 Comparison of the conductivity of MEG base and 1.5%w/w metronidazole MEG.

2.4 Birefringent property of microemulsion gel

Among all available techniques, polarized light microscopy provides the easiest way to qualitatively identify the different phases of liquid crystals by their textures. The lamellar and the hexagonal phases are optically anisotropic and can be directly observed under an optical microscope with polarized light. The sample of these phases look radiant when viewed against a light source placed between crossed polarizer, whereas the cubic phase is optically isotropic and consequently not visible in polarized light. Structure of cubic phase is unique and consists of a curved bicontinuous lipid bilayer which spontaneous formation and thermodynamically stable in many lipid/water systems (Jaymin et al, 2001). Under microscope with polarized light, the two phases, the lamellar and hexagonal phase also display different microscopic patterns.

Lamellar phase structure may be call lamellar mesophases that exhibit distinct optical textures, when confined between cross-polarizer microscope. Typically, the texture is "streaky" or mosaic-like and resembles the marbling in freshly cut streak. Alternatively, lamellar can eradicate all edges by folding into vesicles. The formation of lamellar mesophases is almost unavoidable in the majority of amphiphile-water systems. Double chain surfactants typically form lamellar phases on water dilution, and single chain detergents form a lamellar mesostructure under more concentrated conditions. Hexagonal mesophase or columnar mesophase are anisotropic phase which intermediate viscosity to discrete micellar and bicontinuous cubic phases. The standard picture of hexagonal phase consists of a dense packing of cylindrical micelles. It is often identified by a characteristic "fan" texture in the optical microscope, due to focal conic domains of columns (Stephen, 1996).

The formation and the structure of various microemulsion gel and liquid crystal were identified by polarized light microscopy according to the classification established by Makai et al (1999), Yamamoto et al. (2001). Liquid crystal and microemulsion gel system from IPM : T_{80}: W system had a lamellar liquid crystalline structure especially in formulation (1/1), (1/3), (1/5) had butterfly pattern in lamellar phase as shown in Figure 21, 22 and 23 respectively whereas formulation (1/2), (1/4) and (1/6) appeared dark when viewed under cross polarizer indicating of non-birefringent property. Thus, they would be classified as microemulsion.

The CO:C_{EL}:W: PG(4:1) system appeared dark in every ratio under cross-polarizer. They would be classified as cubic phase or transparent oil in water system. The lutrol system, IPM : T_{80} : L_{68} : W(T_{80}:L_{68}=2:1); in formulation (3/1)-(3/6) had a well-organized hexagonal phase structure in almost every ratio in this study as shown in Figure 24-29. In IPM : C_{EL} : W: PG (4:1) system, lamellar liquid crystalline phase was found in formulation (4/2) to (4/4) as shown in Figure 30, 31 and 32 whereas formulation (4/1) were appeared dark, this would be microemulsion system.

The IPM : C_{RH} : W: PG (4:1) system had lamellar structure in formulation (5/1) and (5/2) as shown in Figure 33 and 34. Both lamellar phase structure and hexagonal phase structure could be obtained in formulation (5/3) to (5/5) when viewed with the same sample at interval time as shown in Figure 35-37. The initial

time, hexagonal structure could be seen at 40x polars then come after with lamellar phase structure when used 100x polars in such formulation.

The IPM : T_{80} : C_{EL} : W system almost all formulation (6/1), (6/3), (6/4) had lamellar phase structure especially formulation (6/4) which exhibited most butterfly pattern in lamellar phase structure of liquid crystalline while formulation (6/2) were appeared dark that exhibited microemulsion property as shown in Figure 38, 39 and 40.

The system that composed of IPM : T_{80} : B_{35} : W had a specific microscopic pattern as shown in Figure 41. which exhibited unique lamellar phase with butterfly pattern. The last system; SBO : T_{80} : W exhibited microemulsion property that were appeared dark when viewed with cross-polarizing microscope. The relationship between appearance and microscopic pattern described that the systems that appeared dark under cross-polarizer were transparent yellow, liquid gel and the system that appeared birefringent, lamellar phase and butterfly pattern were viscoelastic lamellar and highly viscous lamellar gel when investigated by visual observation.

The results showed that when the oil was changed from isopropyl myristate to castor oil or soybean oil, the non-birefringent property occurred. In pharmaceuticals and cosmetics, combination between two or more emulsifiers or the presence of co-surfactant tended to increase formulation stability (Trotta, 1999). Hence, the combining effect of two surfactants on the formation and structures of liquid crystals between two surfactants was also studied by using tween80:lutrol F-68, tween80: cremophor EL and tween80 : Brij35. The results show that the combination of two surfactants caused more occurrence of both lamellar phase and hexagonal phase structure. According to the microscopic pattern of each system, the formation and structures of liquid crystalline phase depended on the characteristic of the amphiphillic compound, the other component in the system, the type and ratio of the components and time.

The advantages of these microemulsion gel and liquid crystal mesophase were that these characteristic of mesophase could be controlled drug delivery. Upon contact with liquid from gingival fluid the composition may change from lamellar phase gel structure to a reverse hexagonal or cubic phase gel which contacted with the site of action and released the active agent in controlled fashion.

The method of delivery was particularly useful for the treatment of periodontal disease by insertion of the lamellar gel phase (less viscous and flowable) which known to absorb water and body fluids in situ and directly into the periodontal pocket by using injectable syringes, where water from the gingival fluid induces the spontaneous in-situ formation of the reverse hexagonal or cubic phase structure that have a high-viscosity, the biodegradability, ability to incorporate and deliver drugs of varying size, water solubility, physical stability (Jaymin et al, 2001).

The high viscosity and stiffness of the hexagonal or cubic phase gel limited the potential used as the delivery system by itself. However, the ability of the less viscous lamellar phase gel to form cubic or hexagonal phase gel upon absorbed more water had resulted in novel drug delivery opportunities in term of routes of administration and applications that could inject into the periodontal pocket where it

would transform into a stiff highly viscous gel and release antibiotic locally preventing infection (Esposito et al, 1996).

The lamellar phase of lyotropic liquid crystals is utilized as a model of biological membranes because of its structural similarly with such membranes. Amphiphilic polar lipid such as phospholipids when placed in water spontaneously forms thermodynamically stable lipid bilayers, which can assume various geometric shapes and structures. Liposome is one such example in which amphiphile lipids reorganize into closed circular lipid bilayer enclosing an aqueous phase. However, the spontaneous reorganization of amphiphile lipids in aqueous environment can result in other three-dimensional structures such as the lamellar phase, the cubic phase and hexagonal phase which can be used as drug delivery (Cordobes et al, 1997). The lamellar phase has a long-range order in one dimension. Its structure consists of a linear arrangement of alternating lipid bilayers and water channels. The reverse hexagonal phase consists of water rods arranged in a two-dimentional lattice and separated by lipid bilayers. The cubic phase is usually observed between the lamellar phase and reverse hexagonal phase as the water content is increased (Jaymin et al, 2001).

Non-aqueous liquid crystals; which formed by lecithin is capable of self organizing into liquid crystalline state in such organic solvents as glycerol, ethylene glycol and formamide. The main feature of them is that they are prone to a formation of numerous hydrogen bonds. Furthermore, organogel which is the most common phenomenon for the non-aqueous lecithin solutions induced by dissolved water is transformation of the spherical micelles into cylindrical aggregates (Kantaria et al, 1999). The lecithin organogel are transparent, viscoelastic liquid which formed by three-dimensional network without fixed contact between entangled polymer like micelles. It should be mentioned that the lecithin organogel exist in a rather narrow range of water concentrations. If this amount has been exceeded, there is phase separation (Norling et al, 2000).

Surprisingly, in this study the self organizing into liquid crystal of three component could be obtained liquid crystal which derived from pharmaceutical acceptable material. This could occur in specific type and ratio of oil, surfactant and cosurfactant used in formulation without adding gelling agent into system. These three component were presented lamellar phase structure, hexagonal phase structure including phase transformation brought about by increasing water content. Since the lamellar phase is less viscous and could be injectable, it could be used to deliver the gel, which upon contact with excess water from body fluids form the stiff viscous gel providing sustained released.

The unique structure and physicochemical property of this LC gel make it suitable as local drug delivery system. In summary, the high viscosity, biodegradable, ability to incorporated drug and chemical and physical stability of lamellar and hexagonal phase make this novel surfactant LC structure gel an excellent candidate for use a drug delivery system which may be limited to specific application such as periodontal, mucosal, vaginal (Osmond et al, 2001).

The TOW gels were rigid, highly viscous systems while the micellar solution were of low viscosity. The difference between the TOW gels and the anisotropic liquid crystal phase was noted. The TOW gels were perfectly isotropics, while the anisotropic nature of the liquid crystal phase was apparent from the textures they exhibited under polarized light. Since the TOW gels are optically isotropic, no attempt was made to subject the preparations to centrifugation.

The phase area defined as anisotropic liquid crystal phase also included the mixtures of this phase with TOW gels. Nor was a distinction made between the lamellar and hexagonal liquid crystal phases, so mixture of these two phases might be present as well in the liquid crystal area as illustrated by the photomicrograph from polarized light microscopy showing the structure of lamellar phase, hexagonal phase and butterfly pattern are shown and discuss in the next topic (Nina et al, 1999).

The typical hexagonal liquid-crystalline texture are shown in Figure 42. This phase behavior composed of Heptane heptane/ polyoxyethylene glycol nonylphenyl ether and water systems exhibited a direct hexagonal liquid-crystalline structure (Cordobes et al, 1997). The similar hexagonal phase structure has been revealed by using only lecithin: water (40:60) only adding water to the system in order to induce the complete hydration of lecithin. This might be found that the common phenomenon for non-aqueous lecithin solution induced by dissolved water is transformation of the spherical micelles into cylindrical aggregates (Vitoria et al, 1999).

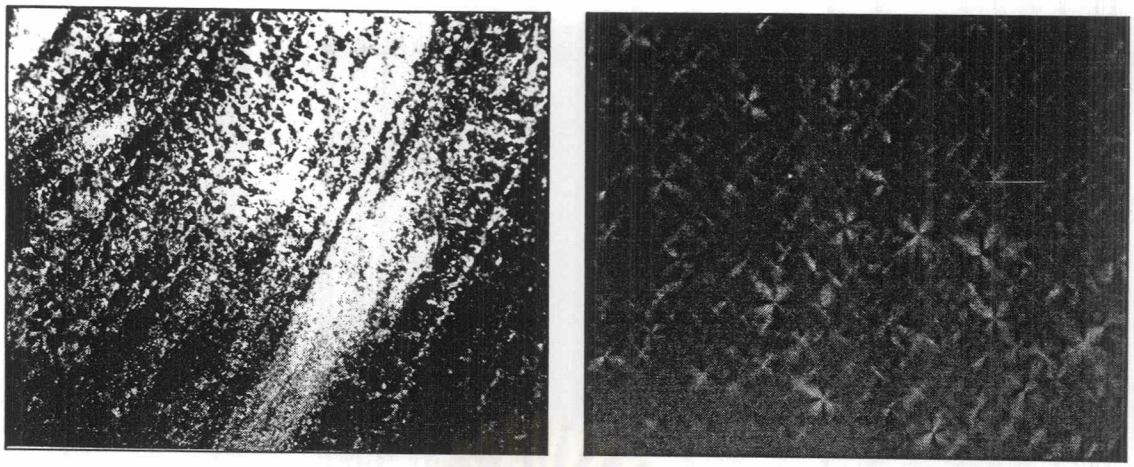


Figure 21 The microscopic pattern under cross-polarized light microscope showing the birefringent property of system (1/1) IPM:T_{80}:W (10%), (IPM:T_{80}=5:5) exhibited lamellar phase structure (left: 40X crossed polars and right: 100X crossed polars).

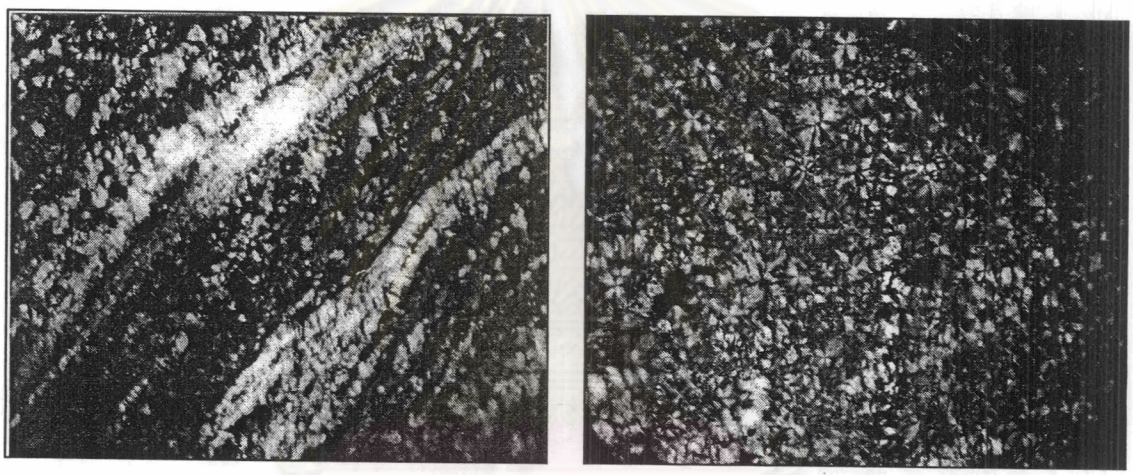


Figure 22 The microscopic pattern under cross-polarized light microscope showing the birefringent property of system (1/3) IPM:T_{80}:W(10%), (IPM:T_{80}=4:6) exhibited lamellar phase structure (left: 40X crossed polars and right: 100X crossed polars).

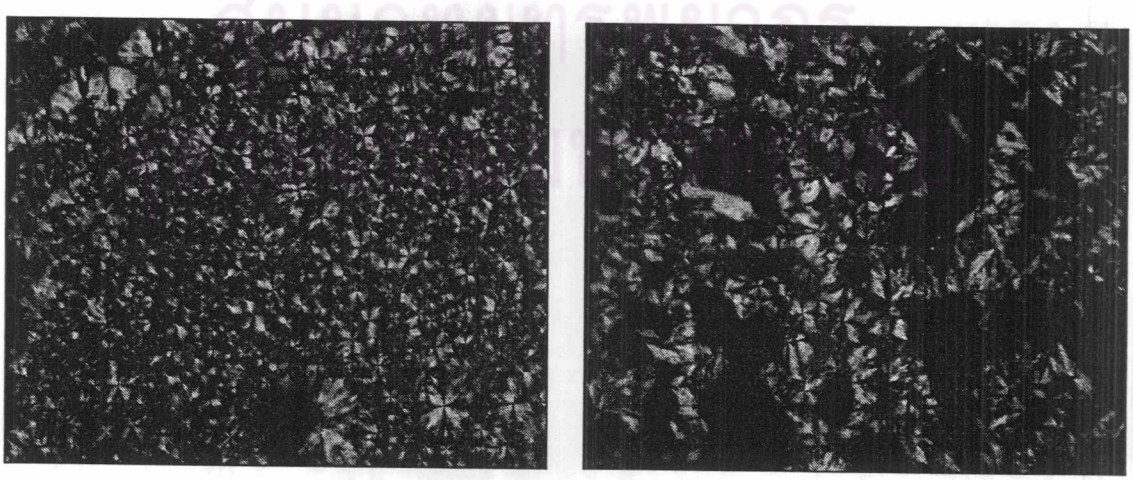


Figure 23 The microscopic pattern under cross-polarized light microscope showing the birefringent property of system (1/5) IPM:T_{80}:W (10%), (IPM:T_{80}=3:7) exhibited lamellar phase structure (left: 40X crossed polars and right: 100X crossed polars).

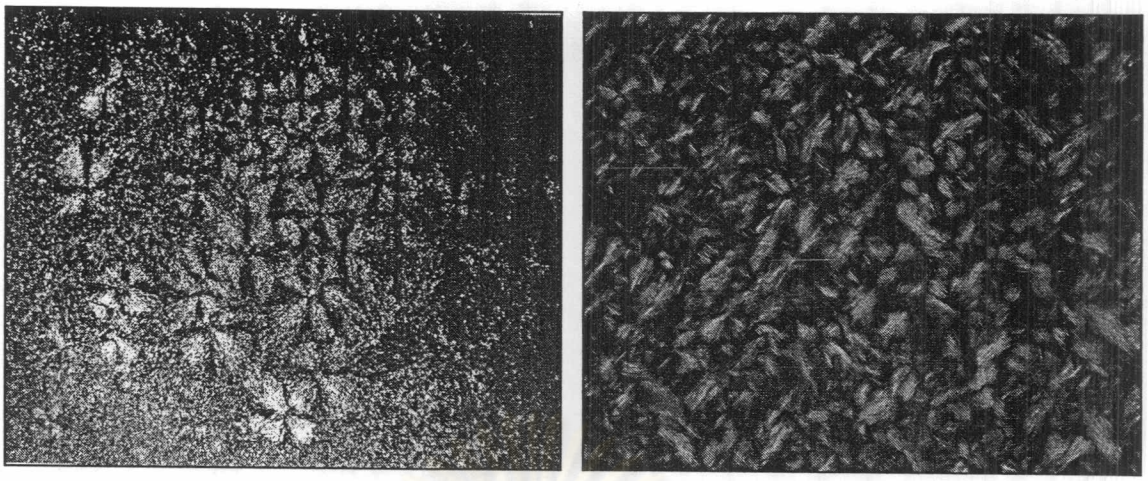


Figure 24 The microscopic pattern under cross-polarized light microscope showing the birefringent property of system (3/1) IPM:T_{80}:L_{68}:W (15%), (T_{80}:L_{68}=2:1) (IPM:T_{80}:L_{68} = 3: 4.67: 2.33) exhibited hexagonal phase structure (left: 40X crossed polars and right: 100X crossed polars).

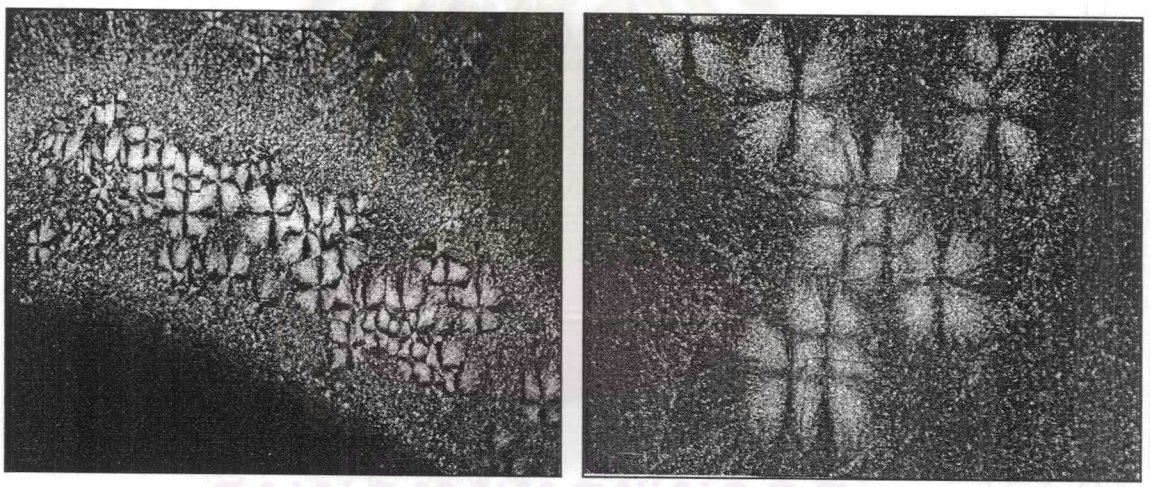


Figure 25 The microscopic pattern under cross-polarized light microscope showing the birefringent property of system (3/2) IPM:T_{80}:L_{68}:W (20%), (T_{80}:L_{68}=2:1) (IPM:T_{80}:L_{68} = 3: 4.67: 2.33) exhibited hexagonal phase structure (left: 40X crossed polars and right: 100X crossed polars).

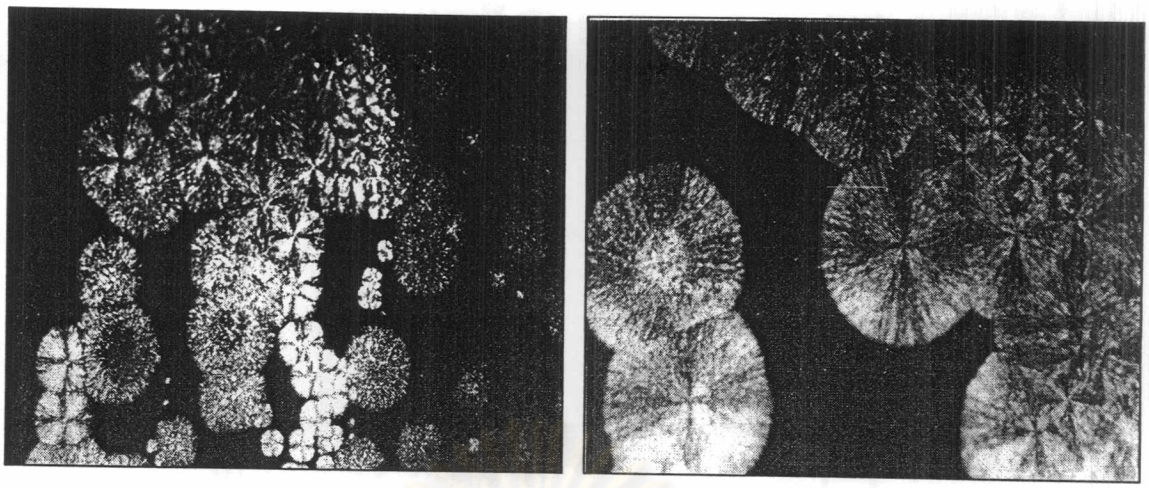


Figure 26 The microscopic pattern under cross-polarized light microscope showing the birefringent property of system (3/3) IPM:T_{80}:L_{68}:W (25%), (T_{80}:L_{68}=2:1) (IPM:T_{80}:L_{68} = 3: 4.67: 2.33) exhibited hexagonal phase structure (left: 40X crossed polars and right: 100X crossed polars).

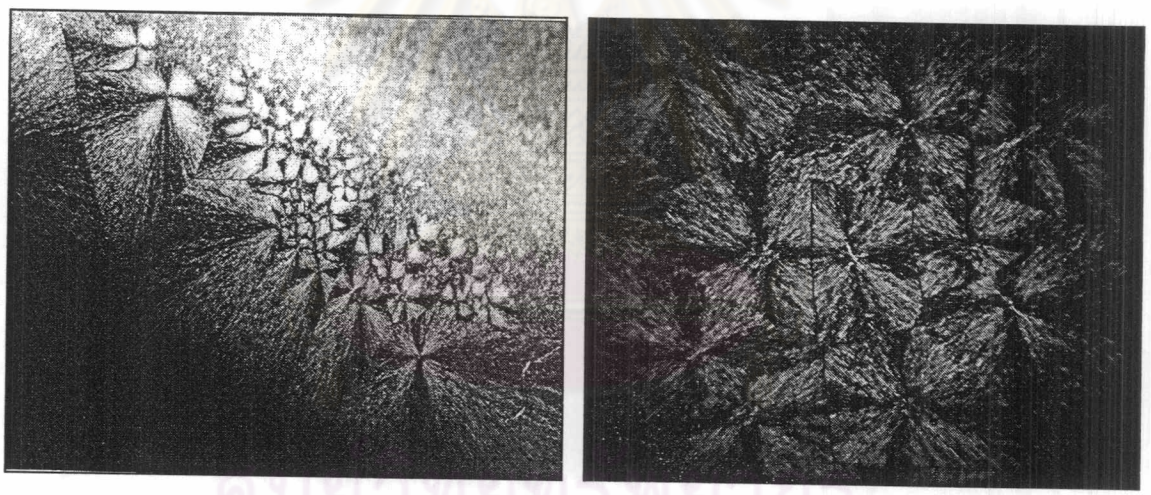


Figure 27 The microscopic pattern under cross-polarized light microscope showing the birefringent property of system (3/4) IPM:T_{80}:L_{68}:W (20%), (T_{80}:L_{68}=2:1) (IPM:T_{80}:L_{68} = 2: 5.33: 2.67) exhibited hexagonal phase structure (left: 40X crossed polars and right: 100X crossed polars).

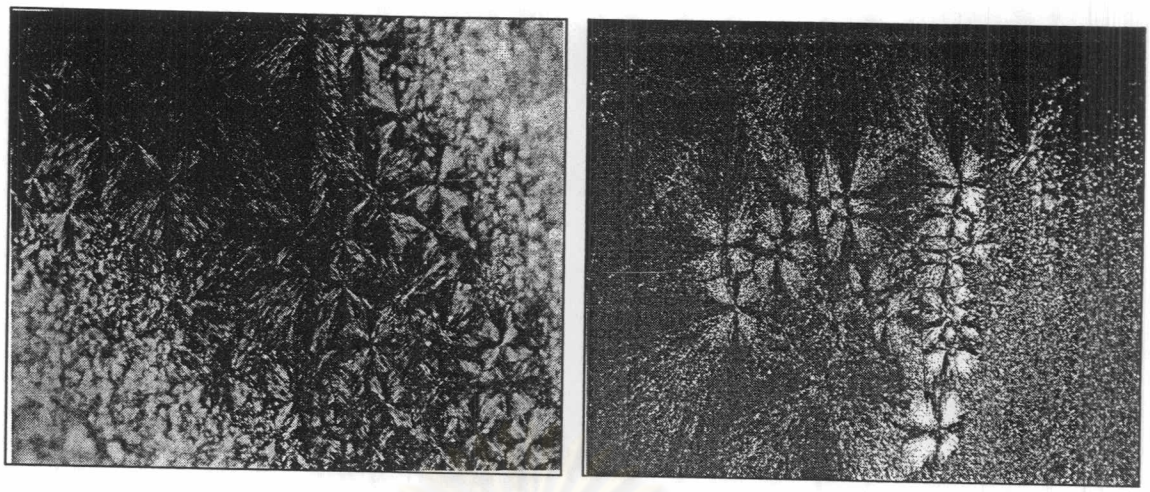


Figure 28 The microscopic pattern under cross-polarized light microscope showing the birefringent property of system (3/5) IPM:T_{80}:L_{68}:W (25%), (T_{80}:L_{68}=2:1) (IPM:T_{80}:L_{68} = 2: 5.33: 2.67) exhibited hexagonal phase structure (left: 40X crossed polars and right: 100X crossed polars).

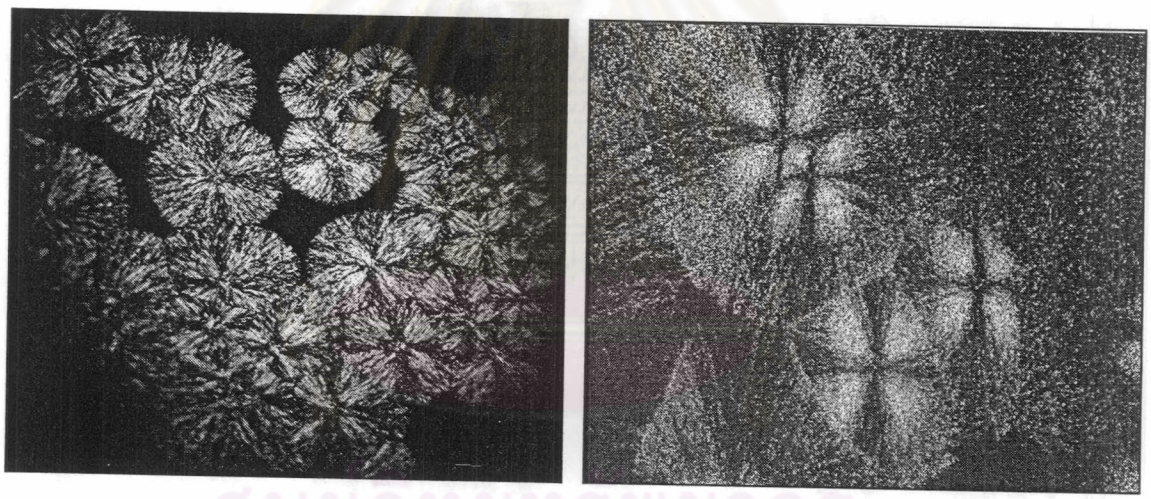


Figure 29 The microscopic pattern under cross-polarized light microscope showing the birefringent property of system (3/6) IPM:T_{80}:L_{68}:W (17%), (T_{80}:L_{68}=2:1) (IPM:T_{80}:L_{68} = 2: 5.33: 2.67) exhibited hexagonal phase structure (left: 40X crossed polars and right: 100X crossed polars).

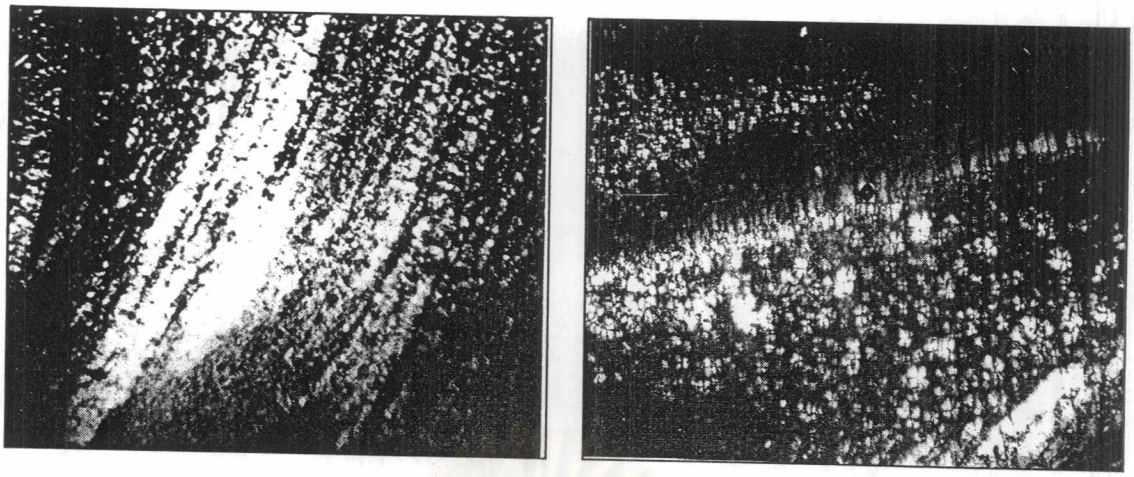


Figure 30 The microscopic pattern under cross-polarized light microscope showing the birefringent property of system (4/2) IPM:C_{EL}:W:PG (25%), (IPM:C_{EL}=2:8) exhibited lamellar phase structure (left: 40X and right: 100X crossed polars).

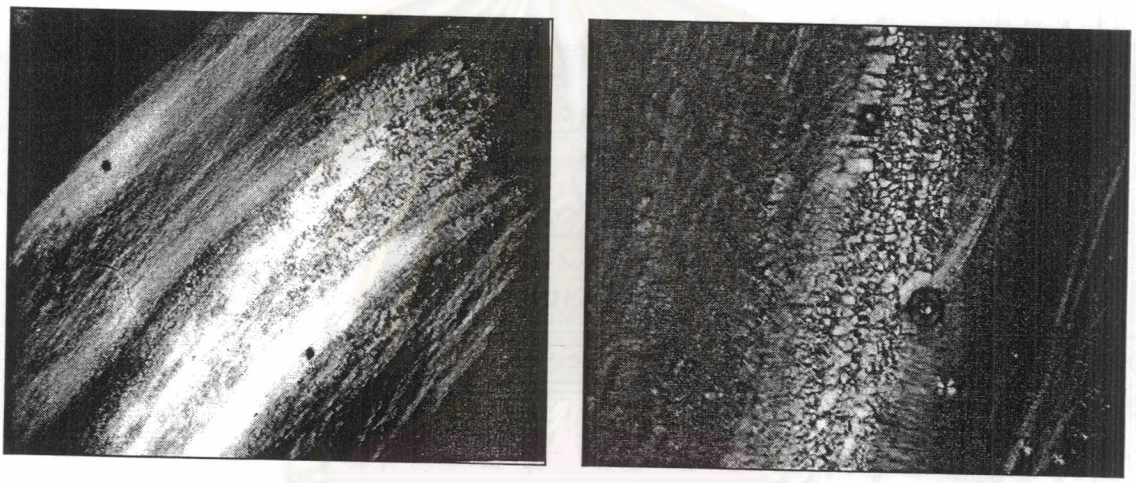


Figure 31 The microscopic pattern under cross-polarized light microscope showing the birefringent property of system (4/3) IPM:C_{EL}:W:PG (25%), (IPM:C_{EL}=3:7) exhibited lamellar phase structure (left: 40X and right: 100X crossed polars).

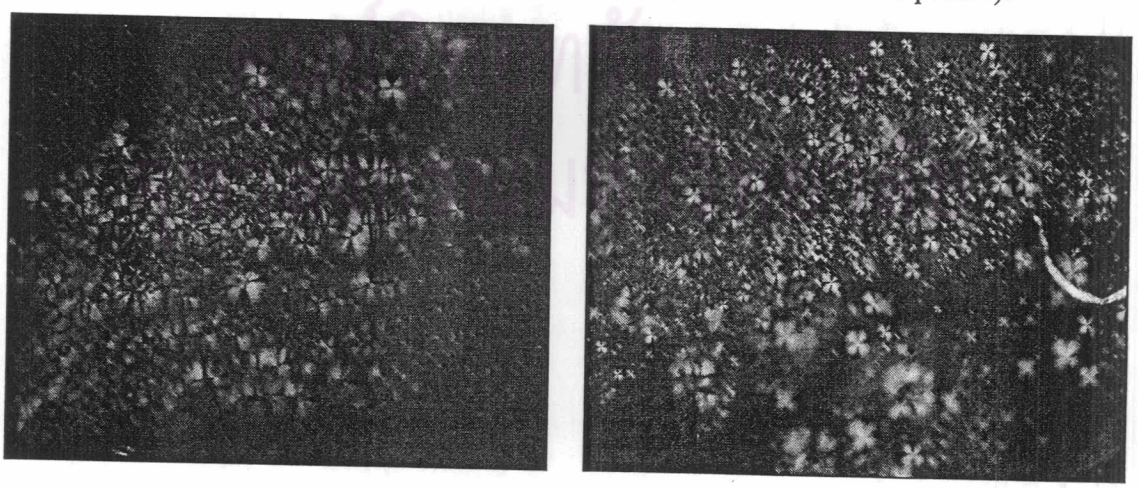


Figure 32 The microscopic pattern under cross-polarized light microscope showing the birefringent property of system (4/4) IPM:C_{EL}:W:PG (20%), (IPM:C_{EL}=3:7) exhibited lamellar phase structure (left: 40X and right: 100X crossed polars).

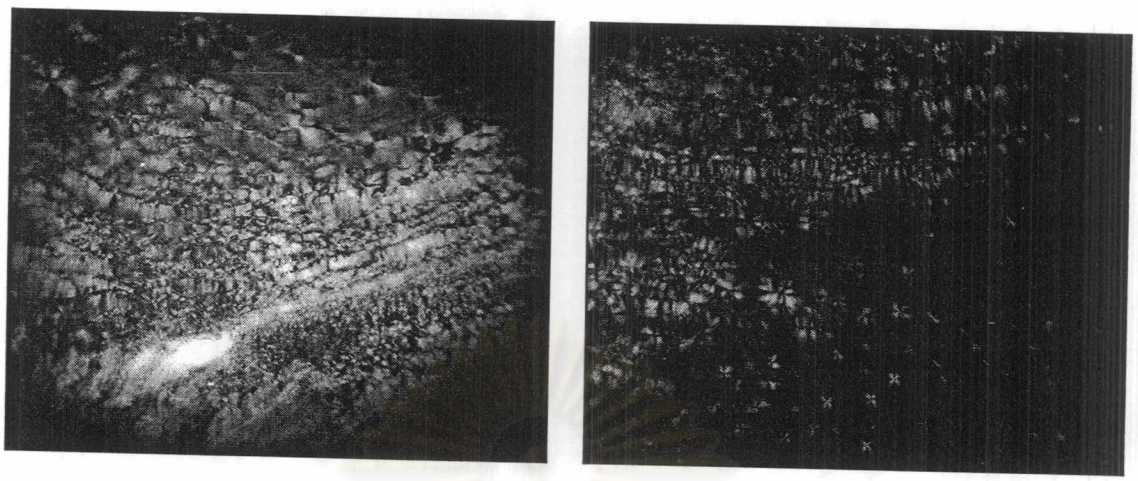


Figure 33 The microscopic pattern under cross-polarized light microscope showing the birefringent property of system (5/1) IPM:C_{RH}:W:PG (14.52%), (IPM:C_{RH}=3:7) exhibited lamellar phase structure (left: 40X and right: 100X crossed polars).

Figure 34 The microscopic pattern under cross-polarized light microscope showing the birefringent property of system (5/2) IPM:C_{RH}:W:PG (4:1) (20%), (IPM:C_{RH}=4:6) exhibited lamellar phase structure (left: 40X and right: 100X crossed polars).

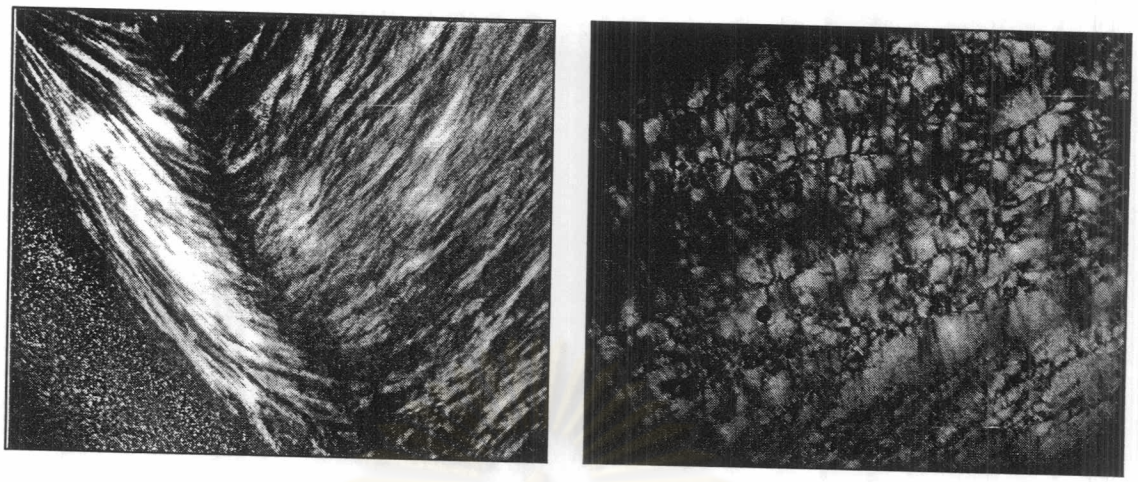


Figure 35 The microscopic pattern under cross-polarized light microscope showing the birefringent property of system (5/3) IPM:C_{RH}:W:PG (4:1) (15%), (IPM:C_{RH}= 4:6) their texture could be found both lamellar and hexagonal phase structure (left: 40X, hexagonal phase structure and right: 100X crossed polars, lamellar phase structure).

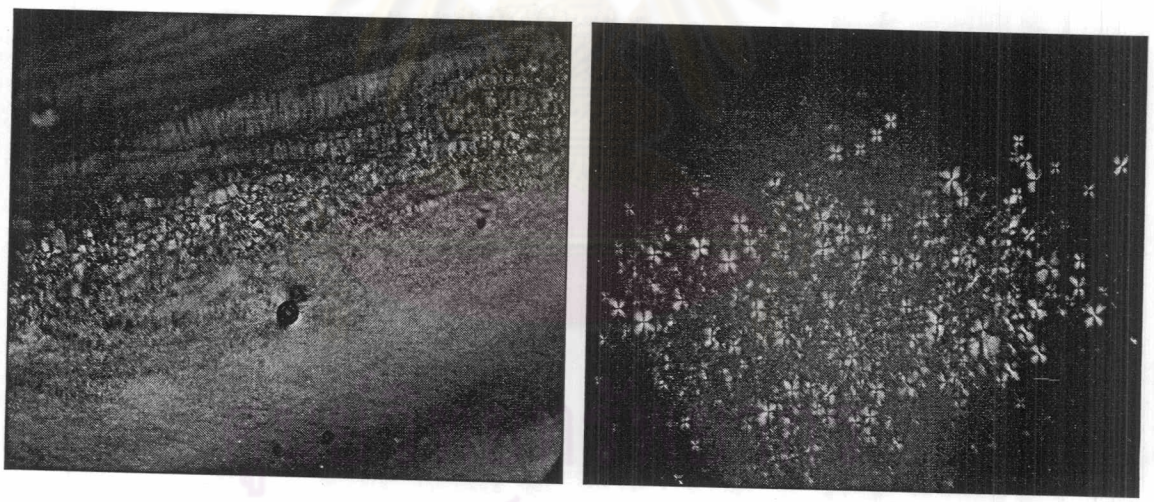


Figure 36 The microscopic pattern under cross-polarized light microscope showing the birefringent property of system (5/4) IPM:C_{RH}:W:PG (4:1) (15%), (IPM:C_{RH}=5:5) their texture could be found both lamellar and hexagonal phase structure (left: 40X, hexagonal phase structure and right: 100X crossed polars, lamellar phase structure).

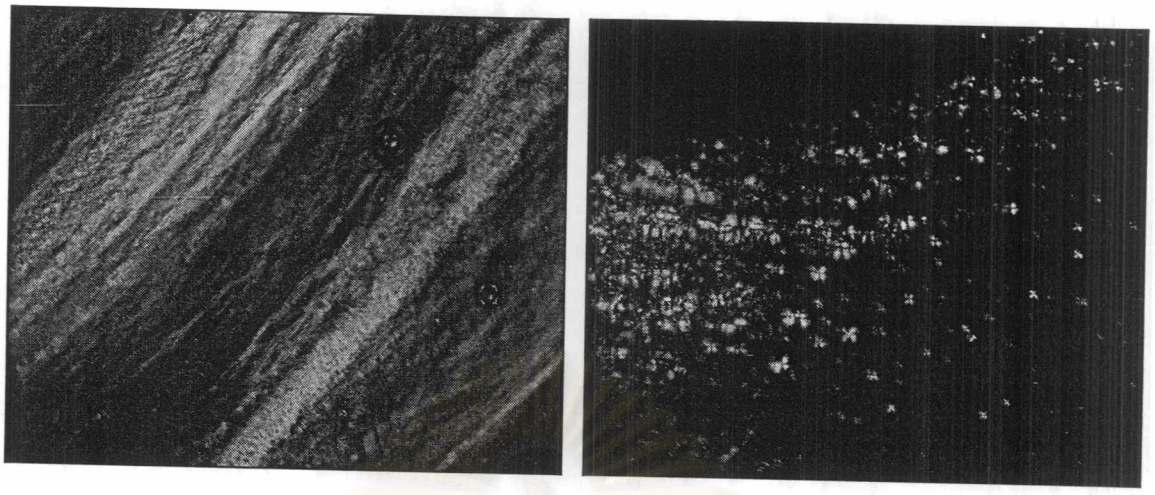


Figure 37 The microscopic pattern under cross-polarized light microscope showing the birefringent property of system (5/5) IPM:C_{RH}:W:PG (4:1) (15%), (IPM:C_{RH}=5:5) their texture could be found both lamellar and hexagonal phase structure (left: 40X, hexagonal phase structure and right: 100X crossed polars, lamellar phase structure).

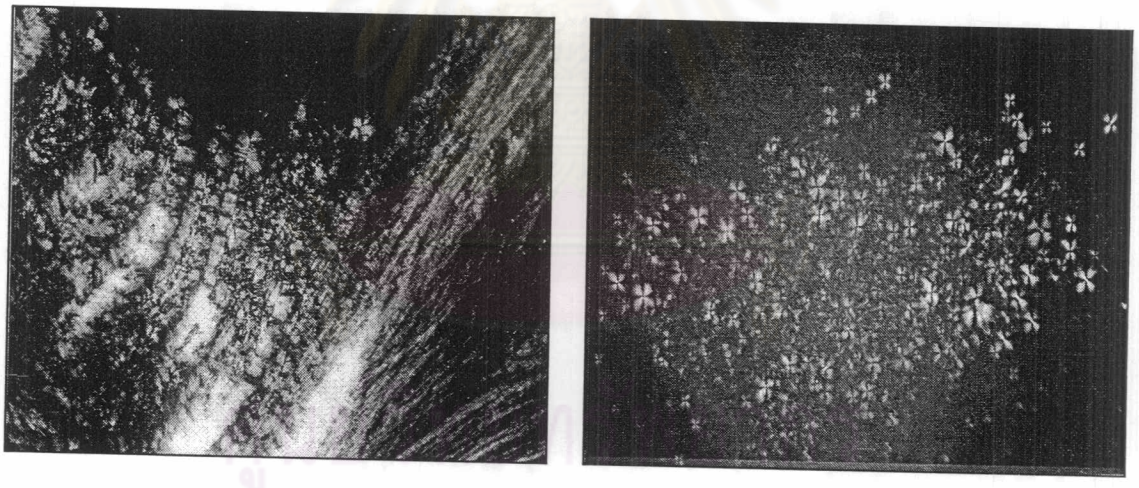


Figure 38 The microscopic pattern under cross-polarized light microscope showing the birefringent property of system (6/1) IPM:T_{80}:C_{EL}:W (15%), (IPM:T_{80}:C_{EL}=3: 3.5: 3.5) exhibited lamellar phase structure (left: 40X and right: 100X crossed polars).

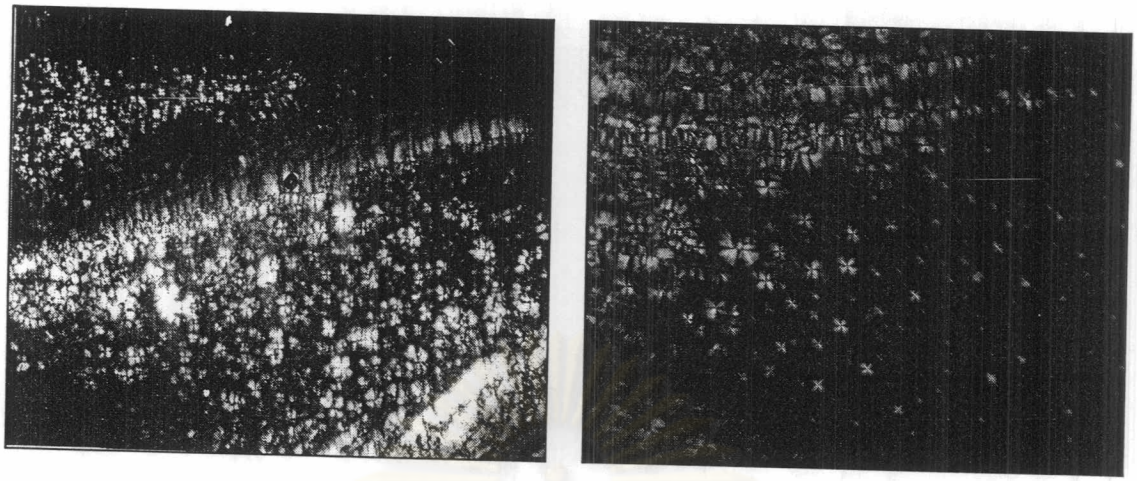


Figure 39 The microscopic pattern under cross-polarized light microscope showing the birefringent property of system (6/3) IPM:T_{80}:C_{EL}:W (15%), (IPM:T_{80}:C_{EL}=1: 4.5: 4.5) exhibited lamellar phase structure (left: 40X and right: 100X crossed polars).

Figure 40 The microscopic pattern under cross-polarized light microscope showing the birefringent property of system (6/4) IPM:T_{80}:C_{EL}:W (20%), (IPM:T_{80}:C_{EL}=1: 4.5: 4.5) exhibited lamellar phase structure (left: 40X and right: 100X crossed polars).

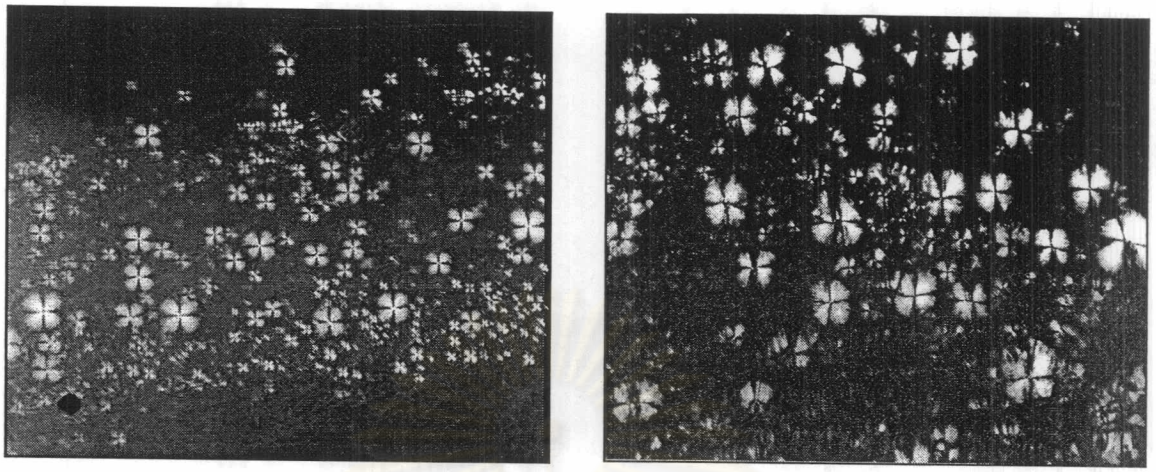


Figure 41 The microscopic pattern under cross-polarized light microscope showing the birefringent property of system (7/1) IPM : T_{80} : B_{35} : W (15%), (IPM:T_{80} :B_{35} = 3 : 3.5 : 3.5) exhibited lamellar phase with butterfly pattern structure (left: 40X crossed polars and right: 100X crossed polars).

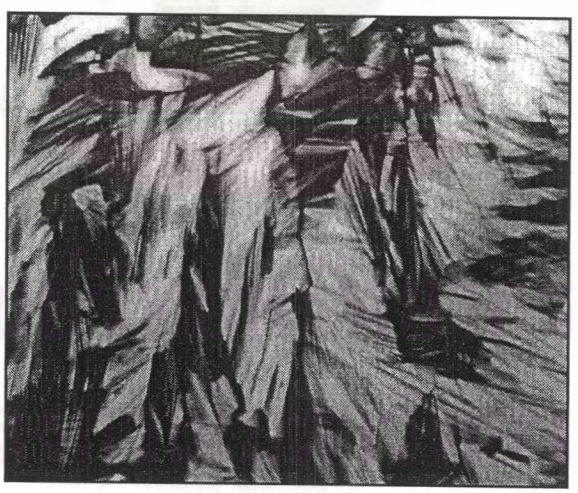


Figure 42 Typical hexagonal liquid-crystalline texture formed by heptane: non-ionic surfactant and water system (from Cordobes et al, 1997).

2.5 pH

The value of pH in each formulation are shown in Table12 and comparison pH of MEG base and MEG containing metronidazole are shown in Figure 43. The pH of MEG in IPM:T_{80}:W system without drug were varied in the range of 6.20 to 6.95. After the incorporation of metronidazole, the pH were decreased in most preparations to the range of 5.89 to 6.46 except in formulation 1/1 and 1/2 that pH of formulation were slightly increased. In CO: C_{EL}: W: PG (4:1) system without drug the pH were varied in the range of 5.97 to 6.65 while the pH of drug-loaded system was increased. In IPM : T_{80} : L_{68} : W (T_{80}:L_{68} = 2:1) system without drug were varied in the range of 6.75 to 7.52, the pH of drug loaded were decreased in all formulations when compared with MEG base. The pH of MEG base of system IPM:C_{EL}: W:PG (4:1), IPM:C_{RH}:W:PG (4:1), IPM:T_{80}:C_{EL}:W, IPM:T_{80}:B_{35}:W and SBO:T_{80}:W were similar to drug-loaded MEG as shown in Figure 43 except in formulation 5/2 and 6/1 that the pH of MEG base was less than MEG containing metronidazole about 0.68 and 1.14, respectively. These could be explained by the wide range pH of final preparations were depended on the pH of nature of each composition in formulation such as the pH of L_{68} was about 5.0-7.4 whereas the pH of C_{EL}, C_{RH} and T_{80} were about 6.0-8.0 (Kibbe, 2000). Furthermore, the overall pH indicated that incorporated drug did not affect the optical texture of MEG formulation and did not influence significantly pH values of the vehicles.

It was also found that the formulation 5/5 of IPM: C_{RH}: W: PG (4:1) system in both MEG base and drug-loaded system had the highest pH whereas formulation 6/1 of IPM:T_{80}:C_{EL}:W MEG base system and formulation 2/1 of CO:C_{EL}:W:PG(4:1) drug loaded system, respectively, had the lowest pH. In formulation 2/1, the drug loaded MEG had lower pH than MEG base might due to the degradation of free fatty acid in formulation similar to formulation 1/3 and 1/5. In contrast, in formulation 6/1 the drug loaded MEG had higher pH than MEG base. This latter results would be further investigated at molecular level in order to detect the changing of pH. The results could be indicated that the pH of investigated MEG base (5.76 to 8.13) and MEG containing metronidazole (5.58-7.96) were in the range that could be compatible with biological tissue and non-irritation resulted in biologic membrane (Jones et al, 1997).

2.6 Syringeability

Syringeability described the ability of MEG to pass easily through a hypodermic needle or transfer from the container prior to injection. The syringeability are closely related to the viscosity, flow characteristic and particle characteristics of MEG. Syringeability of this study was measured in term of rate of injection. This means the higher rate of syringeability, the greater performance of flowability of sample which consequenced in the ease of application of dosage form. The syringeability of each formulation is shown in Table12 and comparison between syringeability of MEG base and MEG containing metronidazole are shown in Figure 44. The results indicated that the lowest and highest syringeability of MEG base were 0.0046 and 0.0772 ml/second in formulation 5/2 and 6/1, respectively. The syringeability of 1.5% w/w metronidazole were varied in the range 0.1686 ml/second in formulation 1/1 and 0.0039 ml/second in formulation 2/4.

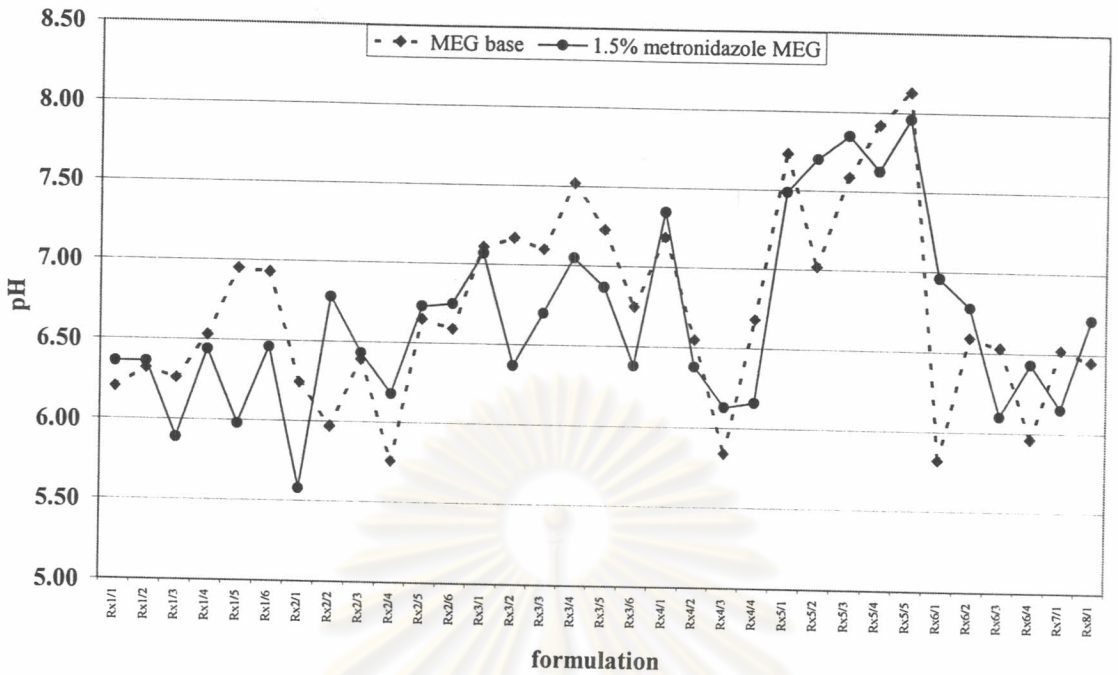


Figure 43 Comparison of the pH of MEG base and 1.5% w/w metronidazole MEG (before stability study).

