CHAPTER IV
RESULTS AND DISSCUSSION

1. Determination of solubility of Glucosamine hydrochloride

The solubilitiy of GS HCl in various solvents are displayed in Table 4-1.

Table 4-1 Solubility of glucosamine hydrochloride in various solvents

Solvents	Polarity	Dielectric Constant (ε)	GS HCl Solubility (mg/ml)
Water	Polar	80.4[a]	110.28
PBS 7.4	Polar	-[c]	98.07
Ethanol	Semi Polar	24.3[a]	4.63
Isopropyl myristate	Non Polar	3.3[b]	4.60

[a] From the Pharmaceutical Codex Principles and Practice of Pharmaceutics 12th ed. 1994

[b] From Suwanpidokkul, 2002

[c] No information

The solvents have been classified according to their dielectric constants (ε) into non-polar solvent (ε ≈ 2.3), semi-polar solvent (ε ≈ 12.46), and polar solvent (ε ≈ 80) (Suwanpidokkul, 2002). According to Table 4-1, greatest solubility of GS HCl is in the polar solvent (water) reaching a solubility of 110.28 mg/ml, and the least solubility of GS HCl was found in non-polar solvent (isopropyl myristate), with the solubility of 4.60 mg/ml. The solubility characteristics of GS HCl confirms again that it is a very hydrophilic compound, and can be easily dissolved in water and PBS 7.4. The GS HCl solubility informations obtained could be applied in the future for the

determination of sink condition for receptor medium in the *in vitro* permeation study of GS HCl.

2. Preparation of transdermal formulations

2.1-2.3 Glucosamine hydrochloride solution, hydroalcoholic solution and gel formulation

After preparation the GS HCl solution and hydroalcoholic solution have a similar appearance, colorless and transparent. GS HCl gel appearance is also colorless, with high viscosity. For GS HCl solution and GS HCl hydroalcoholic solution, change in color was observed from colorless to be brown after preparation for 7 days.

Table 4-2 Appearance and physical properties of glucosamine hydrochloride in various dosage forms

Formulation	Composition(%w/w)		Dosage form	Appearance	Physical properties	
	GS HCl	Ethanol			pH	Viscosity[a] (cP)
F1	0.5	-	Aqueous solution	Transparent solution	4.32	< 100
F2	2	-			4.33	
F3	10	-			4.37	
F4	2	9.8	Hydroalcoholic solution	Transparent solution	4.15	< 100
F5	2	58.8			3.91	
F6	2	-	HPMC gel	Transparent gel	4.09	28,554

[a] measured with spindle number 63 and 64 at ambient temperature.

Glucosamine hydrochloride microemulsion

2.4.1 The pseudo-ternary phase diagram

The investigated surfactants can be divided into four groups. (1) anionic surfactant : AOT (2) cationic surfactant : CTAB (3) zwittering ionic surfactant : lecithin and (4) non-ionic surfactant : Cremophore EL, Lutrol E400, Lutrol F 68, Lutrol F 127, span 20, span 40, span 60 and Tween 80. The results obtained by water titration showed the surfactants that could produce microemulsion were AOT, CTAB, Cremophore EL, Lecithin, Solutol HS 15, Tween 80 as shown in Table 4-3.

Table 4-3 The surfactants used in this study and microemulsion formation

Surfactant	Microemulsion formation*
AOT	✓
CTAB	✓
Cremophore EL	✓
Lutrol E 400	-
Lutrol F 68	-
Lutrol F 127	-
Lecithin	✓
Span 20	-
Span 40	-
Span 60	-
Solutol HS 15	✓
Tween 80	✓

* ✓ found

- not found

From Table 4-3, when IPM was used as the oil phase, the microemulsion could be formed by all groups of the surfactants but not all surfactants in each group. The surfactants and systems selected for preparation of the pseudo-ternary phase diagram, and for investigation of the phase behavior were as following :

Phase diagram 1 (P1) : AOT / IPM / Water

Phase diagram 2 (P2) : CTAB / butanol / IPM / Water

Phase diagram 3 (P3) : Lecithin / IPM / Water

Phase diagram 4 (P4) : Tween 80 / IPM / Water

Phase diagram 5 (P5) : Cremophore EL / IPM / Water

Phase diagram 6 (P6) : Solutol HS 15 / IPM / Water

When the pseudo-ternary phase diagram was prepared using the six surfactants above, in the pseudo-ternary phase diagram, the microemulsion system are shown in different regions. An example of the system P6 of Solutol HS 15 / IPM / water is shown in figure 4-1.