The overall syringeability of most formulation of MEG base were similar to 1.5%w/w metronidazole MEG and varied with in the optimum range of 0.0040 to 0.0500 ml/second except in formulation 1/1, 1/3 and 1/4 that the syringeability of drug loaded were greater than MEG base. This was due to the consequent occurrence of phase separation in these systems after the break down of MEG structure due to the presence of metronidazole could change the dimentional distribution and aggregate structure of system. This behavior could be attributed to the amphiphilic property of metronidazole molecule that might be act as co-surfactant located at the interfacial between the two phases which had not enough surfactant for stabilized system (Esposito et al, 1997). Therefore, increasing in syringeability was obtained due to the decreasing in viscosity. Similar with syringeability study of loading of metronidazole into HEC and Carbopol which the syringeability increasing with lower concentration and viscosity of system was obtained (Hatefi and Amsden, 2002).

The syringeability results also revealed that the syringeability of non-newtonian behavior system were more than those from newtonian system as shown in Table 12. In CO: C_{EL} : W: PG (4:1) system which was newtonian system, the rate of syringeability were lower than those of other systems in this study. The lower syringeability was meant the inconvenient of injection and it could not easily pass through the syringes hence could not ease of application. These might be assumed that the shear-thinning behavior of non-newtonian fluid cause the breakdown of MEG base and MEG containing 1.5% w/w metronidazole structure when shear stress or force was applied (Jones et al, 1997).

One of the major obstacles in the direct administration of drug incorporated cubic phase gel is its extremely high viscosity. This makes the gel impossible to inject for periodontal administration. One of the ways to circumvent this problem has been the use of considerably less viscous lamellar phase gel, which is known to absorb water and gingival fluid in situ and converted to the cubic phase. Lamellar phase possess the non-newtonian and shear-thinning properties that mean the inherently fluid which can be injected using syringes. Moreover, the shear-thinning behavior of these non-newtonian systems will ease the application due to the breakdown structure when increasing the shear stress or force. The break down structure of MEG by shear stress brought about formulation to be easily injectable and to have good syringeability property (Esposito et al, 1996).

All non-newtonian system in this study had good syringeability and also was easily administered by syringes equipped with needles appropriated for intrapocket delivery. The schematic of this flow behavior coupling with less viscous lamellar phase structure made this system able to be injected using syringes (confirmed by viscosity study in next topic) which known to absorb water at gingival fluid and converted to highly viscous phase. Moreover, this formulation had Cremophor RH40 as surfactant which exhibited excellently clear white gel, good appearance and physical stability.

Table 12 The pH, syringeability and flow behavior of MEG base and MEG containing 1.5%w/w metronidazole.

Composition of formula	System	Ratio of oil:surfactant (percentage of water)	pH		Syringeability (ml/sec)		Flow behavior	
			MEG base	1.5%w/w MTZ	MEG base	1.5%w/w MTZ	MEG base	1.5%w/w MTZ
IPM : T_{80} : W	1/1	5:5 (10%)	6.20	6.36	0.0557	0.1686	N^-	**
	1/2	4:6 (8%)	6.32	6.36	0.0527	0.0702	~	~
	1/3	4:6 (10%)	6.26	5.89	0.0435	0.1486	N^-	**
	1/4	3:7 (8%)	6.53	6.44	0.0217	0.0554	~	~
	1/5	3:7 (10%)	6.95	5.98	0.0482	0.0482	N^-	**
	1/6	1:9 (7%)	6.93	6.46	0.0208	0.0403	~	~
CO : C_{EL} : W: PG (4:1)	2/1	2:8 (23%)	6.24	5.58	0.0182	0.0091	~	~
	2/2	2:8 (20%)	5.97	6.78	0.0063	0.0115	~	~
	2/3	3:7 (10%)	6.39	6.43	0.0132	0.0160	~	~
	2/4	3:7 (25%)	5.76	6.18	0.0052	0.0039	~	~
	2/5	5:5 (13%)	6.65	6.73	0.0020	0.0060	~	**
	2/6	5:5 (20%)	6.59	6.75	0.0086	0.0143	~	**
IPM : T_{80} : L_{68} : W (T_{80}:L_{68} = 2:1)	3/1	3 : 4.67 : 2.33 (15%)	7.11	7.07	0.0334	0.0175	N^-	N^-
	3/2	3 : 4.67 : 2.33 (20%)	7.17	6.37	0.0133	0.0175	N^-	N^-
	3/3	3 : 4.67 : 2.33 (25%)	7.10	6.70	0.0089	0.0093	N^-	N^-
	3/4	2 : 5.33 : 2.67 (20%)	7.52	7.05	0.0208	0.0187	N^-	N^-
	3/5	2 : 5.33 : 2.67 (25%)	7.23	6.87	0.0217	0.0169	N^-	N^-
	3/6	2 : 5.33 : 2.67 (17%)	6.75	6.38	0.0278	0.0172	N^-	N^-
IPM : C_{EL} : W: PG (4:1)	4/1	1:9 (10%)	7.19	7.35	0.0233	0.0257	~	~
	4/2	2:8 (25%)	6.55	6.38	0.0075	0.0072	N^-	N^-
	4/3	3:7 (25%)	5.84	6.13	0.0091	0.0045	N^-	N^-
	4/4	3:7 (20%)	6.68	6.16	0.0204	0.0139	N^-	N^-
IPM : C_{RH}: W: PG (4:1)	5/1	3:7 (14.52%)	7.73	7.49	0.0286	0.0045	N^-	N^-
	5/2	4:6 (20%)	7.02	7.70	0.0046	0.0221	N^-	N^-
	5/3	4:6 (15%)	7.59	7.85	0.0137	0.0132	N^-	N^-
	5/4	5:5 (15%)	7.92	7.63	0.0097	0.0103	N^-	N^-
	5/5	5:5 (15%)	8.13	7.96	0.0054	0.0164	N^-	N^-

Table 12 (Continue).

Composition of formula	System	Ratio of oil:surfactant (% water)	pH		Syringeability (ml/sec)		Flow behaior	
			MEG base	1.5%w/w MTZ	MEG base	1.5%w/w MTZ	MEG base	1.5%w/w MTZ
IPM : T_{80} : C_{EL} : W	6/1	3 : 3.5 : 3.5 (water 15%)	5.82	6.96	0.0772	0.0644	N^-	N^-
	6/2	2 : 4 : 4 (water 9%)	6.59	6.78	0.0286	0.0301	~	~
	6/3	1 : 4.5 : 4.5 (water 15%)	6.53	6.10	0.0286	0.0213	N^-	**
	6/4	1 : 4.5 : 4.5 (water 20%)	5.96	6.43	0.0435	0.0301	N^-	N^-
IPM : T_{80} : B_{35} :W	7/1	3 : 3.5 : 3.5 (water 15%)	6.52	6.15	0.0179	0.0177	N^-	N^-
SBO : T_{80} : W	8/1	1:9 (water 7%)	6.45	6.71	0.0331	0.0328	~	~

~ = Newtonian behavior

N^- = non- Newtonian, shear-thinning behavior

** = phase separation

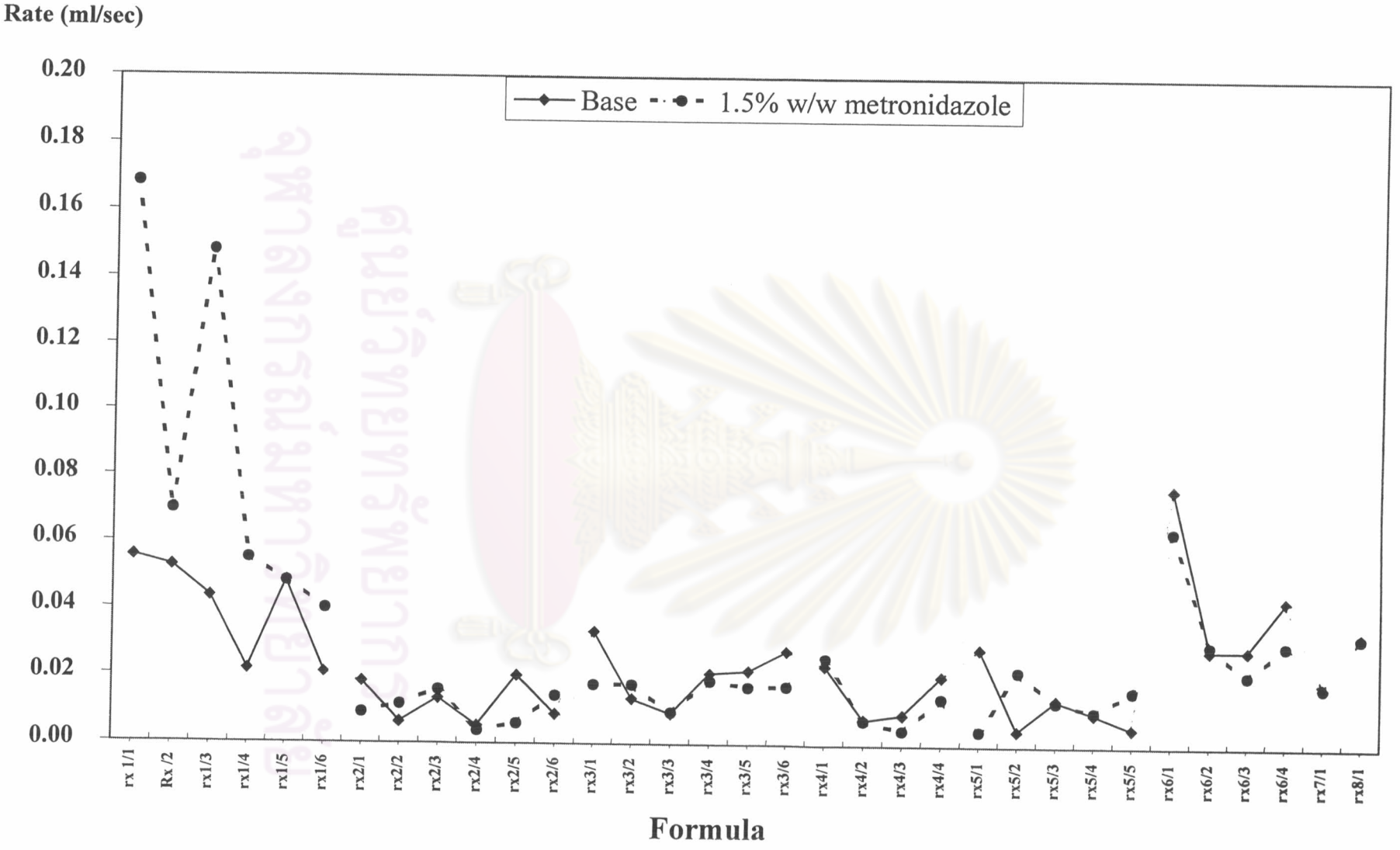


Figure 44 Comparison of the syringeability of MEG base and 1.5% metronidazole MEG.

2.7 Viscosity of microemulsion gel

I. Viscosity of microemulsion gel base

The viscosity of MEG base and MEG containing 1.5% w/w metronidazole are shown in Table 13 In this study, the rheology of MEG system was found to be both newtonian and non-newtonian systems which were classified according to the type of flow and deformation. Newtonian system had a constant viscosity upon changing in shear rate or time whereas non-newtonian system exhibited shear-thinning or shear-thickening system which was time-dependent condition. For non-newtonian system, the viscosity value was detected at 1 minutes after viscometer was operated. The rheology of the obtained MEG would involve in the mixing and flow of material, the removal prior to use, extrusion from tube or passage through a syringe needle. The viscosity of these systems could range in consistency from fluid to semi-solid viscous systems.

For the formulation of MEG base, the viscosity of IPM : T_{80} : W system; formulation 1/1, 1/3 and 1/5 were 21,600 cps, 1,080 cps and 1,567 cps whereas the viscosity of formulation 1/2, 1/4 and 1/6 were 382 cps, 387 cps and 435 cps, respectively. In these systems, the viscosity of MEG was decreased when decreasing the ratio of oil: surfactant from 5:5 to 3:7 at the same percentage of water (10% water) in formulation 1/1 and 1/5. In contrast, for formulation 1/2, 1/4 and 1/6, the viscosities were increased when the ratio of oil: surfactant decreased from 4:6 to 3:7 and 1:9 at the equal percentage of water (7-8% water). The results also found that the flow behavior of MEG base was changed from newtonian to non-newtonian system when the percentage of water in formulation was increased from 8 to 10% water at both ratio of oil: surfactant as 4:6 and 3:7. Therefore, it could be concluded that in lamellar phase or non- newtonian system, the viscosity decreased when the amount of oil in formulation was decreased. For newtonian system the viscosity increased when the amount of oil in formulation was decreased and the amount of surfactant in formulation was consequently increased.

The relationship between time and viscosity of these non-newtonian systems (formulation 1/1, 1/3 and 1/5) as shown in Figure 45 It is well known that the viscosity of MEG depended on the composition of the system. There was a linear relationship between emulsion viscosity and the viscosity of continuous phase. And the greater volume of the internal phase, the greater is apparent viscosity. In addition, the viscosities of formulations 1/1, 1/3 and 1/5 were classified to be a time dependent non-newtonian behavior that called "shear-thinning system" whereas formulations 1/2, 1/4 and 1/6 were classified as newtonian systems which had constant viscosity even changing in the rate of shear and time (Martin, 1993). These time-dependent non-newtonian behavior may be called as thixotropic or pseudoplastic flow behavior, this structure referred some degree of rigidity on the system and it resembled as gel. As shear was applied and flow started, this structure began to break down as the point of contact was disrupted and the particle became aligned (Berni et al, 2002). The material underwent a gel to sol transformation and exhibited shear-thinning system. Upon removal of the stress, the structure started to reform. This process was not instantaneous, rather it was a progressive restoration of consistency as asymmetric particle came into contact with one another by undergoing random Brownian

movement. Thixotropic system was highly dependent on time of shear, the rate of shear and degree of structure in the sample (Jones et al, 1997).

For CO: C_{EL}: W: PG (4:1) system; the viscosities of formulation 2/1 to 2/6 were 895 cps, 1,080 cps, 858 cps, 3,078 cps, 1,605 cps and 2,346 cps, respectively. The results revealed that formulation 1/4 had the highest viscosity eventhough the percentage of water was the highest (25% water). These may be described together with the results from polarized light microscopy technique that this system could be cubic phase of liquid crystal system which would absorb amount of water and transform into a stiff and more viscous gel. Therefore, this formulation would achieve the highest viscosity due to transformation of structure by absorbing more water. Similar results was observed by Jaymin (2001) and Trotta (1999), the effect of oil: surfactant ratio in the formulation on viscosity found that increasing the ratio of oil: surfactant would increase the viscosity especially at the ratio oil: surfactant of 2:8 and 3:7 in formulation 2/1-2/2 and 2/3-2/4 , respectively. This observation was not noted in the formulation 2/5 and 2/6 due to phase separation. In addition, the viscosity of MEG formulation increased when the percentage of water was increasing from 10% to 25% water in formulation 2/3 and 2/4 and from 13% to 20% water in formulation 2/5 and 2/6 at the same ratio of oil: surfactant in formulation as 3:7 and 5:5 except the formulation 1/1 and 1/2 that percentage of water had no effect on the viscosity of system. All of formulation in these systems showed time-independent newtonian behavior that had constant viscosity in every changing shear rate and time interval as shown in Table 13.

In IPM: T_{80} : L_{68} : W (T_{80}:L_{68} = 2:1) system; the viscosity of MEG system was increased when increasing the percentage of water in formulation as shown in Table 13 and Figure 46 At the ratio of oil: surfactant: cosurfactant of 3: 4.67: 2.33, when the percentage of water were increasing from 15% to 20% and 25% water, the viscosity of formulation 3/1, 3/2 and 3/3 were 6,120 cps, 12,900 cps and 19,080 cps, respectively. At the ratio of oil: surfactant: cosurfactant of 2: 5.33: 2.67, the viscosity of MEG was increased from 7,020 cps to 18,330 cps and 25,800 cps in formulation 3/6, 3/4 and 3/5, respectively when the percentage of water was increasing from 17% to 20% and 25% water. At the same ratio of component, the viscosity increasing with the amount of water content in formulation. In this case might be explained by the cross link polymer network which could be occurred upon water induced hydration as shown in Figure 44 by strongly bound with water of head group of resulting in rigidity of system (Paul and Moulik, 1997).

On the other hand, the viscosity of MEG system was decreased when increasing amount of oil phase in formulation and consequently decreasing amount of surfactant in formulation as shown in formulation 3/1, 3/2 and 3/3 which had the ratio of oil: surfactant: cosurfactant of 3: 4.67: 2.33 and amount of oil phase of 25.5, 24.8 and 22.5%. At the ratio of oil: surfactant: cosurfactant 2: 5.33: 2.67, the same results of decreasing viscosity was also exhibited. When the amount of oil phase was increased from15% to 16%, 25% oil phase respectively. In MEG system that had the equal amount of 20% water as in formulation 3/2 and 3/4, The viscosity of MEG was decreased from 18,330 cps to 12,900 cps when the ratio of oil: surfactant: cosurfactant was increased from 2: 5.33: 2.67 to 3: 4.67: 2.33. Like the 25% equal amount of water system in formulation 3/3 and 3/5, the viscosity of MEG was also decreased from 25,800 cps to 19,080 cps when the ratio of oil: surfactant: cosurfactant was increased.

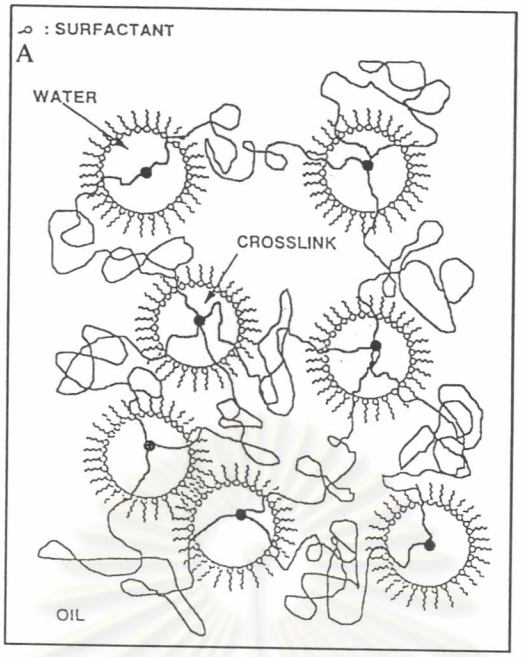


Figure 44 The possibility mechanism of cross link polymer network (from Paul and Moulik, 1997).

Most formulations in this system were lamellar phase microstructure which composed of non-ionic surfactant and block copolymer which exhibited pseudoplastic flow and thixotropic flow behavior as shown in Figure 46 and 47 . From the relationship between time and viscosity of these non-newtonian systems showed the characteristic of time-dependent and shear-thinning system. Microstructural transition was also observed in all formulations in these systems. These would be described that the shear induced transition from sheet-like lamellar to dispersed multilamellar vesicle phase (call droplets, onion structure or vesicle phase) which recently had been observed in block-copolymer (Moore et al, 2000). So, a sharp decreased in viscosity was presented when the increasing in shear rate, suggesting a changed in lamellar orientation (Berni et al, 2002). Furthermore, the observed behavior could be assigned to a structure which broke down arising from the shear flow that brought to decrease the viscosity of system (Jones et al, 1997).

For IPM : C_{EL} : W: PG (4:1) system; the viscosity of MEG was increased from 630 cps to 3,900 cps, 7,860 cps and 35,230 cps in formulation 4/1, 4/4, 4/2 and 4/3, respectively when increasing the percentage of water. At higher ratio of oil: surfactant, the greater of viscosity was obtained as shown in formulation 4/1, 4/2 and 4/3 excepted formulation 4/4. Furthermore, the viscosity of MEG base was markedly decreased from 35,250 cps to 7,860 cps and 630 cps when the amount of surfactant increased from formulation 4/3, 4/2 and 4/1, respectively.

Table 13 Viscosity of MEG base and microemulsion gel containing metronicazole.

Composition of formula	System	Ratio of oil: surfactant (percentage of water)	Viscosity (cps)	
			MEG base	1.5% metronidazole
IPM : T_{80} : W	1/1	5:5 (10%)	*21,600	*2,670
	1/2	4:6 (8%)	382	294
	1/3	4:6 (10%)	*1,080	*3,000
	1/4	3:7 (8%)	387	350
	1/5	3:7 (10%)	*1,567	380
	1/6	1:9 (7%)	435	458
CO : C_{EL} : W: PG (4:1)	2/1	2:8 (23%)	895	1,378
	2/2	2:8 (20%)	1,080	1,134
	2/3	3:7 (10%)	858	930
	2/4	3:7 (25%)	3,072	3,078
	2/5	5:5 (13%)	1,605**	1,170
	2/6	5:5 (20%)	2,346**	2,032
IPM : T_{80} : L_{68} : W (T_{80}:L_{68} = 2:1)	3/1	3 : 4.67 : 2.33 (15%)	*6,120	*8,850
	3/2	3 : 4.67 : 2.33 (20%)	*12,900	*19,500
	3/3	3 : 4.67 : 2.33 (25%)	*19,080	*96,100
	3/4	2 : 5.33 : 2.67 (20%)	*18,330	*37,500
	3/5	2 : 5.33 : 2.67 (25%)	*25,800	*33,600
	3/6	2 : 5.33 : 2.67 (17%)	*7,020	*12,150
IPM : C_{EL} : W: PG (4:1)	4/1	1:9 (10%)	630	754
	4/2	2:8 (25%)	*7,860	*7,868
	4/3	3:7 (25%)	*35,230	*47,500
	4/4	3:7 (20%)	*3,900	*3,980
IPM : C_{RH}: W: PG (4:1)	5/1	3:7 (14.52%)	45,600	60,000
	5/2	4:6 (20%)	200,000	280,000
	5/3	4:6 (15%)	124,000	125,334
	5/4	5:5 (15%)	105,000	107,666
	5/5	4.9:5 (15%)	110,000	160,000
IPM : T_{80} : C_{EL} : W	6/1	3 : 3.5 : 3.5 (water 15%)	*11,610	*12,140
	6/2	2 : 4 : 4 (water 9%)	480	536
	6/3	1 : 4.5 : 4.5 (water 15%)	*13,000	728**
	6/4	1 : 4.5 : 4.5 (water 20%)	*21,840	*24,620
IPM : T_{80} : B_{35} : W	7/1	3 : 3.5 : 3.5 (water 15%)	*10,200	*93,000
SBO : T_{80} : W	8/1	1:9 (water 7%)	480	492

* = non-newtonian behavior of system, the viscosity value were detected at 1 minutes after measurement

** = separation

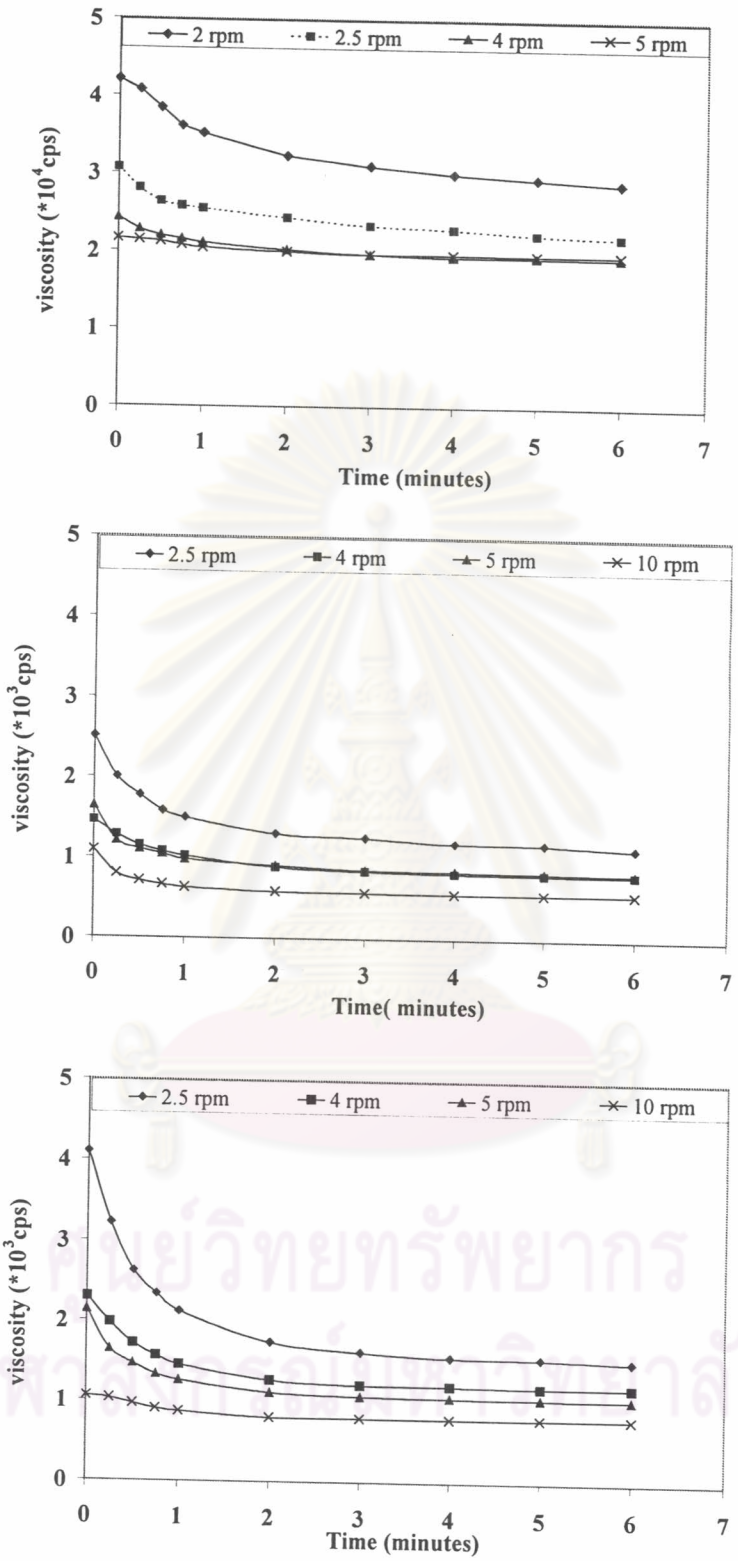


Figure 45 Time-viscosity relationship of IPM: T80: W system; formulation 1/1 (IPM:T_{80}=5:5, 10%water) (top), 1/3 (IPM:T_{80}=4:6, 10%water) (middle) and 1/5 (IPM:T_{80}=3:7, 10%water) (bottom). Using spindle no.62.

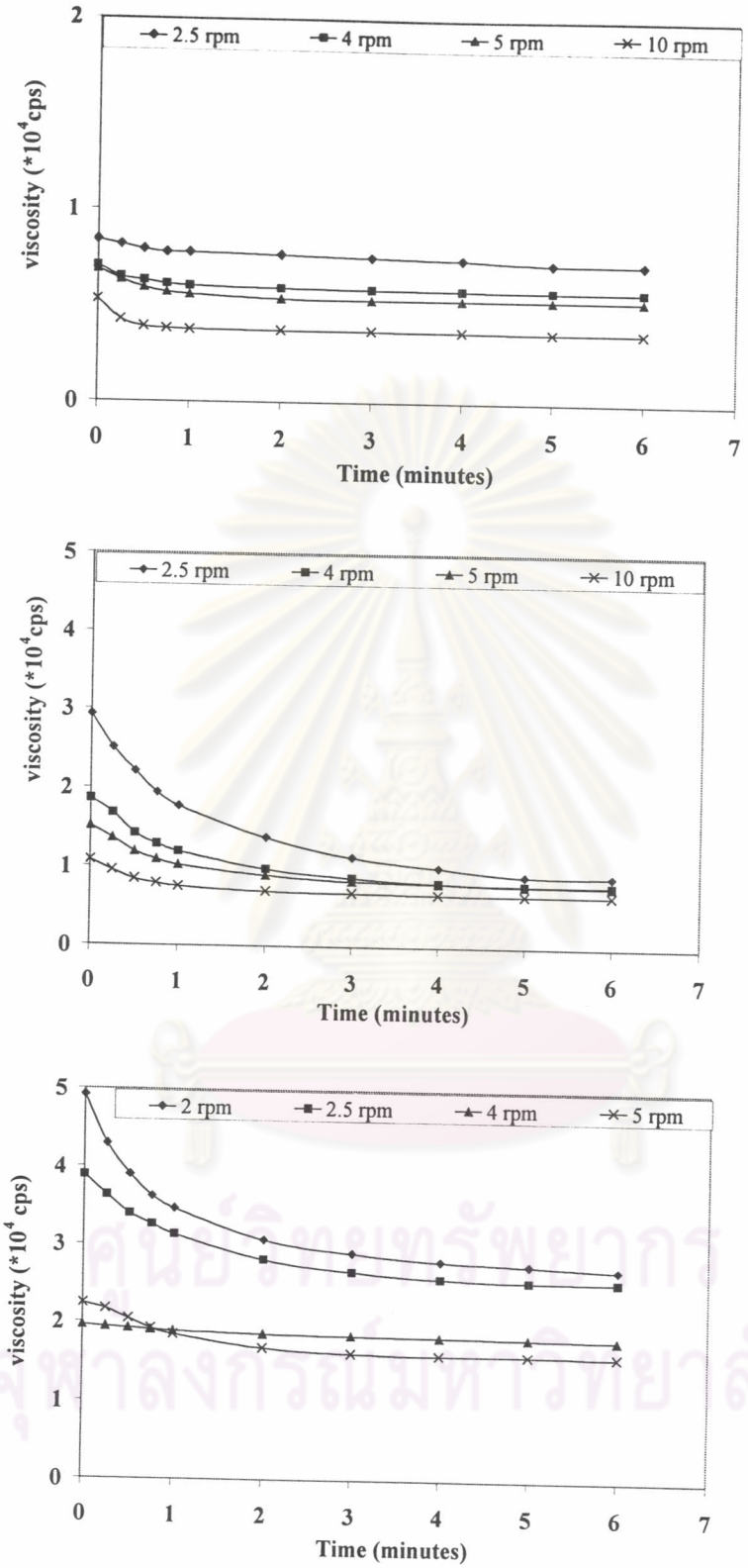


Figure 46 Time-viscosity relationship of IPM : T_{80} : L_{68} : W (T_{80}:L_{68}=2:1) system, (IPM:T_{80}:L_{68} = 3 : 4.67 : 2.33); formulation 3/1 (15% water) (top), 3/2 (20%water) (middle) and 3/3 (25%water) (bottom). Using spindle no.62.

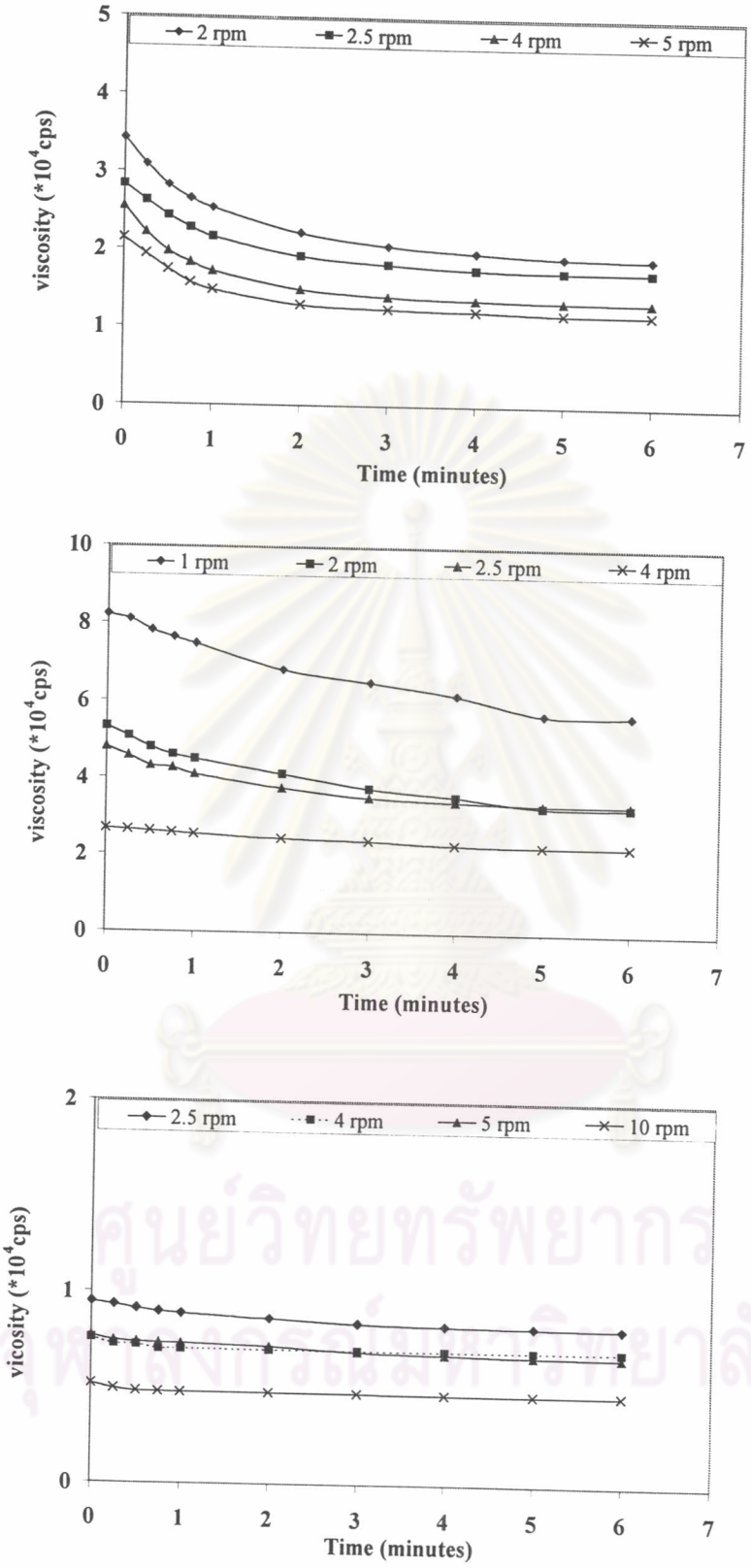


Figure 47 Time-viscosity relationship of IPM : T_{80} : L_{68} : W (T_{80}:L_{68}=2:1) system, (IPM:T_{80}:L_{68} = 2: 5.33: 2.67); formulation 3/4 (20% water) (top), 3/5 (25%water) (middle) and 3/6 (17%water) (bottom). Using spindle no.62.

From these studies, it was likely that at higher percentage of water presented in the formulation, the structure of system may transform lamellar phase which had low viscosity into highly viscous cubic phase or hexagonal phase (Jaymin et al, 2001). Cubic phase was presented between lamellar and reversed hexagonal phase with the phase transformation brought about by increasing water content. Therefore, the viscosity of the formulation 4/3 was much higher than 4/2, 4/4 and 4/1. In addition, there was linear relationship between MEG viscosity and the viscosity of the continuous phase. And the greater volume of the internal phase, the greater apparent viscosity was obtained (Kuneida et al, 1999). In this study, the high viscosity of formulation 4/3 additionally resulted from the increasing in the amount of oil phase which was internal phase.

The time-dependent non-newtonian behavior was also observed in formulation 4/2, 4/3 and 4/4 except in formulation 4/1 which exhibited newtonian fluid system as shown in Table 13 and Figure 48 It could be explained that when shear was applied and flow started, this structure begins to break down as the point of contact was disrupted and the particle became aligned (Kantaria et al, 1999). The material underwent a gel to sol transformation and exhibited shear-thinning system. The results were similar the IPM : T_{80} : L_{68} : W (T_{80}:L_{68} = 2:1) system.

Interestingly, poloxamer or Lutrol is a commercially available poly (oxyethylene) poly (oxypropylene) block copolymer containing 73% polyoxyethylene units. Lutrol has low toxicity, high compatibility with other chemical. Moreover, Poloxamer possessed interesting rheological properties. When used at the concentration above 20% the polymer formed thermoreversible gels characterized by a critical temperature (T_c). At temperature below T_c the Lutrol was low-viscosities sol, whilst above T_c a transparent viscous gel could be formed. The sol-gel trasition is a reversible process and it occurs whenever cooling and heating cycles are conducted, without any appreciable alteration in the gel characteristic and viscosity (Moore et al, 2000). In this respect, Lutrol formed liquid at room temperature and viscous gels when approaching body temperature, T_c of the average 20% poloxamer in formulation were 15.7° C (Esposito et al, 1996). Therefore, when using this formulation wherever above this temperature, transparent viscous gel was formed in mount cavity which has average temperature about 37.5 ° C. (Rawrence and Rees, 2000).

In IPM : C_{RH}: W: PG (4:1) system; the viscosity of MEG was increased from 45,600 cps to 105,000 cps, 110,000 cps, 124,000 cps and 200,000 cps in formulation 5/1, 5/4, 5/5, 5/3 and 5/2, respectively when increasing the percentage of water. Similarly, the viscosity was increased when increasing the amount of surfactant from 5:5 to 4:6. At the same amount of oil: surfactant was 4:6; the viscosity increasing with the higher water content in formulation except formulation 5/1, syneresis were observed after storage for 7 days after preparation.

From the results, it could be verified as in other system that at higher percentage of water in the formulation, the structure of system may transform lamellar phase to higher viscous cubic phase (Jaymin et al, 2001). Therefore, the formulation that had the highest percentage of water was the most viscous system (20% water, 200,000 cps) as in formulation 5/2. In contrast, the effect of oil: surfactant ratio in system was unlike the former system [IPM: C_{EL}: W: PG (4:1)], these may explained

by the viscosity of MEG depended on the nature of composition in system. In these systems, C_{RH} were used as surfactant and the characteristics of this excipient was white, semi-solid viscous material paste. The material contained mainly ricinoleyl glycerol ethoxylated with 40 molecules of ethylene oxide, approximately 75% of the component of the mixture were hydrophobic (HLB value=14-16). Accordingly, the viscosities of this system was depended on the amount of this viscous surfactant present in the formulation. The higher amount of surfactant in formulation, the greater in viscosity were obtained (Kibbe, 2000).

The time-dependent non-newtonian behavior also observed in formulation 5/1-5/5 as shown in Table 13. Eventhough these systems possessed highly viscous MEG, shear-thinning thixotropic system could be observed under this experiment. This indicated that a breakdown of structure of MEG occurred shear-thinning behavior that gradually reformed.

The IPM : T_{80} : C_{EL} : W system showed similar results as the IPM : C_{RH}: W: PG (4:1) system. Formulation 6/4 had highest viscosity and were decreased following by formulation 6/3, 6/1 and 6/2, respectively which depended on the higher amount of water in formulation. In addition, the viscosity of MEG was increased at lower ratio of oil: surfactant excepted in the formulation 6/2. When oil: surfactant ratio was decreased from 3: 3.5: 3.5 to 1: 4.5: 4.5, the viscosity was increased in formulation 6/1, 6/3 and 6/4, respectively. Therefore, the viscosity of MEG system depended on the main composition of systems especially surfactant and co-surfactant in formulation.

These systems composed of two-different surfactant and co-surfactant which highly affected the entire viscosity of formulation. Therefore, the formulation containing higher surfactant and co-surfactant would have higher viscosity as shown in formulation 6/1 (T_{80} : C_{EL}= 59.5%), 6/3 (T_{80} : C_{EL}= 76.5%) and 6/4 (T_{80} : C_{EL}= 72%) from Table 13. Surprisingly, formulation 6/2 had the lowest viscosity even at high ratio of oil: surfactant than some formulation. This might be explained by the existence of newtonian time-independent behavior.

The exitence of non-newtonian behavior was observed under this study, especially in formulation 6/1, 6/3 and 6/4. The relationship between viscosity and time were illustared in Figure 49.

In IPM : T_{80} : B_{35} : W system; (IPM : T_{80} : B_{35}= 3 : 3.5 : 3.5, 15% water), the viscosity was 10,200 cps and also exhibited non-newtonian time-dependent behavior. Shear-thinning property could only be seen at lower shear rate (1 rpm). When performing the measurement with higher shear rate, constant viscosity was observed as shown in Figure 50.

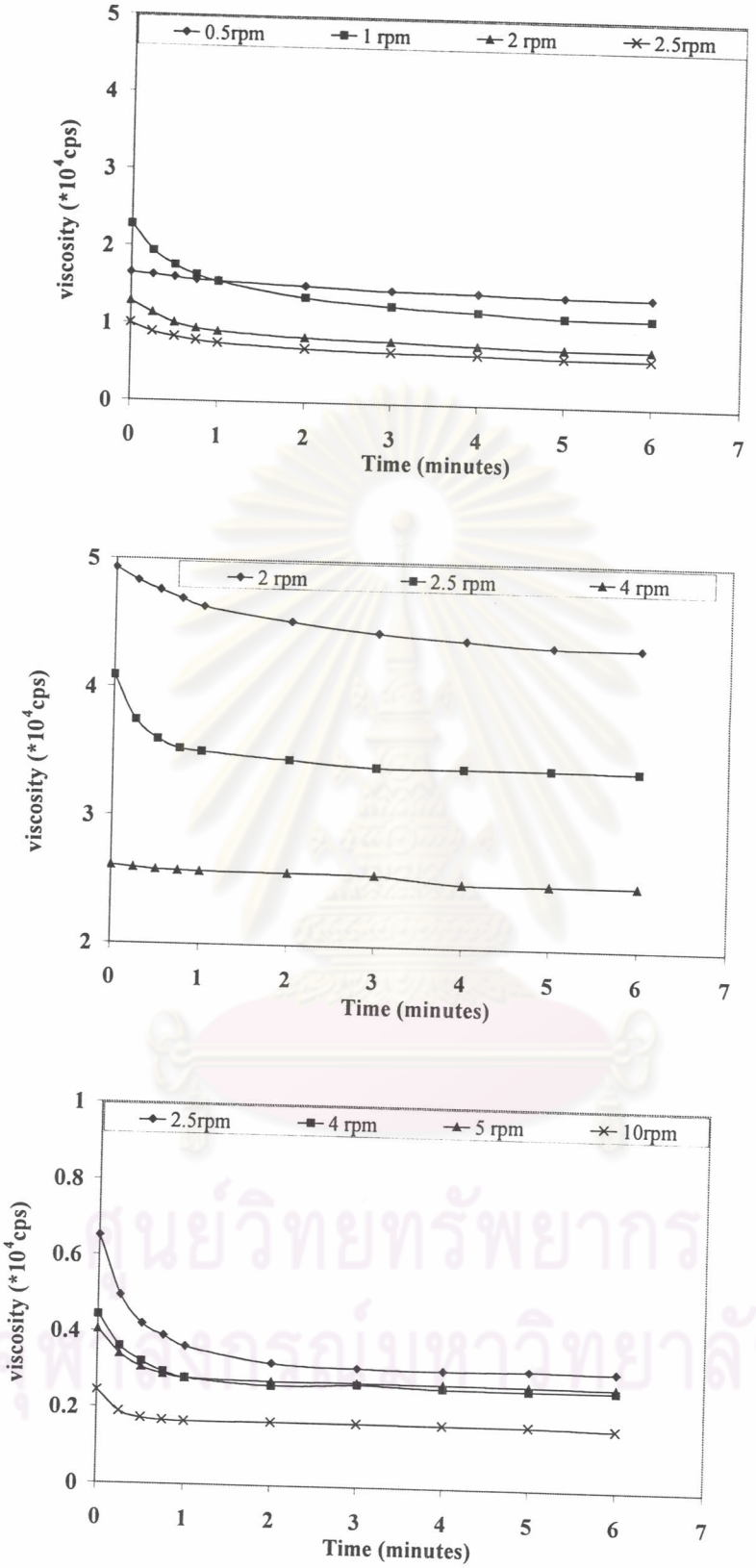


Figure 48 Time-viscosity relationship of IPM: C_{EL} : W: PG (4:1) system; formulation 4/2 (IPM: C_{EL} =2:8, 25%water) (top), 4/3 (IPM: C_{EL} =3:7, 25%water) (middle) and 4/4 (IPM: C_{EL} =3:7, 20%water) (bottom). Using spindle no.62.

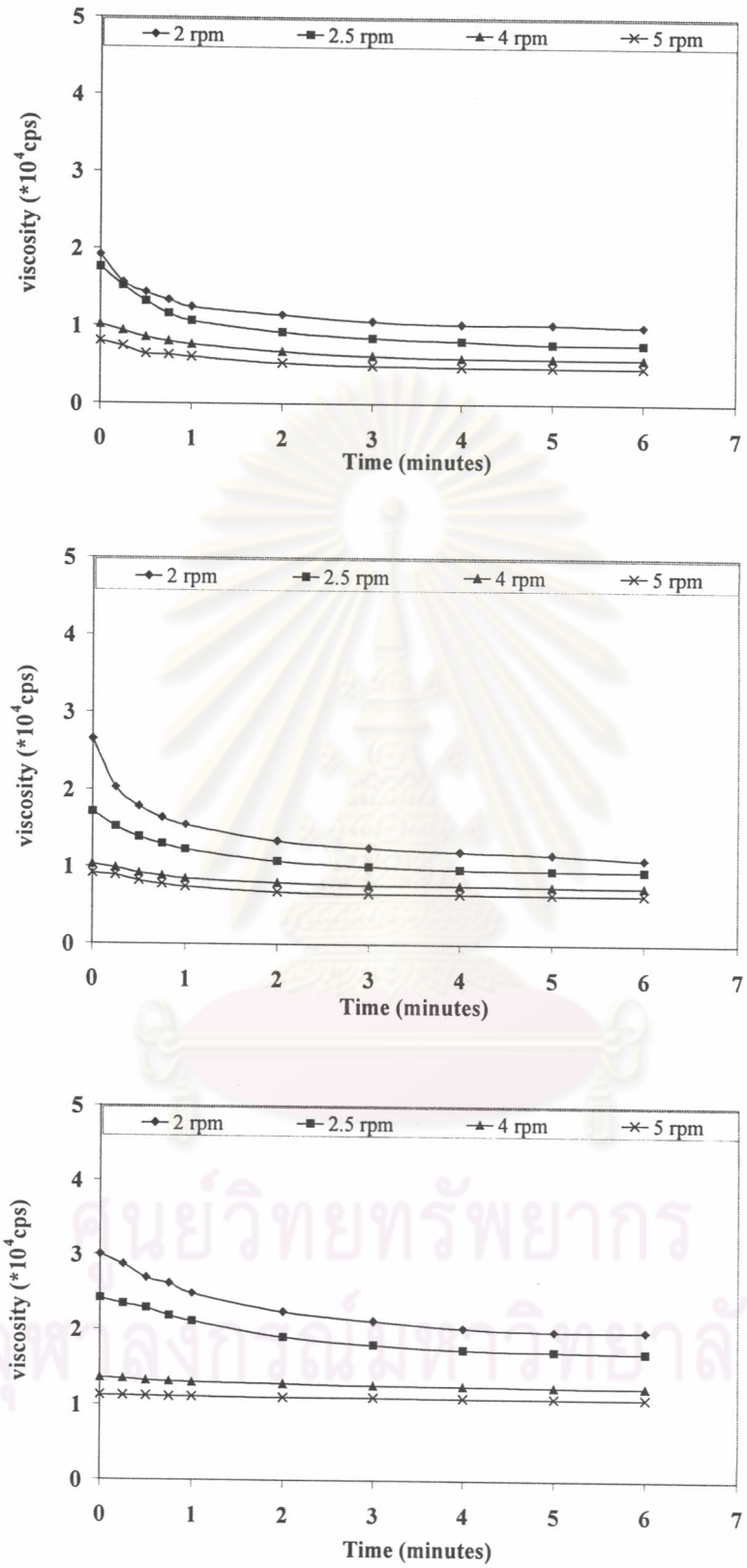


Figure 49 Time-viscosity relationship of IPM: T_{80}: C_{EL}: W system; formulation 6/1 (IPM: T_{80}: C_{EL} =3: 3.5: 3.5, 15%water) (top), 6/3 (IPM: T_{80}: C_{EL} =2: 4: 4, 9%water (middle) and 6/4 (IPM: T_{80}: C_{EL} =1: 4.5: 4.5, 15%water) (bottom). Using spindle no.62.

The last system in this study was SBO: T_{80} : W (SBO: T_{80}=1: 9). The viscosity was 480 cps. The system also showed newtonian time-independent behavior that provided constant value of viscosity while shear rate and time had changed. This might be the nature of soybean oil had viscosity only 50.09 cps at 25°C and could not form liquid crystal structure when mixing with the tween 80 in formulation. Therefore, the lower viscosity than other system could be obtained.

The schematic illustration of effect of shear rate on the viscosity for non-newtonian and shear-thinning system of studied formulation are shown in Figure 51-55. Formulation 1/1, 1/3 and 1/5 had shear-thinning behavior as illustrated by the decreasing in viscosity when increased shear rate were observed in this experiment in Figure 51. Similar to [IPM:T_{80}: L_{68}:W (T_{80}:L_{68}=2:1)], [IPM: T_{80}: C_{EL}: W], [IPM: C_{EL}: W: PG (4:1)] and [IPM: T_{80}: B_{35}: W] system that illustrated from Figure 52 to 55.

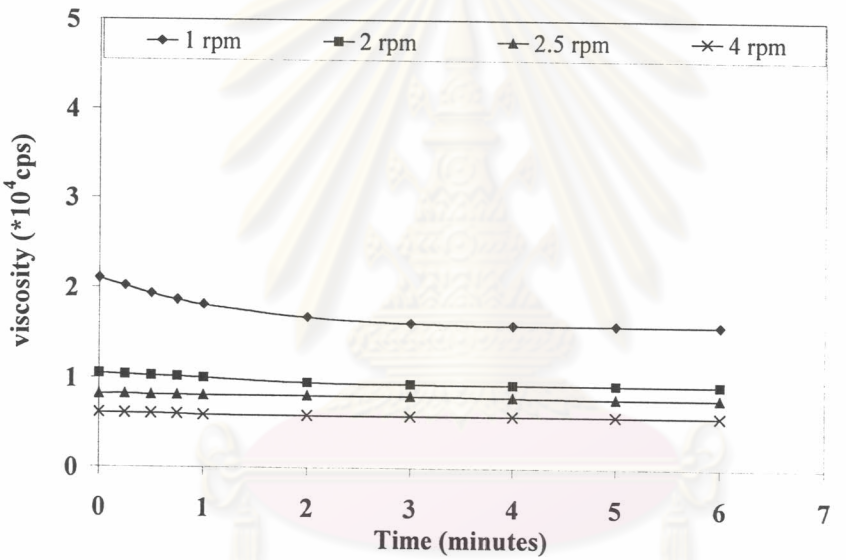


Figure 50 Time-viscosity relationship of IPM: T_{80}: B_{35}: W system; (IPM: T_{80}: B_{35} =3: 3.5: 3.5, 15%water) of formulation 7/1 . Using spindle no.62.

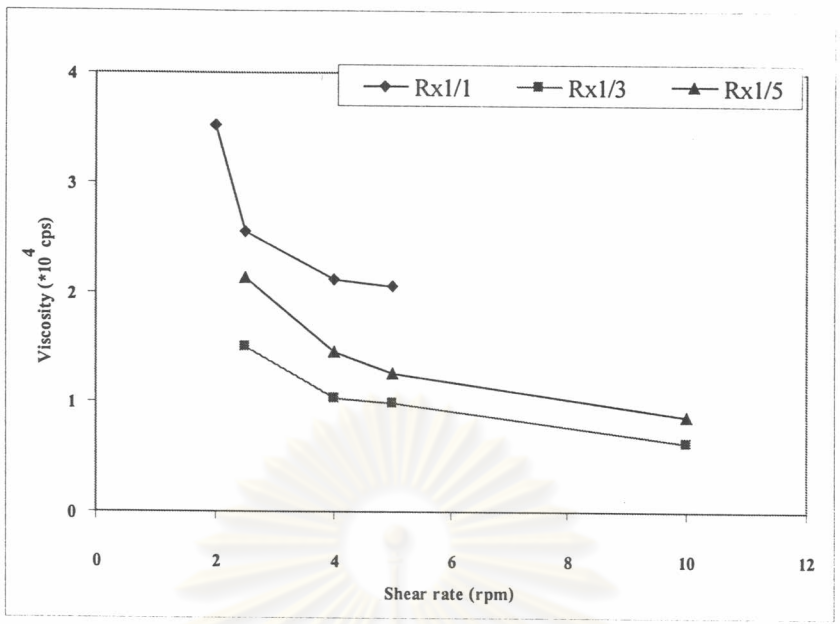


Figure 51 Schematic illustration of effect of shear rate on the viscosity for non-newtonian and shear-thinning system of IPM: T80: W system; formulation 1/1 (IPM:T_{80}=5:5, 10%water), 1/3 (IPM:T_{80}=4:6, 10%water) and 1/5 (IPM:T_{80}=3:7, 10%water); using spindle no.62 and detected at 1 minutes after start measuring.

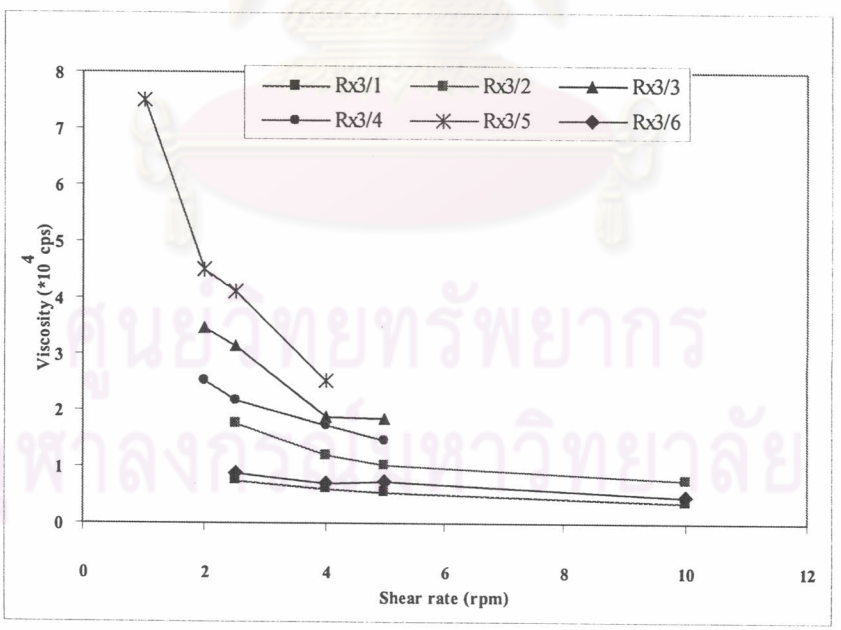


Figure 52 Schematic illustration of effect of shear rate on the viscosity for non-newtonian and shear-thinning system of IPM:T_{80}:L_{68}:W (T_{80}:L_{68}=2:1) system, formulation 3/1, 3/2, 3/3, 3/4, 3/5 and 3/6; Using spindle no.62. and detected at 1 minutes after start measuring.

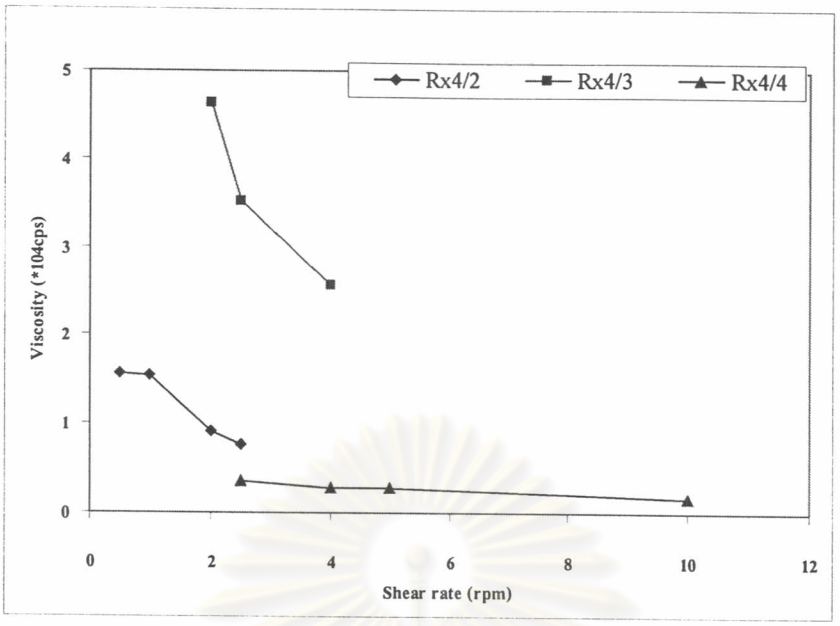


Figure 53 Schematic illustration of effect of shear rate on the viscosity for non-newtonian and shear-thinning system of IPM: C_{EL} : W: PG (4:1) system; formulation 4/2 (IPM: C_{EL} =2:8, 25%water), 4/3 (IPM: C_{EL} =3:7, 25%water) and 4/4 (IPM: C_{EL} =3:7, 20%water); using spindle no.62 and detected at 1 minutes after start measuring.

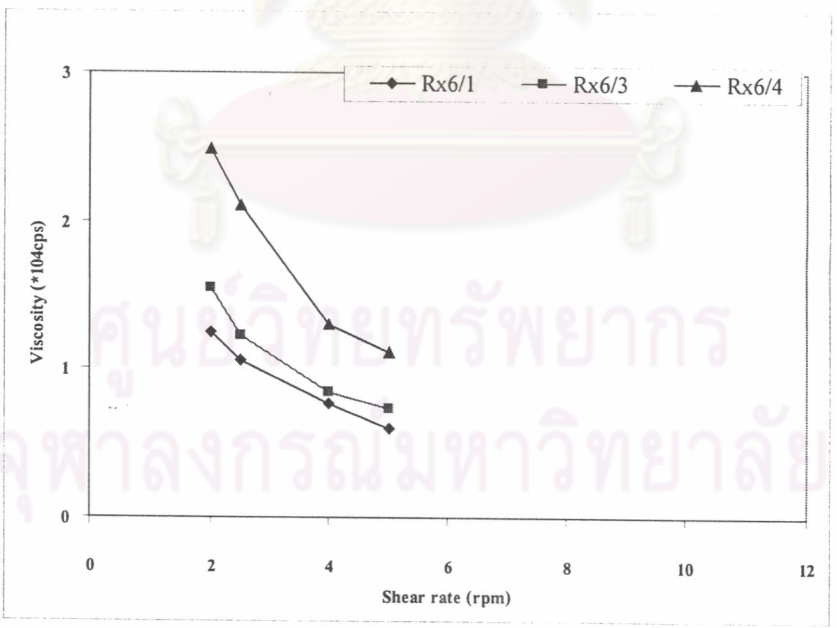


Figure 54 Schematic illustration of effect of shear rate on the viscosity for non-newtonian and shear-thinning system of IPM: T_{80}: C_{EL}: W system; formulation 6/1 (IPM: T_{80}: C_{EL} =3: 3.5: 3.5, 15%water), 6/3 (IPM: T_{80}: C_{EL} =2: 4: 4, 9%water) and 6/4 (IPM: T_{80}: C_{EL} =1: 4.5: 4.5, 15%water); using spindle no.62 and detected at 1 minutes after start measure.

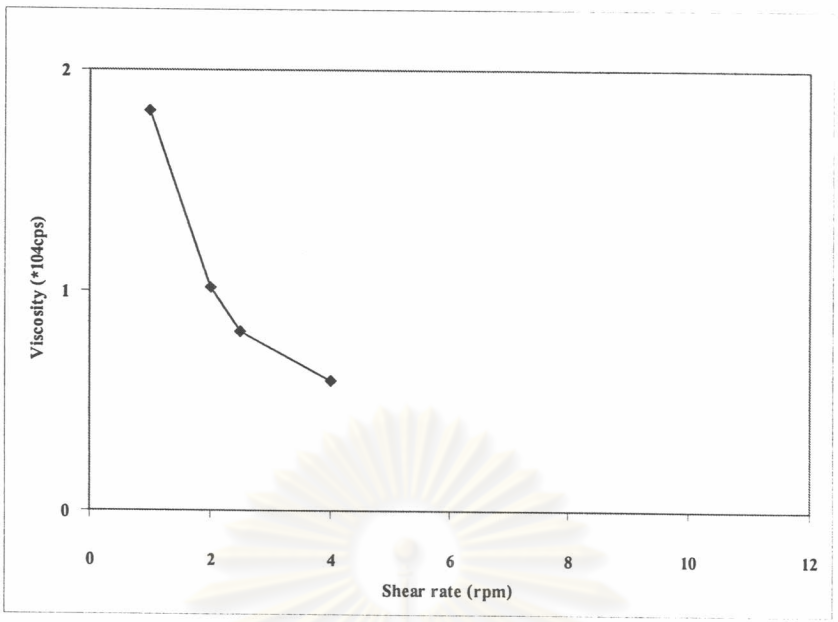


Figure 55 Schematic illustration of effect of shear rate on the viscosity for non-newtonian and shear-thinning system of IPM: T_{80}: B_{35}: W system; (IPM: T_{80}: B_{35} =3: 3.5: 3.5, 15%water) of formulation 6/1. Using spindle no.62 and detected at 1 minutes after start measuring.

Effect of oil type on viscosity

There were three types of oil were used in this study; isopropyl myristate (IPM), castor oil (CO) and soybean oil (SBO). The viscosity of IPM was about 5-7 cps whereas SBO was 50.09 cps and CO was about 1,000 cps. Comparative study of differrent oil types was illustrated by 2 systems; formulation 1/6 [IPM : T_{80} : W (IPM: T_{80} =1:9, water = 7%)] and formulation 8/1 [SBO : T_{80} : W (SBO:T_{80}=1:9, water = 7%)]. These two systems had different types of oil (IPM-SBO) but the same amount of surfactant (T_{80}) and water (7%). The results showed that system with SBO as an oil phase had higher viscosity than system of IPM as shown in Table 13. These concluded that the viscosity of system depended on the main composition in the system.

The system of CO : C_{EL} : W: PG (4:1) and IPM : C_{EL} : W: PG (4:1) also had the same amount of surfactant and water phase in the 2/4 and 4/3 but lower viscosity was observed in formulation 2/4 that had castor oil as oil phase. Similar result was observe in formulation 2/6 and 4/4 that lower viscosity was observed in formulation 2/6 that contained castor oil as oil phase. These may explained by the occurrence of lamellar or hexagonal liquid crystal in IPM : C_{EL} : W: PG (4:1) system that had IPM as oil phase while castor oil as an oil phase could not form lamellar and hexagonal liquid crystal in at all. IPM had a more possibility to form liquid crystal system than castor oil and could form lamellar and hexagonal phase in most ratio and other type of oil (Kuneida et al, 2001). Most formulations in system of IPM: C_{EL} : W: PG (4:1) formed lamellar and hexagonal phase including non-newtonian behavior thus induced greater viscosity (Makai et al, 2002).

Effect of co-surfactant type on viscosity

To study the effect of co-surfactant on viscosity, system IPM : T_{80} : C_{EL} : W (6/1) and IPM : T_{80} : B_{35} : W (7/1) at the same amount of oil: surfactant: co-surfactant (3: 3.5: 3.5)and 15% water in formulation were selected to be discussed. The results found that the system with C_{EL} as co-surfactant had more viscosity than that of Brij 35.

The structural formula of Brij 35 (Polyoxyl 23 lauryl ether) is long chain polymer with this structure $CH_3(CH_2)_X$ $(OCH_2CH_2)_Y$ OH. It had 23 polyoxyethylene glycol ether group of lauryl alcohol. C_{EL} (polyoxyethylene 35 castor oil) had more chain length than Brij 35 that induced more viscosity of system IPM : T_{80} : C_{EL} : W than system IPM : T_{80} : B_{35} : W (Kale and Allen, 1989).

Effect of surfactant type on viscosity

System IPM: C_{EL} : W: PG (4:1) and IPM : C_{RH}: W: PG (4:1) had different surfactant type. The viscosity of system that used C_{RH} as surfactant was 10 fold more viscous than that of C_{EL} as surfactant in at any ratio. These may be the characteristic of C_{RH} that was viscous, semi-solid paste material. Furthermore it could form lamellar, hexagonal or cubic phase with the appropriated type and amount of oil, surfactant, co-surfactant and water in individual formulation. The viscosity of C_{EL} was about 650-850 cps which was lower than semi-solid viscous paste of C_{RH} due to their structural formula.

C_{EL} (polyoxyethylene 35 castor oil) and C_{RH} (polyoxyethylene 40 hydrogenated castor oil) also had the different amount of chain length. C_{RH} had 40 polyoxyethylene groups, whereas C_{EL} that had only 35 polyoxyethylene groups.

II. Viscosity of microemulsion gel containing 1.5% w/w metronidazole

The physical appearance of drug-loaded MEG was similar to drug free MEG and no precipitation of metronidazole crystal could be seen in all formulations. The overall trend of viscosity in drug-loaded MEG was increased when metronidazole was incorporated into MEG base. The viscosity of drug loaded MEG and LC of each system were investigated and comparative value with drug free MEG as shown in Table 13. and Figure 56 and 57.

In formulation of MEG containing drug, the viscosity of IPM : T_{80} : W system at the ratio of oil:surfactant 5:5, 4:6 and 3:7 in formulation 1/1, 1/2, 1/4 and 1/5 were 2,670 cps, 294 cps, 350 cps and 380 cps whereas the viscosity of formulation 1/3 and 1/6 were 3,000 cps and 458 cps, respectively. The viscosity of MEG with drug was decreased in formulation 1/1, 1/2, 1/4 and 1/5 and lower than the formulation without metronidazole while the viscosity of drug loaded MEG in formulation 1/3 and 1/6 was increased from 1,080 to 3,000 cps and 435 to 458 cps respectively. This was due to the formulation had a phase separation upon addition of drug in formulation 1/1, 1/2, 1/4 and 1/5 which could lower the viscosity of system. In addition, it was found that the flow behavior of MEG base was changed from non- newtonian to newtonian in formulation 1/5 at the both ratio of oil: surfactant as 3:7 whereas formulation 1/1 and

1/3 still exhibited non- newtonian behavior but had less viscosity than the MEG base without drug. The relationship between time and viscosity of these non-newtonian systems (formulation 1/1 and 1/3) are shown in Figure 58. In addition, the viscosity of formulations 1/1 and 1/3 were classified to be a time dependent non-newtonian behavior that called "shear-thinning system" whereas formulations 1/2, 1/4, 1/5 and 1/6 were classified as newtonian systems (Martin, 1993). This time-dependent non-newtonian behavior (thixotropic or pseudoplastic flow behavior) did not change after metronidazole was incorporated in formulation. This structure resulted in some degree of rigidity on the system and it resembled as gel referred that the stability of 1.5%w/w metronidazole MEG.

For CO: C_{EL}: W: PG (4:1) containing drug system; the viscosities of formulations 2/1 to 2/4 were increased from 895 cps to 1,378 (2/1), 1,080 to 1,134 cps (2/2), 858 cps to 930 (2/3) and 3,072 cps to 3,078 (2/4) when the drug was incorporated into MEG base. In contrast, the viscosity was decreased in formulation 2/5 and 2/6 when 1.5%w/w metronidazole was incoporated. These could be assumed that the occurrence of phase separation in such formulation at the ratio of oil: surfactant 5:5 water content 13% and 20%, respectively. In formulations 2/5 and 2/6, phase separation was observed before the drug was incorporated. All formulations in these systems still exhibited time-independent newtonian behavior (Martin, 1993) as shown in Table 13. It is important to realize that the presence of drug in MEG may affect the stability and structure of MEG. These study, metronidazole is insoluble drug formed by non-ionic surfactant and co-surfactant that are less sensitive to the presence of drug.

For drug loaded IPM : T_{80} : L_{68} : W (T_{80}:L_{68} = 2:1) system; the viscosity of all formulations with drug were increased at every ratio of oil: surfactant. The viscosity of drug loaded MEG was 8,850 cps, 19,500 cps, 96,100 cps, 37,500 cps, 33,600 cps and 12,150 cps in formulation 3/1-3/6, respectively. As expected, addition of metronidazole would increase the concentration of both internal phase and disperse phase due to its solubility both in surfactant layer and internal micelles. Therefore, the viscosity of all formulations would increase when metronidazole was incorporated into MEG base (Macdonald et al, 1972). Furthermore, the drug did not interfere thermodynamic behavior and breakdown structure of system did not occurred. Therefore, the lamellar phase microstructure still observed at every ratio similar to MEG without drug. This indicated that the combination of these non-ionic surfactant and block copolymer which exhibited pseudoplastic flow and thixotropic flow behavior was stable enough to persist their dominant characteristic even after the incorporation of the large and insoluble drug as shown in Figure 59 and 60. The relationship between time and viscosity of these non-newtonian systems showed the characteristic of time-dependent and shear-thinning system.

Microstructural transition was observed in all formulation in these systems. These would be described that the shear induced transition from sheet-like lamellar to dispersed multilamellar vesicle phase (call droplets, onion structure or vesicle phase) which recently had observed in block-copolymer (Ziptel et al, 1998). Structure was broken down arising from the change of thermodynamic system brought to decrease in viscosity of system was not observed in all formulations.

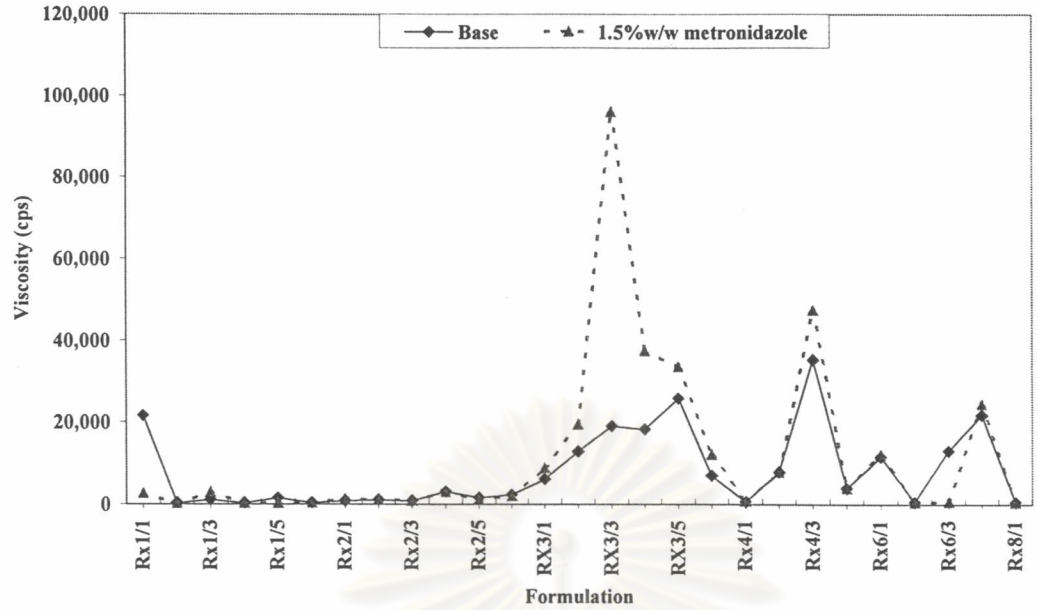


Figure 56 Comparison of the viscosity between MEG base and MEG containing 1.5% w/w metronidazole.

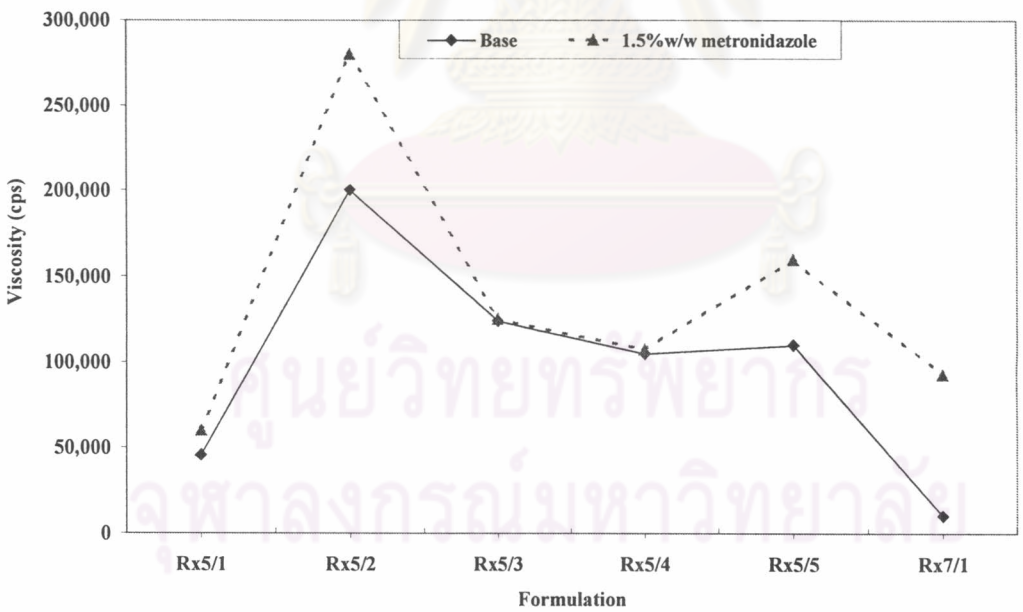


Figure 57 Comparison viscosity between MEG base and MEG containing 1.5% w/w metronidazole in formulation 5/1-5/5 and 7/1 (continue).

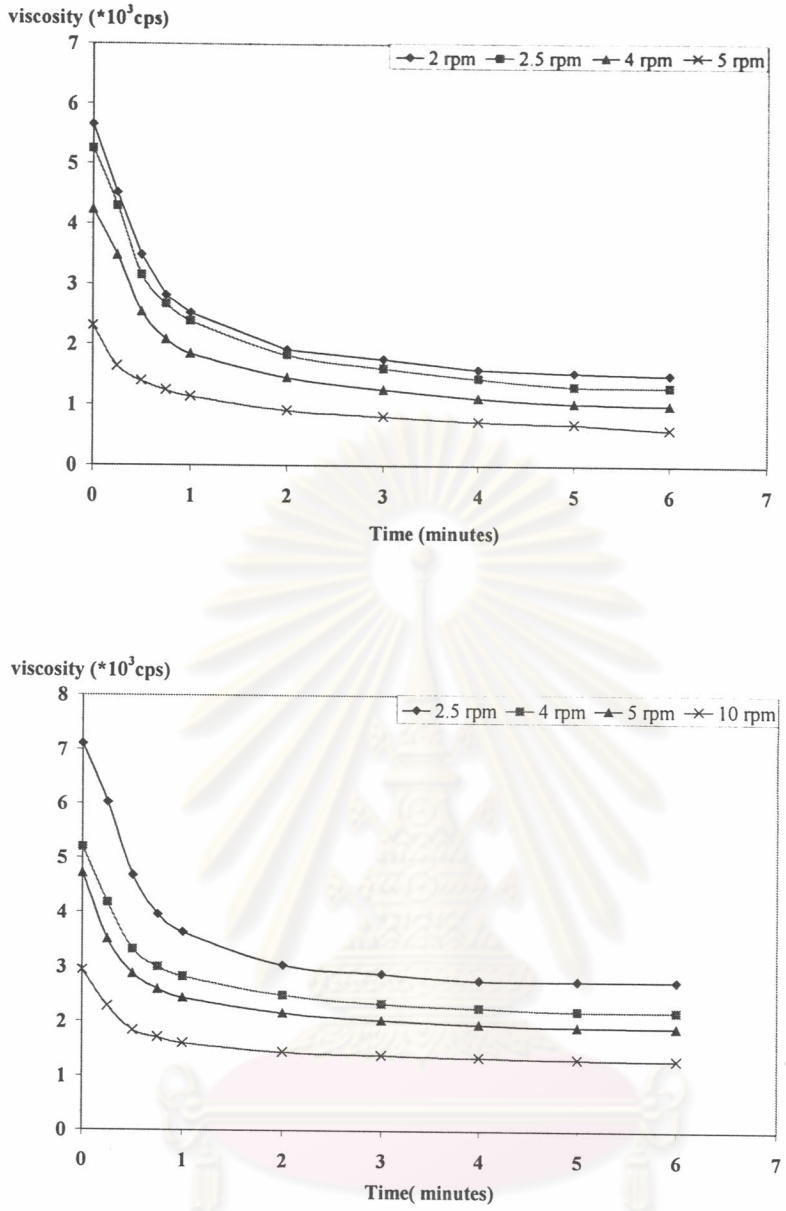


Figure 58 Time-viscosity relationship of IPM: T80: W system containing 1.5% metronidazole ; formulation 1/1(IPM:T_{80}=5:5, 10%water) (top) and 1/3 (IPM:T_{80}=4:6, 10%water). Using spindle no.62.

When the drug was loaded in IPM: C_{EL}: W: PG (4:1) system; their viscosity at the ratio of oil: surfactant of 1:9, 2:8, 3:7, 3:7 at 10, 25, 25 and 20% water content was increased as shown in Table13. The viscosity of drug loaded MEG in formulation 4/1-4/4 was higher than the corresponding formula without metronidazole as compared in Figure 56. Similar to aforementioned, incorporation of metronidazole into MEG base would increase the concentration of drug both in polarity part of amphiphile surfactant molecule and between the inter lamellar space (Makai et al, 2003). It could be supposed that MEG with large concentration of water phase came to forming aggregates of the drug molecules, which reflects on the apparent viscosity of system. In the formulations which are proved to have cylindrical and bicontinuous structure, metronidazole is probably located in the interfacial film of tensides and it could not

affect the viscosity of the system. In the system with low water content and high amphiphiles concentrations, metronidazole is most likely solubilized in the oil-continuous system (Djordjevic et al, 2004). This results had also revealed by using diclofenac diethanolamine incorporated into IPM, water and capric triglyceride with similarly in the increasing in viscosity.

Lamellar structure and time-dependent non-newtonian behavior was also observed in formulation 4/2, 4/3 and 4/4 similar to the MEG without drug as shown in Figure 61 whereas formulation 4/1 exhibited newtonian fluid system. The figure illustrated that the remain of this lamellar liquid crystal structure still remained and the break down did not appear (Malmsten, 1999). The material underwent a gel- to-sol transformation and exhibited shear-thinning system. It could be found that these systems could maintain the thermodynamic behavior and stability even with interference of the system with drug.

In drug loaded, IPM: C_{RH}: W: PG (4:1) system; an obviously increase in viscosity of MEG in formulation 5/1-5/5 was obtained. At the every ratio of oil:surfactant as 3:7, 4:6, 4:6, 5:5, 5:5 as water content was 14.52, 20, 15, 15 and 15%, the viscosity of all formulations as dramatically increased in formulations 5/1-5/5 (60,000, 280,000, 125,334, 107,666 and 160,000 cps) as shown in Figure 57. Comparison of MEG and drug-loaded MEG showed that the viscosity of the latter was makedly increased and higher than the corresponding formulation without metronidazole. These might be explained by the incorporating of metronidazole into MEG base would increase the concentration of drug both in polarity part of amphiphile surfactant molecule and between the inter lamellar space (Malcomson and Lawrence, 1993). Phase separation and syneresis was also not observed in these systems.

These may explain that the viscosity and stability of MEG were depended on the nature of composition in system. In these systems, Cremophor RH40 were used as surfactant and the characteristic of this excipient was white, semi-solid viscous material paste. This excipient are used as solubilizing agent and emulsifying agent such as vitamin, essential oil and certain drug and also improve the solubility of drug. This excipient is stable and compatible with other pharmaceutical excipients or electrolite. Therefore, the use of cremophor RH40 as cosurfactant had no significant for product instability (Kibbe, 2000).

The time-dependent non-newtonian behavior was also observed in formulation 5/1-5/5 after incoporating metronidazole into MEG. Eventhough these systems possessed highly viscous MEG, shear-thinning thixotropic system could be observed under this experiment. This indicated that a breakdown of structure and hence shear-thinning was gradually reformed. Moreover, this formulation had Cremophor RH40 as surfactant which exhibited excellent clearly white gel and good appearance and physical stability.

IPM : T_{80} : C_{EL} : W containing drug showed similar results as the IPM : C_{RH}: W: PG (4:1) system. The viscosity of drug loaded MEG of formulation 6/1, 6/2 and 6/4 was higher than the corresponding formulation without metronidazole. The viscosity of formulation 6/1, 6/2 and 6/4 was 12,140, 536, 24,620, respectively and was higher than MEG base without metronidazole at the ratio of oil:surfactant:co-

surfactant=3: 3.5: 3.5 (15%water), 2: 4: 4 (9%water) and 1: 4.5: 4.5 (20%water) whereas formulation 6/3 at oil: surfactant: co-surfactant of 1: 4.5: 4.5 (15%water) showed a dramatic decrease in viscosity when the drug was incorporated. Furthermore, shear-thinning behavior of this non-newtonian fluid was changed to newtonian fluid that the viscosity decreased from 13,000 cps (time-dependent non-newtonian) to 728 cps which had constant viscosity even when shear rate and time had changed. These might be the occurrence of phase separation in formalution 6/3 which brought to breakdown the lamellar structure and unstable formulation. The existence of non-newtonian behavior was still observed under this study especially in formulation 6/1and 6/4. The relationship between viscosity and time were illustrated in Figure 62.

In drug loaded, IPM : T_{80} : B_{35} : W system; (IPM : T_{80} : B_{35}= 3 : 3.5 : 3.5, 15% water), the viscosity of formulation 7/1 illustrated an obvious increase in viscosity when drug was added. The viscosity was increased from 10,200 cps to 93,000 cps as shown in Table13. In addition, shear-thinning and non-newtonian time-dependent behavior still exhibited as shown in Figure 63. Furthermore, more transparent and clearly ringing gel was also obtained when the drug was incorporated.

The viscosity of the drug loaded system in this study; SBO: T_{80}: W (SBO: T_{80}=1:9) in formulation 8/1 revealed that the viscosity of MEG containing metronidazole was increased and slightly higher than that of the MEG without drug. The viscosity was increased from 480 cps to 492 cps when the drug was incorporated into MEG base. The unchanged behavior was also observed and still showed newtonian time-independent behavior system similar to corresponding formulation without drug that provided constant value of viscosity while shear rate and time had changed. Soybean oil had viscosity only 50.09 cps at 25°C. This might affect the viscosity of MEG base and drug loaded MEG.

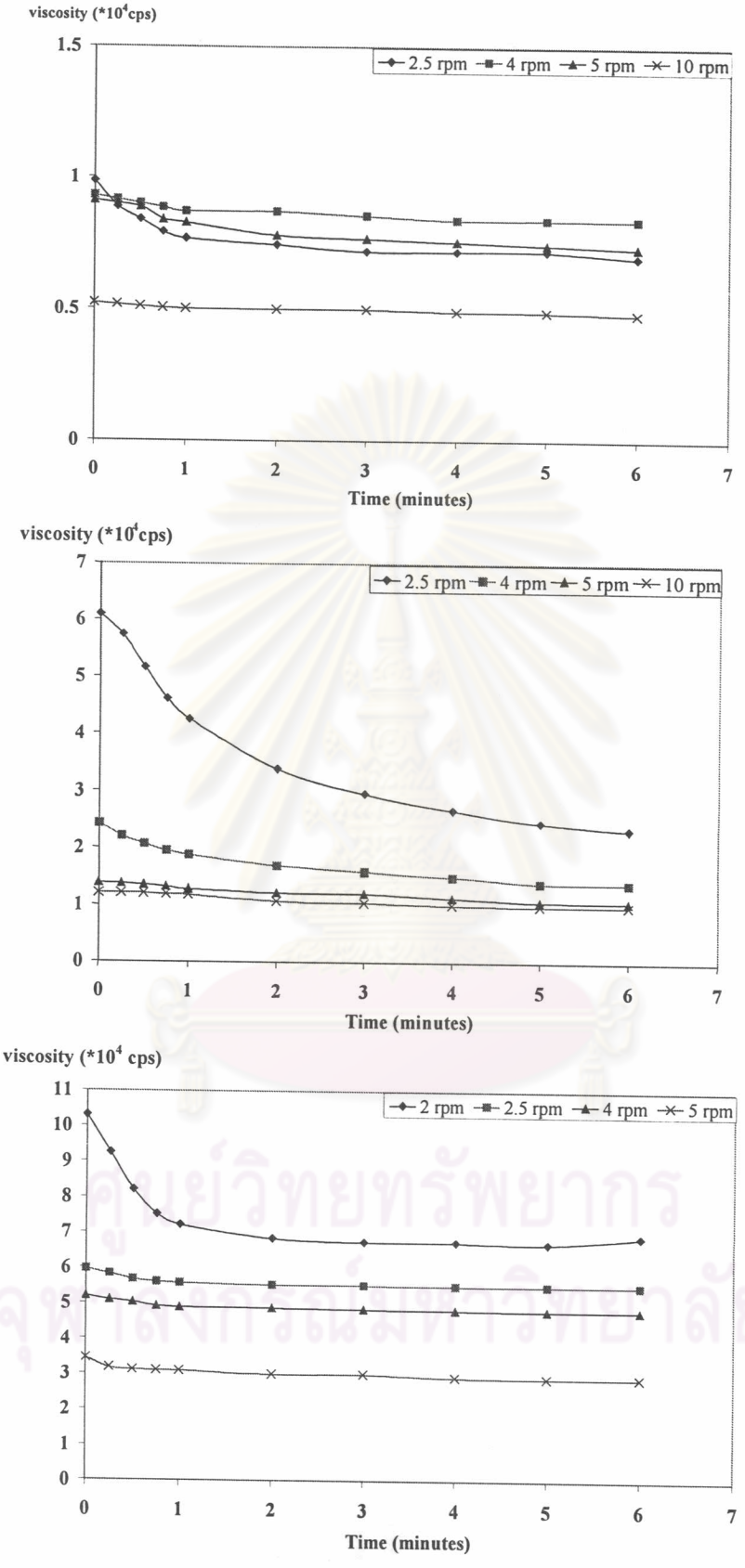


Figure 59 Time-viscosity relationship of drug loaded IPM : T_{80} : L_{68} : W (T_{80}:L_{68}=2:1) system, (IPM:T_{80}:L_{68} = 3 : 4.67 : 2.33); formulation 3/1 (15% water) (top), 3/2 (20%water) (middle) and 3/3 (25%water) (bottom). Using spindle no.62.

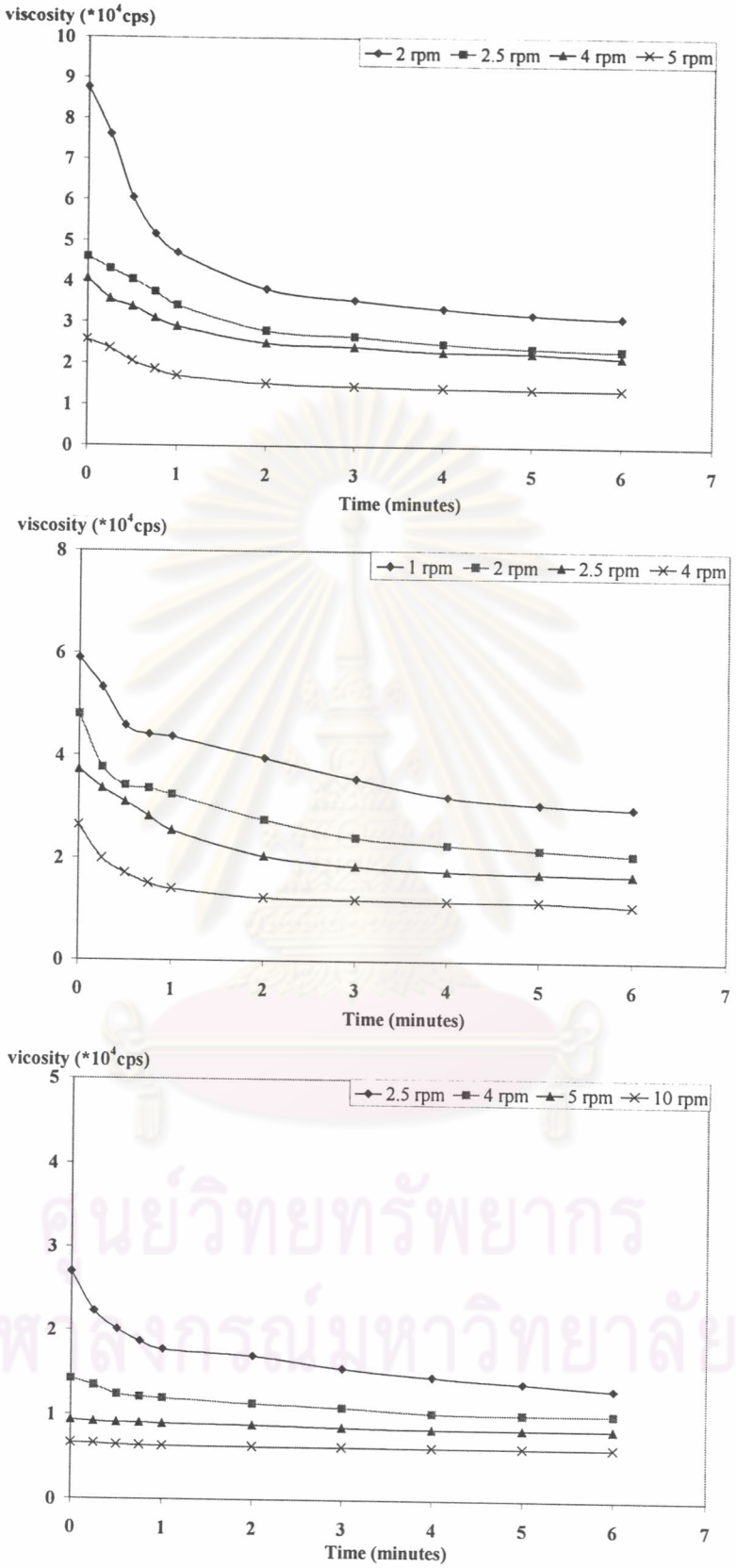


Figure 60 Time-viscosity relationship of drug loaded IPM : T_{80} : L_{68} : W (T_{80}:L_{68}=2:1) system, (IPM:T_{80}:L_{68} = 2: 5.33: 2.67); formulation 3/4 (20% water) (top), 3/5 (25%water) (middle) and 3/6 (17%water) (bottom). Using spindle no.62.

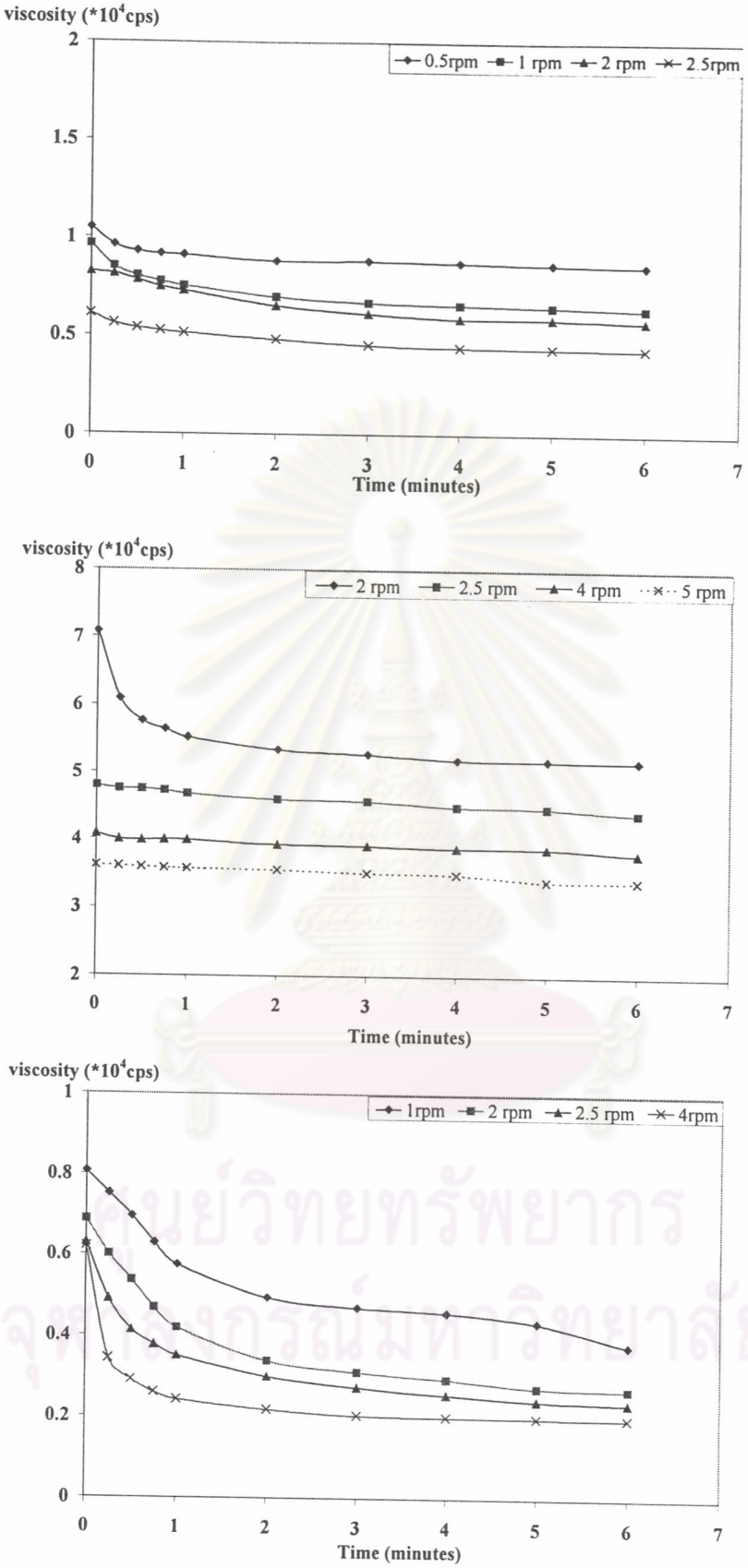


Figure 61 Time-viscosity relationship of IPM: C_{EL} : W: PG (4:1) system; formulation 4/2 (IPM: C_{EL} =2:8, 25%water) (top), 4/3 (IPM: C_{EL} =3:7, 25%water) (middle) and 4/4 (IPM: C_{EL} =3:7, 20%water) (bottom). Using spindle no.62.

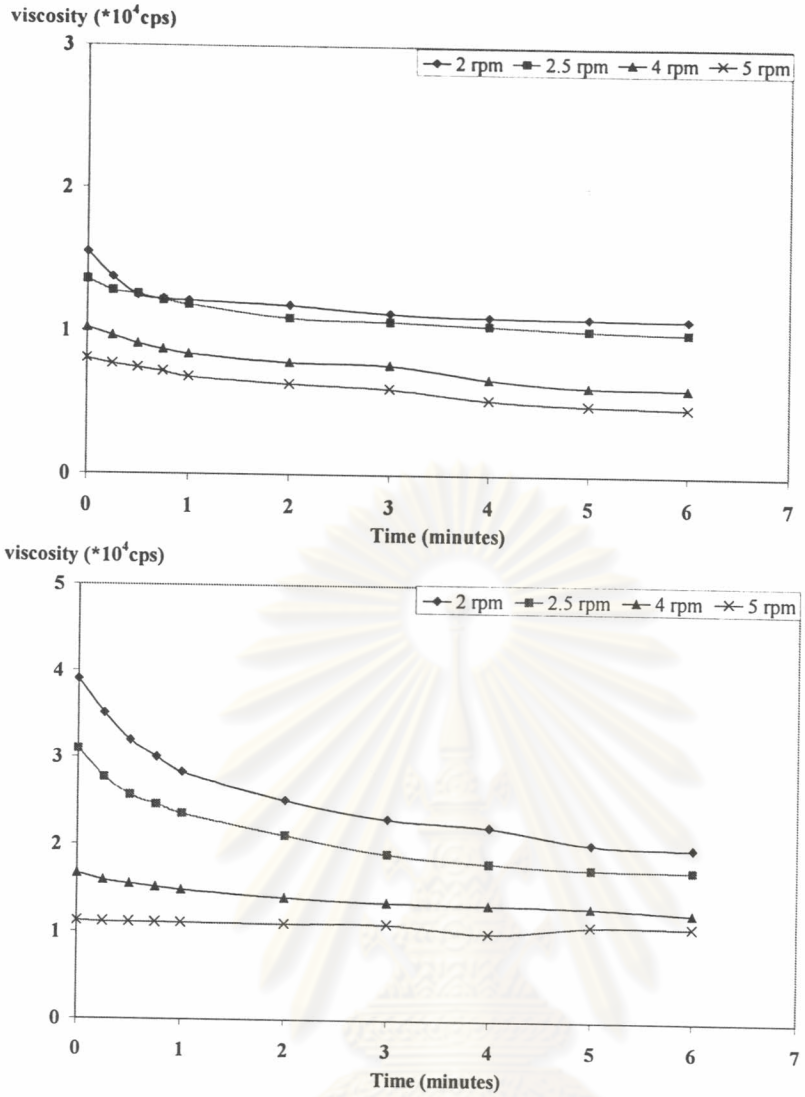


Figure 62 Time-viscosity relationship of IPM: T_{80}: C_{EL}: W system; formulation 6/1 (IPM: T_{80}: C_{EL} =3: 3.5: 3.5, 15%water) (top), and 6/4 (IPM: T_{80}: C_{EL} =1: 4.5: 4.5, 15%water) (bottom). Using spindle no.62.

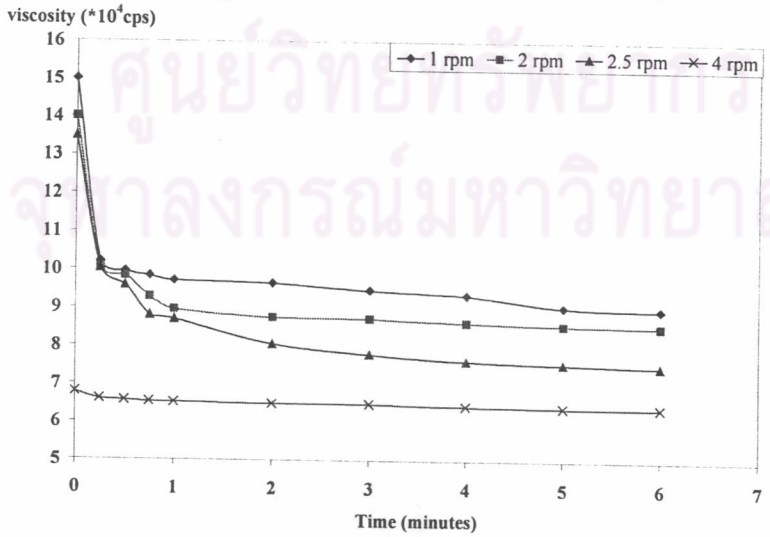


Figure 63 Time-viscosity relationship of IPM: T_{80}: B_{35}: W system; (IPM: T_{80}: B_{35} =3: 3.5: 3.5, 15%water) of formulation 6/1. Using spindle no.62.

2.8 Particle size and shape of MEG

Droplet size is one of the properties which is usually used to characterize ME. TEM technique is primarily concerned with the examination of bulk specimen and is the most important technique for the study microstructures because it directly produced images at high resolution and it can capture any coexistence of structures and microstructural transition. During the experiment, the images were viewed at various magnification to see overall of the samples. Then the droplet sizes were analyzed using SemAfore to count and calibrate their sizes.. The mean particle diameter could be obtained from SemAfore program after setting the scale before measuring had performed, the image scale had to be determined. Scaling requires that there was something of accurately known size visible in the image. Usually a size calibration bar ('micron bar') was available in the TEM image. The scaling was started by selecting Settings/ Scale in the menu. The cursor shape was changed to indicate that scaling was in progress. A reference line was next drawn with the mouse. The cursor was moved to one end of the calibration bar (or other image object of known size), the left mouse button was pressed and hold it down while dragging with the mouse to the other end of the bar. Then the mouse button was released. A dialog box was appeared, the length of the calibration bar in micrometers was entered, and pressed <Enter> or clicked OK. The length suggested when the dialog box opened was the value used last time so that SemAfore could calculate the magnification. The smallest allowed scale in SemAfore was 0.23 nm/pixel. The frequency, mean, mode, median, summation and histogram were obtained using the SPSS version 11.0 and are shown in Appendix D.

The particle sizes of MEG determined by TEM listed in Table 14 and Figure 66-99 shows the TEM photomicrographs and particle size distribution of MEG system without metronidazole both before and after stability testing. The mean particle diameter of each preparation was compared in Figure 65. The shapes of particles in most formulations were spherical particles whereas particle shapes of some were irregular of cluster shape. All formulations had mean particle size in the range of microemulsion which was under 100 nm Furthermore, wide particle size distribution were obtained in formulations (1/3) IPM: T_{80}: W (10%), (1/6) IPM: T_{80}: W (7%), (5/1) IPM: C_{RH}: (W: PG=4:1) (14.52%) and (6/1) IPM: T_{80}: C_{EL}: W (15%) while particle size distributions of formulations (1/1) IPM: T_{80}: W (10%), (1/5) IPM: T_{80}:W (10%), (3/1) IPM:T_{80}:L_{68}:W (15%) and (3/2) IPM:T_{80}:L_{68}:W (20%) were narrower (SD <10). The largest particle size was obtained from formulation (7/1) IPM: T_{80}: B_{35}: W while the smallest particle size was obtained from formulation (1/5) IPM: T_{80}: W (10%). After stability testing, the mean particle diameter was increased in most formulations except in formulations (5/1) and (6/1). The mean particle diameter after stability testing was decreased from 81.63 to 54.28 nm and 82.09 to 27.28 nm in formulations (5/1) and (6/1), respectively as shown in Table 14 and Figure 65. The overlapping of microstructures in a thicker sample would also make the image difficult to interpret. The high viscosity of both two formulations which had 45,600 cps and 11,610 cps (from characterization of viscosity in Topic 2.6) in formulations (5/1) and (6/1), respectively resulting in the excess could hardly be drained off thus netlike or an overlapping picture were obtained. Therefore, the mean particle diameter appeared larger than it should be in formulations (5/1) and (6/1).

However, MEG could not be easily imaged with TEM due to the difficulty to prepare the viscous, oily sample which had to be prepared by specialist. Furthermore, electron may induce chemical reaction in MEG systems that could change the microstructure. There was insufficient contrast between the MEG structures and their surroundings. The undetectable results might be due to some sample had low internal phase. In addition, high viscosity which the excess could hardly be drained off thus netlike or an overlapping picture were obtained. The microemulsion were also examined for their particle size by the light scattering technique. Unfortunately, the component of these MEG systems could not be detected by this technique due to they could not be diluted with the external phase or other solvent used in light scattering technique. When the sample was diluted with water, the transformation of microstructure from lamellar to hexagonal phase would resulting in the stiff viscous gel phase. The TEM photomicrographs and particle size distribution of these formulations both before and after stability testing are shown in Figure 66-99. All formulations produced spherical particles with wide particle size distribution.

IPM : T_{80} : W

The TEM photomicrographs of MEG base and particle size distribution of formulations (1/1), (1/3), (1/5) and (1/6) before and after freeze-thawing are shown in Figure 66-73. The shapes of particles in all formulations were spherical. Furthermore, wide particle size distribution was obtained in formulations (1/3) and (1/6) whereas particle size distribution of formulations (1/1) and (1/5) was narrower than that of formulations (1/3) and (1/6). The largest particle size was obtained from formulation (1/6) which was 78.53±20.48 nm. In contrast, the smallest one (27.68± 6.13) was obtained from formulation (1/5). The mean particle diameter was increased when increasing the ratio of oil: surfactant as shown in formulations (1/1), (1/3), (1/5) from Table 14. The mean particle diameter of formulation (1/5) was lower than formulations (1/1) and (1/3) except for formulation (1/6) that not followed by this pattern. On the other hand, the mean particle diameter was decreased with the increasing amount of surfactant in formulation.

This results indicated that the higher surfactant in system causes the interfacial film to condense while an addition of the cosurfactant would cause that film to expand. Similar result was found in the study on microemulsion using Brij 96 as cosurfactant and glycerin, ethylene glycol and propylene glycol as cosurfactant. Results suggested that the higher surfactant: cosurfactant ratio, the decreasing in particle size was obtained (Constantinides and Scalart, 1997). Another proposed was that the diameter was found to decrease when the surfactant: cosurfactant or surfactant: oil was increased when use mineral oil, Brij 96 and PEG 400. These might be explained by the decrease in the internal phase diameter could be attributed to the solubilization of oil within a lager number of surfactant micelles, which were consequently swollen to lesser extent (Rosano et al, 1988).

After freeze-thawing, the mean particle diameter was dramatically increased in all formulations in this system. Moreover, the wider particle size distribution than before stability testing was obtained in all formulations as shown in Table 14 and Figure 65. The TEM photomicrographs and particle size distribution of these MEG base after freeze-thawing are shown in Figure 67, 69, 71 and 73. All formulations still remained spherical particle with wider particle size distribution than before stability

testing. Similarly, statistically evaluation showed statistical significant difference in mean particle diameter of all formulations in these system between before and after freeze-thawing ($P<0.05$, test by 2 tailed pared-sample T test).

This indicated that upon freeze-thawing the rapid change in temperature and stress condition occurred including the high temperature produced high kinetic energy. This might affect or destroy the layer of surfactant film. Consequently, the high activation kinetic energy had increased with increasing the droplets size of system. The possibly mechanism of component redistribution among droplets in MEG was occurred to maintain dynamic condition after high kinetic energy from high temperature, the mechanism in exchange and transfer of mass among droplet by fusion and fission including with agglomeration between droplet had been proposed by Moulik and Paul (1998) as shown in Figure 64.

Experiments revealed that mass-exchange, mass transfer and chemical reactions could occur through interdroplet fusion. According to experimental evidences, the redistribution of components among droplets in microemulsions were fairly rapid which had been attributed to two distinct type of processes as shown in Figure 64 (A) Droplets collided, temporarily merged, fused into larger droplets and then broke and fissioned into smaller droplets. This dynamic process led to reaction by way of mass exchange and transfer, (B) Droplets broke with loss of fragments which subsequently associated or coagulated with other droplets. This dynamic process also helped in chemical reaction and mass distribution (Moulik and Paul, 1998).

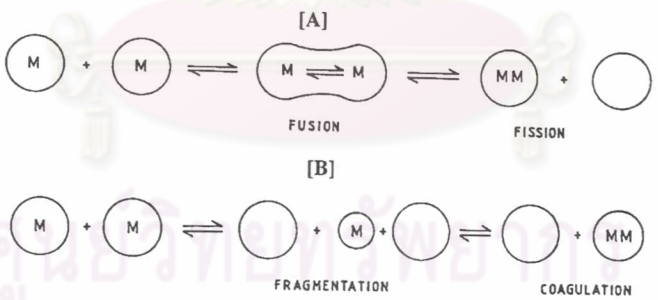


Figure 64 (A) Collision, fusion and fission with mass transfer and (B) fragmentation followed by coagulation causing mass transfer (from Moulik and Paul, 1998).

CO : C_{EL} : W: PG (4:1)

The most stable formulation which had a good physicochemical stability was found in formulation (2/1). Figures 74 illustrated the TEM photomicrographs and particle size distribution of MEG base of formulation (2/1) compared with that

formulation after freeze-thawing as shown in Figures 75. The shape of particles of this formulation both before and after freeze-thawing were spherical. The mean particle diameter of this formulation was increased from 72.94±19.67 to 83.84±22.04 nm after freeze-thawing. The particle size distribution of this formulation was wide as shown in Table 14. Furthermore, the shape of particle remained spherical and slightly wider distribution was obtained after freeze-thawing as shown in Figure 75. Comparison of the mean particle diameter between before and after freeze-thawing showed that after freeze-thawing the mean particle diameter was significantly increased ($p<0.05$, test by 2 tailed pared-sample T test).

Accordingly, the new layer of surfactants film of microemulsions was reformed thereby the mean particle diameter of microemulsion was altered. Similarly, the mean particle diameter of formulations in this system showed an increase after freeze-thawing. Furthermore, coalescence of some particle might occur during freeze-thawing cycle thereby the mean particle diameter increased after freezing and thawing cycle (Attwood et al, 1992).

IPM : T_{80} : L_{68} : W(T_{80}:L_{68}=2:1)

In this system, Figure 76-83 illustrated the TEM photomicrographs of MEG base before and after freeze-thawing of formulations (3/1)-(3/5). The shapes of particle of all formulations were spherical. The particle size distribution of formulations (3/4) and (3/5) was wider than formulations (3/1) and (3/2) as shown in Table 14. The mean particle diameter was 41.77, 47.67, 39.73, 58.33 nm. In formulations (3/1), (3/2), (3/4) and (3/5), respectively. The largest particle size was obtained from formulation (3/5) whereas formulation (3/4) gave the smallest. The mean particle diameter of MEG at the ratio of surfactant: cosurfactant 4.67: 2.33 in formulations (3/1) and (3/2) had mean particle diameter more than formulation (3/4) which containing higher amount of surfactant: cosurfactant (5.33: 2.67) in formulation as shown in Table 14.

The results indicated that the mean particle diameter decreased with the increasing of the surfactant: cosurfactant ratio in formulation. 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 the smaller particle was obtained. While the addition of cosurfactant caused the film expand due to the amount of cosurfactant that penetrated into the interfacial film increased therefore larger particle was obtained. Similar results were obtained from Kale and Allen (1989), Attwood et al (1992), Ho et al (1996) and Corswant et al (1998).

The mean particle diameter was decreased when decreasing the ratio of oil: surfactant in formulation as shown in Table 14. The mean particle diameter of formulation (3/4) was less than formulations (3/1) and (3/2) which had higher amount of oil in formulation. Exception in formulation (3/5) the largest mean particle diameter was obtained eventhough the higher amount of surfactant and cosurfactant in formulation. Furthermore, the mean particle diameter of formulation (3/2) that contained 20% water was more than 15% water content of formulation (3/1). Similarly, the mean particle diameter of 25% water content in formulation (3/5) was more than 20% water content in formulation (3/4). The percentage of water in systems

showed that the higher percentage of water in formulation, the larger mean particle diameter of MEG was obtained at the same oil: surfactant ratio. This was due to the increasing the percentage of water in formulation resulted in the decrease amount of surfactant in formulation or dilution effect was occurred (Kale and Allen, 1989). Therefore, the mean particle diameter was increased with the percentage of water in formulation as indicated by effect of decreasing surfactant: oil ratio like aforementioned above.