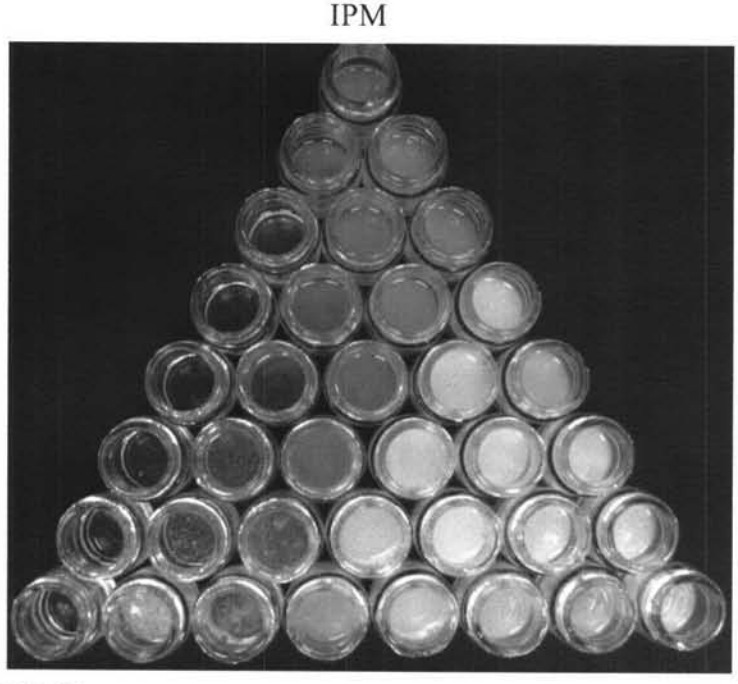

Figure 4-1 Photograph the system of Solutol HS15 / IPM / Water showing the different appearances. The right, left and top angles of the triangle were 100% water,surfactant and oil, respectively.

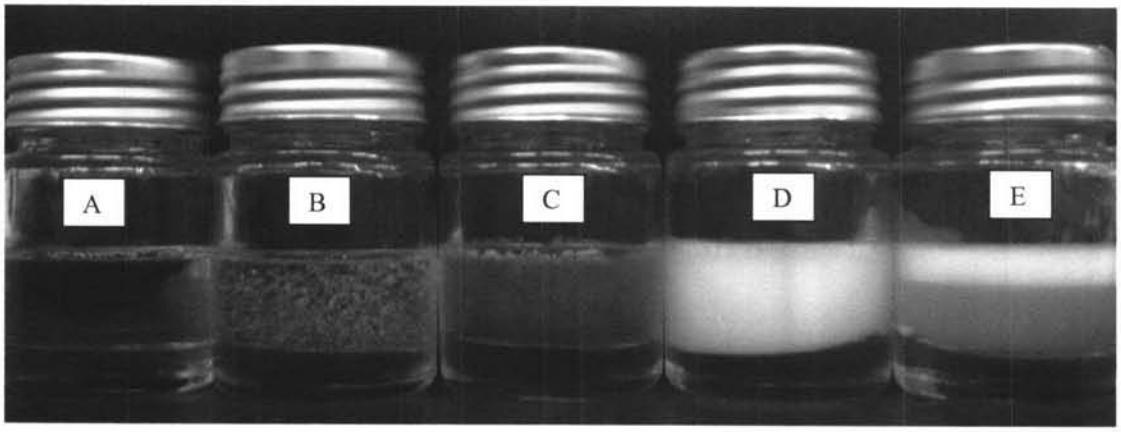

Figure 4-2 Photographs of different regions taken from the ternary phase diagram of solutol HS15 / IPM / water : (A) the transparent and low viscosity area ; (B, C) the transparent and high viscosity area ; (D) the milky white area ; and (E) the separation into two phases area.