After freeze-thawing, their mean particle diameter was 84.81, 52.94, 57.87, 73.54 nm, respectively. It was found that the mean particle diameter of all formulations was dramatically increased after freeze-thawing as shown in Figure 65. The TEM photomicrographs of MEG base revealed that spherical particle were remained in all formulations after freeze-thawing as seen in Figure 77, 79, 81 and 83. The particle size distributions of all formulations were wider than before stability testing. Comparison of the mean particle diameter between before and after freeze-thawing showed that after freeze-thawing the mean particle diameter was significantly increased ($p<0.05$, test by 2 tailed pared-sample T test). These could be explained by thermodynamical stability mechanism aforementioned by Attwood (1994) and Moulik and Paul (1998).

IPM : C_{EL} : W: PG (4:1)

The TEM photomicrographs of MEG base and particle size distribution of formulations (4/2) and (4/4) before and after freeze-thawing are shown in Figure 37-44. The shapes of particles in both formulations were spherical. The mean particle diameter was 49.03 and 57.33 nm with the particle size distribution were 10.57 and 14.82 in formulations (4/2) and (4/4), respectively. The larger particle size was obtained from formulation (4/4) whereas the smaller was obtained from formulation (4/2). The results showed that the mean particle diameter was increased with the increasing the ratio of oil: surfactant as shown in Table 14. These might be the effect of surfactant: oil ratio and amount of surfactant in formulation similar as described in the first system.

After freeze-thawing, the mean particle diameter of formulations (4/2) and (4/4) were 99.17 and 99.89 nm, respectively. It was found that the mean particle diameters of both formulations were extensively increased including the wider particle size distribution than before stability testing was obtained as shown in Table 14 and Figure 84 and 86. The TEM photomicrographs of MEG revealed that spherical particle were remained in both formulations after freeze-thawing as seen in Figure 85 and 87. Comparison of the mean particle diameter between before and after freeze-thawing showed that after freeze-thawing the mean particle diameter was significantly increased ($p<0.05$, test by 2 tailed pared-sample T test). These could also be explained by thermodynamical stability mechanism aforementioned by Prince (1977); Moulik and Paul (1998).

IPM : C_{RH}: W: PG (4:1)

The TEM photomicrographs of MEG base and particle size distribution of formulations (5/1) and (5/3) before and after freeze-thawing are shown in Figure 88-

91. The shapes of particles in both formulations were spherical. The mean particle diameter was 81.63 and 50.70 nm with the particle size distribution were 21.69 and 16.62 in formulations (5/1) and (5/3), respectively. The larger particle size was obtained from formulation (5/1) whereas the smaller was obtained from formulation (5/3). According to the theory smaller particle should be obtained from formulation (5/1) but in this study mean particle diameter of formulation (5/1) was larger than (5/3), unlike the mean particle diameter after freeze-thawing that followed other results in this study. The overlapping of microstructures in a thicker sample would make the image difficult to interpret. The high viscosity of sample could be resulting in the preparation of sample before measure which excess could hardly be drained off thus netlike or an overlapping picture were obtained. Therefore, the mean particle diameter appeared lager than it should be in formulation (5/1). Furthermore,

From the results showed that the mean particle diameter was increased with the increasing ratio of oil: surfactant as shown in Table 14. These might be the effect of surfactant: oil ratio and amount of surfactant in formulation similar as described in the first system.

After freeze-thawing, the mean particle diameter of formulation (5/1) and (5/3) were 54.28 and 60.70 nm, respectively. It was found that the mean particle diameter of formulation (5/3) was increased except for formulation (5/1) as shown in Table 14, Figure 89 and 91. The TEM photomicrographs of MEG revealed that spherical particle were remained in both formulations after freeze-thawing as seen in Figure 88-91. Comparison of the mean particle diameter between before and after freeze-thawing showed that after freeze-thawing the mean particle diameter was significantly increased ($p<0.05$, test by 2 tailed pared-sample T test). These could be also explained by thermodynamical stability mechanism aforementioned by Gasco (1997); Moulik and Paul (1998).

IPM : T_{80} : C_{EL} : W

In this system, Figure 92-95 illustrated the TEM photomicrographs of MEG base before and after freeze-thawing of formulation (6/1) and (6/4). The shapes of particle of both formulations were spherical. The particle size distribution of formulation (6/1) was wider than formulation (6/4) as shown in Table 14. The mean particle diameter was 82.09 and 47.25 nm in formulation (6/1) and (6/4), respectively. The larger particle size was obtained from formulation (6/1) whereas formulation (6/4) gave the smaller particle. The mean particle diameter of MEG at the ratio of surfactant: cosurfactant 3.5: 3.5 in formulation (6/1) was more than formulation (6/4) which containing higher amount of surfactant: cosurfactant (4.5: 4.5) in formulation as shown in Table 14. Moreover, the mean particle diameter was decreased when decreasing the ratio of oil: surfactant in formulation as shown in Table 14. The mean particle diameter of formulation (6/4) was less than formulation (6/1) which had higher amount of oil in formulation.

The results indicated that the mean particle diameter decreased with the increasing of the surfactant: cosurfactant ratio in formulation. 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 the smaller particle was obtained, while

the addition of cosurfactant caused the film to expand due to the amount of cosurfactant that penetrated into the interfacial film increased therefore larger particle was obtained. Similar results were obtained from Kale and Allen (1989); Attwood et al (1992); Goa et al (1998) and Kuneida et al (2001).

After freeze-thawing, their mean particle diameter was 27.28 and 158.89 nm, respectively. It was found that the mean particle diameter of formulation (6/4) was dramatically increased after freeze-thawing. Surprisingly, the mean particle diameter of formulation (6/4) was extensively decreased including with narrow particle size distribution as shown in Figure 95. The TEM photomicrographs of MEG base revealed that spherical particle were remained in both formulations after freeze-thawing as seen in Figure 92 and 94. Comparison of the mean particle diameter between before and after freeze-thawing showed that after freeze-thawing the mean particle diameter of formulation (6/4) was significantly increased ($p<0.05$, test by 2 tailed pared-sample T test) whereas significantly decreased in formulation (6/1). These could be explained by thermodynamical stability mechanism aforementioned by Rosano et al (1988) and Moulik and Paul (1998) for the formulation (6/4). The mean particle diameter of formulation (6/1) should be larger than before stability testing but in this case showed the opposite result. These might be cause by the other mechanism which should be further investigated.

IPM : T_{80} : B_{35} : W

Figure 96 illustrated the TEM photomicrographs and particle size distribution of MEG base of formulation (7/1) compared with that formulation after freeze-thawing as shown in Figure 97. The shape of particles of formulation before and after freeze-thawing were spherical. The mean particle diameter of this formulation was dramatically increased from 84.37±16.04 to 112.56±31.97 after freeze-thawing. The particle size distribution was wider than before stability testing as shown in Table 14. Comparison of the mean particle diameter between before and after freeze-thawing showed that after freeze-thawing the mean particle diameter was significantly increased ($p<0.05$, test by 2 tailed pared-sample T test). This indicated that the mean particle diameter increased after freezing and thawing cycle.

Accordingly, the new layer of surfactants film of microemulsions was reformed thereby the mean particle diameter of microemulsion was altered. Similarly, the mean particle diameter of formulations in this system showed an increased after freeze-thawing. Furthermore, coalescence of some particle might occur during freeze-thawing cycle thereby the mean particle diameter was increased after freezing and thawing cycle (Attwood et al, 1992).

SBO : T_{80} : W

The TEM photomicrographs and particle size distribution of MEG base of formulation (8/1) compared with that formulation after freeze-thawing are shown in Figure 98-99. The shape of particles of formulation before and after freeze-thawing were similarly spherical. The mean particle diameter of this formulation was slightly increased from 78.33±20.00 to 79.58±26.52 nm after freeze-thawing. The particle size distribution was wider than before stability testing as shown in Table 14. Comparison of the mean particle diameter between before and after freeze-thawing showed no

statistical significant difference in mean particle diameter before and after freeze-thawing ($p>0.05$, test by 2 tailed pared-sample T test). This indicated that the mean particle diameter was not difference after freezing and thawing cycle. These might be the highly amount of surfactant in formulation (oil: surfactant=1:9) could stabilize the thermodynamic of system.

Comparison MEG gel system before and after freeze-thawing

The TEM photomicrographs and particle size distribution showed that mean particle sizes and standard deviation of most formulations were increased after freeze-thawing as compared in Figure 64-99. Formulation (1/3) IPM: T_{80}: W (10%) and (6/4) IPM: T_{80}: C_{EL}: W (20%) exhibited the most increasing in mean particle diameter and wide size distribution formulation as 2 and 4 folds compared with before stability testing respectively. From this study, the results indicated that the mean particle diameters were increased after the freeze-thawing process. These may occurred by rapid temperature changing between freezing process (-4°C) and thawing process (45°C) could affect the structure and viscoelastic property of formulation especially around the droplet of internal phase during 6 cycles of freeze-thawing process. Thus the droplets may change into large diameter to maintain thermodynamic of the system. Firstly, the new layer of surfactants film of microemulsions was reformed thereby the mean particle diameter of microemulsion was altered. Furthermore, coalescence of some particle might occur during freeze-thawing cycle thereby the mean particle diameter increased after freezing and thawing cycle (Alany et al, 2001).

Secondly, upon freeze-thawing, the rapid change in temperature and stress condition occurred including the high temperature produced high kinetic energy. This might affect or destroy the layer of surfactant film. Consequently, the high activation kinetic energy had increased with increasing the droplets size of system. According to the possibly mechanism of redistribution of components among droplets in MEG, in order to maintain dynamic condition after high kinetic energy from high temperature. The mechanism in exchange and transfer of mass among droplet by fusion and fission including with agglomeration between droplet were proposed as shown in Figure 64 (Moulik and Paul, 1998).

Comparison between ratio of surfactant: co-surfactant

The results indicated that the mean particle diameter decreased with the increasing of the surfactant: cosurfactant ratio in formulation. 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 the smaller particle was obtained. While the addition of cosurfactant caused the film to expand due to the amount of cosurfactant that penetrated into the interfacial film increased therefore larger particle was obtained. Similar results were obtained from Kale and Allen (1989), Attwood et al (1992), Ho et al (1996) and Corswant et al (1998).

Another proposed was that the diameter was found to decrease when the surfactant: cosurfactant or surfactant: oil was increased. This could be explained that the decrease in the internal phase diameter could be attributed to the solubilization of

oil within a lager number of surfactant micelles, which are consequently swollen to lesser extent (Zheliaskova et al, 1999).

Comparison between surfactant: co-surfactant system

Formulation that had the similar system (type and ratio of surfactant) and percentage amount of water but different type of oil, showed equal mean particle diameter as shown in formulation (1/6) and (8/1) that contained T_{80} as surfactant and 7% water. Both formulations had mean particle diameter of 78.53±20.48 and 78.33±20.00 nm, respectively. Similarly, in formulation (6/1) and (7/1) that had the ratio of surfactant: co-surfactant of 3: 3.5: 3.5 and 15% water had similar mean particle diameter before stability testing of 82.09 nm and 84.37 nm, respectively. The ratio of surfactant: cosurfactant also affected the mean particle diameter of formulation as shown in Table14. Comparison of formulation (6/1) and (7/1) that had ratio of surfactant: cosurfactant of 1:1 with formulation (3/1) that had ratio of surfactant of 2:1 and the same 15% water found that the mean particle diameter was decreased when increasing the ratio of surfactant: cosurfactant.

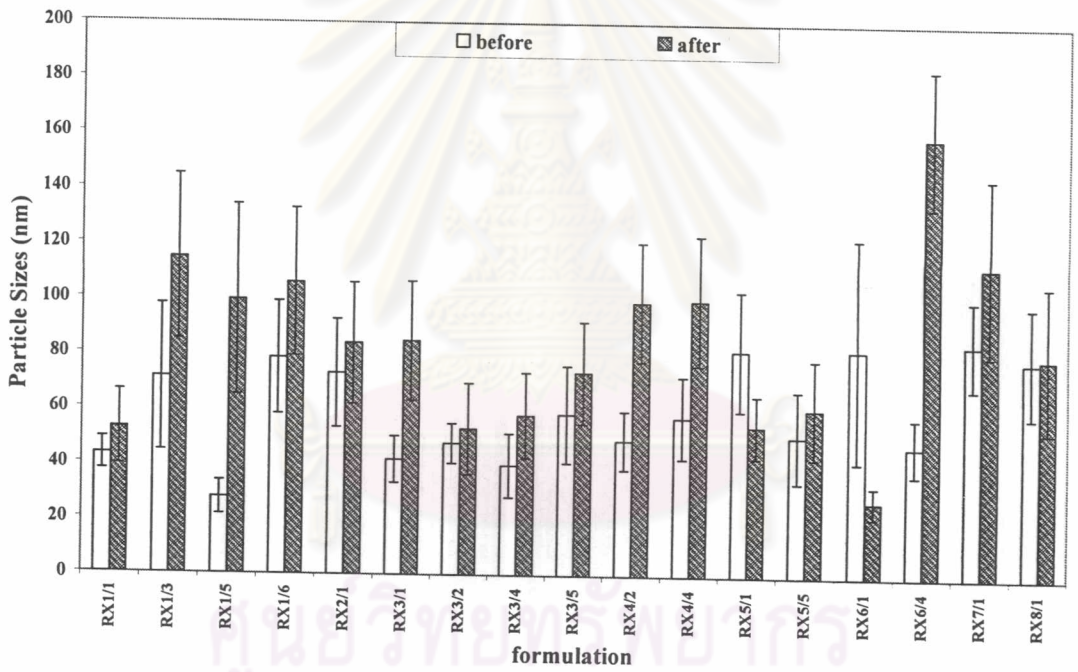


Figure 65 Comparison of the mean particle size of microemulsion gel system between before and after freeze-thawing.

Overall results indicated that most MEG and LC systems which contained suitable surfactant, cosurfactant, oil: surfactant ratio were thermodynamically stable for a long period of time. The mean particle diameter of these systems was in nanometer size range that less than 100 nm The surfactant to surfactant ratio had influence on the mean particle diameter of MEG and LC. Moreover, the particle size decreased with the increasing of surfactant: cosurfactant and surfactant: oil ratio. Obviously, after freeze-thawing the mean particle diameter was statistically increased ($p<0.05$) in all formulation except in formulation (8/1) that showed no statistical significant difference in mean particle diameter ($P>0.05$) between before and after freeze-thawing.

Table 14 Mean particle size of the representative MEG base system before and after stability testing.

Composition and ratio		Mean particle diameter (nm)			
		Before stability	SD	After stability	SD
IPM : T_{80} : W					
1/1)	5:5 (10%)	43.31	5.80	52.86*	13.47
1/2)	4:6 (8%)	-	-	-	-
1/3)	4:6 (10%)	71.27	26.64	114.83*	29.85
1/4)	3:7 (8%)	-	-	-	-
1/5)	3:7 (10%)	27.68	6.13	99.50*	34.47
1/6)	1:9 (7%)	78.53	20.48	105.90*	26.79
CO : C_{EL} : W: PG (4:1)					
2/1)	2:8 (23%)	72.94	19.67	83.84*	22.04
2/2)	2:8 (20%)	-	-	-	-
2/3)	3:7 (10%)	-	-	-	-
2/4)	3:7 (25%)	-	-	-	-
2/5)	5:5 (13%)	-	-	-	-
2/6)	5:5 (20%)	-	-	-	-
IPM : T_{80} : L_{68} : W(T_{80}:L_{68}=2:1)					
3/1)	3 : 4.67 : 2.33 (15%)	41.77	8.40	84.81*	21.64
3/2)	3 : 4.67 : 2.33 (20%)	47.64	7.19	52.94*	16.52
3/3)	3 : 4.67 : 2.33 (25%)	-	-	-	-
3/4)	2 : 5.33 : 2.67 (20%)	39.73	11.62	57.87*	15.50
3/5)	2 : 5.33 : 2.67 (25%)	58.33	17.56	73.54*	18.52
3/6)	2 : 5.33 : 2.67 (17%)	-	-	-	-
IPM : C_{EL} : W: PG (4:1)					
4/1)	1:9 (10%)	-	-	-	-
4/2)	2:8 (25%)	49.03	10.57	99.14*	21.58
4/3)	3:7 (25%)	-	-	-	-
4/4)	3:7 (20%)	57.33	14.82	99.89*	23.47
IPM : C_{RH}: W: PG (4:1)					
5/1)	3:7 (14.52%)	81.63	21.69	54.28*	11.20
5/2)	4:6 (20%)	-	-	-	-
5/3)	4:6 (15%)	50.70	16.62	60.70*	17.85
5/4)	5:5 (15%)	-	-	-	-
5/5)	5:5 (15%)	-	-	-	-
IPM : T_{80} : C_{EL} : W					
6/1)	3 : 3.5 : 3.5 (water 15%)	82.09	40.45	27.28*	5.49
6/2)	2 : 4 : 4 (water 9%)	-	-	-	-
6/3)	1 : 4.5 : 4.5 (water 15%)	-	-	-	-
6/4)	1 : 4.5 : 4.5 (water 20%)	47.25	10.25	158.89*	24.81
IPM : T_{80} : B_{35} : W					
7/1)	3 : 3.5 : 3.5 (water 15%)	84.37	16.04	112.56*	31.97
SBO : T_{80} : W					
8/1)	1:9 (water 7%)	78.33	20.00	79.58	26.52

(-) = exclude from the representative formula due to poor physical stability testing.

* = statistically significant difference ($p<0.05$) of mean particle diameter between before and after freeze-thawing that was calculated from 300 particles.

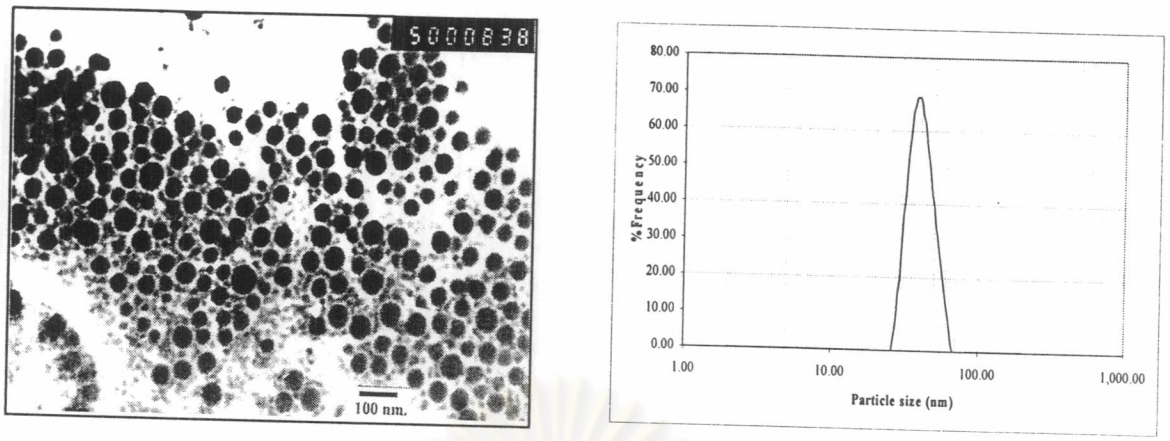


Figure 66 The TEM photomicrograph and particle size distribution of formulation (1/1) IPM : T_{80} : W (10%) (IPM:T_{80}=5:5) before stability testing.

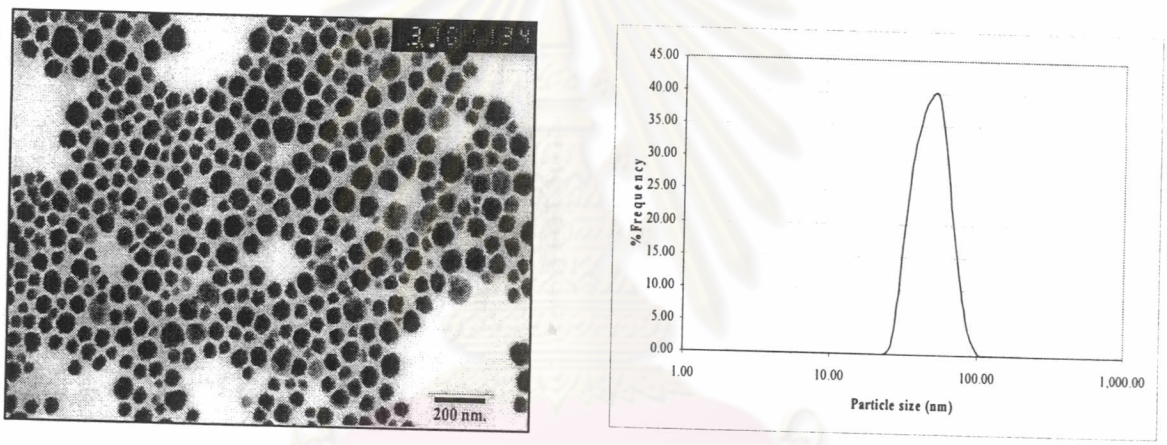


Figure 67 The TEM photomicrograph and particle size distribution of formulation (1/1) IPM : T_{80} : W (10%) (IPM:T_{80}=5:5) after stability testing.

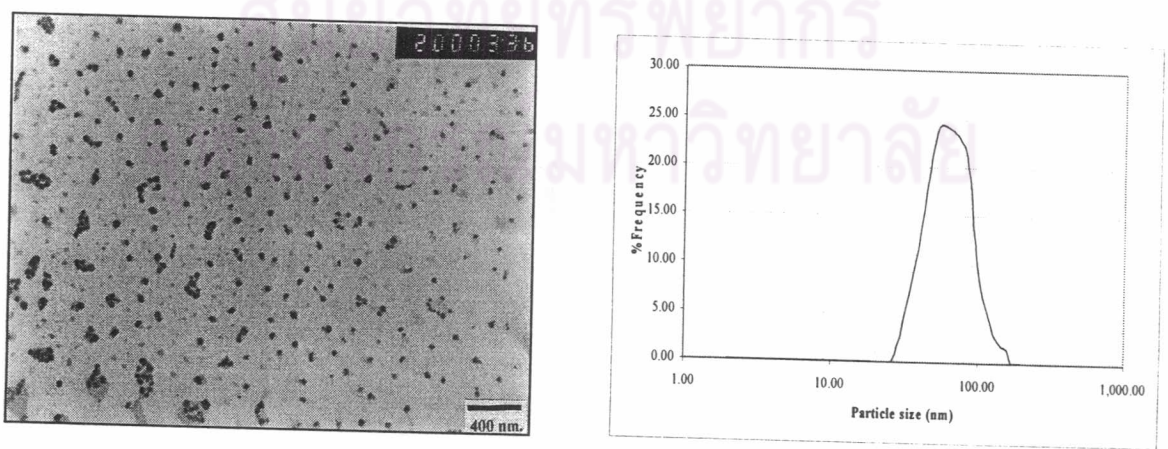


Figure 68 The TEM photomicrograph and particle size distribution of formulation (1/3) IPM : T_{80} : W (10%) (IPM:T_{80}=4:6) before stability testing.

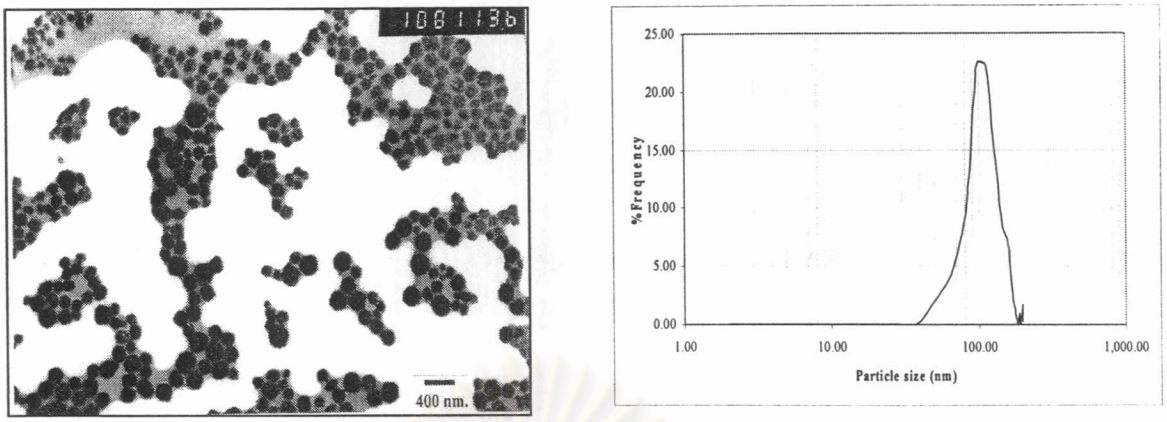


Figure 69 The TEM photomicrograph and particle size distribution of formulation (1/3) IPM : T_{80} : W (10%) (IPM:T_{80}=4:6) after stability testing.

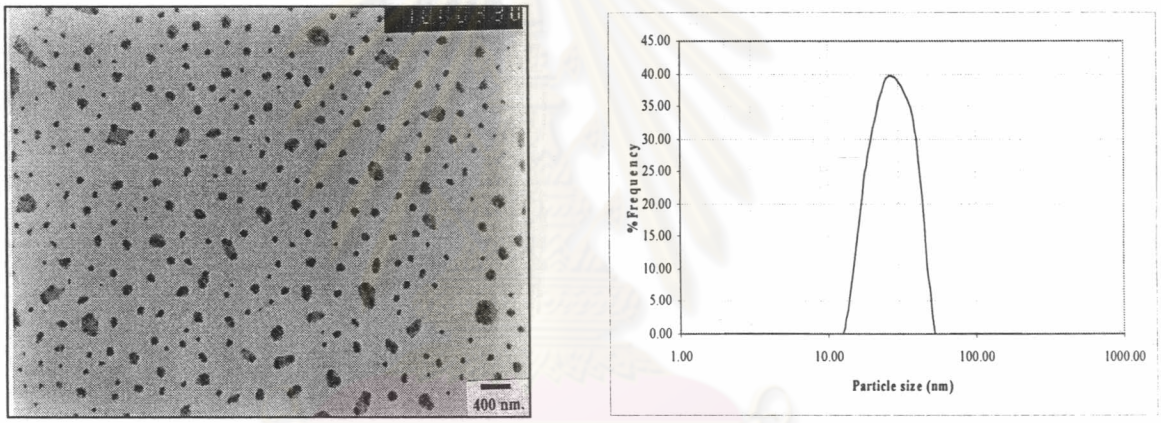


Figure 70 The TEM photomicrograph and particle size distribution of formulation (1/5) IPM : T_{80} : W (10%) (IPM:T_{80}=3:7) before stability testing.

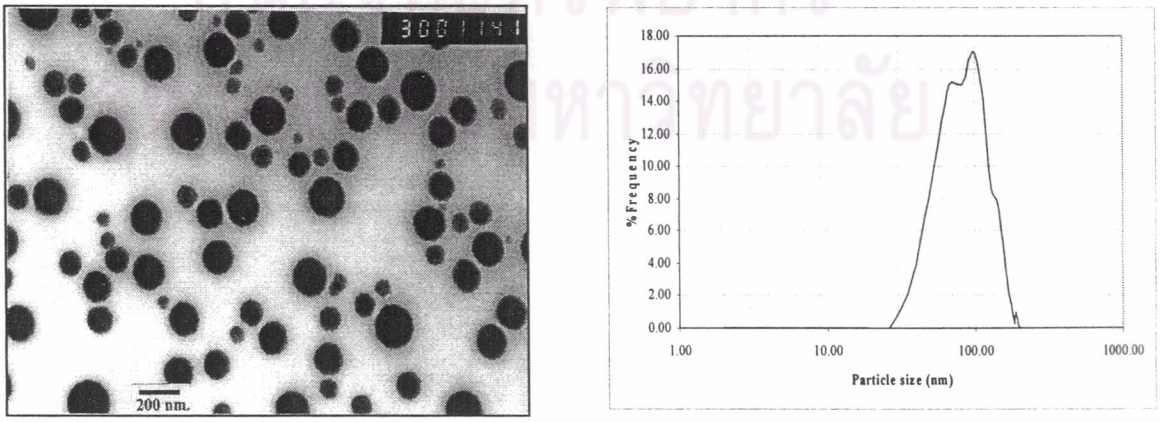


Figure 71 The TEM photomicrograph and particle size distribution of formulation (1/5) IPM : T_{80} : W (10%) (IPM:T_{80}=3:7) after stability testing.

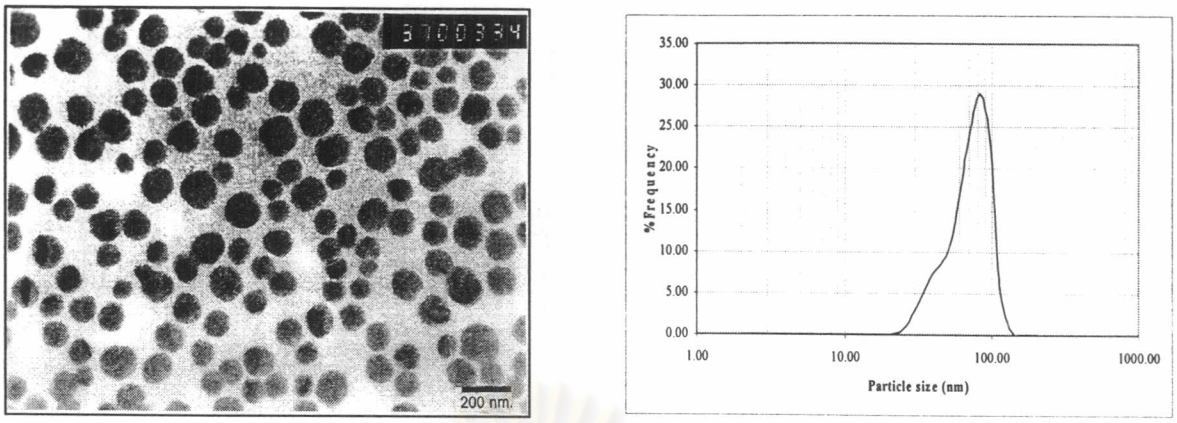


Figure 72 The TEM photomicrograph and particle size distribution of formulation (1/6) IPM : T_{80} : W (7%) (IPM:T_{80}=1:9) before stability testing.

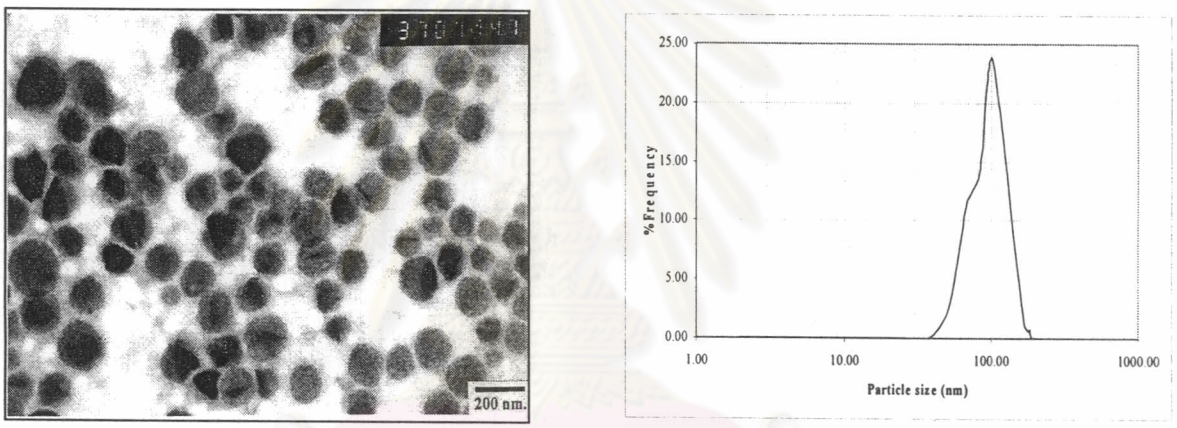


Figure 73 The TEM photomicrograph and particle size distribution of formulation (1/6) IPM : T_{80} : W (7%) (IPM:T_{80}=1:9) after stability testing.

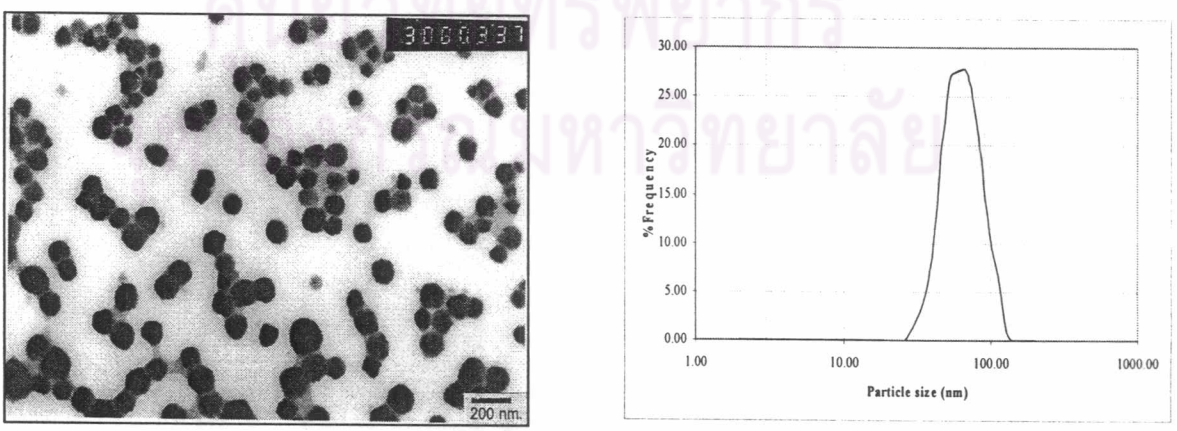


Figure 74 The TEM photomicrograph and particle size distribution of formulation (2/1) CO:C_{EL}:W: PG(4:1) (23%) (CO:C_{EL} = 2:8) before stability testing.

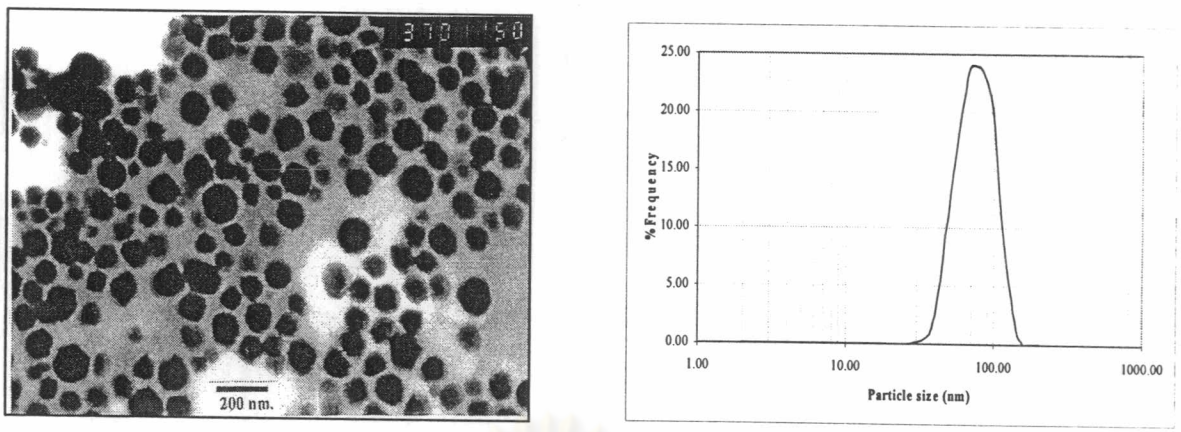


Figure 75 The TEM photomicrograph and particle size distribution of formulation (2/1) CO:C_{EL}:W: PG(4:1) (23%) (CO:C_{EL} = 2:8) after stability testing.

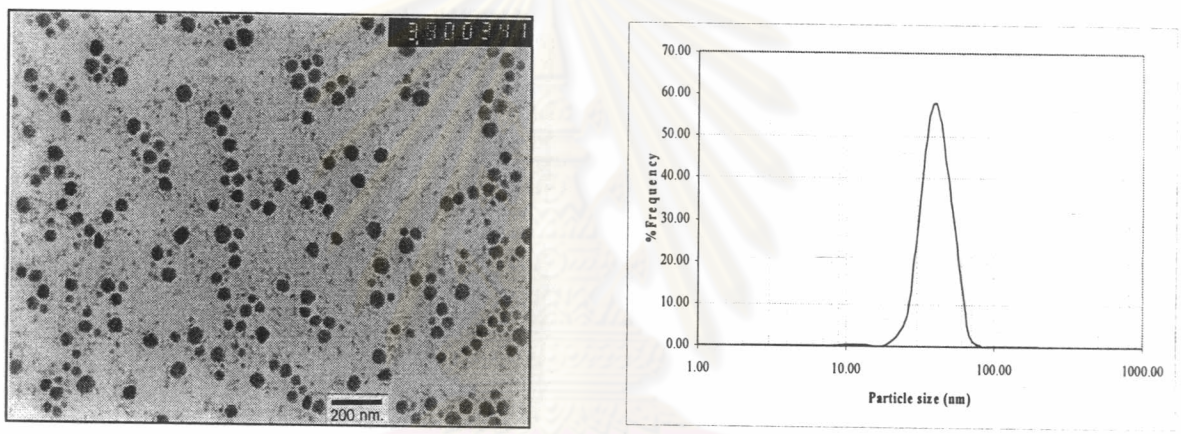


Figure 76 The TEM photomicrograph and particle size distribution of formulation (3/1) IPM : T_{80} : L_{68} : W(T_{80}:L_{68}=2:1) (W=15%) (IPM:T_{80}:L_{68} = 3 : 4.67 : 2.33) before stability testing.

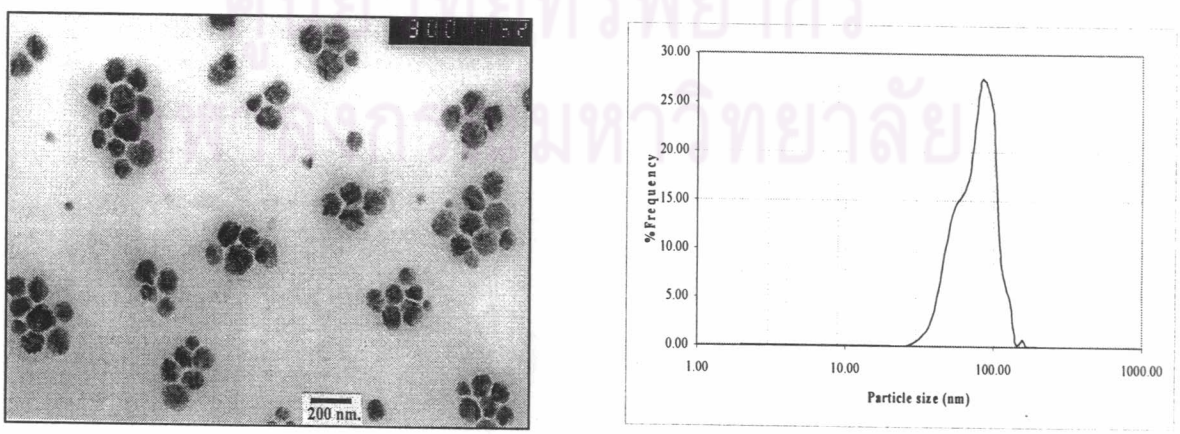


Figure 77 The TEM photomicrograph and particle size distribution of formulation (3/1) IPM : T_{80} : L_{68} : W(T_{80}:L_{68}=2:1) (W=15%) (IPM:T_{80}:L_{68} = 3 : 4.67 : 2.33) after stability testing.

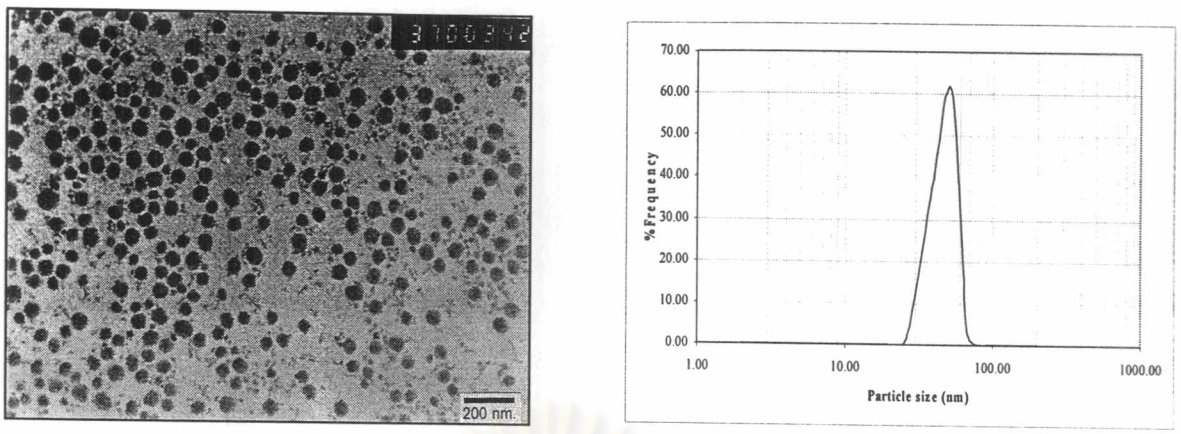


Figure 78 The TEM photomicrograph and particle size distribution of formulation (3/2) IPM : T_{80} : L_{68} : W(T_{80}:L_{68}=2:1) (W=20%) (IPM:T_{80}:L_{68} = 3 : 4.67 : 2.33) before stability testing.

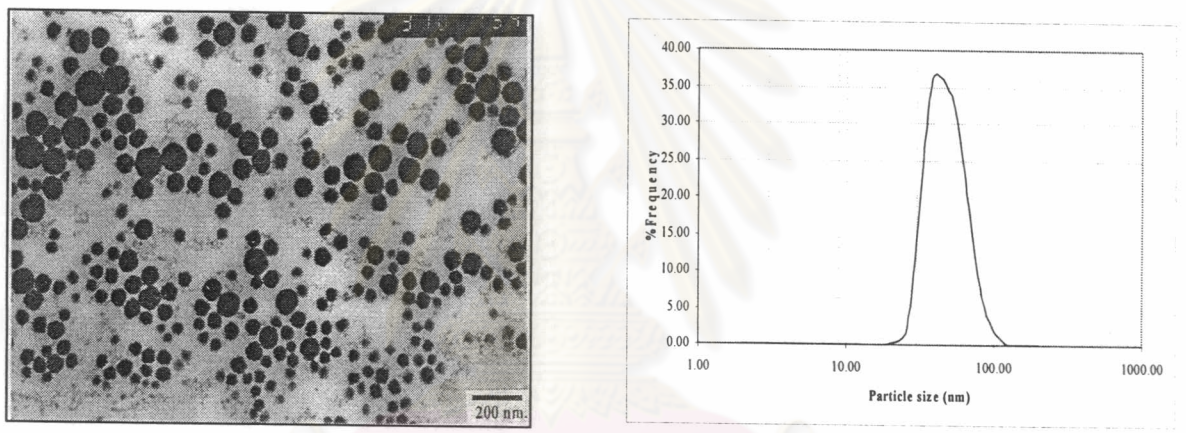


Figure 79 The TEM photomicrograph and particle size distribution of formulation (3/2) IPM : T_{80} : L_{68} : W(T_{80}:L_{68}=2:1) (W=20%) (IPM:T_{80}:L_{68} = 3 : 4.67 : 2.33) after stability testing.

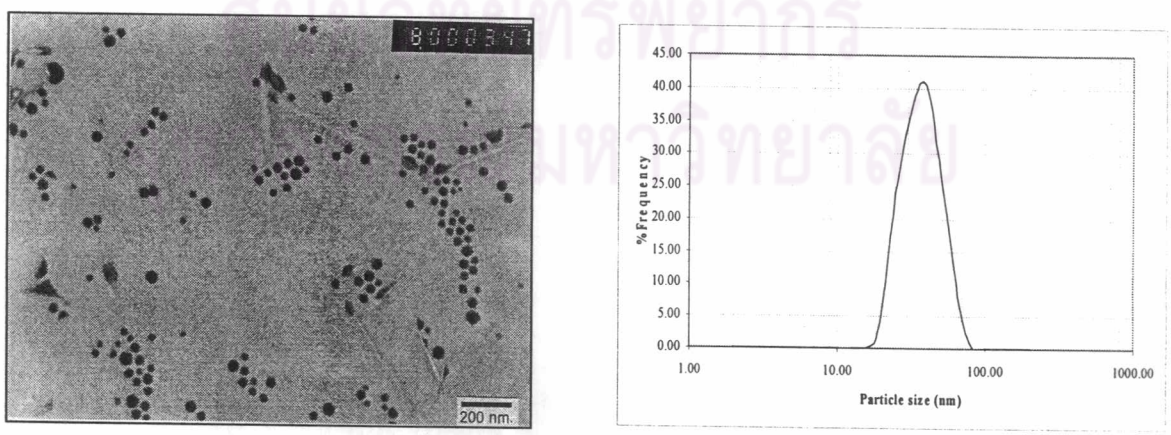


Figure 80 The TEM photomicrograph and particle size distribution of formulation (3/4) IPM : T_{80} : L_{68} : W(T_{80}:L_{68}=2:1) (W=20%) (IPM:T_{80}:L_{68} = 2 : 5.33 : 2.67) before stability testing.

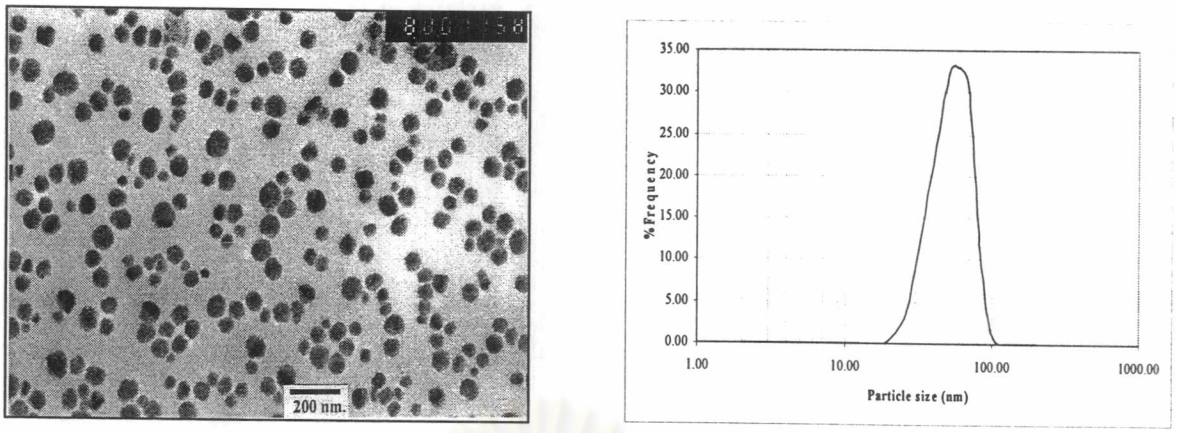


Figure 81 The TEM photomicrograph and particle size distribution of formulation (3/4) IPM : T_{80} : L_{68} : W(T_{80}:L_{68}=2:1) (W=20%) (IPM:T_{80}:L_{68} = 2 : 5.33 : 2.67) after stability testing.

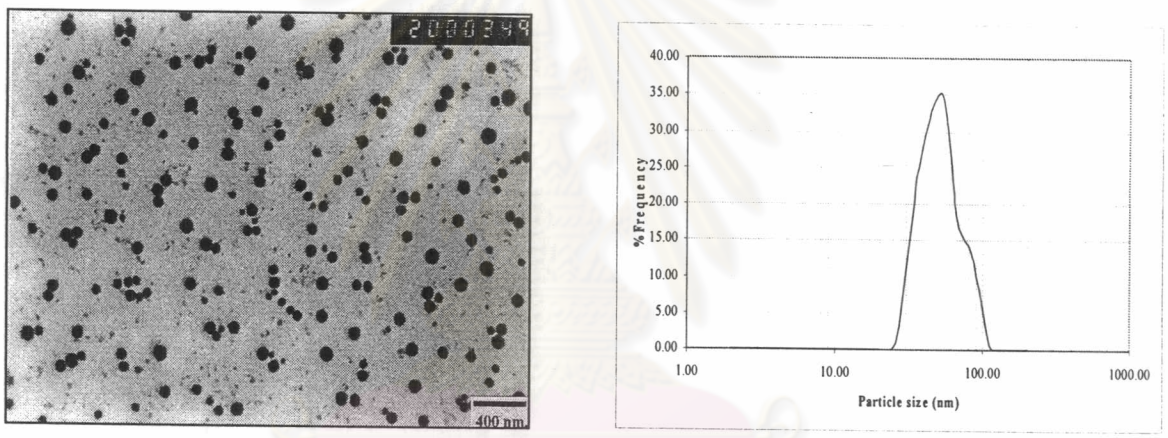


Figure 82 The TEM photomicrograph and particle size distribution of formulation (3/5) IPM : T_{80} : L_{68} : W(T_{80}:L_{68}=2:1) (W=25%) (IPM:T_{80}:L_{68} = 2 : 5.33 : 2.67) before stability testing.

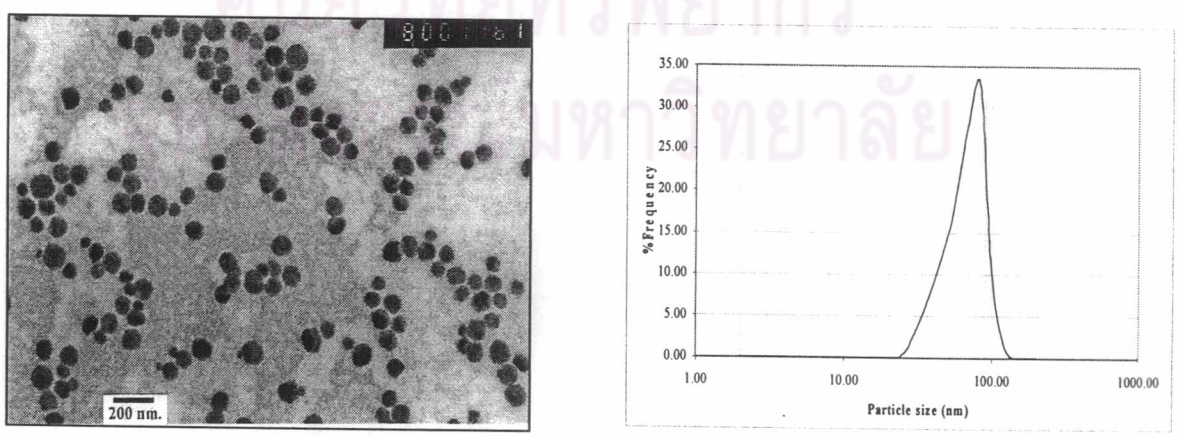


Figure 83 The TEM photomicrograph and particle size distribution of formulation (3/5) IPM : T_{80} : L_{68} : W(T_{80}:L_{68}=2:1) (W=25%) (IPM:T_{80}:L_{68} = 2 : 5.33 : 2.67) after stability testing.

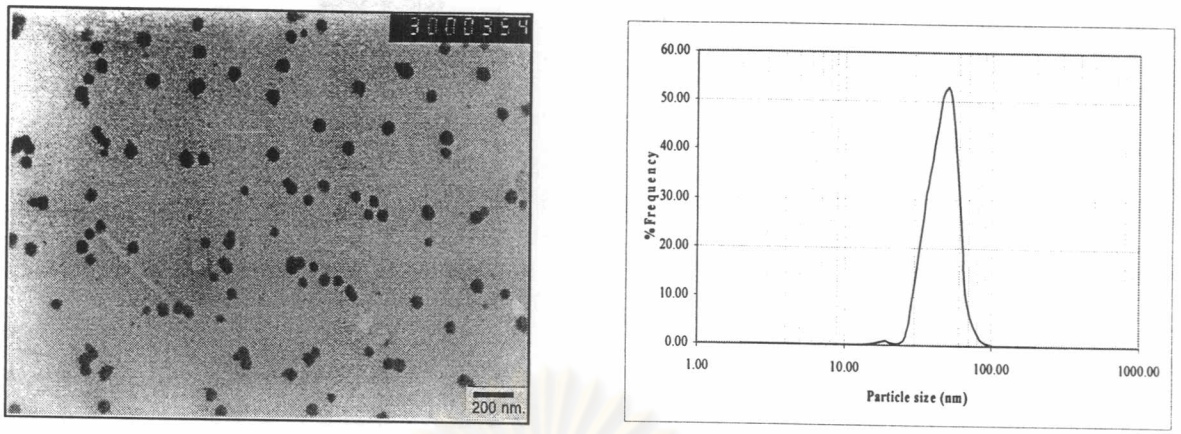


Figure 84 The TEM photomicrograph and particle size distribution of formulation (4/2) IPM : C_{EL} : W: PG (4:1) (25%) (IPM:C_{EL} = 2:8) before stability testing.

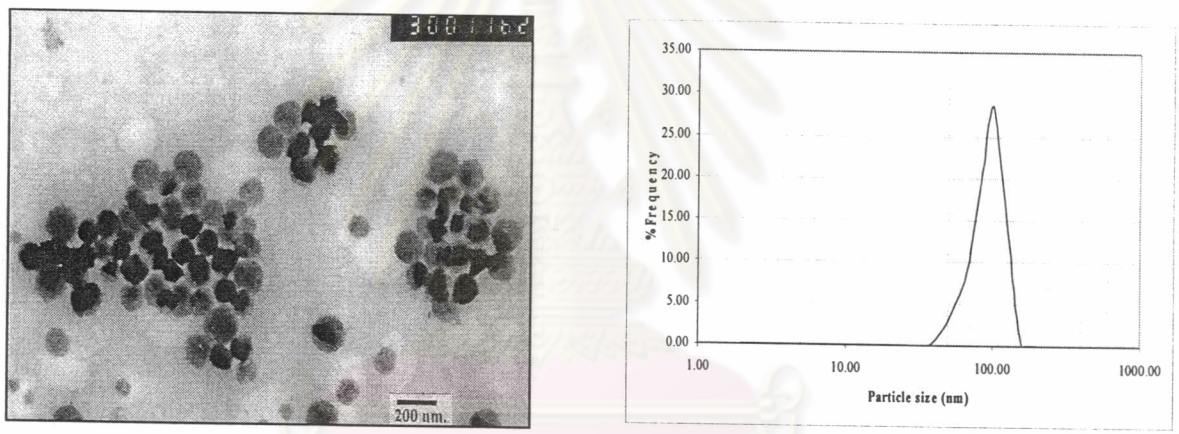


Figure 85 The TEM photomicrograph and particle size distribution of formulation (4/2) IPM : C_{EL} : W: PG (4:1) (25%) (IPM:C_{EL} = 2:8) after stability testing.

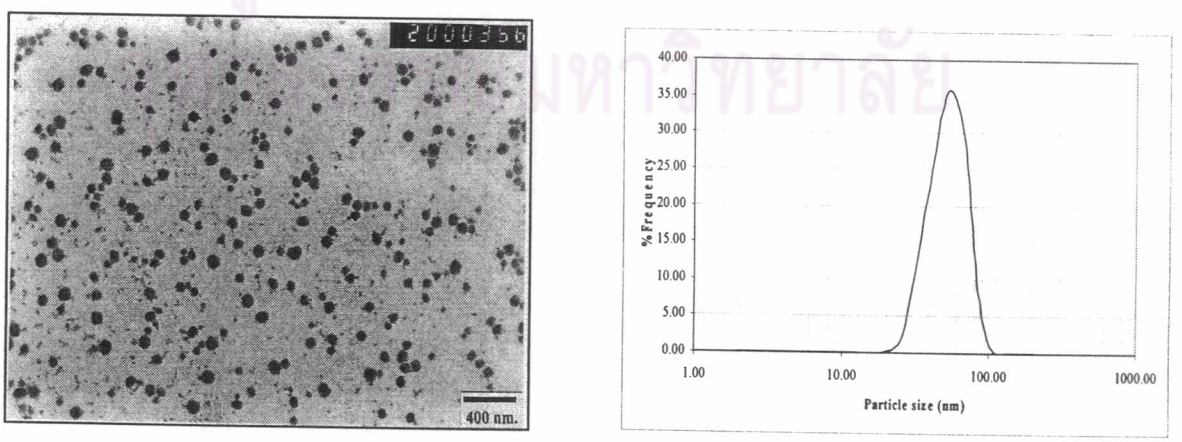


Figure 86 The TEM photomicrograph and particle size distribution of formulation (4/4) IPM : C_{EL} : W: PG (4:1) (20%) (IPM:C_{EL} = 3:7) before stability testing.

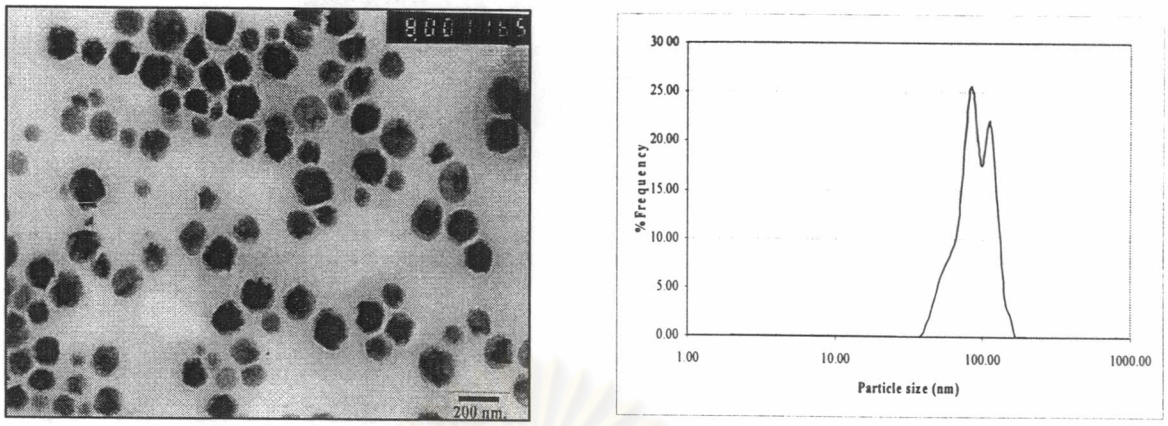


Figure 87 The TEM photomicrograph and particle size distribution of formulation (4/4) IPM : C_{EL} : W: PG (4:1) (20%) (IPM:C_{EL} = 3:7) after stability testing.

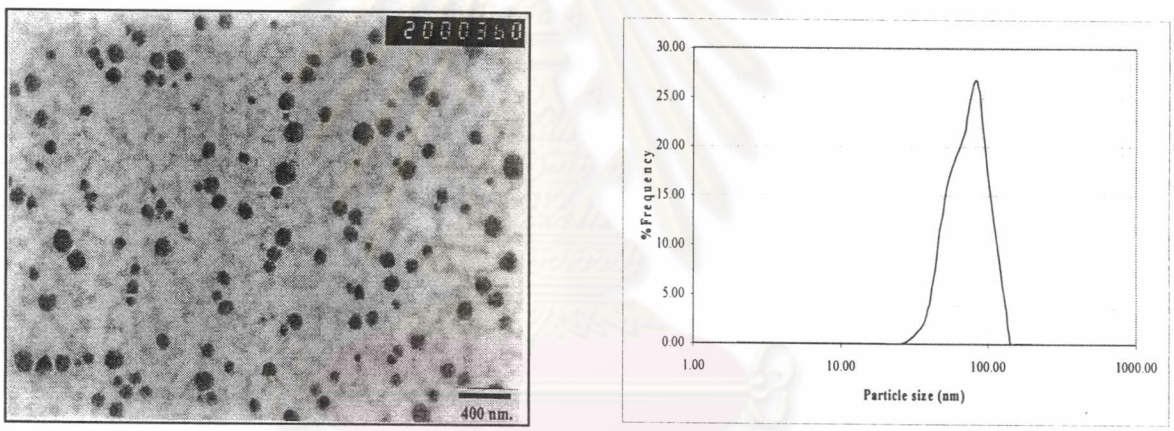


Figure 88 The TEM photomicrograph and particle size distribution of formulation (5/1) IPM : C_{RH} : W: PG (4:1) (W=14.52%) (IPM:C_{RH} = 3:7) before stability testing.

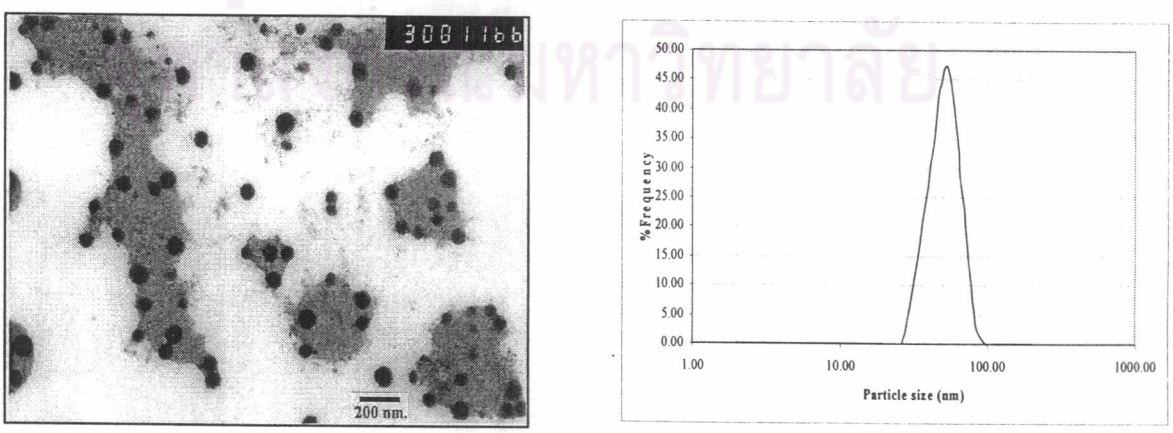


Figure 89 The TEM photomicrograph and particle size distribution of formulation (5/1) IPM : C_{RH} : W: PG (4:1) (W=14.52%) (IPM:C_{RH} = 3:7) after stability testing.

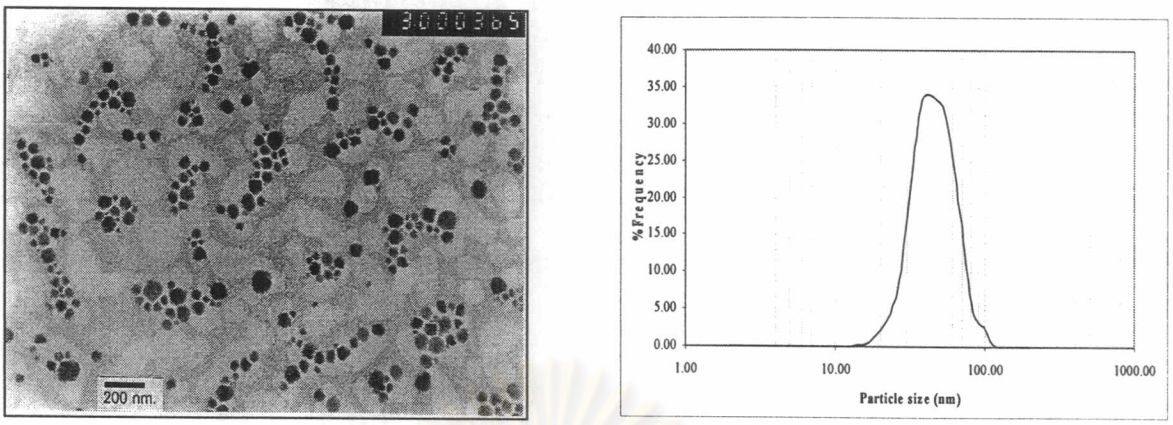


Figure 90 The TEM photomicrograph and particle size distribution of formulation (5/3) IPM : C_{RH} : W: PG (4:1) (W=15%) (IPM:C_{RH} = 5:5) before stability testing.

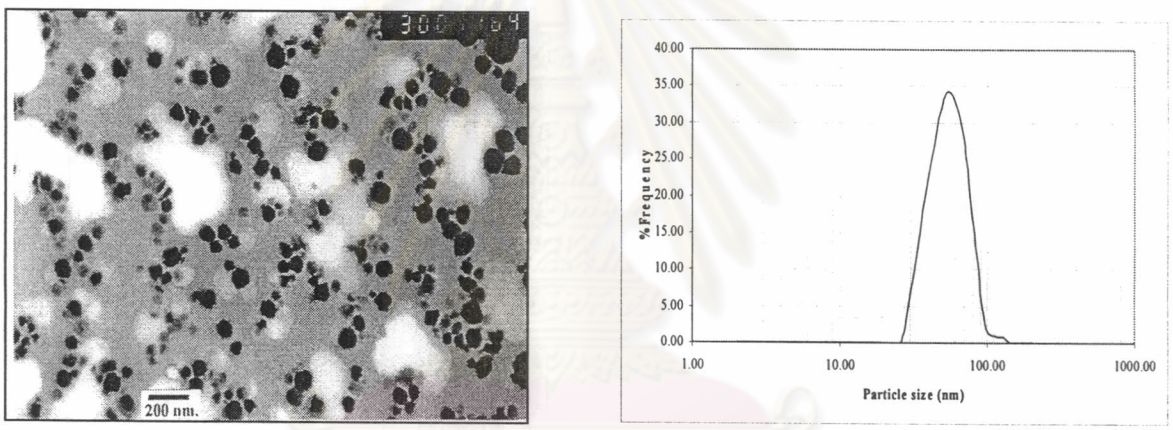


Figure 91 The TEM photomicrograph and particle size distribution of formulation (5/3) IPM : C_{RH} : W: PG (4:1) (W=15%) (IPM:C_{RH} = 5:5) after stability testing.

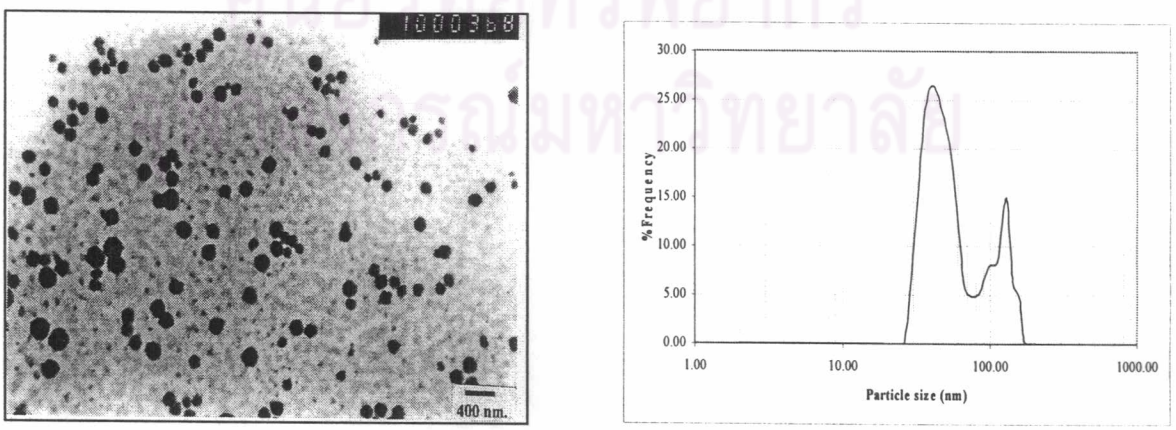


Figure 92 The TEM photomicrograph and particle size distribution of formulation (6/1) IPM : T_{80} : C_{EL} : W(W=15%) (IPM: T_{80} : C_{EL} = 3 : 3.5 : 3.5) before stability testing.

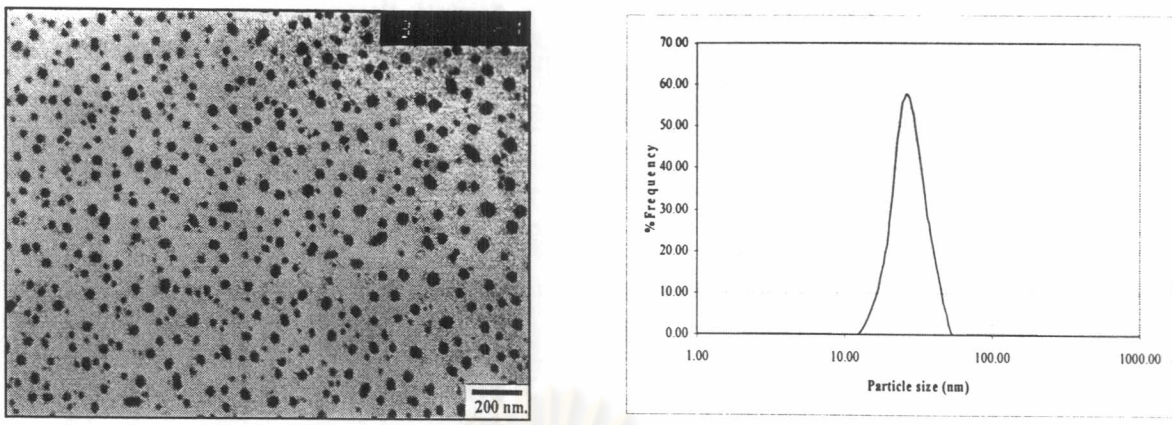


Figure 93 The TEM photomicrograph and particle size distribution of formulation (6/1) IPM : T_{80} : C_{EL} : W(W=15%) (IPM: T_{80} : C_{EL} = 3 : 3.5 : 3.5) after stability testing.

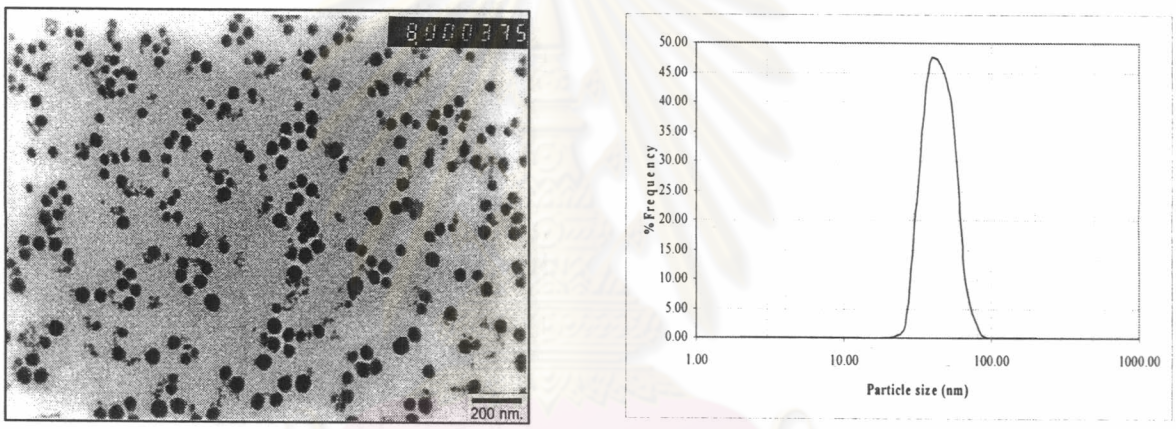


Figure 94 The TEM photomicrograph and particle size distribution of formulation (6/4) IPM : T_{80} : C_{EL} : W(W=20%) (IPM: T_{80} : C_{EL} = 1 : 4.5 : 4.5) before stability testing.

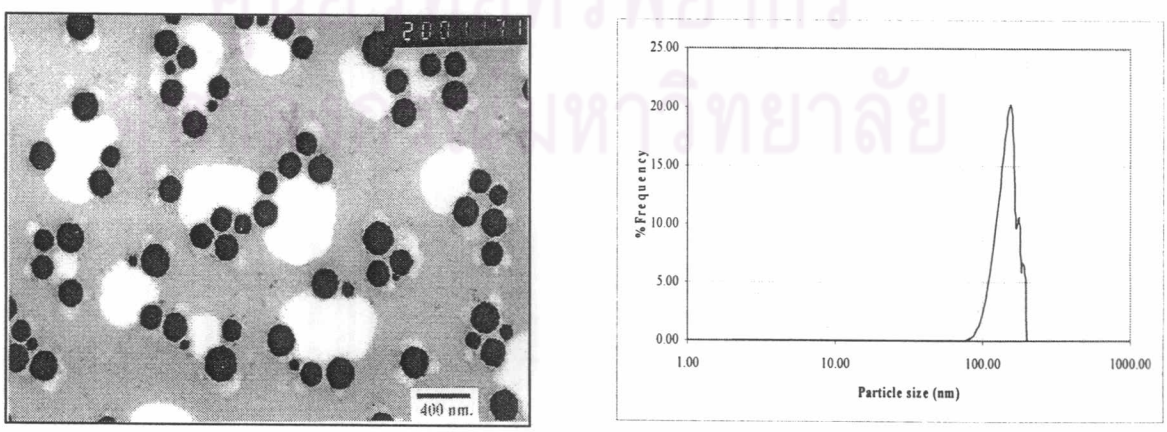
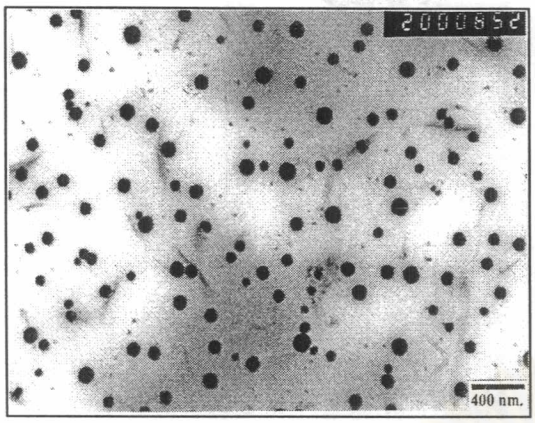


Figure 95 The TEM photomicrograph and particle size distribution of formulation (6/4) IPM : T_{80} : C_{EL} : W(W=20%) (IPM: T_{80} : C_{EL} = 1 : 4.5 : 4.5) after stability testing.



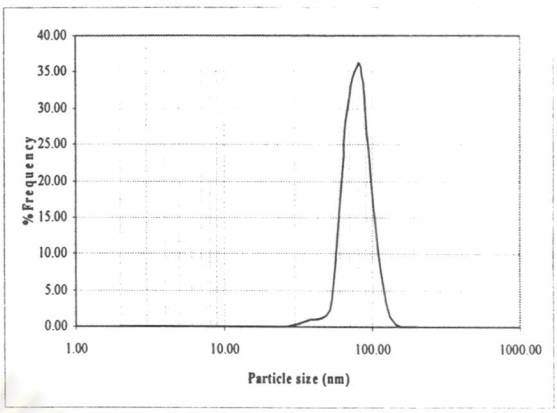
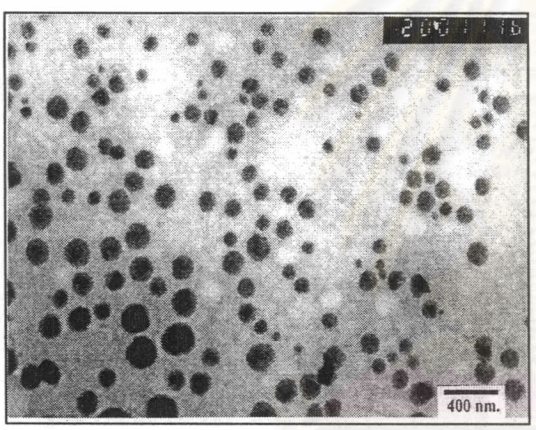


Figure 96 The TEM photomicrograph and particle size distribution of formulation (7/1) IPM : T_{80} : B_{35} : W(W=15%) (IPM: T_{80}: B_{35} = 3 : 3.5 : 3.5) before stability testing.



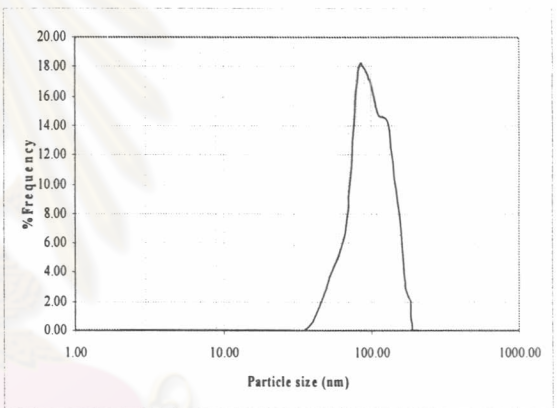
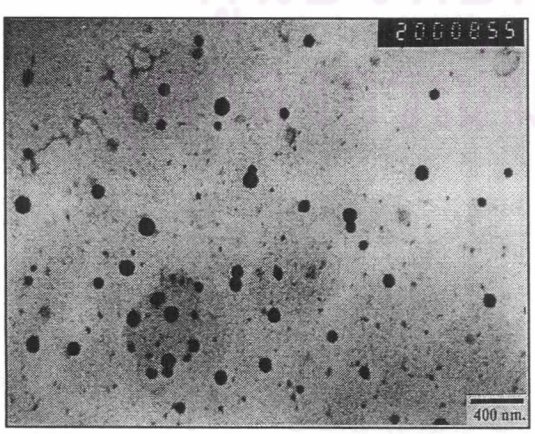


Figure 97 The TEM photomicrograph and particle size distribution of formulation (7/1) IPM : T_{80} : B_{35} : W(W=15%) (IPM: T_{80} : B_{35} = 3 : 3.5 : 3.5) after stability testing.



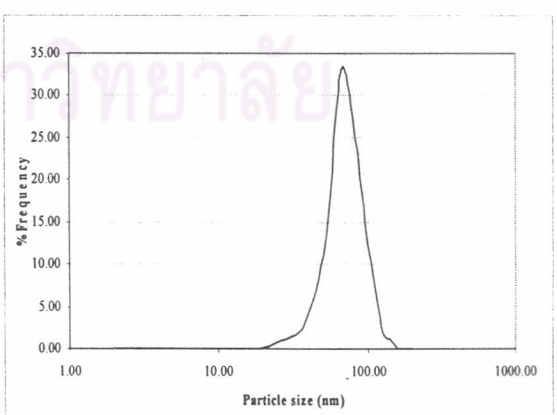


Figure 98 The TEM photomicrograph and particle size distribution of formulation (8/1) SBO : T_{80} : W (7%) (SBO:T_{80}=1:9) before stability testing.

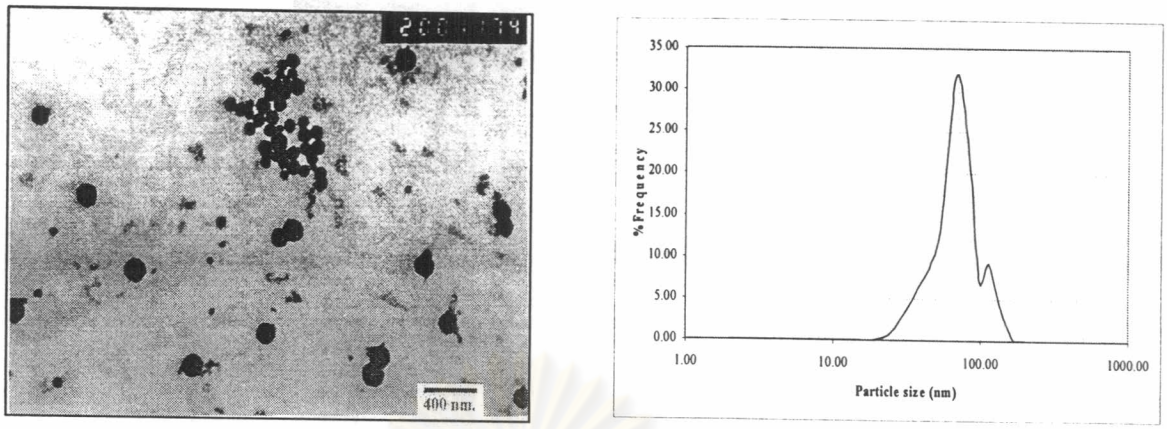


Figure 99 The TEM photomicrograph and particle size distribution of formulation (8/1) SBO : T_{80} : W (7%) (SBO:T_{80}=1:9) after stability testing.

2.9 Physical property when contacted with water

During the release study of MEG containing metronidazole from various MEG Base, their unique characteristics were noticed when contacted amount of water that diffused from the released medium. The microemulsion transformed into emulsions, liquid crystals or remained as microemulsion. This transformation of phase microstructure was confirmed by polarized light microscopy. From this point, these characteristics could be exploited. The lamellar phase was less viscous which phase transformation could be obtained by increasing water content. Since the lamellar phase is less viscous and injectable, it could be used to deliver the gel. Upon contact with excess water from gingival of body fluid lamellar phase formed stiff viscous liquid crystalline phase providing sustained release.

Most formulations formed viscous gel when diluted with one, double and triple folds of water. More dilution could result in transformation into solution or milky emulsion. Polarizing microscope also confirmed their transformation structure. Macroscopic observation of system IPM: T_{80}: W showed an increase in viscosity after diluted with equal amount of water in all formulations. The higher viscosity when diluted with double folds of water could be obtained in formulation 1/3, 1/4 and 1/6 whereas formulation 1/1, 1/2 and 1/5 became milky emulsion and solution after diluted with double, triple and ten folds of water. Cross polarizing microscope confirmed the phase transformation of liquid phase of microemulsion to lamellar phase structure as shown in Table 15 and Figure 101.

For CO: C_{EL}: W: PG (4:1) system, an increase in the viscosity after diluted with one, double and triple folds of water was found in formulation 2/1-2/4 whereas milky emulsion could observed when diluted with ten folds of water. Formulation 2/5 and 2/6 became milky emulsion and after diluted with double, triple and ten folds of water. These might be the occurrence of phase separation in these formulations. Cross polarizing microscope confirmed the phase transformation of liquid phase of microemulsion to hexagonal and lamellar phase structure as shown in Figure 102.

For IPM: T_{80}: L_{68}: W (T_{80}:L_{68} = 2:1) system; the increasing in viscosity upon higher water dilution was also observed. The viscosity of formulation 3/1-3/6 was higher when increasing the water dilution from one to double folds. After water dilution triple and ten folds formulation 3/1-3/3 and 3/6 bacame colloidal solution whereas formulation 3/4 and 3/5 were transform to solution. Cross polarizing microscope confirmed the phase transformation of lamellar phase to hexagonal phase structure as shown in Figure 103.

In IPM: C_{EL}: W: PG (4:1) system; the increasing in viscosity upon higher water dilution was also observed. The viscosity of formulation 4/1 and 4/3 was higher when increasing water dilution from one, double and triple folds before the forming solution in water dilution of ten folds. In formulation 4/2 and 4/4, the increasing in viscosity could only found when water dilution of one fold. After water dilution double, triple and ten folds, formulation 4/2 and 4/4 were transform to solution and milky emulsion, respectively. Cross polarizing microscope confirmed the phase transformation of liquid microemulsion phase to lamellar phase structure as shown in Figure 104.

The viscosity of IPM: C_{RH} : W: PG (4:1) system showed the increasing in the viscosity after diluted with one and double folds of water in formulation 5/1-5/5 whereas colloidal solution could observed when diluted with triple and ten folds of water. Cross polarizing microscope confirmed the phase transformation of lamellar phase structure phase to hexagonal phase structure as shown in Figure 105.

The viscosity of IPM: T_{80}: C_{EL}: W system showed the increasing in the viscosity after diluted with water up to triple folds which finally bacame a rigid viscous gel in formulation 6/1-6/4 whereas clear solution could observed when diluted with ten folds of water. Cross polarizing microscope confirmed the phase transformation of liquid microemulsion structure phase to lamellar phase structure as shown in Figure 106. Similarly, viscosity also increased in IPM: T_{80}: B_{35}: W after water dilution of ten folds, colloidal solution was observed. Cross polarizing microscope confirmed the phase transformation of lamellar phase structure phase to hexagonal phase structure as shown in Figure 107. The viscosity of SBO: T_{80}: W system increased with water dilution of one, double and triple folds. They became solution when water dilution of ten folds was performed. Cross polarizing microscope confirmed the phase transformation of liquid microemulsion structure phase to lamellar phase structure as shown in Figure 108.

The relationship between phase structure and release rate was also investigated as shown in Table15. The rate of drug released from hexagonal phase structure system was much lower than those from the other systems. The release observed from the microemulsion that transformed into liquid crystal form which might be lamellar phase structure or hexagonal phase structure was slow release required for drug delivery system. The lowest release rate constant ($1.14*10^{-2}$) was observed in formulation 7/1 which had viscous hexagonal phase structure after transformation. The results also showed slower release rates for other formulations which had hexagonal phase structure in formulation 3/1, 5/2, 5/3 and 6/1. The released rate constant of formulation 3/1, 5/2, 5/3 and 6/1 were ($1.85*10^{-2}$), ($1.86*10^{-2}$), ($2.60*10^{-2}$) and ($1.77*10^{-2}$). The different in release rate might be correlated with the final dilution transformation of each systems. The release rate of system that transform into hexagonal phase structure should be lower than those of lamellar phase structure. Surprisingly, the transform into hexagonal phase structure of formulation 3/5 and 3/6 had higher rate than formulation 5/2 and 5/3 with the same structure. These might be correlated with the final dilution. After water dilution of triple and ten folds, formulation 3/4 and 3/5 transformed into solution whereas formulation 5/2 and 5/3 transformed into colloidal dispersion. Therefore, the release rate of drug release from solution were faster than colloidal dispersion. The release of drug from liquid crystal system was about two or three times lower than that from a solution because liquid crystals have a highly ordered microstructure and high viscosity (Trotta, 1999). The proposed mechanism for drug release was diffusional exchange of water from the external media into matrix of gel, with exchange of drug and water from the interior phase to the external media. The stiffness and high viscosity of the transformed gel phase by slowing diffusion could provide a slow sustained release of incorporated drug (Jaymin et al, 2001).

This study was similar to the phase transformation of indomethacin release from microemulsion which percentage of indomethacin released from liquid crystal phase transformation was much lower than other systems. The transformation of

system composed of IPM, alcohol, lecithin and isolecithin occurred when contacted with released medium. These might be the lipophilicity of lecithin molecule which having critical packing parameter of 0.8. Formation of lamellar phase or bilayers are thus favored (Trotta, 1999).

Interestingly, one of surfactants used in this study "Poloxamer or Lutrol" is thermoreversible gel which is commercially available. The liquid to semi-solid phase change be triggered by increased temperature. This gels are liquid at room temperature (20-25°C) and undergo gelation when contact with body fluids (35-37 °C), due to an increase in temperature. They formed gel by central hydrophobic part (polyoxypropylene) surrounded by hydrophilic part (ethylene oxide). Depending on the ratio and distribution along the chain of the hydrophobic and hydrophilic subunits, several mmolecular weights are available, leading to different gelation property (Esposito et al, 1996; Victoria et al, 1999).

Three principal mechanisms have been proposed to explain the liquid-gel phase transition after an increase in temperature, including the gradual desolvation of the polymer, increased micellar aggregation and increased entanglement of the polymeric network (Andrews et al, 1984). The most occurrence process is that the intramolecular hydrogen bonds might promote gelation. Mucomimetic property of poloxamers is supposed to be due to their hydrophobic and hydrophilic sequences simulating mucin action by adsorption of the aqueous layer on hydrophobid epithelium (Moore et al, 2000). Furthermore, increasing the polymer concentration decreased the release rate of the drug whereas increasing drug lipophilicity decreased the release rate (Kantaria et al, 1999).

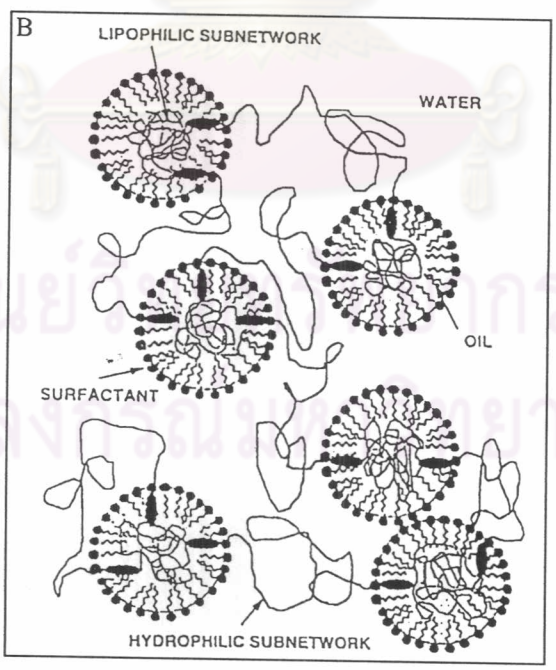


Figure 100 Covalently cross-linked polymer network in o/w microemulsions (from Moulik and Paul, 1998).

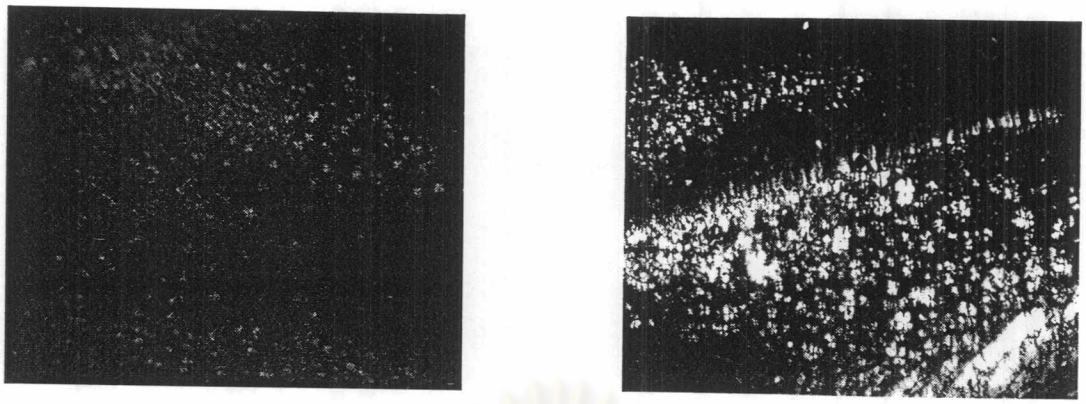


Figure 101 The microscopy patterns of the system IPM : T_{80} : W after dilution with released medium (magnification x 100). The transformation before dilution from liquid microemulsion (left) to lamellar phase structure (right).

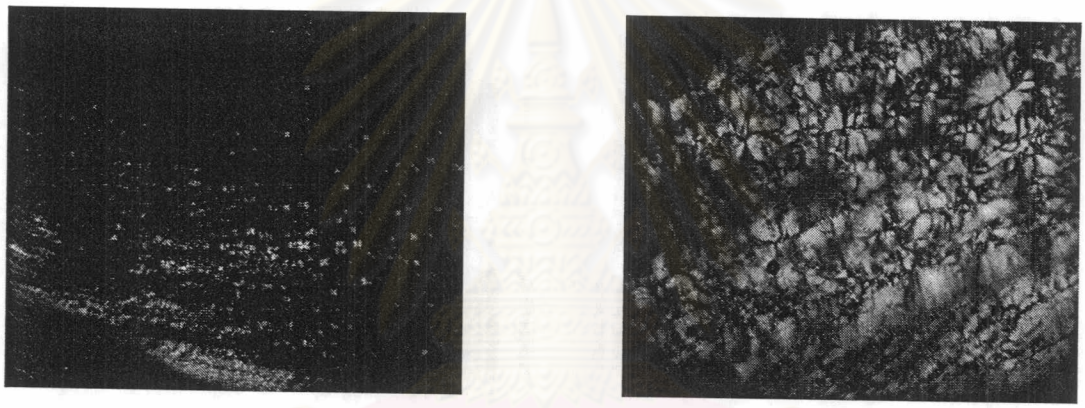


Figure 102 The microscopy patterns of the system CO : C_{EL} : W: PG after dilution with released medium (magnification x 100). The transformation before dilution from liquid microemulsion (left) to lamellar phase structure (right).

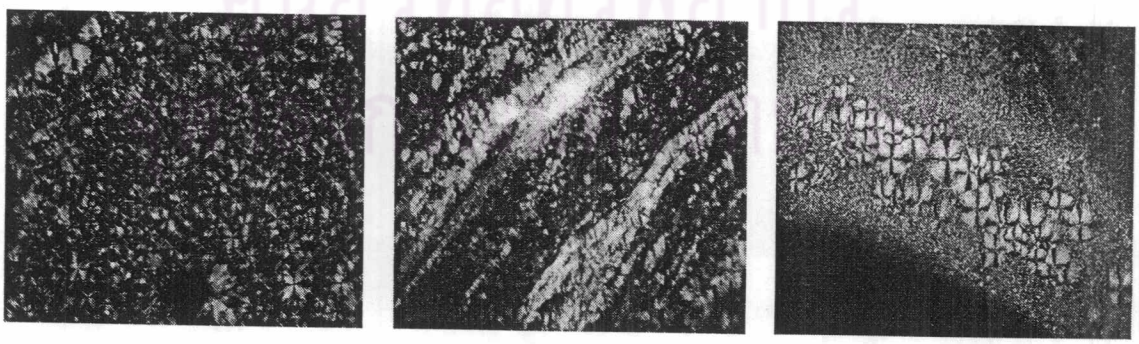


Figure 103 The microscopy patterns of the system IPM : T_{80} : L_{68} : W (T_{80}:L_{68} = 2:1) after dilution with released medium (magnification x 100). The transformation before dilution from liquid microemulsion (left) to lamellar phase structure (middle) and hexagonal phase structure (right).

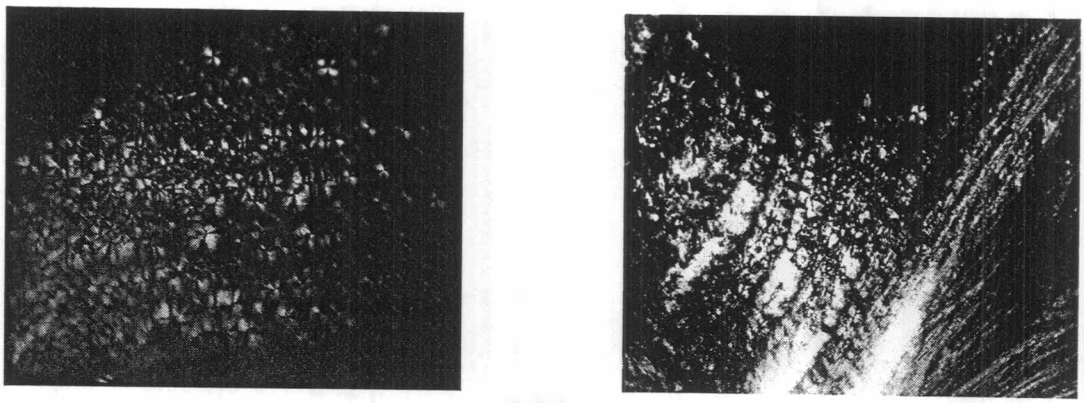


Figure 104 The microscopy patterns of the system In IPM : C_{EL} : W: PG (4:1) after dilution with released medium (magnification x 100). The transformation before dilution from liquid microemulsion (left) to lamellar phase structure (right).

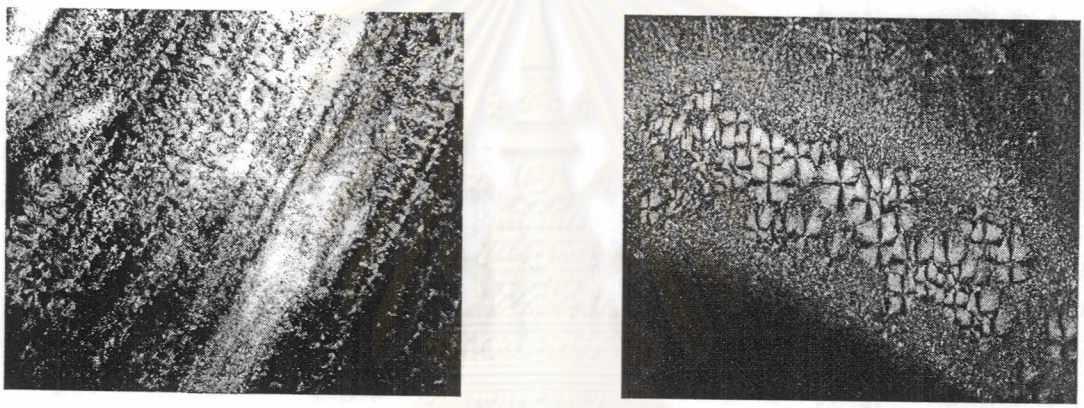


Figure 105 The microscopy patterns of the system IPM : C_{RH} : W: PG (4:1) after dilution with released medium (magnification x 100). The transformation before dilution from lamellar phase structure (left) and hexagonal phase structure (right).

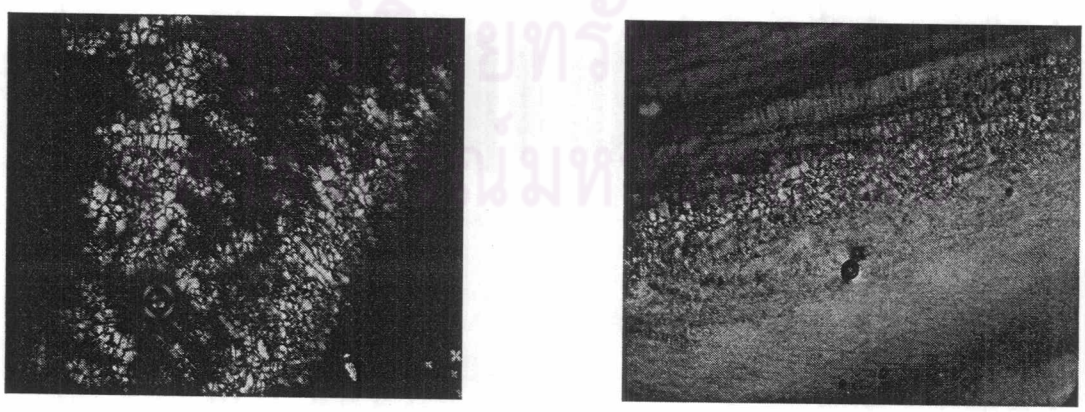


Figure 106 The microscopy patterns of the system IPM : T_{80} : C_{EL} : W after dilution with released medium (magnification x 100). The transformation before dilution from liquid microemulsion (left) to lamellar phase structure (right).

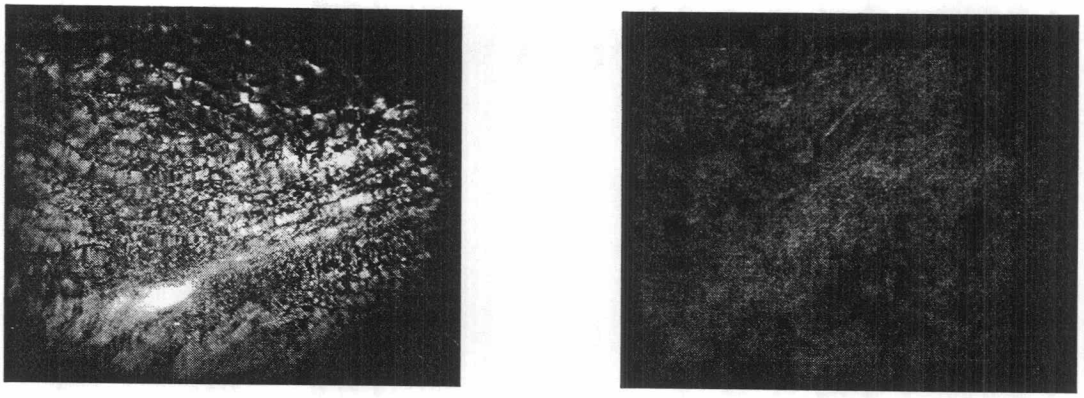


Figure 107 The microscopy patterns of the system IPM : T_{80} : B_{35} :W after dilution with released medium (magnification x 100). The transformation before dilution from liquid microemulsion (left) to lamellar phase structure (middle) and hexagonal phase structure (right).

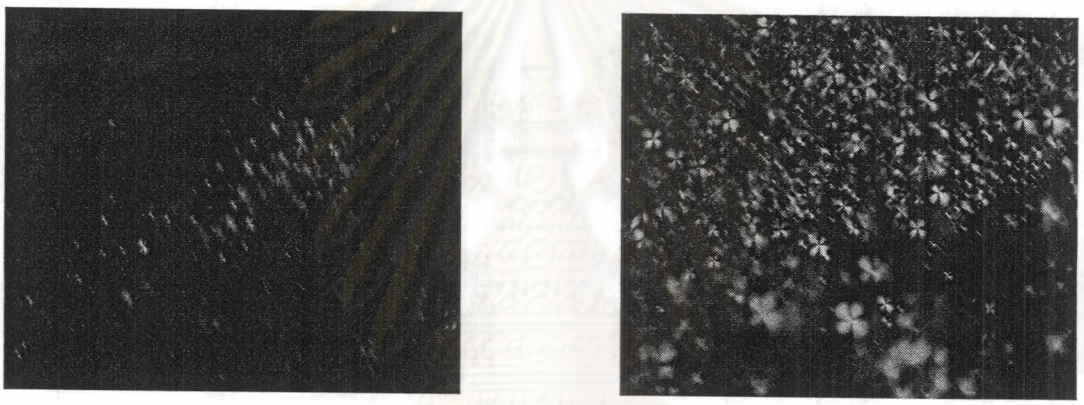


Figure 108 The microscopy patterns of the system SBO : T_{80} : W after dilution with released medium (magnification x 100). The transformation before dilution from liquid microemulsion (left) to lamellar phase structure (right).

Table 15 Rheological change of MEG when diluted with water.

Composition of formula	System	Ratio of oil:surfactant (% water)	Macroscopic property after contact with water (MEG:water)				Phase structure		Rate constant of released (k)
			1: 1	1: 2	1: 3	1: 10	Before	After (1:1)	
IPM : T_{80} : W	1/1	5:5 (10%)	+1	E	E	E	liquid	lamellar	-
	1/2	4:6 (8%)	+1	E	E	E	liquid	lamellar	-
	1/3	4:6 (10%)	+1	+2	E	E	liquid	lamellar	-
	1/4	3:7 (8%)	+1	+2	E	E	liquid	lamellar	-
	1/5	3:7 (10%)	+1	S	S	S	liquid	lamellar	-
	1/6	1:9 (7%)	+1	+2	S	S	liquid	lamellar	**$3.66*10^{-2}$**
CO : C_{EL} : W: PG (4:1)	2/1	2:8 (23%)	+1	+2	+2	S	liquid	hexagonal	-
	2/2	2:8 (20%)	+1	+2	+2	E	liquid	lamellar	-
	2/3	3:7 (10%)	+1	+2	+2	E	liquid	lamellar	-
	2/4	3:7 (25%)	+1	+2	+2	E	liquid	lamellar	**$3.65*10^{-2}$**
	2/5	5:5 (13%)	E	E	E	E	liquid *	emulsion	-
	2/6	5:5 (20%)	E	E	E	E	liquid *	emulsion	-
IPM : T_{80} : L_{68} : W (T_{80}:L_{68} = 2:1)	3/1	3 : 4.67 : 2.33 (15%)	+2	+3	C	C	lamellar	Hexagonal	**$1.85*10^{-2}$**
	3/2	3 : 4.67 : 2.33 (20%)	+2	+3	C	C	lamellar	Hexagonal	-
	3/3	3 : 4.67 : 2.33 (25%)	+2	+3	C	C	lamellar	Hexagonal	-
	3/4	2 : 5.33 : 2.67 (20%)	+2	+3	S	S	lamellar	Hexagonal	**$3.63*10^{-2}$**
	3/5	2 : 5.33 : 2.67 (25%)	+2	+3	S	S	lamellar	Hexagonal	**$3.24*10^{-2}$**
	3/6	2 : 5.33 : 2.67 (17%)	+2	+3	C	C	lamellar	Hexagonal	-
IPM : C_{EL} : W: PG (4:1)	4/1	1:9 (10%)	+1	+2	+3	S	liquid	lamellar	-
	4/2	2:8 (25%)	+1	S	S	S	liquid	lamellar	-
	4/3	3:7 (25%)	+1	+2	+3	S	liquid	lamellar	**$2.83*10^{-2}$**
	4/4	3:7 (20%)	+1	E	E	E	liquid	emulsion	-
IPM : C_{RH}: W: PG (4:1)	5/1	3:7 (14.52%)	+1	+2	C	C	lamellar	Hexagonal	-
	5/2	4:6 (20%)	+1	+2	C	C	lamellar	Hexagonal	**$1.86*10^{-2}$**
	5/3	4:6 (15%)	+1	+2	C	C	lamellar	Hexagonal	**$2.60*10^{-2}$**
	5/4	5:5 (15%)	+1	+2	C	C	lamellar	Hexagonal	-
	5/5	5:5 (15%)	+1	+2	C	C	lamellar	Hexagonal	-

Table 15 (Continue).

Composition of formula	System	Ratio of oil:surfactant (% water)	Macroscopic property after contact with water (MEG:water)				Phase structure		Rate constant of released (k)
			1: 1	1: 2	1: 3	1: 10	before	after	
IPM : T_{80} : C_{EL} : W	6/1	3 : 3.5 : 3.5 (water 15%)	+1	+2	+3	S	liquid	lamellar	$1.77*10^{-2}$
	6/2	2 : 4 : 4 (water 9%)	+1	+2	+3	S	liquid	lamellar	-
	6/3	1 : 4.5 : 4.5 (water 15%)	+1	+2	+3	S	liquid	lamellar	-
	6/4	1 : 4.5 : 4.5 (water 20%)	+1	+2	+3	S	liquid	lamellar	$3.33*10^{-2}$
IPM : T_{80} : B_{35} :W	7/1	3 : 3.5 : 3.5 (water 15%)	+2	+3	+3	C	lamellar	Hexagonal	$1.14*10^{-2}$
SBO : T_{80} : W	8/1	1:9 (water 7%)	+1	+2	+2	S	liquid	lamellar	$4.22*10^{-2}$

+1 = increase in viscosity

+2 = increase higher viscosity

+3 = rigid viscous gel (stiff-viscous liquid crystal (lamellar gel))

** = phase separation

S = solution

C = colloidal solution

E = milky emulsion

3. *In vitro* drug diffusion

Representative formulations from stable system that passed both freeze-thawing and Thai FDA stability testing were selected to evaluate the *in-vitro* drug diffusion. Twelve formulations from excellent appearance and stable system were selected. Their viscosity of MEG before and after metronidazole loading are shown in Table 16. The type and characteristic of these MEG are shown in Table 17. These selected formulations were transparent, semi-solid or viscous, birefringent microemulsion gel and all representative system passed both stability testing; freeze-thawing and Thai FDA stability could be classified as liquid crystal or microemulsion gel (including lamellar, hexagonal or cubic phase gel). The study of drug diffusion from MEG containing metronidazole was performed by using modified Franz diffusion apparatus as it were reported to be the most reliable methodology (Washington, 1990). Table e1 (Appendix B) showed the average percentage of metronidazole diffusion from various MEG base and liquid crystal system compared with each other as a function of time. *In-vitro* drug diffusion of metronidazole from different MEG bases were slow and incomplete. Most MEG base systems could prolonged the release of drug more than two days.

The diffusion profile of drug from MEG base and LC is governed by two main process; drug's transfer from the disperse phase to the continuous phase and drug diffusion through the membrane from the continuous phase to the sink solution (Trotta, 1989). It can be explained by the fact that at the beginning of the experiment the donor compartment contained the drug-loaded MEG while the receiver was filled by a drug-free aqueous medium. Since the drug-loaded MEG was prepared prior before the beginning of the experiment, it could be reasonably assumed that at the equilibrium the drug could be distributed among three different phases; the disperse phase, the continuous phase and surfactant layer. For oil in water type MEG, at the beginning a drug concentration gradient existed between the aqueous of external phase of the donor and receiver so that the drug molecules left the ME aqueous phase to reach the receiver fluid crossing the interposed membrane which resulted in the drug concentration increased in the receiver compartment. It should be noted that during the release experiment, the milky emulsion was seen in the donor compartment. The result was due to the receiver solution penetrated through the donor compartment and then MEG was destroyed into microemulsion and then emulsion. Another aspect that could be seen in this experiment was the rigidity and slow diffusion of some system that could be form hexagonal or cubic phase gel which upon contacted with the excess water from penetration of water from receiver compartment that may transform lamellar phase system to hexagonal or cubic phase system that providing sustained release of metronidazole (Jaymin et al, 2001).

The drug diffusion from MEG system was sustained more than 24 hours. The different amount of drug diffused from solution and from MEG could be attributed to the partition of drug between the dispersed oily droplets and the continuous phase of the microemulsions. Two main processes governed the diffusion of drug from MEG system. The transferring of drug from the disperse phase to the continuous phase and diffusion of drug from 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 drug concentration in external aqueous phase of MEG (Trotta, 1989).

Comparison diffusion profile within the same main composition system

IPM : T_{80} : L_{68} : W (T_{80}:L_{68}=2:1) system

Among formulations containing metronidazole at the concentration of 1.5% w/w, the amount of drug diffusion in formulation 3/1 (IPM:T_{80}: L_{68} = 3: 4.67: 2.33, 15% water) was higher than formulation 3/5 (IPM:T_{80}:L_{68} = 2: 5.33: 2.67, 25% water) and 3/4 (IPM:T_{80}:L_{68} = 2: 5.33: 2.67, 20% water) in which amount of drug diffusion in 24 hours were 12.67 mg, 12.64 mg and 9.86 mg, respectively. The higher percentage of diffusion in this system in 24 hours of about 84.50% and 84.31% were obtained from formulations 3/1 and 3/5 and lower percentage of diffusion of 65.74% from formulation 3/4. From these results, the amount of drug diffusion increased when the amount surfactant (Lutrol F-68) in formulation decreased. Furthermore, comparison between MEG containing 1.5% metronidazole at the equal amount of oil: surfactant ratio in formulation 3/4 (IPM:T_{80}:L_{68} = 2: 5.33: 2.67, 20% water) and 3/5 (25%water) showed that the amount of drug diffusion increased when the percentage of water in formulation increased. These could be explained that the concentration gradient of polymer (Lutrol F-68) in formulation 3/5 was lower by higher water content in formulation that induced the system to lower viscosity. At higher concentration of Lutrol F-68 micellar system formed clear viscous, stiffing gel which accounted for the rigidity and slower diffusion of this system whereas formulation 3/4 when adsorbed more water backward from receptor of Franz diffusion cell could transform to viscous, stiffing MEG that also formed gel-like structure which possibly maintained the diffusion of drug from MEG base. The diffusion profile of 1.5% w/w metronidazole MEG containing IPM: T_{80}: L_{68}: W (T_{80}:L_{68}=2:1) comparison in formulations 3/1, 3/4 and 3/5 are shown in Figure 109.

The higher correlation coefficient of formulations 3/1 and 3/5 were obtained from first order as shown in Table 18. In formulation 3/4, the highest correlation coefficient followed the Higuchi square root law (Moore et al, 2000; Makai et al, 2003). And the that diffusion coefficients of the drug in the gel decreased with the increasing Lutrol F-68 content, which was consistent with a consequent increase in bulk viscosity and gel rigidity. In addition, there was an ability to form hexagonal phase structure of this system that could sustained the diffusion rate of metronidazole. From this results, it could be concluded that the drug diffusion kinetic of 1.5%w/w metronidazole in formulations 3/1 and 3/5 was mostly fitted with first order model.

IPM: C_{RH}: W: PG (4:1) system

The amount of drug diffusion of formulations 5/2 (IPM: C_{RH}=4:6, 20% water) and formulation 5/3 (IPM: C_{RH}=4:6, 15% water) were 15.14 mg and 10.27 mg The higher percentage of diffusion profile 24 hours 84.46% was obtained form formulation 5/2 that more percentage of drug diffusion than formulation 5/3 (68.46%). The ratio of

oil:surfactant in formulations 5/2 and 5/3 were similar (oil:surfactant=4:6) but the different in percentage of water content in formulation. The results showed that the drug diffusion was faster in formulation 5/2 when the percentage of water in formulation was increased from 15% to 20%. On the other hand, when the percentage of water was lower the higher amount of cremophor RH40 was in formulation which could result to increase the viscosity of system. Therefore, drug diffusion was decreased due to drug molecule could not easily diffuse through surfactant layer and viscous medium (Lostritto et al, 1987).

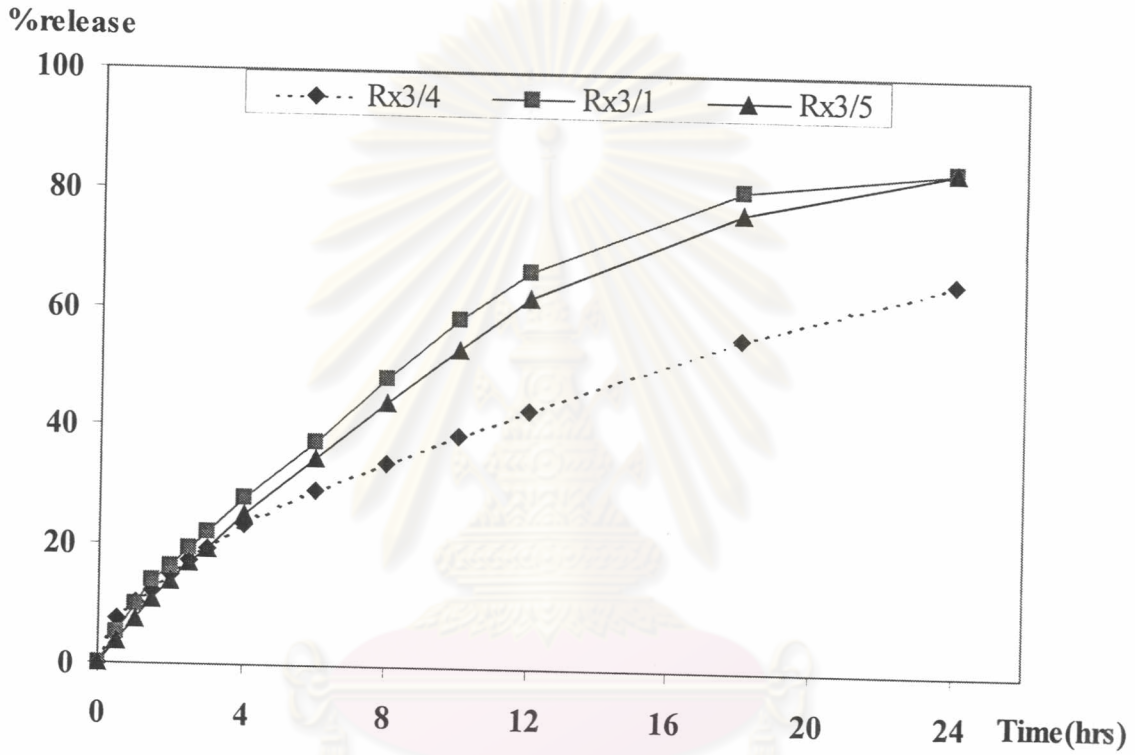


Figure 109 Comparison of the diffusion profile of metronidazole MEG containing IPM : T_{80} : L_{68} : W (T_{80}:L_{68}=2:1) in formulations 3/1 (15%water), 3/4 (20%water) and 3/5 (25%water).

These could result from the higher percentage of water in formulation, the greater to form H-bonding between water and surfactant that brought about the faster and higher drug diffusion, due to the higher surfactant transferring of drug from the disperse phase to the continuous phase and the diffusion of drug from continuous phase through the membrane to diffusion medium.

The drug diffusion kinetic of MEG in both formulations in IPM : C_{RH}: W: PG (4:1) system followed first order model that could sustained the diffusion of drug more than 24 hours.

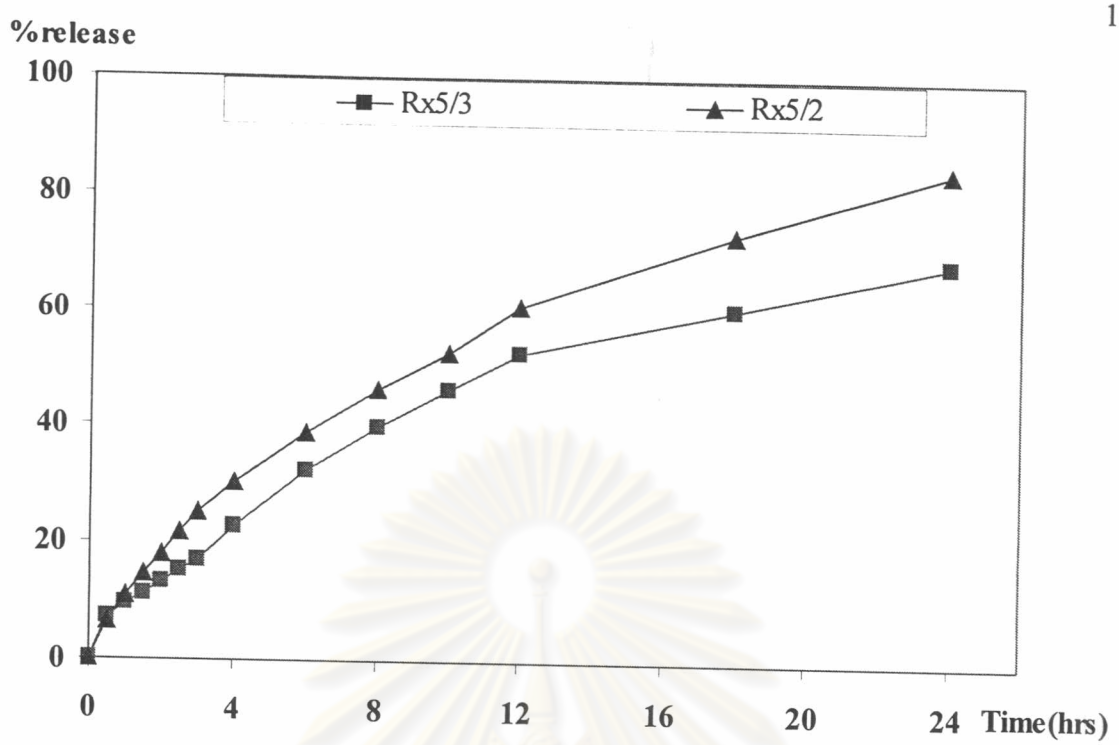


Figure 110 Comparison of the diffusion profile of metronidazole MEG containing IPM : C_{RH}: W: PG (4:1) of formulations 5/2 (IPM:C_{RH}=4:6, 20%water) and 5/3 (IPM:C_{RH}=4:6, 15% water).

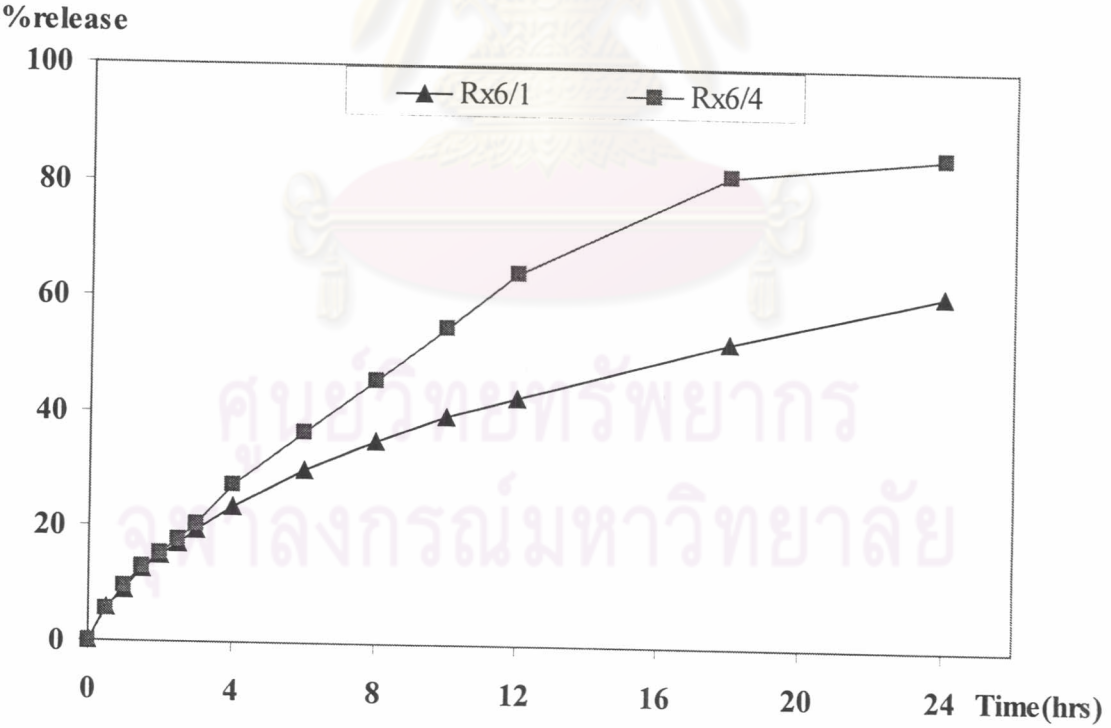


Figure 111 Comparison of the diffusion profile of metronidazole MEG containing IPM: T_{80}: C_{EL}: W system in formulations 6/1 (3: 3.5: 3.5, water 15%) and 6/4 (1: 4.5: 4.5 water 20%).

IPM: T_{80}: C_{EL}: W system

The diffusion profiles of formulations 6/1 (IPM: T_{80}: C_{EL} =3: 3.5: 3.5, 15%water) and 6/4 (IPM: T_{80}: C_{EL} =1: 4.5: 4.5, 15%water) showed that the amount of drug diffusion in formulation 6/4 were more than that of formulation 6/1 as 12.77 mg and 9.19 mg after 24 hours or 85.11% and 61.26%, respectively. The results indicated that the amount of drug diffusion increased when the amount of oil in formulation decreased. On the other hand, when the amount of surfactant in formulation was higher, the greater drug diffusion was observed. Furthermore, the percentage of water content in formulation also affected the drug diffusion. The higher percentage of water content in formulation, the apparently higher amount of drug diffusion was obtained as shown in Figure 111.

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 the retention capacity of dispersed phased oily droplets, the larger amount of which was able to sustain the drug release over longer period of time. In this way a reservoir of the drug was produced and sustained release effect was achieved as the drug continuously transferred from oil droplets to continuous phase to replace drug diffusion from MEG system (Friedman and Benita, 1987).

Table 16 showed the characteristic both MEG base and drug-loaded MEG. The MEG base and drug-loaded were investigated their flow behavior, viscosity including the appearance of obtained MEG. The stable, non-separation formulations after drug-loaded including the formulation which had optimum viscosity from various systems. Moreover, the formulation which passed the freeze-thawing and Thai FDA stability testing were preferable. The highly viscous formulations were more preferable than the liquid system that confirmed with physical changed after contacted amount of water. Therefore, liquid or fluid systems were excluded from further release study. Table 17 Showes the characteristic of representative MEG and LC which selected from system passed freeze-thawing and Thai FDA stability.

Table 17 shows birefringent property, physical characteristics; conductivity and type of the representative formulation. Most representative formulation exhibited non-birefringent property except formulation 1/6, 2/4 and 8/1 which birefringent property could be obtained after diluted with water. Type of MEG also effect the release of drug from the formulation. The type of formulation was confirmed by two different methods; conductivity test and dilution test.

Table 16 Viscosity of investigated microemulsion gel before and after metronicazole loading.

Composition of formula	System	Ratio of oil:surfactant (% water)	Viscosity (cps)	
			MEG base	1.5% metronidazole
IPM : T_{80} : W	1/1	5:5 (10%)	*21,600	-
	1/2	4:6 (8%)	382	-
	1/3	4:6 (10%)	*1,080	-
	1/4	3:7 (8%)	387	-
	1/5	3:7 (10%)	*1,567	-
	1/6	1:9 (7%)	435	458 (+)
CO : C_{EL} : W: PG (4:1)	2/1	2:8 (23%)	895	L
	2/2	2:8 (20%)	1,080	L
	2/3	3:7 (10%)	858	L
	2/4	3:7 (25%)	3,072	3,078(+)
	2/5	5:5 (13%)	1,605	-
	2/6	5:5 (20%)	2,346	-
IPM : T_{80} : L_{68} : W (T_{80}:L_{68} = 2:1)	3/1	3 : 4.67 : 2.33 (15%)	*6,120	*8,850(+)
	3/2	3 : 4.67 : 2.33 (20%)	*12,900	-
	3/3	3 : 4.67 : 2.33 (25%)	*19,080	-
	3/4	2 : 5.33 : 2.67 (20%)	*18,330	*37,500(+)
	3/5	2 : 5.33 : 2.67 (25%)	*25,800	*33,600(+)
	3/6	2 : 5.33 : 2.67 (17%)	*7,020	-
IPM : C_{EL} : W: PG (4:1)	4/1	1:9 (10%)	630	L
	4/2	2:8 (25%)	*7,860	*75,000(+)
	4/3	3:7 (25%)	*35,230	-
	4/4	3:7 (20%)	*3,900	-
IPM : C_{RH}: W: PG (4:1)	5/1	3:7 (14.52%)	45,600	60,000(+)
	5/2	4:6 (20%)	200,000	280,000(+)
	5/3	4:6 (15%)	124,000	125,334(+)
	5/4	5:5 (15%)	105,000	107,666(+)
	5/5	5:5 (15%)	110,000	160,000(+)
IPM : T_{80} : C_{EL} : W	6/1	3 : 3.5 : 3.5 (water 15%)	*11,610	*12,140(+)
	6/2	2 : 4 : 4 (water 9%)	480	L
	6/3	1 : 4.5 : 4.5 (water 15%)	*13,000	-
	6/4	1 : 4.5 : 4.5 (water 20%)	*21,840	*24,620(+)
IPM : T_{80} : B_{35} : W	7/1	3 : 3.5 : 3.5 (water 15%)	*10,200	*93,000(+)
SBO : T_{80} : W	8/1	1:9 (water 7%)	480	492(+)

* = non-newtonian behavior of system, - = phase separation , L = liquid or fluid

(+) = more increased in viscosity

Table 17 Type and characteristic of representative MEG and LC selected after freeze-thawing and Thai FDA stability testing.

System	Polarized light	Conductivity (μs)	Type	Dilution test	
				oil dilution	water dilution
1/6	NB	3.40	W/O	NS	S
2/4	NB	23.20	O/W	S	NS
3/1	B	3.40	M	NS	NS
3/4	B	2.30	M	NS	NS
3/5	B	5.00	M	NS	NS
4/3	B	13.80	O/W	S	NS
5/2	B	8.40	M	NS	NS
5/3	B	2.80	M	NS	NS
6/1	B	3.00	M	NS	NS
6/4	B	12.10	O/W	S	NS
7/1	B	11.0	O/W	S	NS
8/1	NB	3.8	W/O	NS	S

NB = non-birefringent property
B = birefirngent property
S = separation
NS = non-separation
o/w = oil in water
w/o = water in oil
M = bicontinuous or mesh structure

Table 18 The coefficient of determination (R^2) of microemulsion in various drug diffusion kinetics calculated from total drug diffusion data.

Formulation	Coefficient of determination (R^2)		
	Zero order model	First order model	Higuchi model
1/6) IPM : T_{80} : W	0.9922	0.9960	0.9548
2/4) CO : C_{EL} : W: PG (4:1)	0.9856	0.9917	0.9636
3/1) IPM : T_{80} : L_{68} : W (T_{80}:L_{68} = 2:1)	0.9907	0.9943	0.9570
3/4) IPM : T_{80} : L_{68} : W (T_{80}:L_{68} = 2:1)	0.9545	0.9886	0.9936
3/5) IPM : T_{80} : L_{68} : W (T_{80}:L_{68} = 2:1)	0.9939	0.9981	0.9491
4/3) IPM : C_{EL} : W: PG (4:1)	0.9680	0.9942	0.9820
5/2) IPM : C_{RH}: W: PG (4:1)	0.9681	0.9917	0.9854
5/3) IPM : C_{RH}: W: PG (4:1)	0.9874	0.9972	0.9627
6/1) IPM : T_{80} : C_{EL} : W	0.9316	0.9749	0.9954
6/4) IPM : T_{80} : C_{EL} : W	0.9903	0.9975	0.9581
7/1) IPM : T_{80} : B_{35} : W	0.9514	0.9833	0.9958
8/1) SBO : T_{80} : W	0.9898	0.9962	0.9520

(A) Effect of type of oil

The diffusion profiles of metronidazole MEG of formulation 1/6 [IPM : T_{80} : W] and formulation 8/1 [SBO :T_{80} : W] at the ratio of oil: surfactant as 1:9 and 7% water content are compared in Figure 112. The results showed that the drug diffusion from both formulations was sustained for more than 24 hours. A slightly higher percentage of drug diffusion (91.36%) was obtained from formulation 8/1 compared to formulation 1/6 (90.1%). Both formulations of had nearly similar viscosity which was about 458 cps and 492 cps, respectively. The amount of drug diffusion in 24 hours from formulations 1/6 and 8/1 were 13.50 mg and 13.70 mg, respectively. Furthermore, the particle size and size distribution from TEM photomicrographs of both formulations were 78.53±20.48 nm and 78.33±20.09 nm in formulations 8/1 and 1/6, respectively. It could be verified that drug molecule could easily diffuse from continuous phase through the membrane to sink condition due to low viscosity of the formulation. In addition these two systems did not form lamellar or hexagonal phase structure. Thus the faster and higher drug diffusion of these two formulations than other system in this study were observed.

Figure 113 shows the comparison of the diffusion profile of formulation 2/4 [CO : C_{EL} : W: PG (4:1), CO:C_{EL} =3:7, 25% water] and formulation 4/3 [IPM : C_{EL} : W: PG (4:1), IPM:C_{EL}=3:7, 25% water] showed that the higher and faster drug diffusion was observed in formulation 2/4 which had 84.08% drug diffusion whereas formulation 4/3 exhibited 79.15% drug diffusion. These may be explained by the viscosity of these systems. Less viscous formulation resulted in higher and faster drug diffusion. The viscosity of formulation 2/4 was about 3078 cps whereas formulation 4/3 was 47,500 cps. Furthermore, formulation 4/3 tended to form lamellar or hexagonal phase structure that could induce more viscous and stiffing system than formulation 2/4 which could not form liquid crystal in any ratio.

For oil in water type MEG, at the beginning a drug concentration gradient existed between the aqueous of external phase of the donor and receiver so that the drug molecules left the ME aqueous phase to reach the receiver fluid crossing the interposed membrane which resulted to increase the drug concentration in the receiver compartment. It should be noted that during the release experiment, the milky emulsion was seen in the donor compartment. The result was due to the receiver solution penetrated through the donor compartment and then MEG was destroyed into microemulsion and then emulsion (Friedman and Bernita, 1987; Norling et al, 1992).

(B) Effect of type of surfactant

To study the effect of different surfactant on diffusion profile, formulation 4/3 of the system IPM : C_{EL} : W: PG (4:1), IPM:C_{EL}=3:7, 25% water and formulations 5/2 and 5/3 of the system IPM : C_{RH}: W: PG (4:1), (IPM:C_{RH}=6:4) that had 20% water and 15% water content in formulation, respectively were compared in Figure 114. The faster and higher percentage of drug diffusion (79.14%) was obtained from formulation 4/3 that had cremophor EL as surfactant in the system whereas the lower percentage drug diffusion were obtained by the formulations 5/2 and 5/3 (84.46% and

68.46%, respectively) which had cremophor RH40 as surfactant in formulation. These could be explained by the nature of surfactant used in formulation, cremophor RH40 is more viscous (2-4*10^5 cps) than cremophor EL (650-850 cps) (Gasco et al, 1988; Kibbe, 2000). Therefore, formulation 4/3 which contained cremophor EL had low viscosity which could be assumed that the drug molecule would easily diffuse from continuous phase through the membrane in sink solution. In addition, cremophor RH40 had the possibility to form viscous hexagonal or lamellar structure of more than cremophor EL.

In addition, the lower percentage amount of drug diffusion was obtained in formulation 5/3 at the first 12 hours before the higher percentage of drug diffusion was observed after 22 hours. These might attributed to the presence of lower percentage of water in formulation 5/2 induced the more increasing in viscosity than formulation 5/3. Furthermore, receiver solution penetrated through the donor compartment could effect the transformation of MEG to viscous, stiffing hexagonal phase structure that prolonged the diffusion of metronidazole MEG (Gasco et al, 1988; Levy and Bernita, 1990).

(c) Effect of type of co-surfactant

System IPM : T_{80} : L_{68} : W (T_{80}:L_{68} = 2:1), IPM : T_{80} : C_{EL} : W and IPM : T_{80} : B_{35} : W were selected to compare the effect of co-surfactant on drug diffusion as shown in Figure 115. Formulations 3/1, 3/4 and 3/5 were compared with formulations 6/1 and 6/4 including formulation 7/1 that all these system had similar main composition; IPM, tween80 and water but different in the type of cosurfactant; Lutrol F-68 , cremophor EL and Brij 35. The results found that faster and higher percentage of drug diffusion (85.11%) was obtained from formulation 6/4 that used cremophor EL in the system whereas the lower percentage drug diffusion (49.01%) were obtained by the formulation 7/1 which containing Brij 35. These was due to the higher viscosity of formulation 7/1 than the others. On the other hand formulation 7/1 exhibited the most prolonged diffusion. In addition, the distinguish of this system that occurred a "ringing gel" that was an excellent in all characteristics; physical stability, appearance, viscosity. Due to the stiffness and high viscosity of this gel that could provide a slow release of drug to more than 24 hours.

The diffusion profile indicated that the mechanism of release was similarly for matrix-type delivery system. Diffusion of drug was followed by diffusion out of the gel matrix. This could be assumed that the gel controlled the release of drug incorporated or location of drug from the hexagonal phase gel or aqueous channels or lipid bilayers. Therefore, when diffusion across aqueous boundary layer was not released rate limiting, gel controls the release of incorporated drug. Metronidazole would be controlled by this hexagonal liquid crystal gel. However these results suggested that diffusion rate of drug could be controlled to only a limited extent by the liquid crystal gel dependent on drug's solubility and diffusivity in the aqueous diffusion boundary layer. Furthermore, the release kinetics and duration were not influenced significantly by the form of drug incorporation or where it may reside in the phase gel (Costa and Lobo, 2001; Jaymin et al, 2001).

Similar systems from formulations 3/1 and 6/1 as ratio of oil: surfactant: co-surfactant =3: 3.5: 3.5 with 15% water content showed similar diffusion profile pattern as shown in Figure 159. The percentage of drug diffusion of formulations 3/1 and 6/1 were 65.75% and 61.27%, respectively. The results showed that the amount of oil: surfactant: co-surfactant were important for diffusion characteristic eventhough the co-surfactant was change from Lutrol F-68 in formulation 3/1 to cremophor EL in formulation 6/1. The percentage of diffusion profile of formulations 3/4, 3/5 and 6/4 were 84.49%, 84.32% and 85.10%, respectively.

The highest correlation coefficient of formulations 3/1, 3/5 and 6/4 were obtained from first order model as shown in Table 18 whereas the formulation 3/4, 6/1 and 7/1 had the highest correlation coefficient when treated with Higuchi model as shown in Table 18. Furthermore, the higher correlation coefficient of all formulations 4/3, 5/2 and 5/3 were obtained from first order as shown in Table 18. From these results, it could be concluded that the drug diffusion kinetic of 1.5%w/w metronidazole in formulations 3/1 and 3/5 was mostly fitted with first order model. The diffusion profile are illustrated in Figure 115.

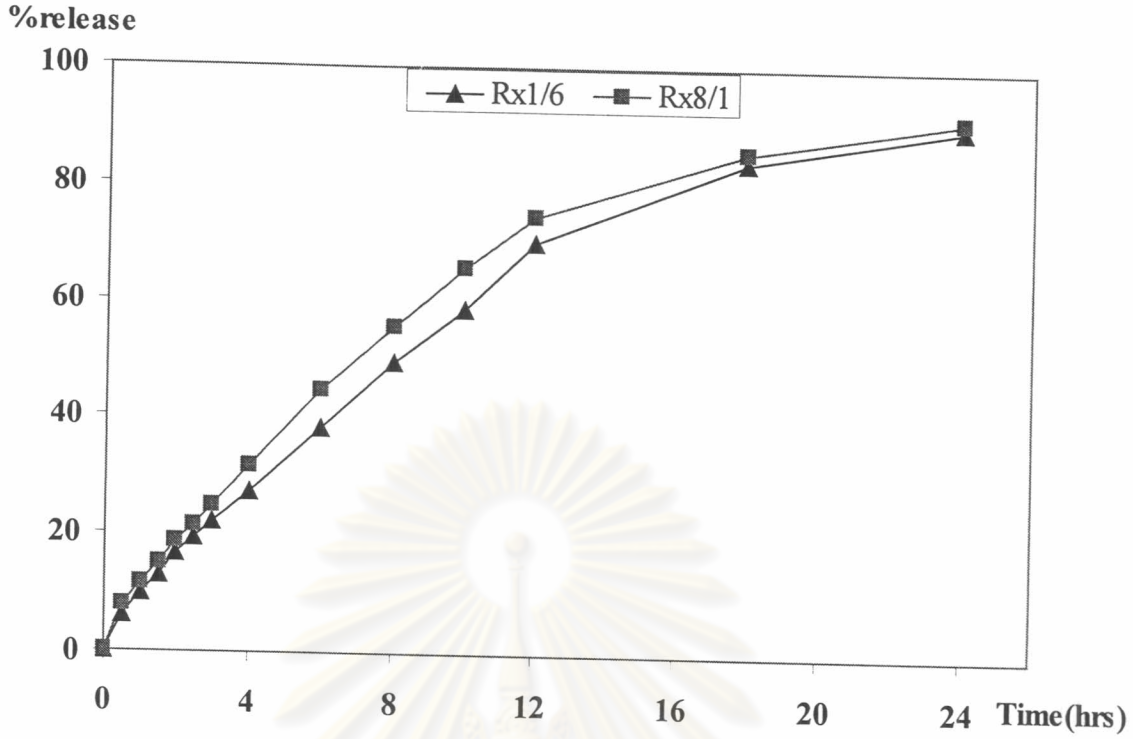


Figure 112 Diffusion profile of metronidazole MEG formulation 1/6 [IPM: T_{80}: W] and formulation 8/1[SBO: T_{80}: W] at the ratio of oil: surfactant ratio as 1:9 and 7% water content.

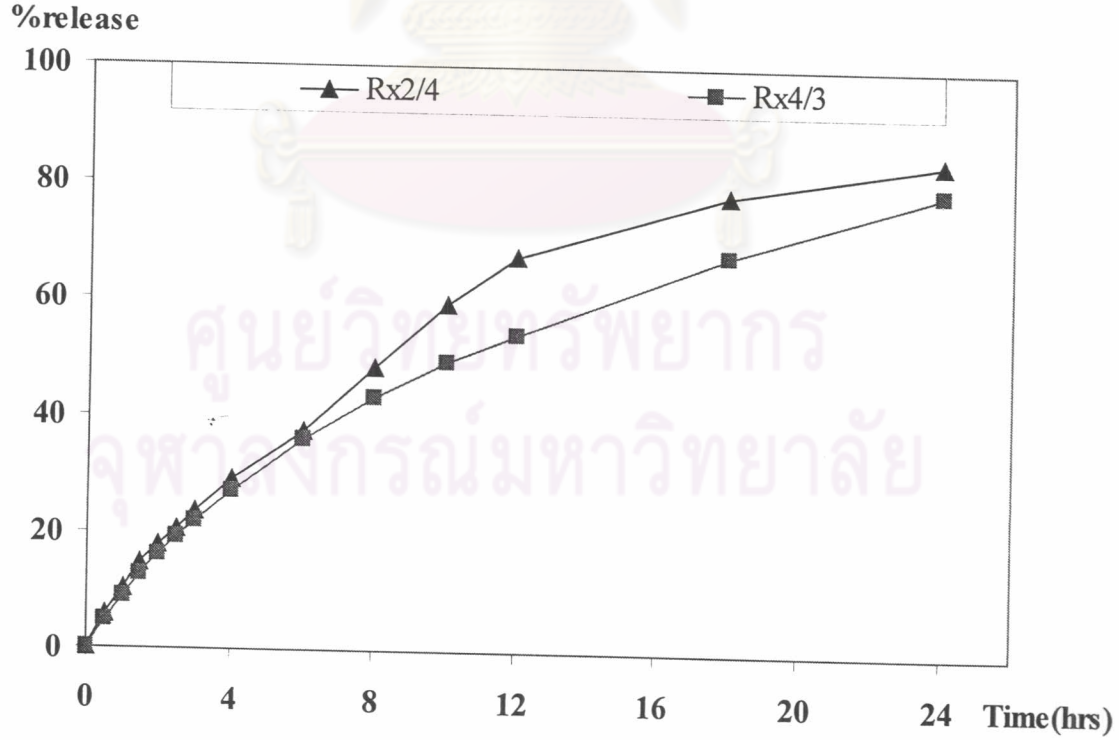


Figure 113 Diffusion profile of 1.5% w/w metronidazole MEG formulation 2/4; [CO : C_{EL} : W: PG (4:1), CO:C_{EL} =3:7, 25% water] and formulation 4/3[IPM : C_{EL} : W: PG (4:1), IPM:C_{EL}=3:7, 25% water].

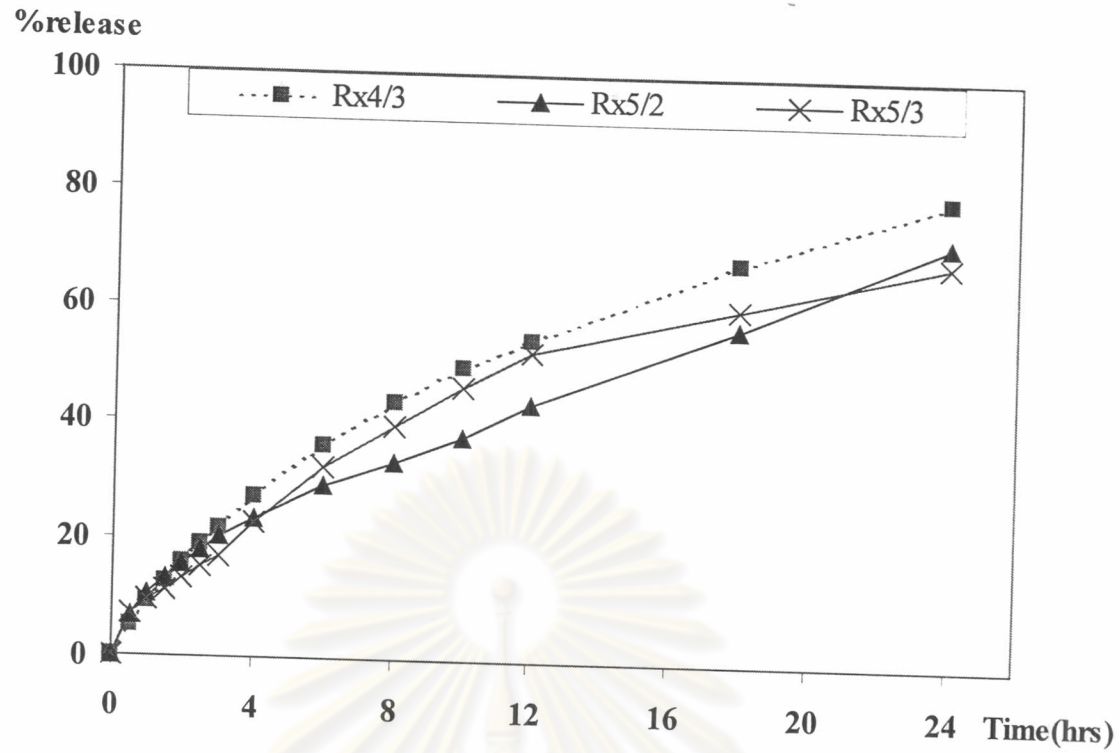


Figure 114 Diffusion profile of 1.5% w/w metronidazole MEG formulation 4/3; IPM : C_{EL} : W: PG (4:1), IPM:C_{EL}=3:7, 25% water and IPM : C_{RH}: W: PG (4:1), IPM : C_{RH} = 6:4 system in formulation 5/2 (20%water) and 5/3 (15%water).

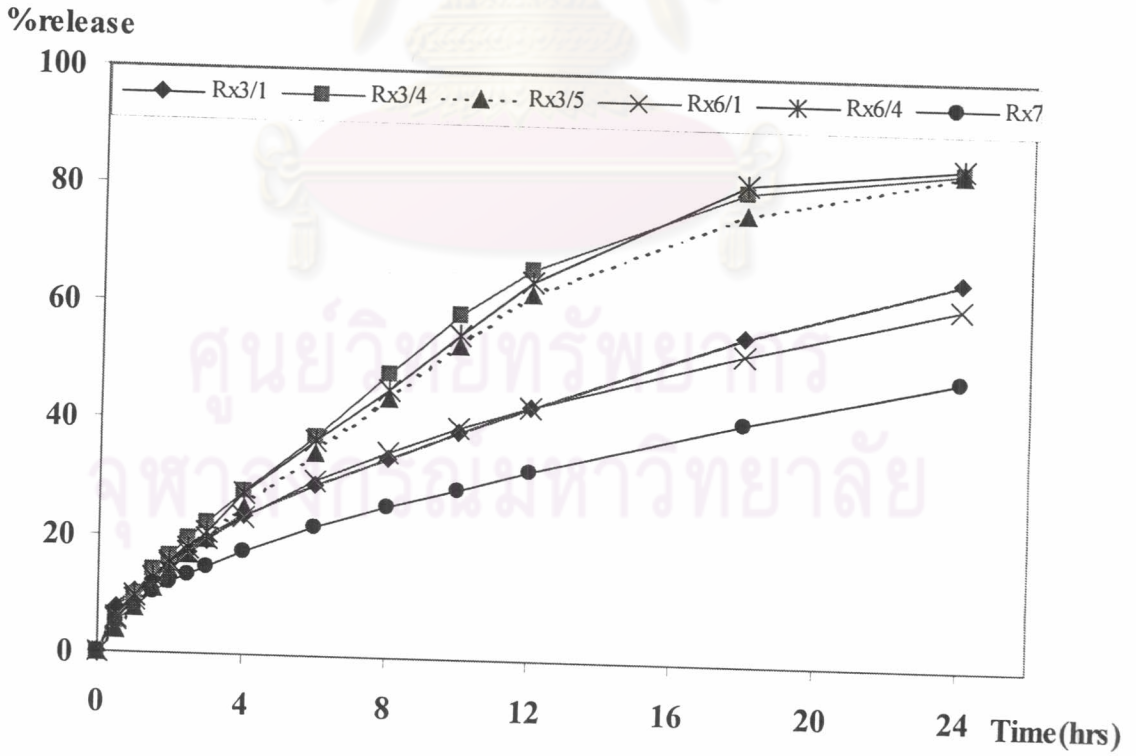


Figure 115 Diffusion profile of 1.5% w/w metronidazole MEG in system IPM : T_{80} : L_{68} : W (T_{80}:L_{68} = 2:1); [formulation 3/1, 3/4, 3/5] and IPM: T_{80}: C_{EL}: W [formulation 6/1, 6/4] and IPM : T_{80} : B_{35} : W [formulation 7/1].

4. Physical stability

The stability of MEG is one of the most important factor to consider. The physicochemical properties of ingredient used may result in instability of these system. All preparations were studied for their stability. Shelf-life stability of the obtained MEG as a function of time and storage temperatures were evaluated by visual inspection including the physical appearance, pH, syringeability, conductivity, viscosity, particle size and microbial activity of MEG and LC after stability testing were undertaken by various technique to confirm the stability of the system. The results are shown in following Table 19-25. All preparations were observed under accelerated conditions at 4 ^{0}C for 48 hours and 45^{0}C for 48 hours about 6 cycles in freeze-thawing study whereas the second groups were selected to observe under temperature 45^{0}C 75% relative humidity which according to Thai FDA stability testing.

4.1 Physical appearance

The physical appearance after stability testing of MEG base and MEG containing metronidazole are shown in Table 19. For MEG base system, the appearance of most systems were not changed except formulation 1/1 to 1/5 of IPM: T_{80}: W system, formulation 2/5 and 2/6 of CO: C_{EL}: W:PG (4:1) system and formulation 4/4 of IPM:C_{EL}:W:PG (4:1) that were separated into two phases after freeze-thawing for 6 cycles. During stability testing by storing at 4^{0}C, all formulations were turbid. When storing at 45^{0}C, phase separation could be seen during first cycle. Although phase separation occurred in such formulations, they were able to recover to one phase MEG by gently shaking. The MEG which containing IPM as an oil phase, T_{80} as surfactant showed the phase separation which upper phase likely to be a mixture of IPM and T_{80} whereas the lower phase was a mixture of water and some surfactant.

In Thai FDA stability testing, formulation 1/1, 1/3, 1/5, 2/5, 2/6, 3/2, 3/3, 3/6, 4/4 and 6/3 were separated into two phases as shown in Table 19. The upper phase was yellow solution while the lower phase also yellow viscous gel which was separated by thin layer between upper phase and lower phase.

The physical appearance of MEG containing 1.5%w/w metronidazole is also shown in Table 19. In IPM : T_{80} : W system, the phase separation and precipitation occurred in formulation 1/1 to 1/5 after freeze-thawing and Thai FDA stability testing. Similar results were also to the formulation 2/5, 2/6, 3/1, 3/6 and 4/4 . Formulation 3/2 and 3/3 were separated when observed under Thai FDA stability testing whereas phase separation was not occurred when performed by freeze-thawing similarly to the observation in formulation 5/4 and 5/5 that synereis were occurred after Thai FDA stability testing. Other formulations that passed both stability testing (freeze-thawing and Thai FDA stability testing) still showed birefringent property in liquid crystal system whereas the birefringent property was not obtained in isotropic system when observed under polarized light microscope similar to before stability testing.

Upon sterilization with high temperature and stress condition, the high temperature produced high kinetic energy. This might be affect or distroy the layer of surfactant film. Hence IPM and CO was separated from surfactant and co-surfactant film (Martin, 1993). Moreover, the hydrogen bonding between hydroxyl group of propylene glycol and polyoxyethylene unit chain of cremophor EL in CO: C_{EL}: W:PG and IPM:C_{EL}:W:PG was possibly broken down by high temperature and stress condition during freeze-thawing stability therefore phase separation could be occurred. However, the preparations were recovered to one phase lamellar gel by gently shaking because MEG were spontaneous formation, which was the unique characteristic of MEG.

4.2 pH

This parameter affects the physical and chemical stability. The pH of MEG base system were considerably increased in formulation 1/1 to 1/6 after freeze-thawing when compared with before stability testing in as shown in Table 20 and Figure 116. The results indicated that prolonged storage of polyoxyethylene surfactant (T_{80}) could lead to the formation of peroxides in IPM: T_{80}: W system which might increase the pH of formulation (Kibbe, 2000). In contrast, the pH of formulation 2/5 and 2/6, 3/1-3/6 and 5/3-5/5 were markedly decreased after freeze-thawing and after Thai FDA stability tesing study as also shown in Figure 160. This could be explained by the pH of MEG falled on freeze-thawing, and also as a function of time during storage. Consequently, the result of glyceride and phosphatide hydrolysis would liberate free fatty acid. This was the main degradation pathway of fat emulsion leads to the formation of fatty acids which gradually reduced the pH of formulation (Levy and Benita, 1990; Lien, 2000). The rate of free fatty acid production was minimal if the pH of the emulsion was between 6 and 7 after stress condition (Floyd and Jain, 1996). Other study also reported that the pH of intraveneous 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 consequent reduction in pH of microemulsions (Lostritto et al, 1987; Levy and Benita, 1990).

The pH of MEG containing 1.5%w/w metronidazole are shown in Figure 117. The pH of all formulations after freeze-thawing and Thai FDA stability testing were slightly changed when compared with before testing. The pH range was also maintained with in the standard deviation of less than ±1. In drug-loaded MEG system, the obtained pH was the pH of saturated solubility of drug in each MEG base system. Most formulations had average pH between 5.5 and 7.5 except formulation 5/1-5/5 had pH between 7.5 and 8.0. This average pH range was the stable pH range for metronidazole. Similarly, commercial metronidazole injection also has the pH between 4.5 and 7.0 which was recommended pH range for stabilized metronidazole (Reynolds, 1996). Metronidazole undergoes hydrolysis in aqueous solution, under alkaline conditions (pH> 8), hydrolysis also observed to yield ammonia, acetic acid and unidentified compound produced a pink to violet colour with ninhydrin reagent (Attwood, 1984). This results indicated that degradation of metronidazole and other component did not occurred during both freeze-thawing and Thai FDA stability testing.

4.3 Syringeability

The syringeability of MEG base are shown in Figure 118. The syringeability of all formulations after freeze-thawing and Thai FDA stability testing were similar to that of before stability study except in formulation 1/3 and 1/5, 4/2-4/4, 5/1-5/5 and 7/1. This could be explained by the phase separation occurred in formulation 1/3, 1/5, 4/4 which could be comfirmed by physical appearance study in aforementioned topic. Surprisingly, formulation 4/2, 4/3, 5/1-5/5 and 7/1 showed an increase in syringeability while the high viscosity of formulation could be observed. These might be the effect of non-newtonian of the lamellar phase behavior including the occurrence in breakdown structure of this formulation during rapid changing in temperature of freezing and thawing cycle (Provost and Kinget, 1988). The shear-thinning behavior could be obtained when increased in a little force or shear stress (Jones et al, 1997). These were the advantage and unique structure of this drug delivery system. Lamellar phase was inherently fluid and could be injected using a syringes that had been the use of considerably less viscous lamellar phase gel, which is known to absorb gingival fluid and transformed to the more stiff, viscous gel phase providing sustained release of drug (Hatefi and Amsden, 2002).

The syringeability of MEG containing 1.5%w/w metronidazole are shown in Figure 119. The pattern of overall syringeability of most system after freeze-thawing and Thai FDA stability testing were similar to corresponding formulation before stability as shown in Figure 119 and Table 21, except formulation 4/3, 4/4 and 5/1-5/5 that the syringeability was increased under accelerated in freeze-thawing stability. These were illustrated by the similar results with those from MEG base system.

4.4 Conductivity

For MEG base system, the conductivity of each formulation after freeze-thawing and Thai FDA stability testing were similar to those before stability system as shown in Table 22, Figure 120 and 121. The different of value of conductivity of each system before stability and after freeze-thawing and Thai FDA stability testing were less than 5 uS. The conductivity of formulation 2/6 and 4/3 after freeze-thawing were higher than those corresponding system before stability. This was resulted from the occurrence of excess water in formulation 2/6 and 4/3 due to phase separation in these formulations.

The conductivity of MEG containing 1.5%w/w metronidazole are shown in Figure 122 and Figure 123. The conductivity of each formulation after freeze-thawing and Thai FDA stability testing was similar to those before stability system. The conductivity of formulation 2/6 and 4/3 after freeze-thawing were higher than those corresponding system before stability. The results were similar to the MEG base as aforementioned.

4.5 Viscosity

Viscosity measurement are also useful in determining MEG stability. The

viscosity during storage at different temperature and time were examined. From the results, the viscosity of most MEG base systems were slightly increased after freeze-thawing and Thai FDA stability testing as shown in Figure 124 and Figure 125. These were explained by the viscosity increased upon aging (Floyd and Jane, 1996). In contrast, the viscosity of formulation 1/1, 1/3, 1/5, 2/5, 2/6, 3/2, 3/3, 3/6, 4/4 and 6/3 after Thai FDA stability testing were dramatically decreased. These was due to the occurrence of phase separation in such formula after Thai FDA stability testing which brought about to breakdown of structure in formulation hence decreasing viscosity in formulation could be observed (Andrews et al, 1984). Furthermore the changed from non-newtonian to newtonian also observed in formulation 1/1, 1/3, 1/5, 3/2, 3/3, 3/6 and 6/3 whereas the formulation 2/5, 2/6 and 4/4 obviously exhibited phase separation. The comparison viscosity of MEG base before stability with after freeze-thawing and Thai FDA stability testing are shown in Table 23.

The viscosity of drug loaded MEG system increased when metronidazole was incorporated and increased upon aging of storage (Kuneida et al, 2001) as shown in Table 23, Figure 126 and 127 showed the phase separation of formulation 1/1-1/5, 2/5-2/6, 3/6, 4/2 and 4/4 after freeze-thawing and Thai FDA stability testing were performed. From the results, MEG composed of IPM: T_{80}: W and IPM: C_{EL}: W: PG were unstable systems. The overall results indicated that the most MEG systems which contained suitable surfactant and cosurfactant, oil: surfactant ratio were thermodynamically stable for long period of time in Thai FDA stability testing and also in accelerated conditions at 4 ^{0}C for 48 hours and 45^{0}C for 48 hours about 6 cycles in freeze-thawing stability.

4.6 Particle size and size distribution

Particle size of MEG was a prominent property normally used to characterize ME. After stability testing, the mean particle diameter were increased in almost all formulations as shown in Table 24. and Figure 128. The shape of MEG remained sphear. but wider distribution was obtained. The results indicated that the mean particle diameters were increased when passed the freeze-thawing stability process. These may occurred by rapid temperature changing between freezing process and thawing process could effect the structure and viscoelstic property of formulation especially at around the droplet of internal phase may changed during 6 cycles of freeze-thawing process, thus the droplets may changing into large diameter to maintain thermodynamic of the system.

4.7 Anti-microbial activity

Comparison of the average minimum inhibition zone against anaerobic bacteria representative by *P. gingivalis* of each MEG base system (mm) showed that system of [IPM: C_{RH}: W: PG (4:1)] which composed of isopropyl as an oil phase, cremophor RH40 and propylene glycol as co-surfactant and co-solvent exhibited the largest clear inhibition zone by histograms in Table 25. All inhibition zone diameters of three group of MEG; freshly prepared, after freeze-thawing and after Thai FDA stability testing MEG system of formulation 2/4, 3/4, 5/1 and 7/1 showed no statistically significant difference after freeze-thawing and Thai FDA stability testing ($p>0.05$). Most systems still had inhibition zone diameters of more than 70 mm. The following representative formulations were

shown in Table 25. Formulation 2/4, 3/4, 4/3, 5/1, 5/2, 6/4 and 7/1 were selected from their good appearance and passed both freeze-thawing and Thai FDA stability testing. This results indicated that 1.5% w/w metronidazole MEG system had an excellent physical and chemical stability.

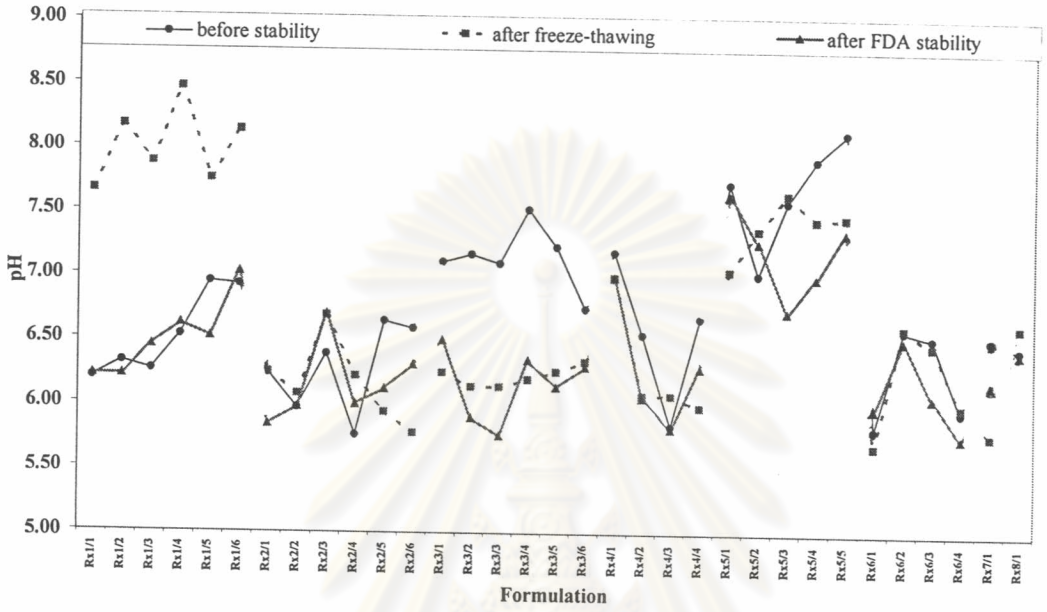


Figure 116 Comparison of pH of MEG base.

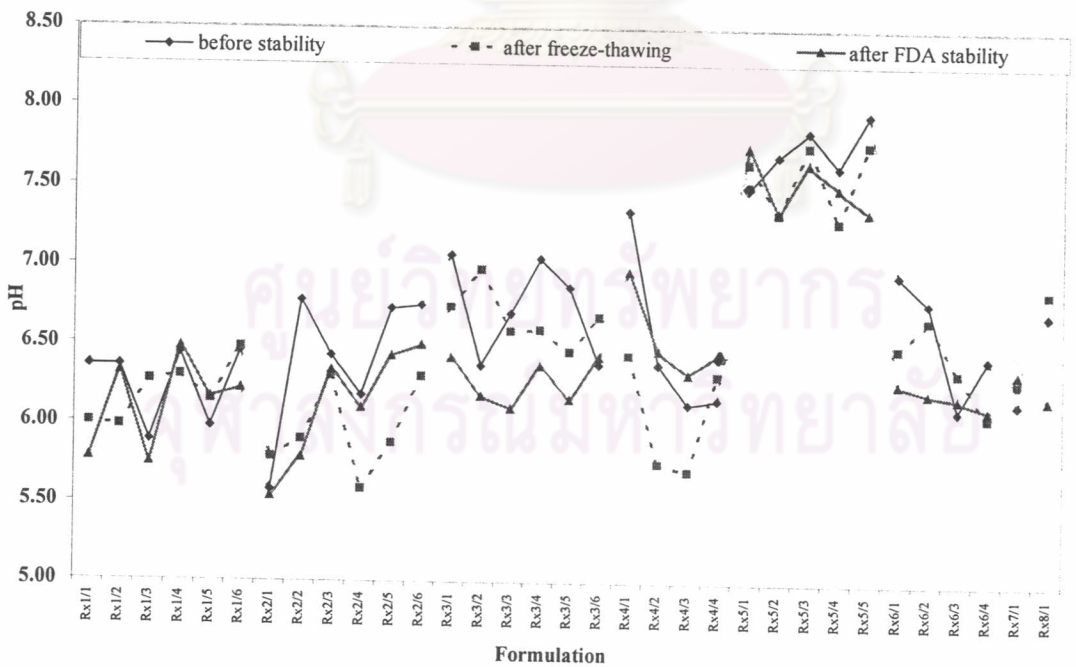


Figure 117 Comparison of pH of MEG containing 1.5%w/w metronidazole.

Table 19 Appearance of MEG base and 1.5% w/w metronidazole MEG before-stability, after freeze-thawing and after Thai FDA stability.

Composition of formula	System	Ratio of oil:surfactant (% water)	Base			1.5% metronidazole MEG		
			before stability	after freeze-thawing	after FDA stability	before stability	after freeze-thawing	after FDA stability
IPM : T_{80} : W	1/1)	5:5 (10%)	LC*	-	-	LC*	-	-,ppt
	1/2)	4:6 (8%)	l	-	l	l	-	-,ppt
	1/3)	4:6 (10%)	LC*	-	-	LC*	-	-,ppt
	1/4)	3:7 (8%)	l	-	l	l	-	-,ppt
	1/5)	3:7 (10%)	LC*	-	-	LC*	-	-,ppt
	1/6)	1:9 (7%)	l	l	l	l	l	l
CO : C_{EL} : W: PG (4:1)	2/1)	2:8 (23%)	l	l	l	l	l	l
	2/2)	2:8 (20%)	l	l	l	l	l	l
	2/3)	3:7 (10%)	l	l	l	l	l	l
	2/4)	3:7 (25%)	l	l	l	l	l	l
	2/5)	5:5 (13%)	l	-	-	l	-	-
	2/6)	5:5 (20%)	l	-	-	l	-	-
IPM : T_{80} : L_{68} : W (T_{80}:L_{68} = 2:1)	3/1)	3 : 4.67 : 2.33 (15%)	LC*	-	LC*	LC*	-	-
	3/2)	3 : 4.67 : 2.33 (20%)	LC*	-	-	LC*	LC*	-
	3/3)	3 : 4.67 : 2.33 (25%)	LCG*	LCG*	-	LCG*	LCG*	-
	3/4)	2 : 5.33 : 2.67 (20%)	LCG*	LCG*	LCG*	LCG*	LCG*	LCG*
	3/5)	2 : 5.33 : 2.67 (25%)	LCG*	LCG*	LCG*	LCG*	LCG*	LCG*
	3/6)	2 : 5.33 : 2.67 (17%)	LC*	-	-	LC*	-	-
IPM : C_{EL} : W: PG (4:1)	4/1)	1:9 (10%)	l	l	l	l	l	l
	4/2)	2:8 (25%)	LC*	LC*	LC*	LC*	-	l
	4/3)	3:7 (25%)	LCG*	LCG*	LCG*	LCG*	LCG*	LCG*
	4/4)	3:7 (20%)	LC*	-	-	LC*	-	-
IPM : C_{RH}: W: PG (4:1)	5/1)	3:7 (14.52%)	LCG*	LCG*	LCG*	LCG*	LCG*	LC*
	5/2)	4:6 (20%)	LCG*	LCG*	LCG*	LCG*	LCG*	LCG*
	5/3)	4:6 (15%)	LCG*	LCG*	LCG*	LCG*	LCG*	LCG*
	5/4)	5:5 (15%)	LCG*	LCG*	LCG*	LCG*	LCG*	S
	5/5)	5:5 (15%)	LCG*	LCG*	LCG*	LCG*	LCG*	S

Table 19 (continue)

Composition of formula	System	Ratio of oil:surfactant (% water)	Base			1.5% metronidazole MEG		
			before stability	after freeze-thawing	after FDA stability	before stability	after freeze-thawing	after FDA stability
IPM : T_{80} : C_{EL} : W	6/1)	3 : 3.5 : 3.5 (water 15%)	*LC	*LC	*LC	*LC	*LC	*LC
	6/2)	2 : 4 : 4 (water 9%)	l	l	l	l	l	l
	6/3)	1 : 4.5 : 4.5 (water 15%)	*LC	l	-	*LC	l	l
	6/4)	1 : 4.5 : 4.5 (water 20%)	*LCG	*LCG	*LCG	*LCG	*LCG	*LCG
IPM : T_{80} : B_{35} :W	7/1)	3 : 3.5 : 3.5 (water 15%)	*LCG	*LCG	*LCG	*LCG	*LCG	*LCG
SBO : T_{80} : W	8/1)	1:9 (water 7%)	l	l	l	l	l	l

* = non-newtonian behavior of system, the viscosity value were detected at 1 minutes after measurement

\- = phase-separation

l = liquid

LC = lamellar liquid crystal fluid

LCG= liquid crystal gel

ppt = precipitate

S = syneresis

Table 20 pH of MEG base and 1.5% w/w metronidazole MEG before-stability, after freeze-thawing and after Thai FDA stability.

Composition of formula	System	Ratio of oil:surfactant (% water)	Base			1.5% metronidazole MEG		
			before stability	after freeze-thawing	after FDA stability	before stability	after freeze-thawing	after FDA stability
IPM : T_{80} : W	1/1)	5:5 (10%)	6.20	7.66	6.22	6.36	6.00	5.78
	1/2)	4:6 (8%)	6.32	8.17	6.22	6.36	5.98	6.32
	1/3)	4:6 (10%)	6.26	7.88	6.45	5.89	6.27	5.75
	1/4)	3:7 (8%)	6.53	8.47	6.62	6.44	6.30	6.48
	1/5)	3:7 (10%)	6.95	7.75	6.52	5.98	6.15	6.16
	1/6)	1:9 (7%)	6.93	8.14	7.03	6.46	6.48	6.22
CO : C_{EL} : W: PG (4:1)	2/1)	2:8 (23%)	6.24	6.25	5.84	5.58	5.79	5.54
	2/2)	2:8 (20%)	5.97	6.08	5.98	6.78	5.90	5.79
	2/3)	3:7 (10%)	6.39	6.69	6.70	6.43	6.30	6.34
	2/4)	3:7 (25%)	5.76	6.22	6.00	6.18	5.59	6.10
	2/5)	5:5 (13%)	6.65	5.94	6.12	6.73	5.88	6.43
	2/6)	5:5 (20%)	6.59	5.78	6.31	6.75	6.30	6.50
IPM : T_{80} : L_{68} : W (T_{80}:L_{68} = 2:1)	3/1)	3 : 4.67 : 2.33 (15%)	7.11	6.25	6.50	7.07	6.74	6.42
	3/2)	3 : 4.67 : 2.33 (20%)	7.17	6.14	5.90	6.37	6.98	6.18
	3/3)	3 : 4.67 : 2.33 (25%)	7.10	6.14	5.76	6.70	6.59	6.10
	3/4)	2 : 5.33 : 2.67 (20%)	7.52	6.20	6.35	7.05	6.60	6.37
	3/5)	2 : 5.33 : 2.67 (25%)	7.23	6.26	6.14	6.87	6.46	6.16
	3/6)	2 : 5.33 : 2.67 (17%)	6.75	6.34	6.30	6.38	6.68	6.43
IPM : C_{EL} : W: PG (4:1)	4/1)	1:9 (10%)	7.19	7.00	7.00	7.35	6.44	6.97
	4/2)	2:8 (25%)	6.55	6.08	6.06	6.38	5.76	6.47
	4/3)	3:7 (25%)	5.84	6.08	5.82	6.13	5.71	6.32
	4/4)	3:7 (20%)	6.68	5.99	6.29	6.16	6.31	6.45
IPM : C_{RH}: W: PG (4:1)	5/1)	3:7 (14.52%)	7.73	7.05	7.64	7.49	7.65	7.75
	5/2)	4:6 (20%)	7.02	7.37	7.27	7.70	7.34	7.35
	5/3)	4:6 (15%)	7.59	7.65	6.73	7.85	7.76	7.65
	5/4)	5:5 (15%)	7.92	7.45	7.00	7.63	7.29	7.50
	5/5)	5:5 (15%)	8.13	7.47	7.35	7.96	7.77	7.35

Table 20 (continue)

Composition of formula	System	Ratio of oil:surfactant (% water)	Base			1.5% metronidazole MEG		
			before stability	after freeze-thawing	after FDA stability	before stability	after freeze-thawing	after FDA stability
IPM : T_{80} : C_{EL} : W	6/1)	3 : 3.5 : 3.5 (water 15%)	5.82	5.69	5.98	6.96	6.49	6.27
	6/2)	2 : 4 : 4 (water 9%)	6.59	6.61	6.51	6.78	6.67	6.21
	6/3)	1 : 4.5 : 4.5 (water 15%)	6.53	6.47	6.07	6.10	6.34	6.18
	6/4)	1 : 4.5 : 4.5 (water 20%)	5.96	6.00	5.76	6.43	6.06	6.11
IPM : T_{80} : B_{35} :W	7/1)	3 : 3.5 : 3.5 (water 15%)	6.52	5.78	6.18	6.15	6.28	6.34
SBO : T_{80} : W	8/1)	1:9 (water 7%)	6.45	6.62	6.42	6.71	6.85	6.18

Table 21 Syringeability of MEG base and 1.5% w/w metronidazole MEG before-stability, after freeze-thawing and after Thai FDA stability.

Composition of formula	System	Ratio of oil:surfactant (% water)	Base (ml/sec)			1.5% metronidazole MEG (ml/sec)		
			before stability	after freeze-thawing	after FDA stability	before stability	after freeze-thawing	after FDA stability
IPM : T_{80} : W	1/1)	5:5 (10%)	0.0557	0.2762	0.2639	0.1686	0.3062	0.2294
	1/2)	4:6 (8%)	0.0527	0.0526	0.0549	0.0702	0.0589	0.0494
	1/3)	4:6 (10%)	0.0435	0.1793	0.1156	0.1486	0.1440	0.1496
	1/4)	3:7 (8%)	0.0217	0.0441	0.0397	0.0554	0.0448	0.0443
	1/5)	3:7 (10%)	0.0482	0.1133	0.0401	0.0482	0.0719	0.0347
	1/6)	1:9 (7%)	0.0208	0.0385	0.0370	0.0403	0.0308	0.0335
CO : C_{EL} : W: PG (4:1)	2/1)	2:8 (23%)	0.0182	0.0203	0.0133	0.0091	0.0106	0.0100
	2/2)	2:8 (20%)	0.0063	0.0118	0.0126	0.0115	0.0107	0.0164
	2/3)	3:7 (10%)	0.0132	0.0166	0.0176	0.0160	0.0171	0.0183
	2/4)	3:7 (25%)	0.0052	0.0040	0.0050	0.0039	0.0040	0.0065
	2/5)	5:5 (13%)	0.0200	0.0112	0.0118	0.0060	0.0167	0.0066
	2/6)	5:5 (20%)	0.0086	0.0062	0.0047	0.0143	0.0053	0.0149
IPM : T_{80} : L_{68} : W (T_{80}:L_{68} = 2:1)	3/1)	3 : 4.67 : 2.33 (15%)	0.0334	0.0321	0.0231	0.0175	0.0346	0.0145
	3/2)	3 : 4.67 : 2.33 (20%)	0.0133	0.0368	0.0168	0.0175	0.0192	0.0119
	3/3)	3 : 4.67 : 2.33 (25%)	0.0089	0.0258	0.0083	0.0093	0.0242	0.0061
	3/4)	2 : 5.33 : 2.67 (20%)	0.0208	0.0166	0.0151	0.0187	0.0134	0.0227
	3/5)	2 : 5.33 : 2.67 (25%)	0.0217	0.0138	0.0182	0.0169	0.0192	0.0062
	3/6)	2 : 5.33 : 2.67 (17%)	0.0278	0.0410	0.0229	0.0172	0.0347	0.0167
IPM : C_{EL} : W: PG (4:1)	4/1)	1:9 (10%)	0.0233	0.0267	0.0286	0.0257	0.0263	0.0262
	4/2)	2:8 (25%)	0.0075	0.0579	0.0202	0.0072	0.0551	0.0117
	4/3)	3:7 (25%)	0.0091	0.0609	0.0106	0.0045	0.0398	0.0174
	4/4)	3:7 (20%)	0.0204	0.1091	0.0170	0.0139	0.0945	0.0129
IPM : C_{RH}: W: PG (4:1)	5/1)	3:7 (14.52%)	0.0286	0.0645	0.0351	0.0045	0.0663	0.0073
	5/2)	4:6 (20%)	0.0046	0.0593	0.0070	0.0221	0.0577	0.0166
	5/3)	4:6 (15%)	0.0137	0.1231	0.0085	0.0132	0.1108	0.0120
	5/4)	5:5 (15%)	0.0097	0.1626	0.0174	0.0103	0.1302	0.0314
	5/5)	5:5 (15%)	0.0054	0.1260	0.0118	0.0164	0.1382	0.0100

Table 21 (continue)

Composition of formula	System	Ratio of oil:surfactant (% water)	Base (ml/sec)			1.5% metronidazole MEG (ml/sec)		
			before stability	after freeze-thawing	after FDA stability	before stability	after freeze-thawing	after FDA stability
IPM : T_{80} : C_{EL} : W	6/1)	3 : 3.5 : 3.5 (water 15%)	0.0772	0.0832	0.0955	0.0644	0.0703	0.0684
	6/2)	2 : 4 : 4 (water 9%)	0.0286	0.0324	0.0196	0.0301	0.0412	0.0305
	6/3)	1 : 4.5 : 4.5 (water 15%)	0.0286	0.0292	0.0193	0.0213	0.0240	0.0235
	6/4)	1 : 4.5 : 4.5 (water 20%)	0.0435	0.0423	0.0303	0.0301	0.0538	0.0273
IPM : T_{80} : B_{35} :W	7/1)	3 : 3.5 : 3.5 (water 15%)	0.0179	0.0955	0.0161	0.0177	0.0130	0.0199
SBO : T_{80} : W	8/1)	1:9 (water 7%)	0.0331	0.0320	0.0284	0.0328	0.0374	0.0336

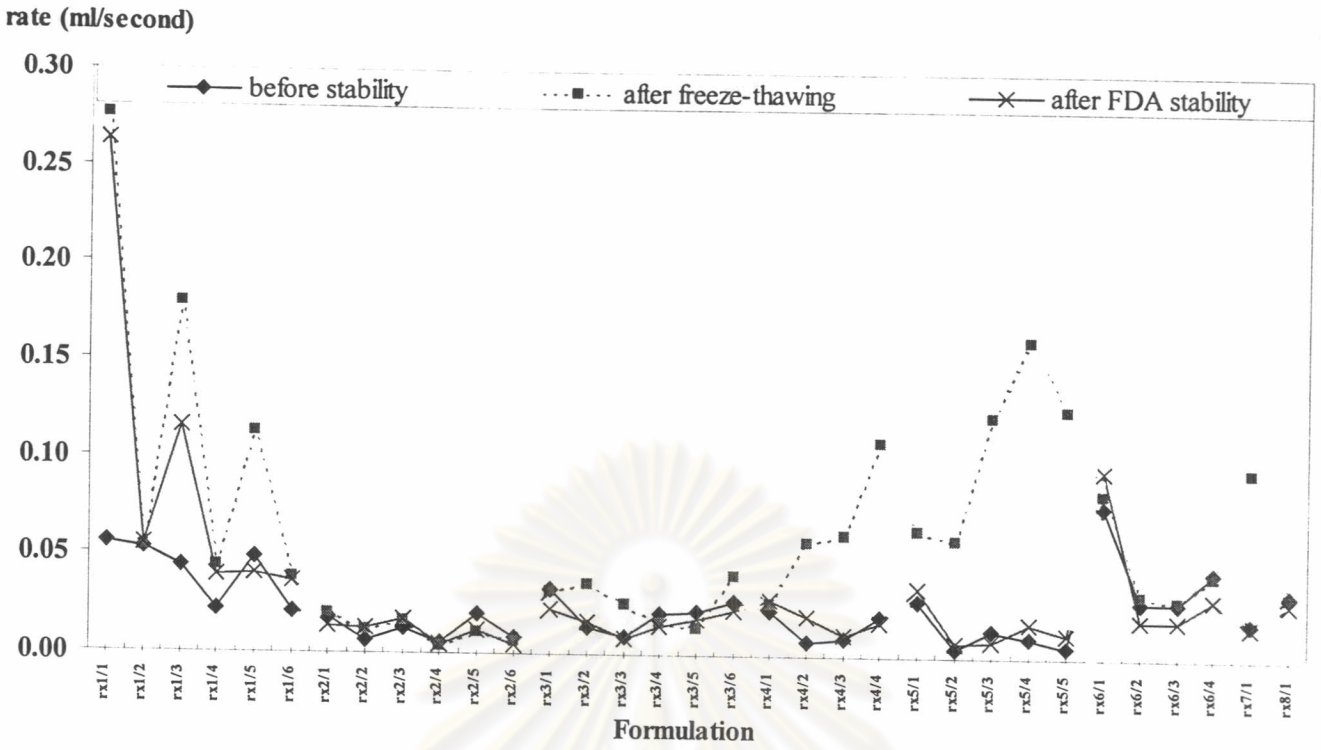


Figure 118 Comparison of syringeability of MEG base.

Figure 119 Comparison of syringeability of 1.5%w/w metronidazole MEG.

Table 22 Conductivity of MEG base and 1.5% w/w metronidazole MEG before-stability, after freeze-thawing and after Thai FDA stability.

Composition of formula	System	Ratio of oil:surfactant (% water)	Base (μS)				1.5% metronidazole MEG (μS)			
			before stability	after freeze-thawing	before stability	after FDA stability	before stability	after freeze-thawing	before stability	after FDA stability
IPM : T_{80} : W	1/1)	5:5 (10%)	2.00	1.60	2.00	2.00	2.00	1.90	0.50	5.90
	1/2)	4:6 (8%)	2.90	2.80	3.10	1.60	2.30	3.00	2.90	3.80
	1/3)	4:6 (10%)	2.00	2.30	2.70	3.00	3.40	3.20	2.40	2.60
	1/4)	3:7 (8%)	2.90	2.90	2.90	2.80	3.30	2.90	3.10	3.30
	1/5)	3:7 (10%)	2.30	2.80	3.10	4.40	3.50	3.50	3.90	4.50
	1/6)	1:9 (7%)	3.10	3.20	3.60	2.60	3.40	3.30	3.10	3.20
CO : C_{EL} : W: PG (4:1)	2/1)	2:8 (23%)	11.10	14.50	12.00	17.60	16.70	16.00	16.70	20.70
	2/2)	2:8 (20%)	11.90	11.30	11.90	13.90	12.20	12.20	12.20	15.50
	2/3)	3:7 (10%)	3.80	4.10	4.00	2.30	3.80	3.90	3.50	4.10
	2/4)	3:7 (25%)	21.30	22.50	23.20	25.70	23.20	21.50	23.20	29.20
	2/5)	5:5 (13%)	1.70	2.90	2.80	2.00	4.60	7.60	2.30	5.40
	2/6)	5:5 (20%)	15.80	1.40	16.10	17.20	14.30	2.30	2.00	7.80
IPM : T_{80} : L_{68} : W (T_{80}:L_{68} = 2:1)	3/1)	3 : 4.67 : 2.33 (15%)	2.60	2.40	4.10	4.80	3.40	3.20	6.60	8.10
	3/2)	3 : 4.67 : 2.33 (20%)	4.30	3.60	15.00	9.20	2.90	4.50	12.70	14.90
	3/3)	3 : 4.67 : 2.33 (25%)	5.40	8.10	19.40	15.20	8.50	12.50	20.10	25.60
	3/4)	2 : 5.33 : 2.67 (20%)	4.00	4.40	6.40	5.30	2.30	2.30	6.00	8.50
	3/5)	2 : 5.33 : 2.67 (25%)	5.00	4.50	10.70	16.30	5.00	5.10	9.80	24.60
	3/6)	2 : 5.33 : 2.67 (17%)	3.40	3.20	4.30	4.40	3.30	3.00	8.00	9.30
IPM : C_{EL} : W: PG (4:1)	4/1)	1:9 (10%)	4.80	4.90	5.50	4.80	5.20	4.00	5.40	5.80
	4/2)	2:8 (25%)	14.70	13.00	20.30	14.30	15.80	16.80	19.30	27.20
	4/3)	3:7 (25%)	12.20	25.50	12.20	21.50	13.80	21.40	20.60	28.10
	4/4)	3:7 (20%)	8.60	9.50	15.80	17.10	11.00	12.20	16.10	19.50
IPM : C_{RH}: W: PG (4:1)	5/1)	3:7 (14.52%)	3.50	3.30	9.00	14.30	3.30	3.30	14.30	13.30
	5/2)	4:6 (20%)	7.20	7.00	20.70	18.20	8.40	9.80	14.60	17.00
	5/3)	4:6 (15%)	2.40	2.20	14.80	13.60	2.80	3.80	13.20	14.00
	5/4)	5:5 (15%)	3.80	4.10	12.70	11.60	4.60	4.70	11.00	11.20
	5/5)	5:5 (15%)	2.80	3.00	15.20	13.50	3.20	4.30	11.00	11.80

Table 22 (continue)

Composition of formula	System	Ratio of oil:surfactant (% water)	Base (μS)				1.5% metronidazole MEG (μS)			
			before stability	after freeze-thawing	before stability	after FDA stability	before stability	after freeze-thawing	before stability	after FDA stability
IPM : T_{80} : C_{EL} : W	6/1)	3 : 3.5 : 3.5 (water 15%)	3.30	3.80	7.80	8.40	3.00	4.00	6.00	9.00
	6/2)	2 : 4 : 4 (water 9%)	4.10	4.30	4.60	5.30	4.30	4.30	4.80	5.60
	6/3)	1 : 4.5 : 4.5 (water 15%)	4.20	4.80	10.90	11.20	11.90	11.70	9.60	11.10
	6/4)	1 : 4.5 : 4.5 (water 20%)	10.10	9.80	12.00	12.80	12.10	11.80	11.90	22.40
IPM : T_{80} : B_{35} :W	7/1)	3 : 3.5 : 3.5 (water 15%)	5.10	4.80	9.10	8.50	11.00	10.60	6.90	7.60
SBO : T_{80} : W	8/1)	1:9 (water 7%)	3.40	3.20	3.50	3.50	3.80	3.60	3.40	3.20

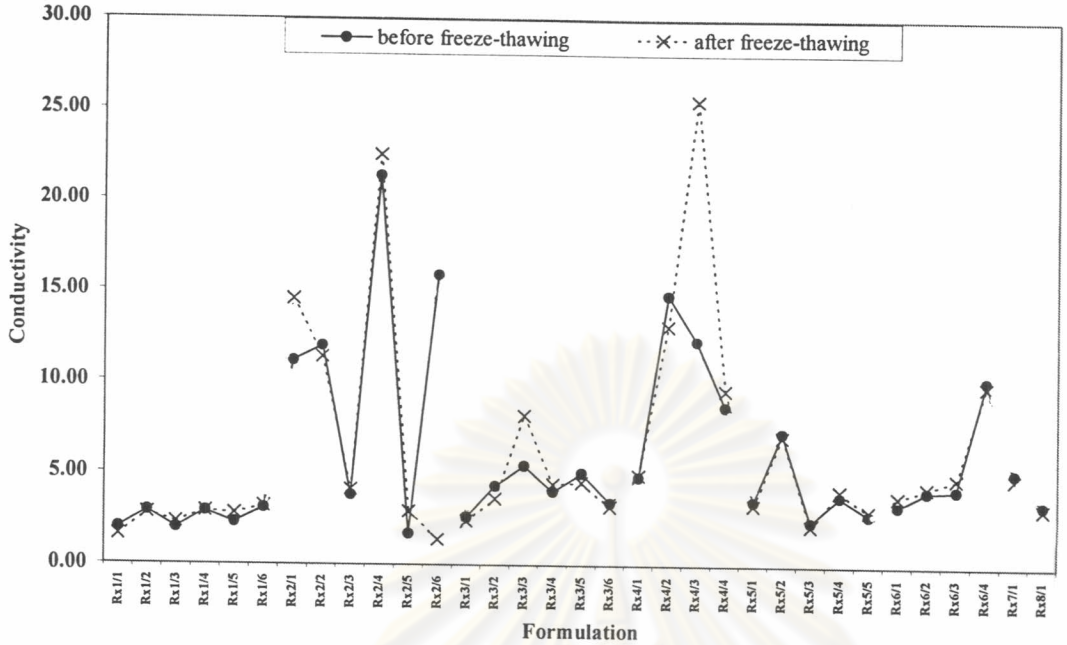


Figure 120 Conductivity of MEG base (from freeze-thawing formula).

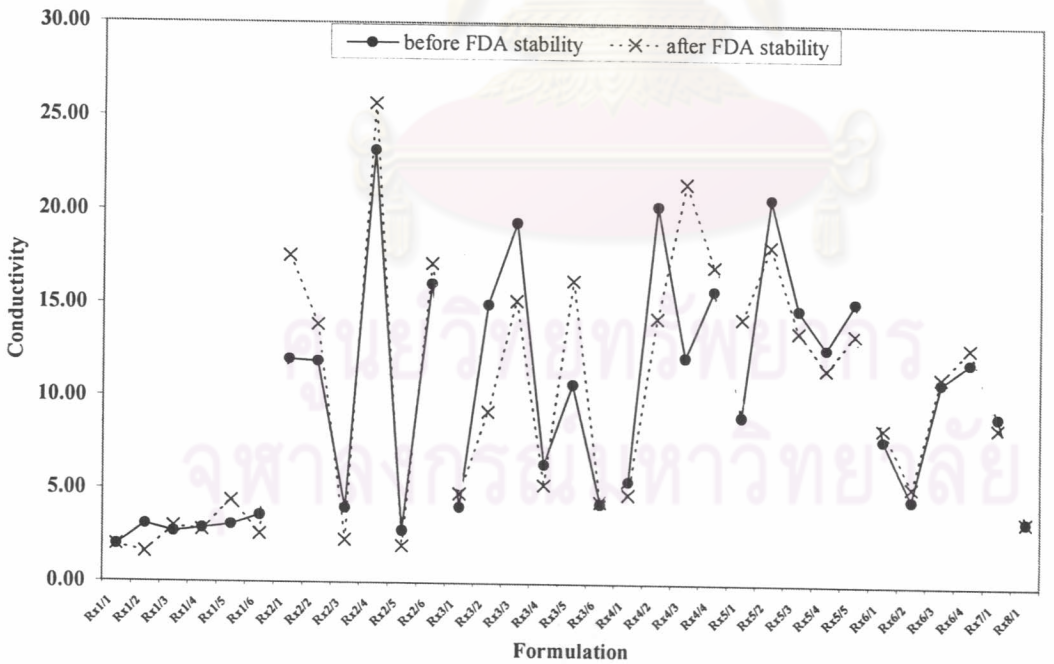


Figure 121 Conductivity of MEG base (from Thai FDA stability tasting formula).

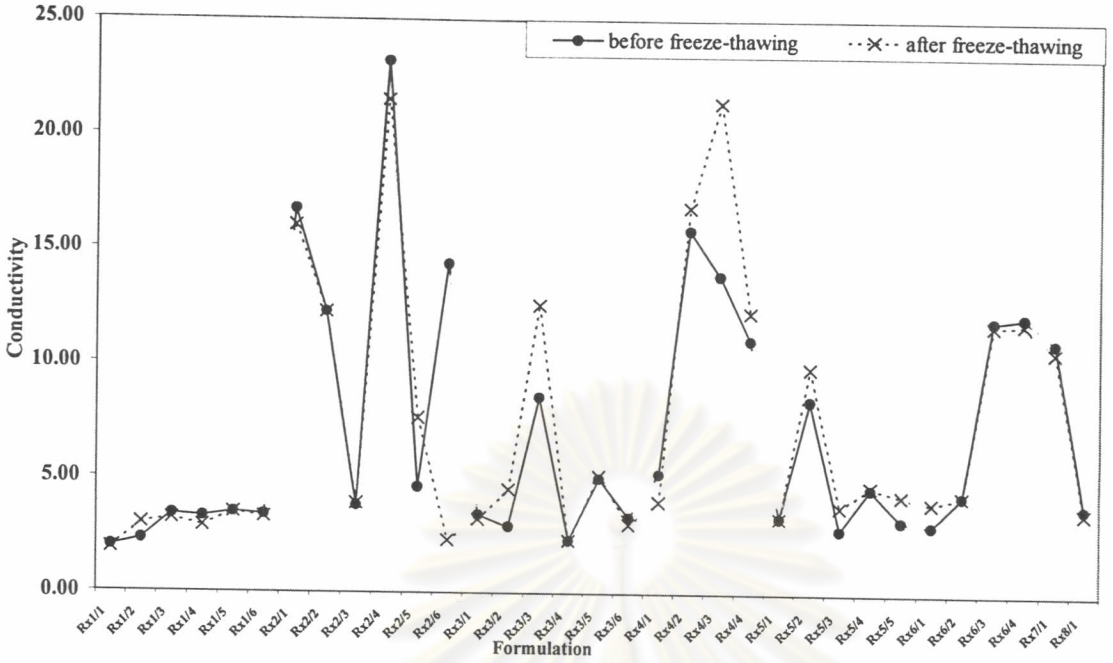


Figure 122 Conductivity of MEG containing 1.5%w/w metronidazole base (from freeze-thawing formula).

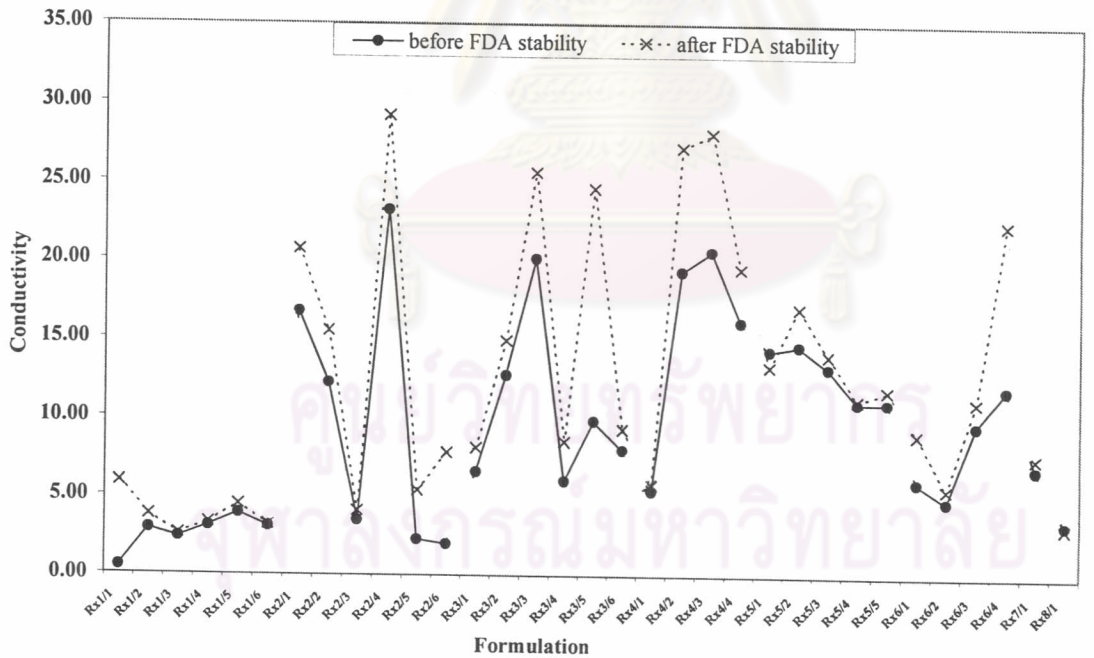


Figure 123 Conductivity of MEG containing 1.5%w/w metronidazole (from Thai FDA stability tasting formula).

Table 23 Viscosity of MEG base and 1.5% w/w metronidazole MEG before-stability, after freeze-thawing and after Thai FDA stability.

Composition of formula	System	Ratio of oil:surfactant (% water)	Base (cps)			1.5% metronidazole MEG (cps)		
			before stability	after freeze-thawing	after FDA stability	before stability	after freeze-thawing	after FDA stability
IPM : T_{80} : W	1/1)	5:5 (10%)	*21,600	*24,630	-	*2,670	-	-
	1/2)	4:6 (8%)	382	382	382	294	-	-
	1/3)	4:6 (10%)	*1,080	*1,130	-	*3,000	-	-
	1/4)	3:7 (8%)	387	450	432	350	-	-
	1/5)	3:7 (10%)	*1,567	*2,175	-	380	-	-
	1/6)	1:9 (7%)	435	540	543	458	445	516
CO : C_{EL} : W: PG (4:1)	2/1)	2:8 (23%)	895	1,300	1,476	1,378	1,450	1,560
	2/2)	2:8 (20%)	1,080	1,294	1,350	1,134	1,200	1,387
	2/3)	3:7 (10%)	858	948	1,038	930	1,020	1,048
	2/4)	3:7 (25%)	3,072	4,022	4,050	3,078	3,078	3,400
	2/5)	5:5 (13%)	1,605	-	-	1,170	-	-
	2/6)	5:5 (20%)	2,346	-	-	2,032	-	-
IPM : T_{80} : L_{68} : W (T_{80}:L_{68} = 2:1)	3/1)	3 : 4.67 : 2.33 (15%)	*6,120	*7,050	*8,100	*8,850	*9,000	*9,000
	3/2)	3 : 4.67 : 2.33 (20%)	*12,900	*12,980	-	*19,500	*19,500	-
	3/3)	3 : 4.67 : 2.33 (25%)	*19,080	*22,400	-	*96,100	*96,500	*96,500
	3/4)	2 : 5.33 : 2.67 (20%)	*18,330	*18,750	*22,650	*37,500	*38,000	*38,000
	3/5)	2 : 5.33 : 2.67 (25%)	*25,800	*26,820	*26,820	*33,600	*34,000	*34,000
	3/6)	2 : 5.33 : 2.67 (17%)	*7,020	*7,230	-	*12,150	-	-
IPM : C_{EL} : W: PG (4:1)	4/1)	1:9 (10%)	630	720	720	754	780	780
	4/2)	2:8 (25%)	*7,860	*9,732	*9,732	*7,868	-	-
	4/3)	3:7 (25%)	*35,230	*39,600	*39,600	*47,500	*48,000	*48,000
	4/4)	3:7 (20%)	*3,900	*3,900	-	*3,980	-	-
IPM : C_{RH}: W: PG (4:1)	5/1)	3:7 (14.52%)	*45,600	*56,400	*56,400	*60,000	*76,800	*76,800
	5/2)	4:6 (20%)	*200,000	*200,000	*200,000	*280,000	*289,000	*289,000
	5/3)	4:6 (15%)	*124,000	*124,000	*124,000	*125,334	*500,000	*500,000
	5/4)	5:5 (15%)	*105,000	*105,000	*105,000	*107,666	*258,000	*258,000
	5/5)	5:5 (15%)	*110,000	*123,000	*123,000	*160,000	*161,000	*161,000

Table 23 (continue)

Composition of formula	System	Ratio of oil:surfactant (% water)	Base (cps)			1.5% metronidazole MEG (cps)		
			before stability	after freeze-thawing	after FDA stability	before stability	after freeze-thawing	after FDA stability
IPM : T_{80} : C_{EL} : W	6/1)	3 : 3.5 : 3.5 (water 15%)	*11,610	*11,610	*11,610	*12,140	*12,250	*12,250
	6/2)	2 : 4 : 4 (water 9%)	480	600	612	536	535	600
	6/3)	1 : 4.5 : 4.5 (water 15%)	*13,000	672	-	728	780	888
	6/4)	1 : 4.5 : 4.5 (water 20%)	*21,840	*24,620	*24,620	*24,620	*24,800	*24,800
IPM : T_{80} : B_{35} :W	7/1)	3 : 3.5 : 3.5 (water 15%)	*10,200	*10,200	*10,200	*93,000	*100,000	*100,000
SBO : T_{80} : W	8/1)	1:9 (water 7%)	480	630	480	492	462	483

* = non-newtonian behavior of system, the viscosity value were detected at 1 minutes after measurement

\- = phase-separation

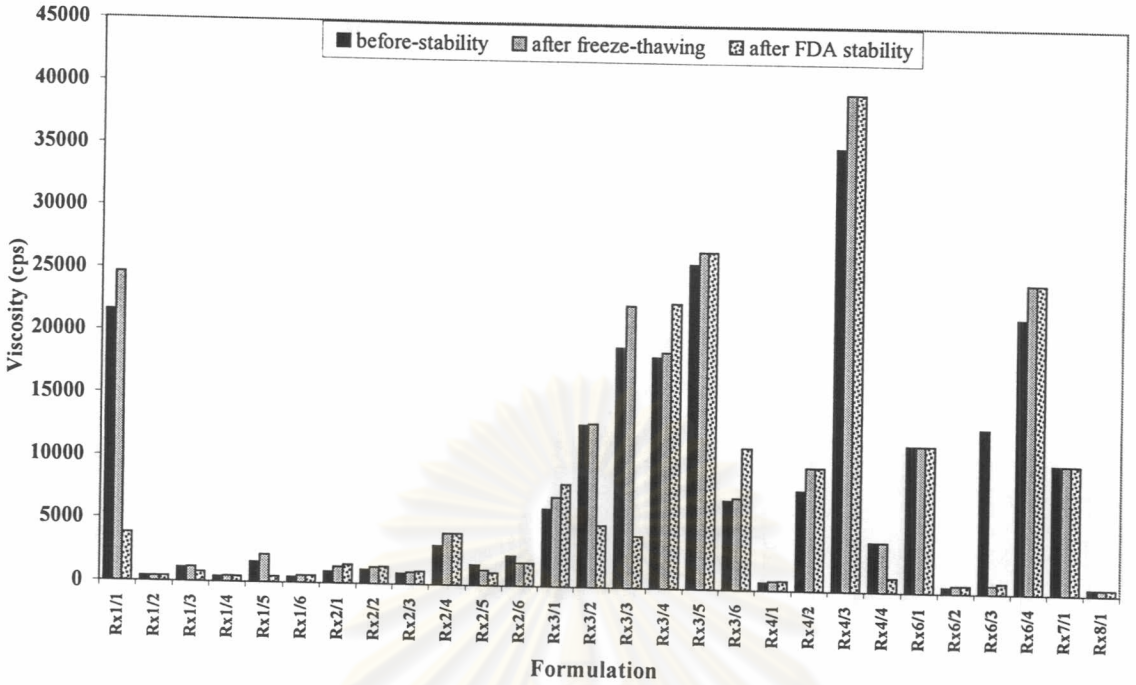


Figure 124 Viscosity of MEG base before-stability, after freeze-thawing and after Thai FDA stability testing.

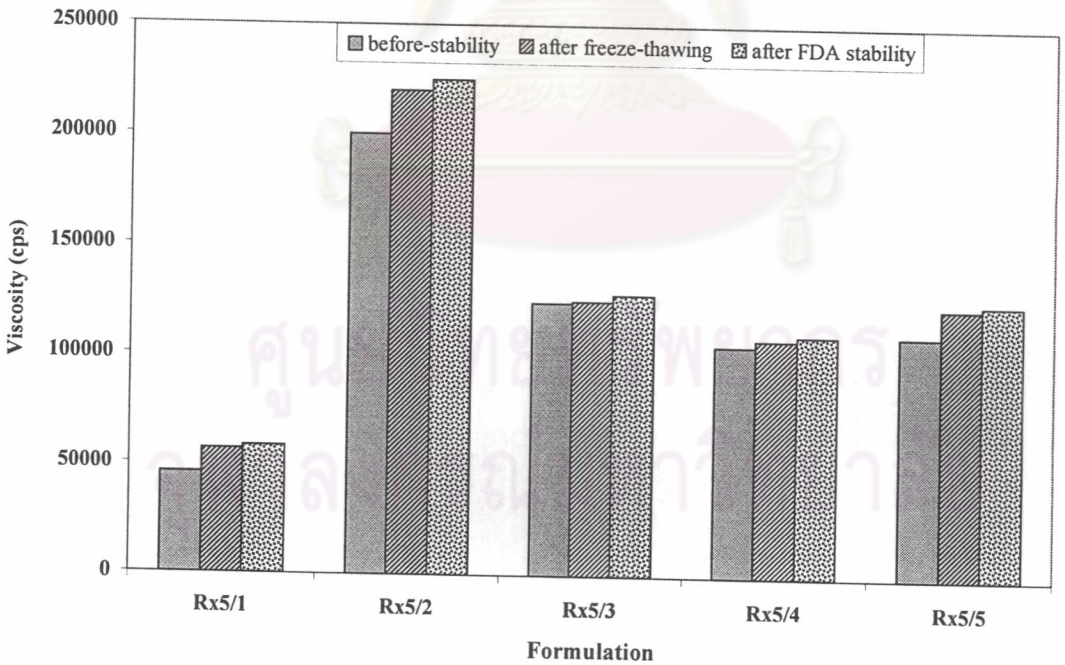


Figure 125 Viscosity of MEG base in IPM:C_{RH}:W:PG system before-stability, after freeze-thawing and after Thai FDA stability testing.

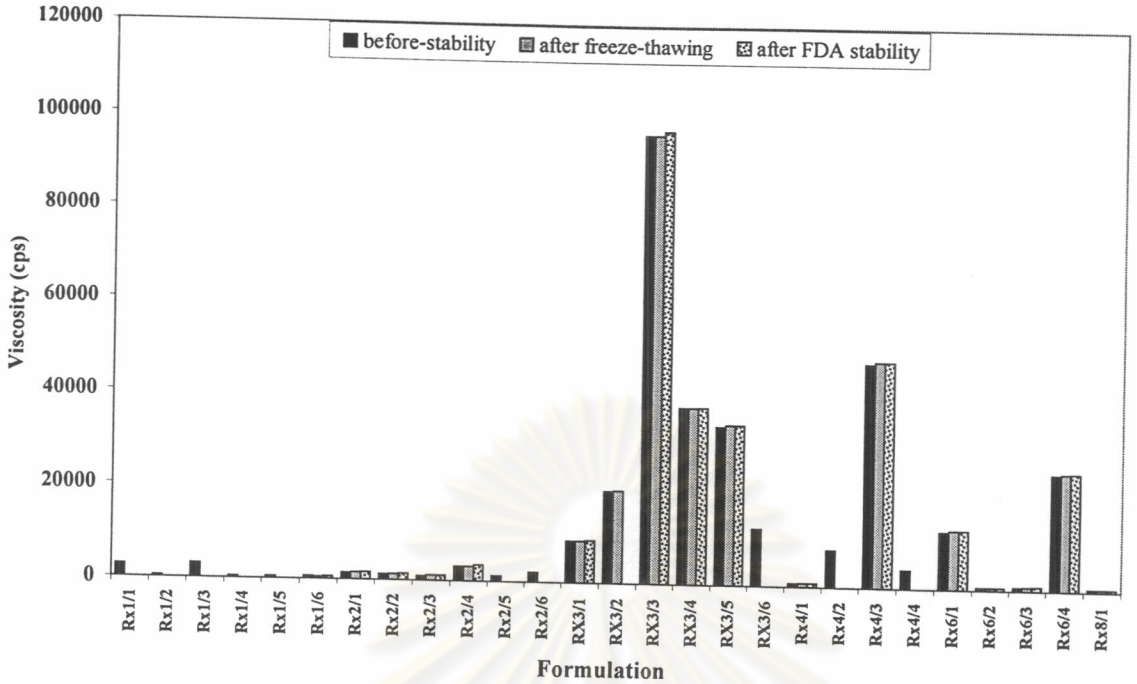


Figure 126 Comparison viscosity of MEG containing 1.5%w/w metronidazole before-stability, after freeze-thawing and after Thai FDA stability.

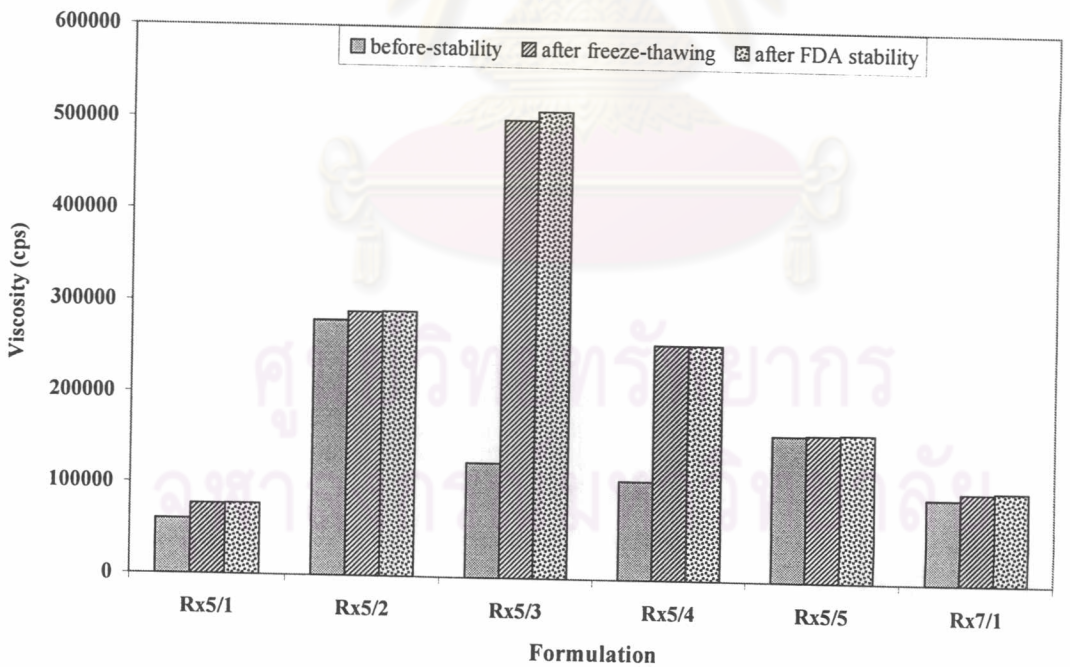


Figure 127 Comparison viscosity of MEG containing 1.5%w/w metronidazole in IPM:C_{RH}:W:PG system before-stability, after freeze-thawing and after Thai FDA stability.

Table 24 Mean particle sizes of the representative MEG base system before and after freeze-thawing.

Composition and ratio		Mean particle diameter (nm)			
		Before stability	SD	After stability	SD
IPM : T_{80} : W					
1/1)	5:5 (10%)	43.31	5.80	52.86	13.47
1/2)	4:6 (8%)	-	-	-	-
1/3)	4:6 (10%)	71.27	26.64	114.83	29.85
1/4)	3:7 (8%)	-	-	-	-
1/5)	3:7 (10%)	27.68	6.13	99.50	34.47
1/6)	1:9 (7%)	78.53	20.48	105.90	26.79
CO : C_{EL} : W: PG (4:1)					
2/1)	2:8 (23%)	72.94	19.67	83.84	22.04
2/2)	2:8 (20%)	-	-	-	-
2/3)	3:7 (10%)	-	-	-	-
2/4)	3:7 (25%)	-	-	-	-
2/5)	5:5 (13%)	-	-	-	-
2/6)	5:5 (20%)	-	-	-	-
IPM : T_{80} : L_{68} : W(T_{80}:L_{68}=2:1)					
3/1)	3 : 4.67 : 2.33 (15%)	41.77	8.40	84.81	21.64
3/2)	3 : 4.67 : 2.33 (20%)	47.64	7.19	52.94	16.52
3/3)	3 : 4.67 : 2.33 (25%)	-	-	-	-
3/4)	2 : 5.33 : 2.67 (20%)	39.73	11.62	57.87	15.50
3/5)	2 : 5.33 : 2.67 (25%)	58.33	17.56	73.54	18.52
3/6)	2 : 5.33 : 2.67 (17%)	-	-	-	-
IPM : C_{EL} : W: PG (4:1)					
4/1)	1:9 (10%)	-	-	-	-
4/2)	2:8 (25%)	49.03	10.57	99.14	21.58
4/3)	3:7 (25%)	-	-	-	-
4/4)	3:7 (20%)	57.33	14.82	99.89	23.47
IPM : C_{RH}: W: PG (4:1)					
5/1)	3:7 (14.52%)	81.63	21.69	54.28	11.20
5/2)	4:6 (20%)	-	-	-	-
5/3)	4:6 (15%)	50.70	16.62	60.70	17.85
5/4)	5:5 (15%)	-	-	-	-
5/5)	5:5 (15%)	-	-	-	-
IPM : T_{80} : C_{EL} : W					
6/1)	3 : 3.5 : 3.5 (water 15%)	82.09	40.45	27.28	5.49
6/2)	2 : 4 : 4 (water 9%)	-	-	-	-
6/3)	1 : 4.5 : 4.5 (water 15%)	-	-	-	-
6/4)	1 : 4.5 : 4.5 (water 20%)	47.25	10.25	158.89	24.81
IPM : T_{80} : B_{35} : W					
7/1)	3 : 3.5 : 3.5 (water 15%)	84.37	16.04	112.56	31.97
SBO : T_{80} : W					
8/1)	1:9 (water 7%)	78.33	20.00	79.58	26.52

(-) = exclude from the representative formula due to poor physical stability testing.

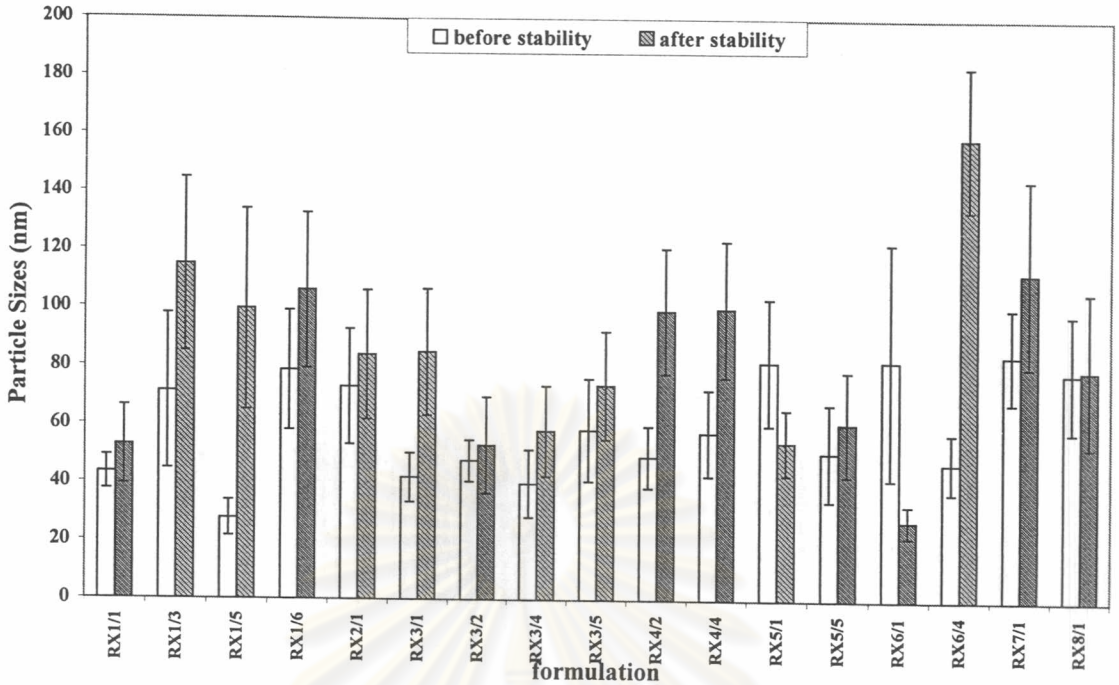


Figure 128 Mean particle size of microemulsion gel system between before and after freeze-thawing stability resting.

Table 25 Antimicrobial activity of the MEG containing 1.5% w/w metronidazole before stability, after freeze-thawing and Thai FDA stability.

Formulation	System	Ratio of oil:surfactant (% water)	Mean diameter inhibition zone (cm.)		
			freshly prepare	after freeze-thawing	after FDA-stability
			Average± SD	Average± SD	Average± SD
CO : C_{EL} : W: PG (4:1)	2/4)	3:7 (25%)	7.33±0.04	7.35±0.00	7.60±0.00
IPM : T_{80} : L_{68} : W (T_{80}:L_{68} = 2:1)	3/4)	2:5.33:2.6(20%)	7.40±0.14	7.54±0.06	7.34±0.06
IPM : C_{EL} : W: PG (4:1)	4/3)	3:7 (25%)	7.55±0.07		
IPM : C_{RH}: W: PG (4:1)	5/1)	3:7 (14.52%)	7.00±0.00	7.55±0.07	7.18±0.11
	5/2)	4:6 (20%)	7.39±0.01		
IPM : T_{80} : C_{EL} :W	6/4)	1:4.5:4.5 (20%)	7.18±0.11		
IPM : T_{80} : B_{35} : W	7/1)	3:3.5:3.5 (15%)	7.30±0.07	7.69±0.01	7.38±0.04

5. Anti-microbial activity of MEG base and 1.5%w/w of metronidazole MEG

Antimicrobial activity is measured *in vitro* in order to determine the potency and sensitivity of a given *P. gingivalis* to know the minimum inhibition zone of each composition, microemulsion gel base and microemulsion gel containing metronidazole. Two general types of tests; dilution test and diffusion method were used. In dilution test; various increasing amount of antimicrobial substances were incorporated into liquid or solid bacteriologic media. The media were subsequently inoculated with test bacteria and incubated. The end point was taken as that amount of microbial substance required to inhibit the growth or to kill the test bacteria. Tube dilution tests were usually more accurate than the diffusion tests but were not done routinely because of time and expense. For diffusion method or modified agar diffusion method, a filter paper disk, well, a porous cup or an open ended cylinder containing accurated quantities of drug is placed on a solid medium that had been heavily seeded with the test organisms. This experiment used the exactly quantity of formulation or test sample placed in the perforated hole of tryptic soy agar plate. A concentration gradient of sample was produced in the medium by diffusion from the perforated hole that placed with sample. After incubation, the diameter of the clear zone of inhibition surrounding the deposit of sample was taken as a measure of inhibitory power of the microemulsion gel base and microemulsion containing metronidazole against P. *gingivalis*. The size of zone growth varied with the type and composition of microemulsion gel base and microemulsion gel containing metronidazole. The diffusion method test measured the ability of an agent to inhibit rather than kill. In vitro study of antimicrobial activity used the modified agar diffusion method.

It is well documented that the progression of periodontal disease through various stages of severity begins with an accumulation of bacteria (plaque). Colonization of the gingival margin and gingival crevice begins with accumulation of bacteria that thrive in the presence of oxygen (aerobic) as well as bacteria that can tolerate low levels of oxygen (facultaive anaerobic bacteria). Gingivitis is associated with an increase in the quantity and complexity of plaque. In adult periodontitis, the total amount of plaque is further increased which characteristically contains anaerobic bacteria. Adult peridontitis lesions contain high proportions of gram negative rod, non-motile anaerobes especially *P. gingivalis, P. intermedia and A. actinomycetemcomitan. P. gingivalis* is closely related to advanced adult peridontitis and seems to be one of the numerically most important pathogens in the disease. *P. gingivalis* posseses one of the highest virulence potantials of any oral organism tested so far. *P. intermedia* is associated with peridontitis as well as gingivalis. *A. actinomycetemcomitans* is mostly associated with juvenile peridontitis. In this study, *P. gingivalis* was a representative of microbials in adult periodontitis.

The antimicrobial activity of MEG base and 1.5%w/w of metronidazole MEG were performed by using *Porphyromonas gingivalis* (strain 381) or *P. gingivalis (Pg)* which represented the anaerobic bacteria in adult periodontal pocket. The effectiveness against *P. gingivalis (Pg)* was evaluated by using the agar diffusion method. First,

gingival fluid of periodontal disease patient was collected from periodontal pocket then was isolated to pure prior collected in plaque as shown in Figure 129.

Deep frozen *Porphyromonas gingivalis* in plaque was recovered from frozen condition and melted then inoculated and sub-cultured onto tryptic soy agar. Figure 130 shows the unique characteristic of short rod-shape gram-negative anaerobe that form black brown to black colonies on blood agar.

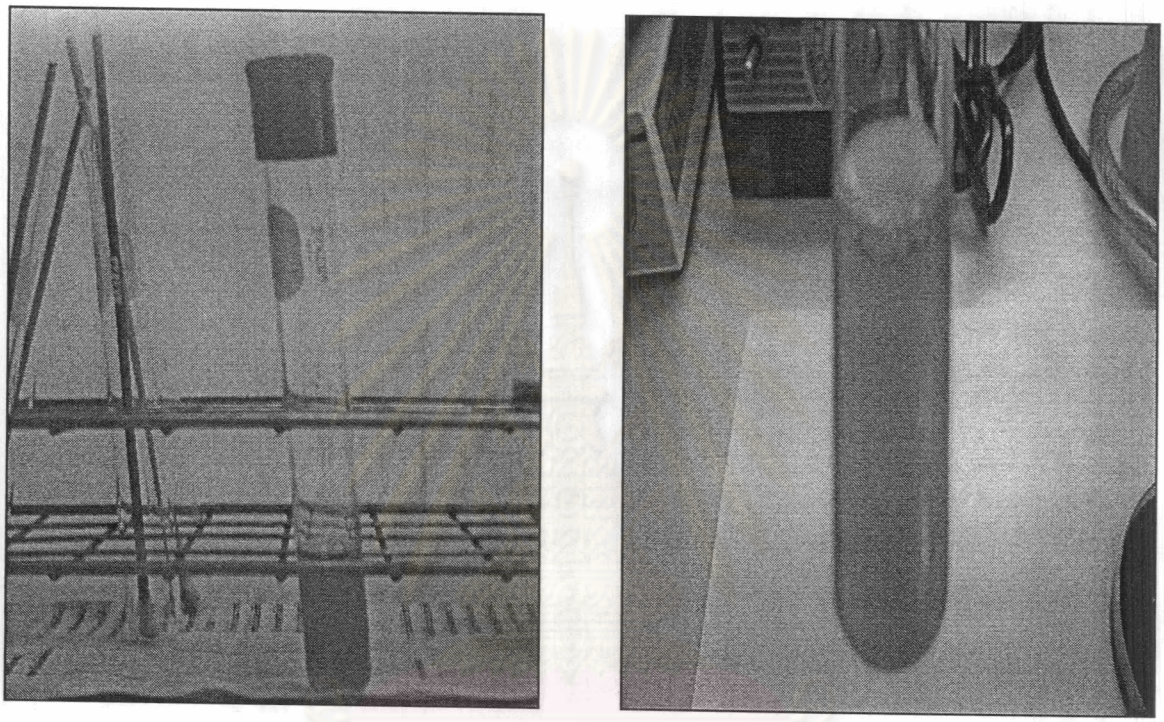


Figure129 *Porphyromonas gingivalis* (strain 381) or *P. gingivalis (Pg)* from periodontal pocket (left) and *P. gingivalis (Pg)* isolated to pure prior collected in plaque (right).

Plaque agar plates were prepared by adding tryptic soy agar (see the composition in Appendix C to each petri-dish with a diameter of 10 cm. The agar plates were perforated as shown in Figure 131. Subcultures and cultures were done in anaerobic glove box to control anaerobic conditions (see in Figure 133)

Figure 130 Unique characteristic of short rod-shape gram-negative on blood agar.

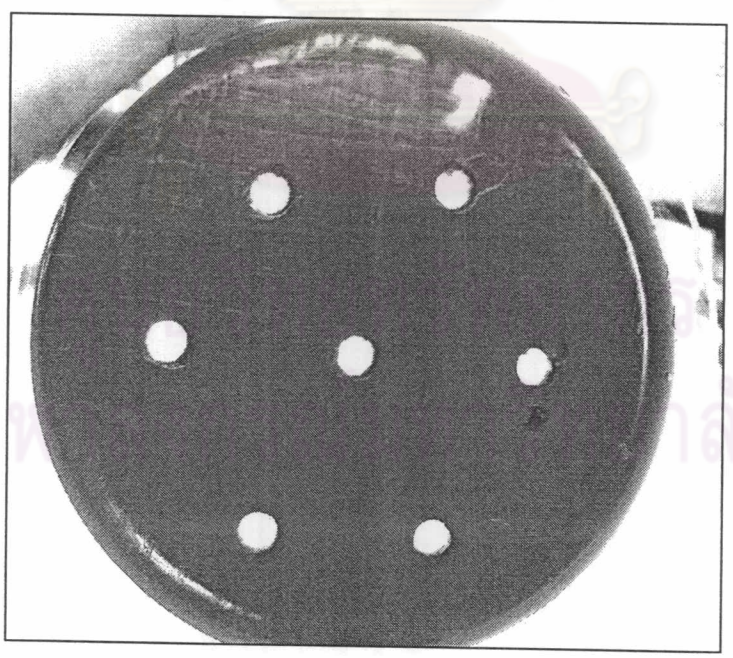


Figure 131 Agar plates perforated for placing the formulation into the hole prior to perform anti-microbial activity test.

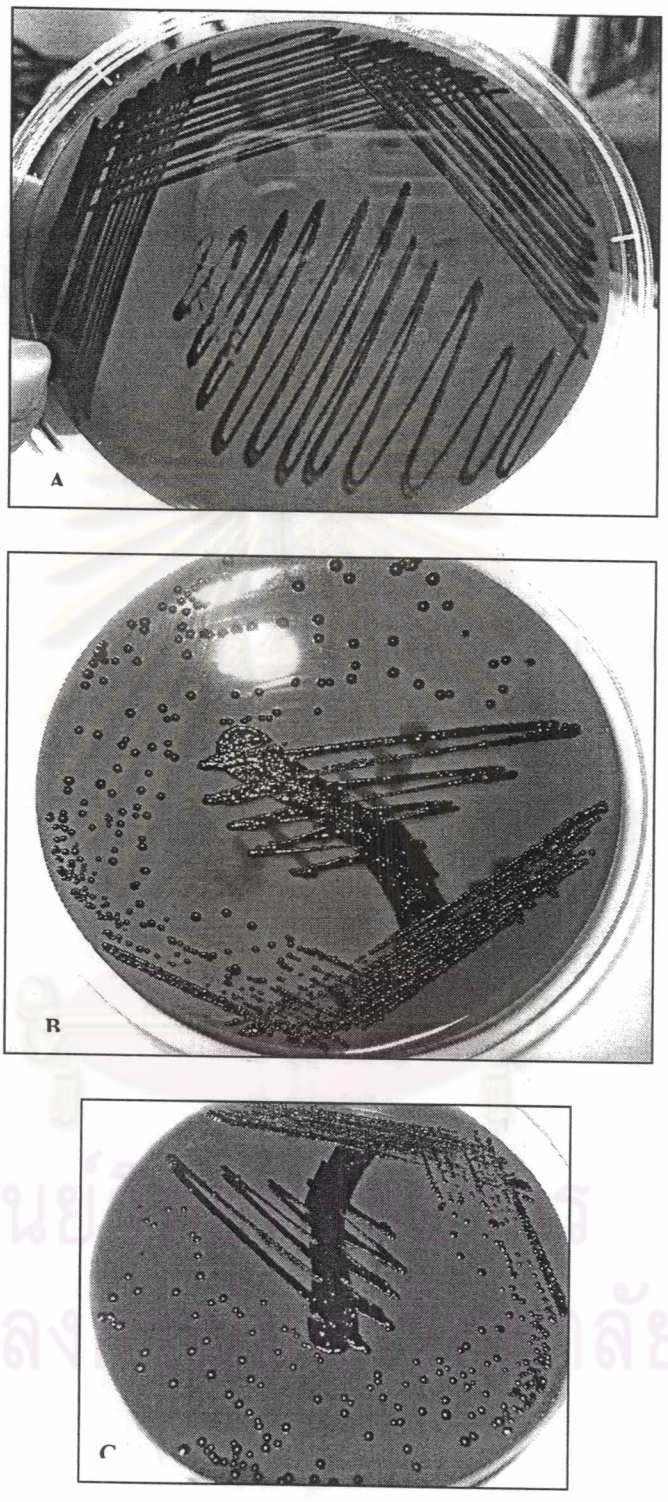


Figure 132 *Porphyromonas gingivalis* in plaque recovered from frozen condition (**A**), inoculated and sub-cultured onto tryptic soy agar (**B**), multiple sub-cultured on plaque agar to obtain isolated colony (**C**).

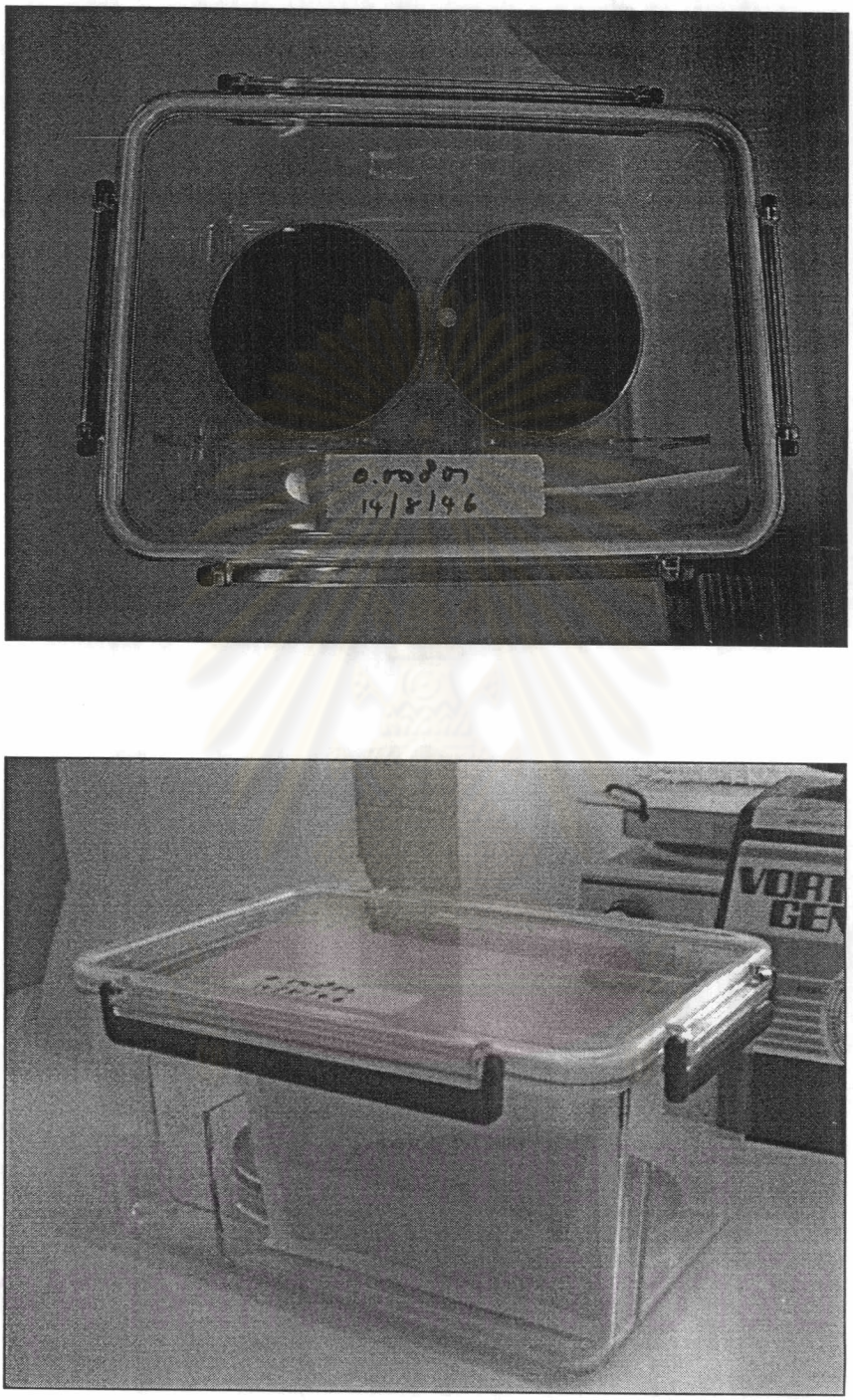


Figure 133 Subcultures and cultures in anaerobic glove box to control anaerobic conditions; the top view (top) and side view (bottom) of anaerobic glove box.

5.1 Antimicrobial activity test of MEG and liquid crystal system using modified agar diffusion method

The formulations from qualified system that passed physicochemical stability test and excellent physical appearance were selected to be formulation for microbial activity test. Individual components, MEG base and MEG containing 1.5% w/w metronidazole were subjected to test microbial activity by modified agar diffusion method. The sample was divided into three groups; first: the composition of each system (castor oil, soybean oil, isopropyl myristate, tween 80, cremophor RH40, cremophor EL and propylene glycol), second: the microemulsion gel base and third group: MEG containing 1.5% w/w metronidazole. The amount of 0.10 gm of each sample was filled into the perforated hole of plaque agar. The plates were incubated in anaerobic glove box at 37°C for 3-5 days. At the end of incubation period, the diameter of inhibition zone was measured. The results were listed into three groups.

The antimicrobial activity of each components of MEG composition (castor oil, soybean oil, isopropyl myristate, tween 80, cremophor RH40, cremophor EL and propylene glycol) showed the minimum inhibition zone of less than 6 mm based on the same quantity and condition. The inhibition zone of cremophor EL was larger than tween 80, cremophor RH40 and castor oil as 5.04, 3.09, 2.04 and 2.03 mm, respectively as shown in Table 26 and Figure 134. Thus, the combination of commonly used pharmaceutical excipients as MEG formulation was shown the ability of anti-microbial activity. The components used for MEG formulation are non-toxic solubilizer for lipophilic drug used in the preparation of a variety of topical, oral and IV and IM injectable medications.

Castor oil, soybean oil, isopropyl myristate, cremophor EL, propylene glycol and lutrol F-68 are widely used parenteral vehicles as non-toxic solubilizer for lipophilic drugs and vitamins. Cremophor EL which did not cause any apparent membrane damage to cell, and lutrol F-68 are being used to enhance absorbtion of drugs through the mucous membranes (Midolo et al, 1995). Microemulsion gel bases were found to exhibit moderate antimicrobial activity as shown in Table 27 and the clear zone of inhibition are shown in Figure 135. The last group; MEG containing 1.5% w/w metronidazole was found to exhibit potent antimicrobial activity as shown in Table 28 and the inhibition zone are shown in Figure 136-137.

Table 26 Antimicrobial activity of each pharmaceutical excipient.

Group 1 pharmaceutical composition	**Mean diameter inhibition zone (mm.)**
control group	0.00± 0.00
isopropyl myristate	1.07± 0.06
castor oil	2.04± 0.04
soybean oil	1.16± 0.14
tween 80	3.09± 0.09
cremophor EL	5.04± 0.05
cremophor RH40	2.02± 0.03

The result of microbial activity test for each excipient in microemulsion gel base by modified agar diffusion, showed that each component of MEG had microbial activity and exhibited the minimum inhibition zone of less than 6 mm.

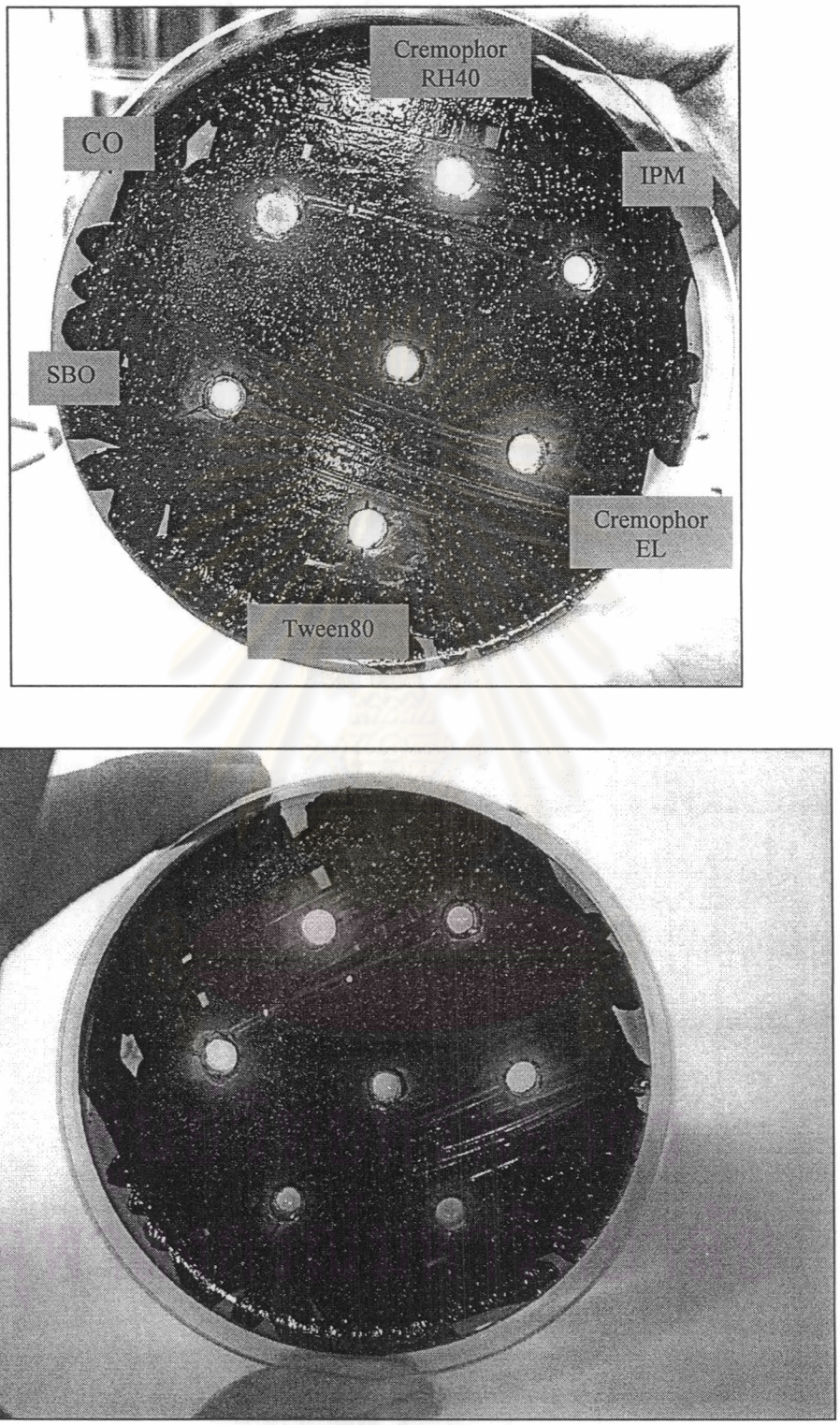


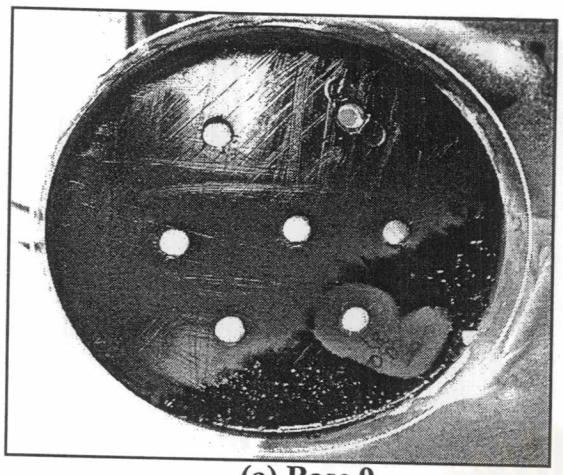
Figure 134 Microbial sensitivity test of the each component of microemulsion gel base; isopropyl myristate, castor oil, soybean oil, tween 80, cremophor EL and cremophor RH40. The duplication of each sample was performed in the same condition.

Table 27 Antimicrobial activity of microemulsion gel base.

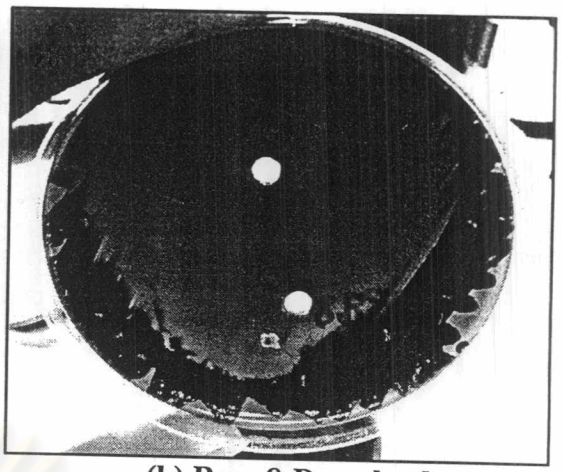
Group 2	MEG base	Mean diameter inhibition zone (mm)
group	Control group	0.00 ± 0.00
Base 2	CO : C_{EL} : W: PG (4:1)	12.12± 0.10
Base 3	IPM : T_{80} : L_{68} : W (T_{80}:L_{68} = 2:1)	14.15± 0.13
Base 4	IPM : C_{EL} : W: PG (4:1)	12.09± 0.09
Base 5	IPM : C_{RH}: W: PG (4:1)	25.03± 0.06
Base 6	IPM : T_{80} : C_{EL} : W	11.04± 0.07
Base 7	IPM : T_{80} : B_{35} : W	19.25± 0.25
Base 8	SBO : T_{80} : W	18.07± 0.12

Table 28 Antimicrobial activity of the MEG containing 1.5% w/w metronidazole.

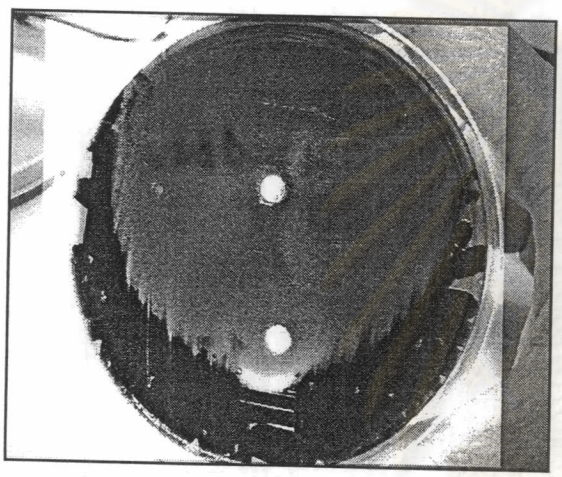
Group 3 1.5% w/w MTZ	System	Ratio of oil:surfactant (% water)	Mean diameter inhibition zone (cm.)		
			freshly prepare	After freeze-thawing	After FDA-stability
CO : C_{EL} : W: PG (4:1)	2/4	3:7 (25%)	7.33± 0.04	7.35± 0.00	7.60± 0.00
IPM : T_{80} : L_{68} : W (T_{80}:L_{68} = 2:1)	3/4	2:5.33:2.6(20%)	7.40± 0.14	7.54± 0.06	7.33± 0.06
IPM : C_{EL} : W: PG (4:1)	4/3	3:7 (25%)	7.55± 0.07	-	-
IPM : C_{RH}: W: PG (4:1)	5/1	3:7 (14.52%)	7.00± 0.00	7.55± 0.07	7.13± 0.11
	5/2	4:6 (20%)	7.39± 0.01	-	-
IPM : T_{80} : C_{EL} : W	6/4	1:4.5:4.5 (water20%)	7.18± 0.11	-	-
IPM : T_{80} : B_{35} : W	7/1	3:3.5:3.5 (water15%)	7.30± 0.07	7.69± 0.01	7.38± 0.04



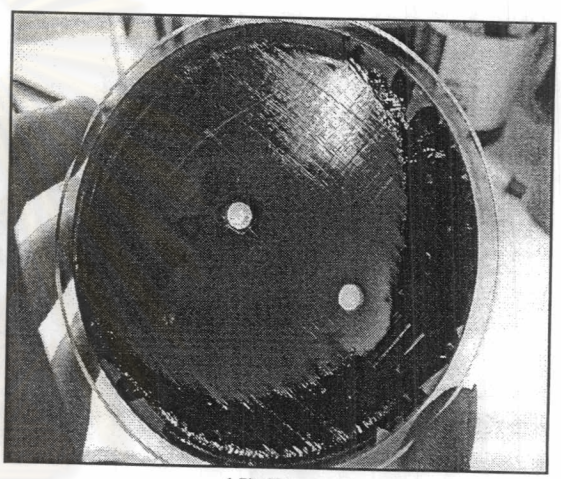
(a) Base 9



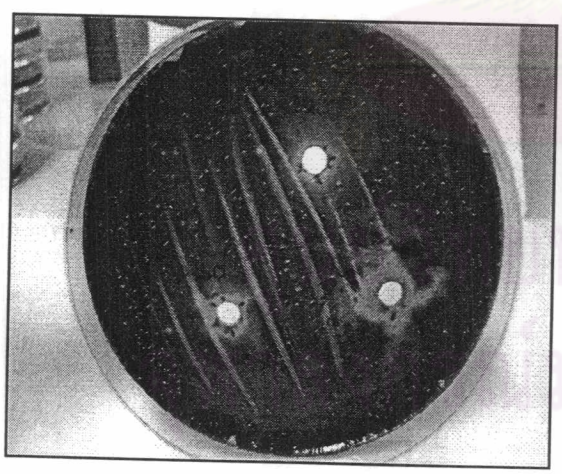
(b) Base 8-Drug load



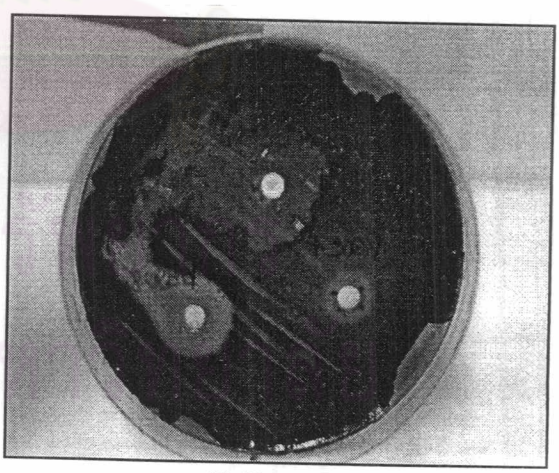
(c) Base 4 - drug load



(d) Base 4



(e) Base 2, 3, 4



(f) Base 5, 6, 7

Figure 135 Inhibition zone diameter of various drug and base system (a) base 8; SBO : T_{80} : W system, (b) base 7; IPM : T_{80} : B_{35} :W system, (c)and (d) base 3; IPM : T_{80} : L_{68} : W(T_{80}:L_{68} = 2:1), (e) inhibition zone of base 2, base 3 and base 4 (f) inhibition zone of base 5, base 6 and base 7.

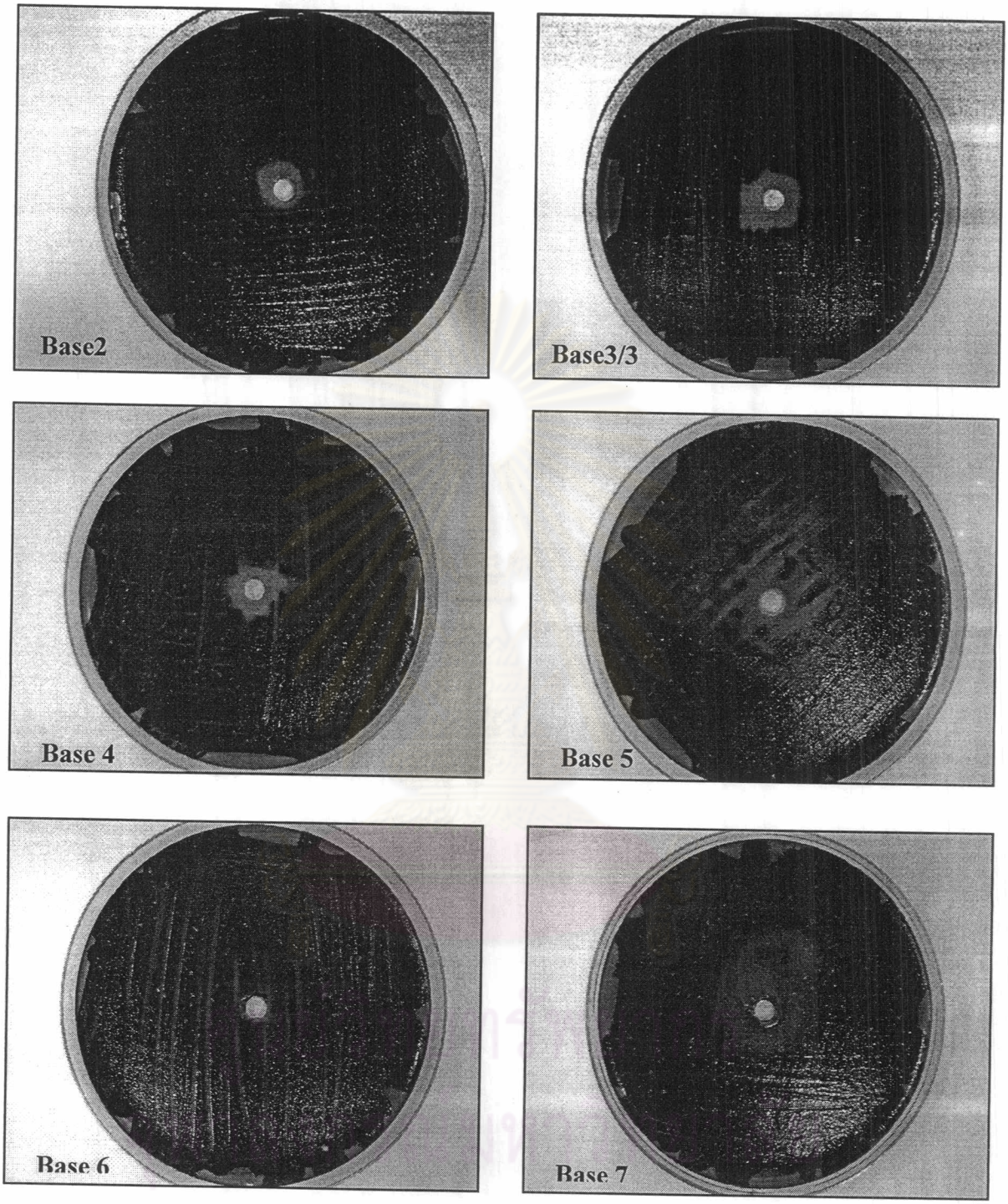
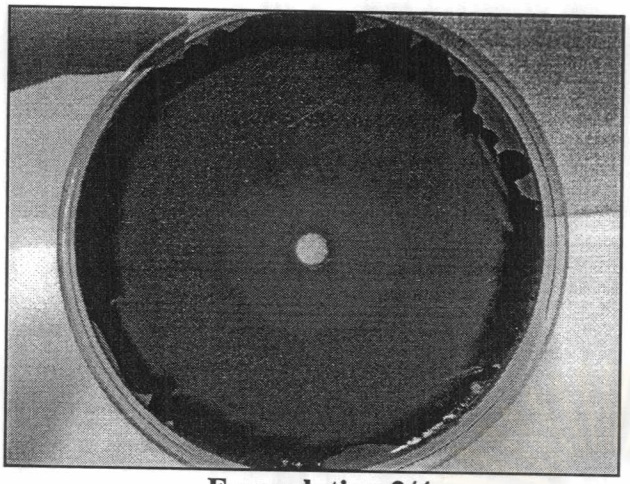
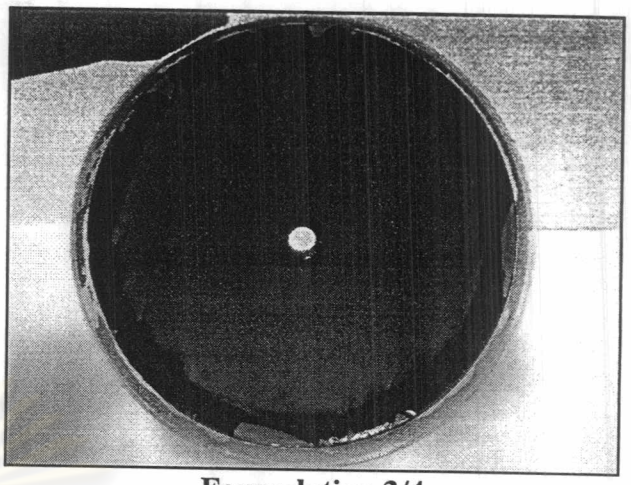


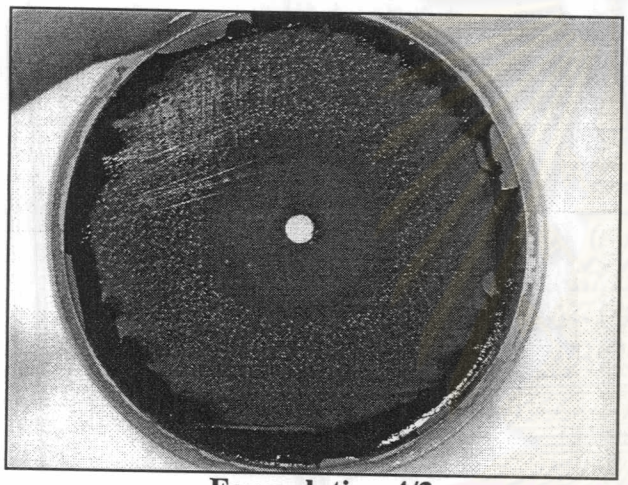
Figure 136 Microbial activity test of microemulsion gel base; base 2 [CO :C_{EL} : W:PG(4:1)], base 3 [IPM : T_{80} : L_{68} : W, (T_{80}:L_{68} = 2:1)], base 4 [IPM : C_{EL} : W: PG(4:1)], base 5 [IPM : C_{RH}: W: PG (4:1)], base 6 IPM : T_{80} : C_{EL} : W,and base 7 [IPM :T_{80} : B_{35} :W].The duplication of each sample were performed in the same condition.



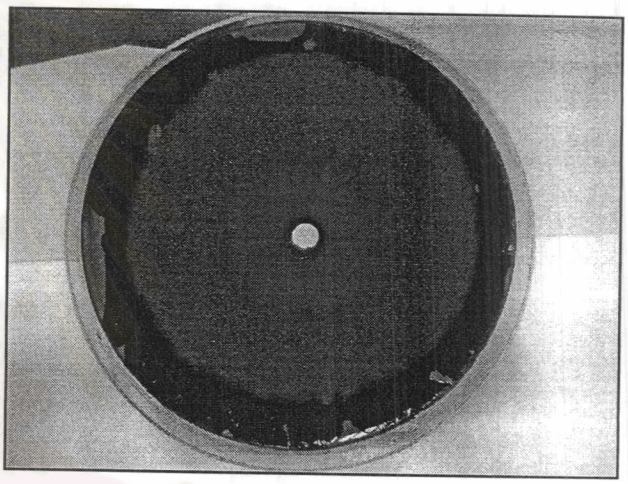
Formulation 2/4



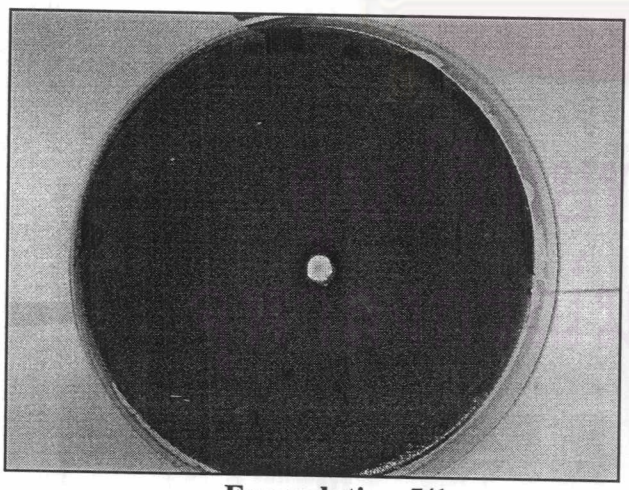
Formulation 3/4



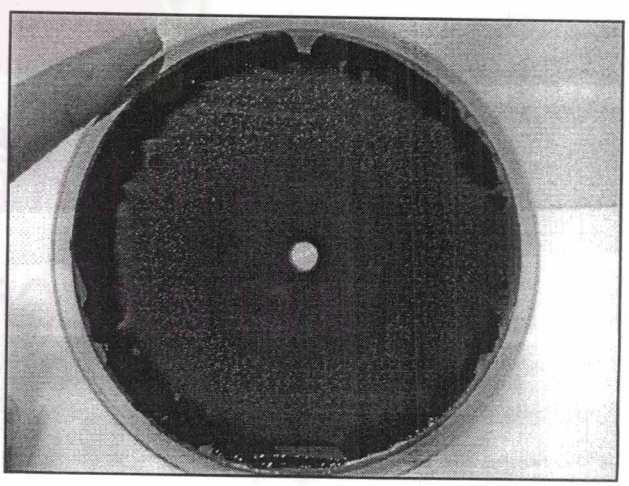
Formulation 4/3



Formulation 5/1

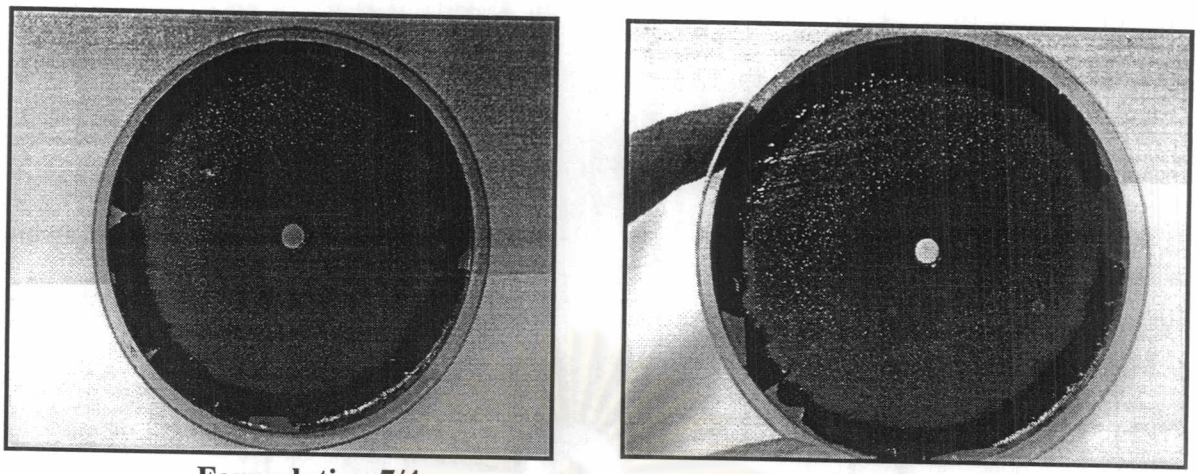


Formulation 5/1



Formulation 5/2

Figure 137 Microbial activity test of freshly prepared 1.5% metronidazole microemulsion: formulation (2/2); CO : C_{EL} : W: PG(4:1), formulation (4/4); IPM : C_{EL} :W: PG(4:1), formulation (5/3); IPM : C_{RH}: W: PG(4:1); formulation (6/1) and (6/2) ; IPM : T_{80} : C_{EL} : W.



Formulation 7/4 **Formulation 8**

Figure 137 (continue) Microbial activity test of freshly prepared 1.5%metronidazole microemulsion; formulation (7/4); IPM : T_{80} : B_{35} :W and formulation 8; SBO : T_{80} : W.

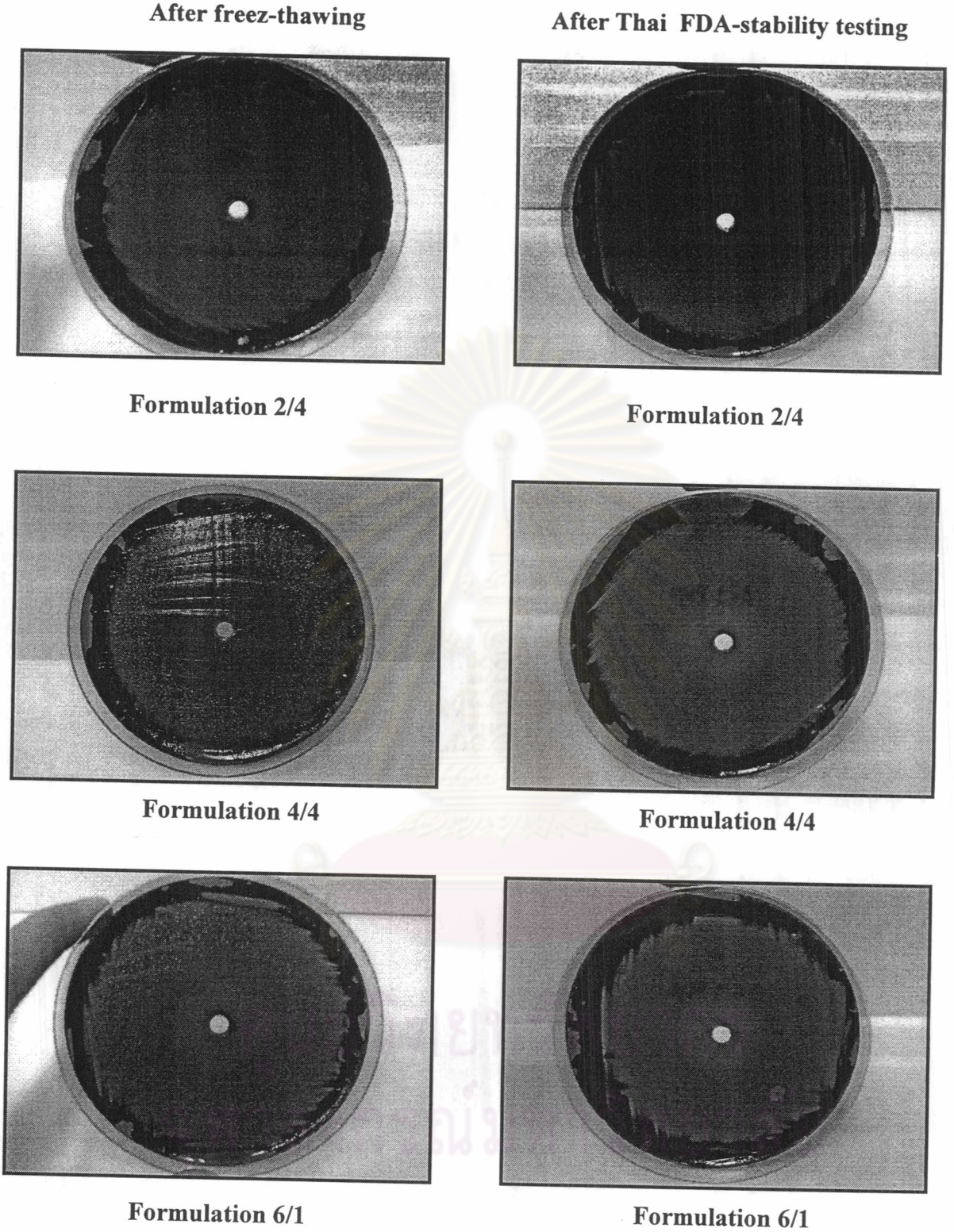
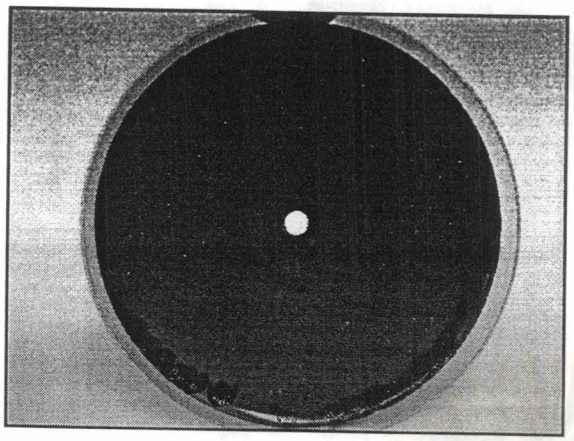
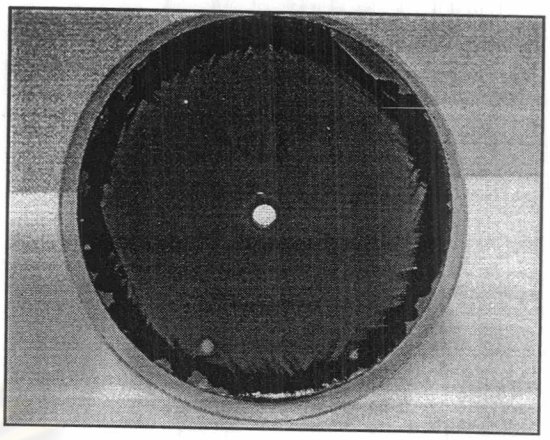


Figure 138 Microbial activity test of 1.5%metronidazole microemulsion after freeze-thawing and FDA stability testing; formulation (2/4), (4/4) and (6/1) after freeze-thawing compared with the same formulation after Thai FDA stability testing.

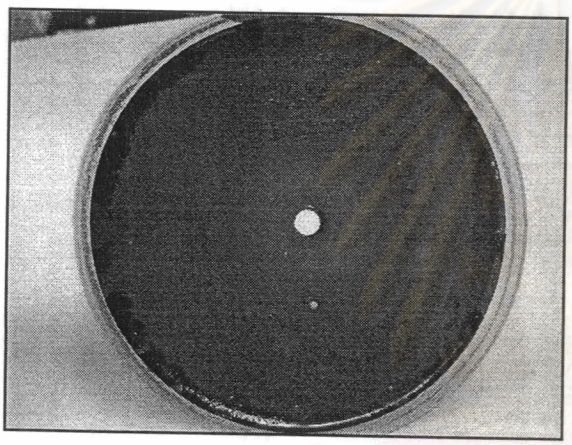
Thai FDA stability testing



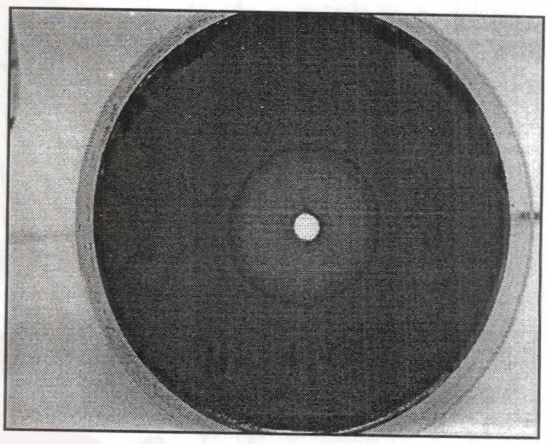
Formulation 8



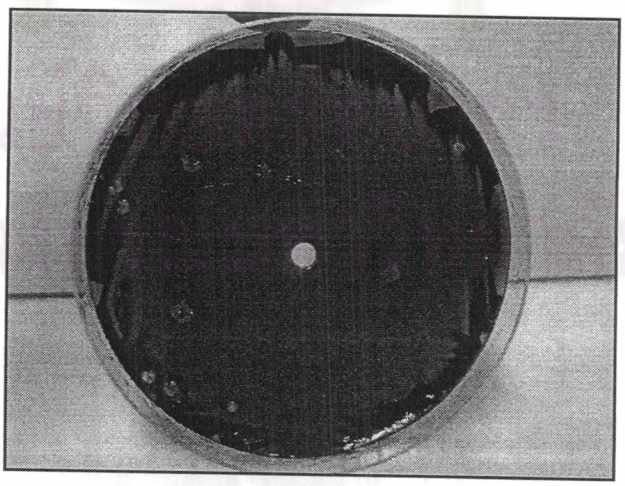
Formulation 8



Formulation 6/1



Formulation 4/4



Formulation 8 (after freeze thawing)

Figure 139 Microbial activity test of 1.5%metronidazole microemulsion after freeze-thawing and FDA stability testing.

Comparison of the average minimum inhibition zone of each MEG base system (mm) by histograms showed that base5; IPM : C_{RH}: W: PG (4:1) which composed of isopropyl as an oil phase, cremophor RH40 and propylene glycol as co-surfactant and co-solvent showed the largest clear inhibition zone by histograms in Figure 175. Comparison of the microbial activity between the base and 1.5%metronidazole MEG found that the clear zone of 1.5% w/w metronidazole MEG in every system was larger than clear zone of MEG base system as shown by histograms in Figure 142. Furthermore, the range of inhibitory zone diameters obtained from all 1.5% w/w metronidazole MEG systems were quite similar and more than 70 mm. (as shown in Table 28 and histograms in Figure 143) which meant that systems had similar antimicrobial activity against anaerobic bacteria representative by *P. gingivalis.* The inhibition zone diameters of three groups of MEG; freshly prepared, after freeze-thawing and after Thai FDA stability testing were not changed significantly. Most systems still had inhibition zone diameters of more than 70 mm is was because of the excellent chemical and physical stability of 1.5% w/w metronidazole MEG.system. Comparison of each component from MEG base by histograms are shown in Figure 140.

Figure 144 shows comparison of inhibition zone of microemulsion gel systems; freshly prepared, freeze-thawing and FDA stability testing.

The inhibition zone of formulation 2/4, 3/4 , 5/1 and 7/1showed no statistically significanly different when compared with both after freeze-thawing and after Thai FDA stability testing. (P>0.05) as shown in Appendix E.

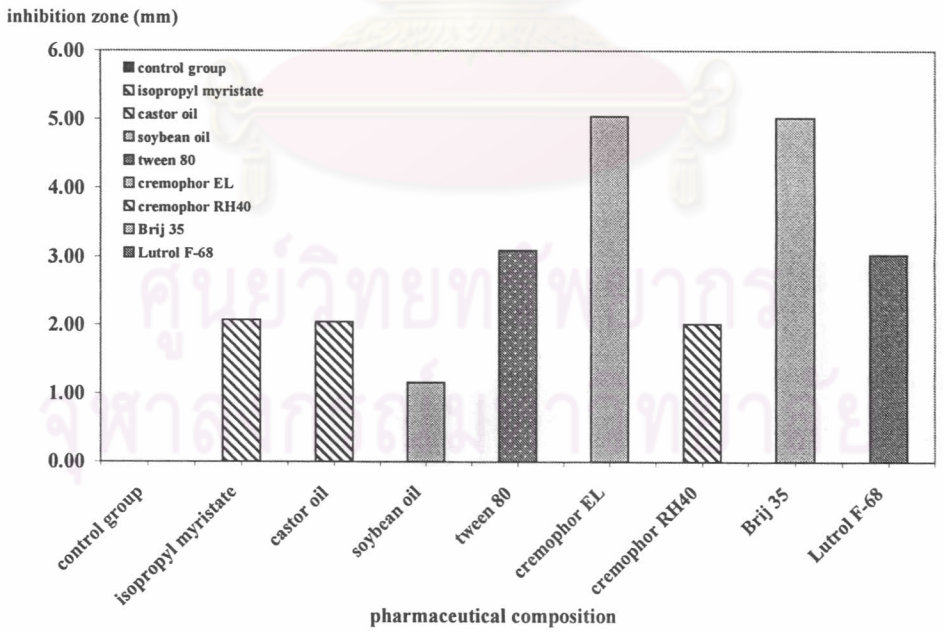


Figure 140 Average minimum inhibition zone of individual components of MEG base.

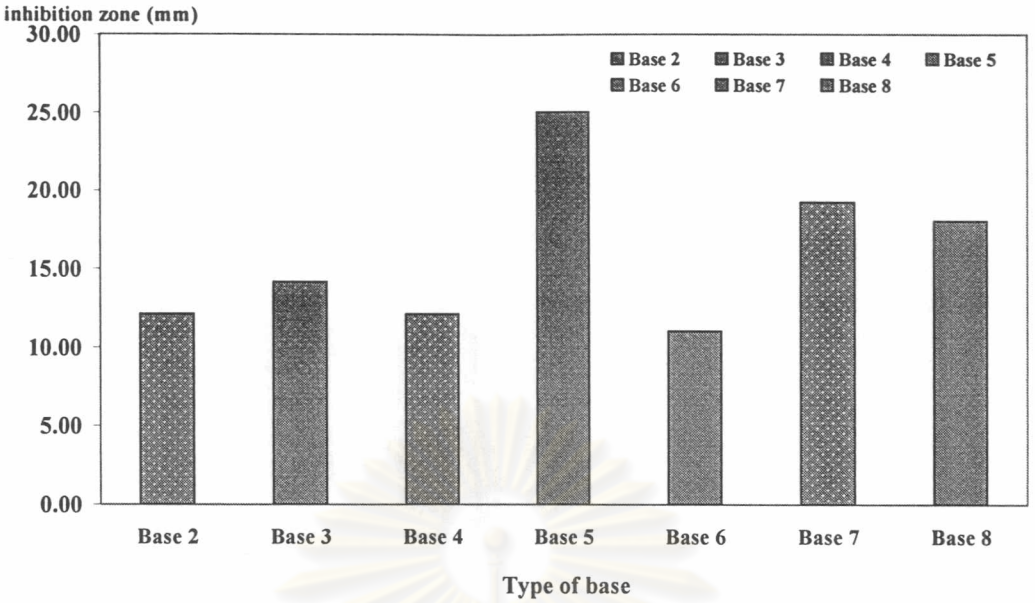


Figure 141 Average minimum inhibition zone of MEG base system (mm).

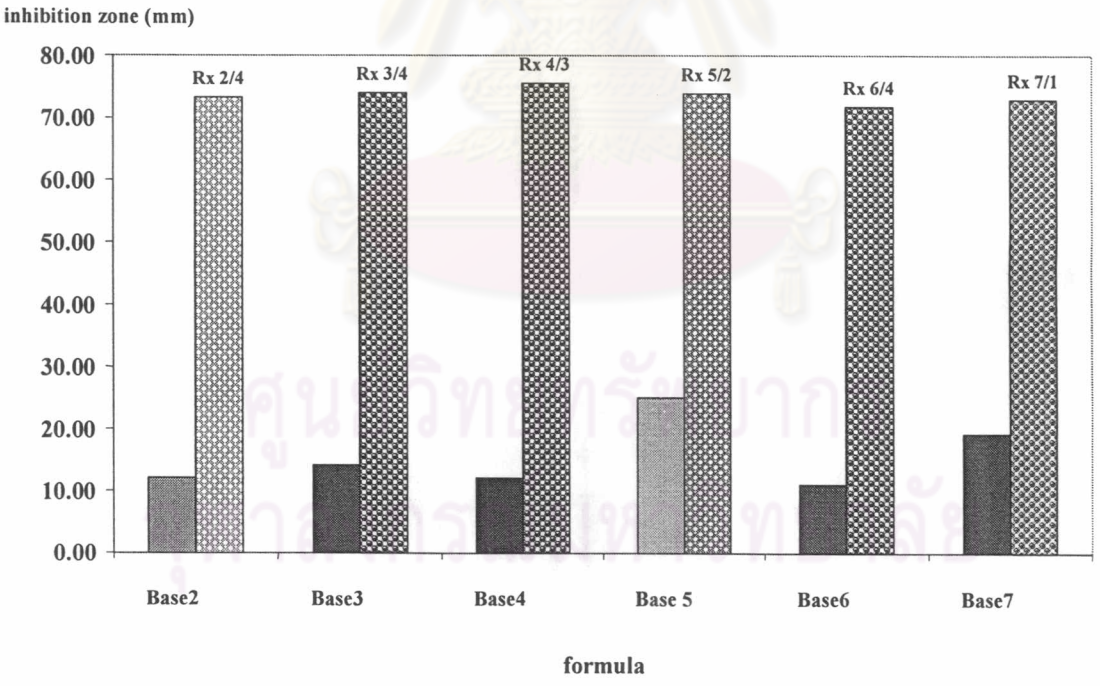


Figure 142 Comparison of microbial activity between base and 1.5% metronidazole MEG.

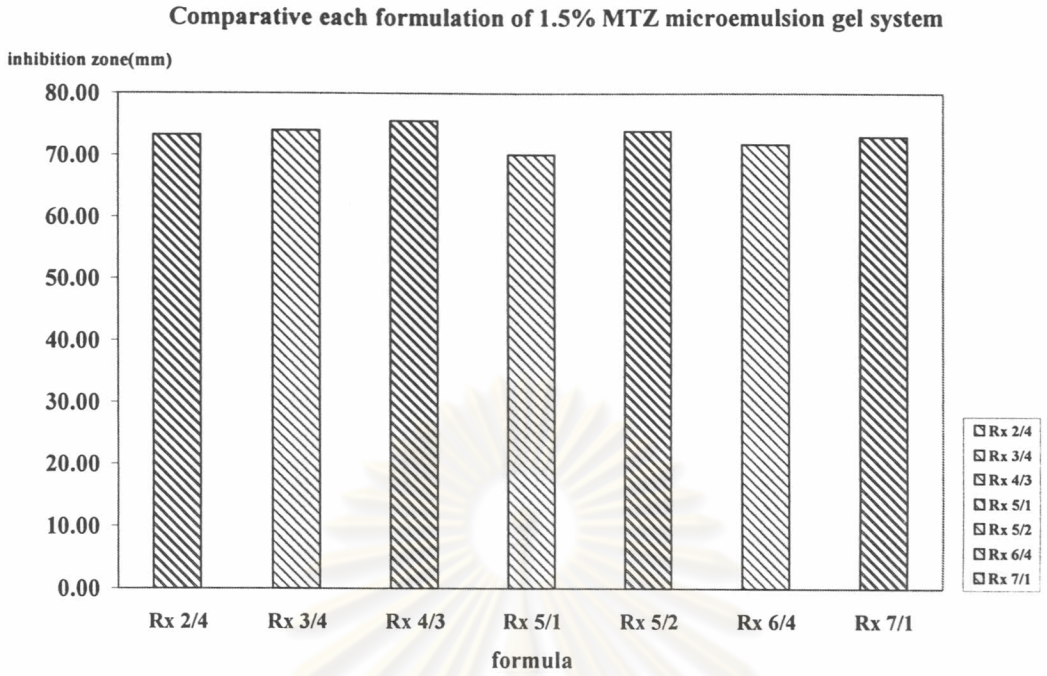


Figure 143 Comparison of microbial activity of 1.5% metronidazole MEG system.

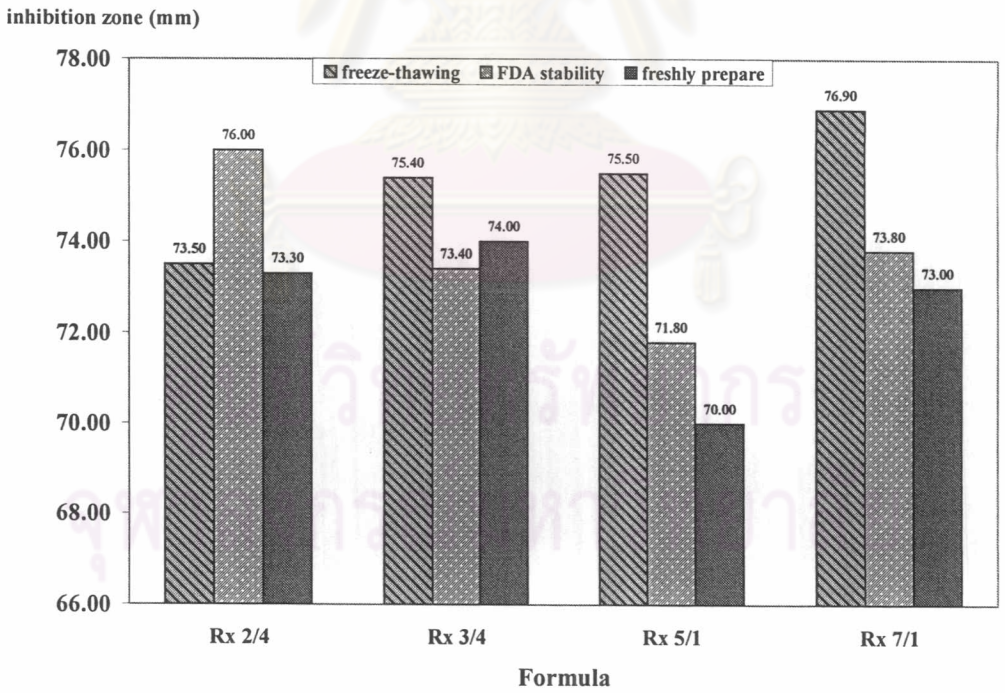


Figure 144 Comparison of inhibition zone of microemulsion gel systems; freshly prepared, freeze-thawing and FDA stability testing.