When taken from the different area of the pseudo-ternary phase diagram, the products exhibited many systems as shown in Figure 4-2. The transparent and low viscosity area presented in the phase diagram (Figure 4-2 ;A) was the area of microemulsion. The clear and high viscosity region (Figure 4-2 ; B,C) was the area of microemulsion gel. The emulsion region was an area in a milky white (Figure 4-2 ; D) and the other areas outside beyond the above areas are separated into two phases (Figure 4-2 ; E). The phase behavior from the different microemulsion systems exhibited different behaviors. As shown in Figures 4-3-4-6, In the system of AOT / IPM /water, microemulsion and microemulsion gel formation was favorable at high surfactant concentrations over 70%. Also, at higher water concentration, the system tended to separate into two phases as shown in Figure 4-3. In the system of the CTAB / IPM / water that, the ratio of surfactant and co-surfactant (CTAB and butanol) was kept constant (2:1). The microemulsion was formed in the area of high contents of the surfactant and water. At the higher surfactant concentration, the microemulsion gel was found. In this phase diagram, the turbid region was found when concentration of oil was increased as shown in Figure 4-4.

In the system of lecithin (PC) / IPM / water, it did not exhibit area of microemulsion because this surfactant needs a co-surfactant to form microemulsion. So this system in which co-surfactant was not used, exhibited only a small area of liquid crystal. At the ratio of lecithin to IPM 50:40 as shown in Figure 4-5.

For the three non-ionic surfactant, Tween 80, Cremophore EL and Solutol HS 15, they exhibited similar phase behavior. The microemulsion was formed at very low concentration of water and wide range of concentration of surfactant and IPM. In addition when the concentration of surfactant was increased, the microemulsion gel region was found.

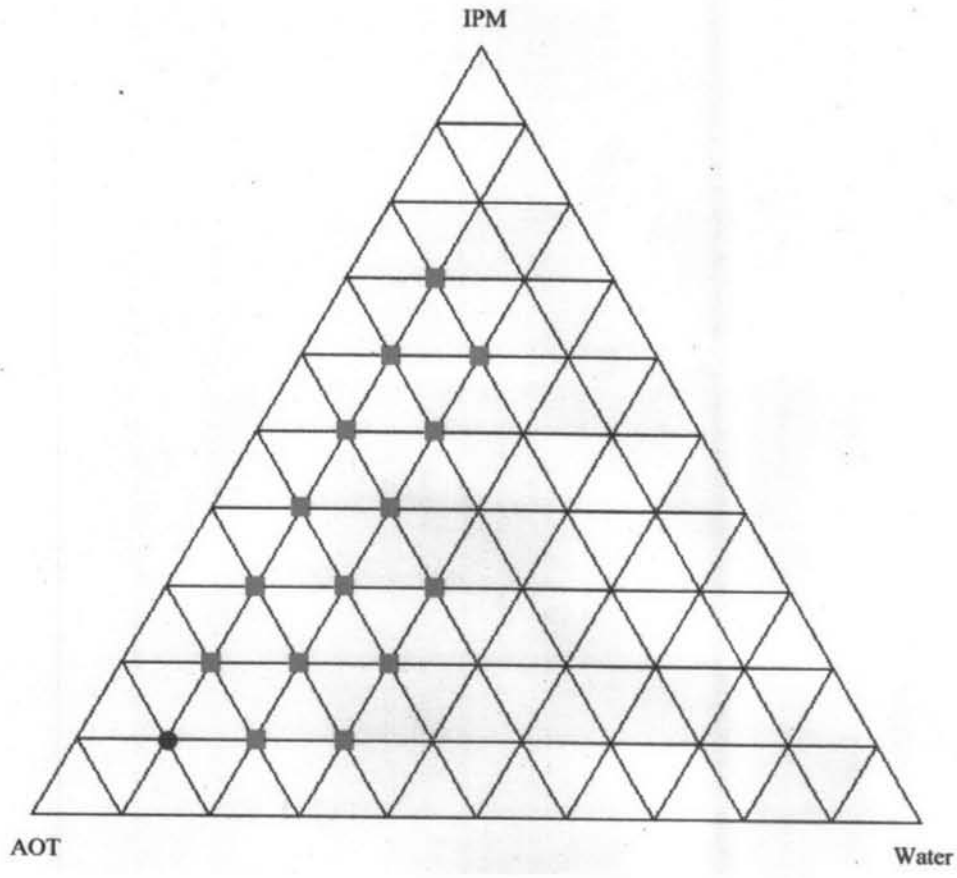

Figure 4-3 Pseudo-ternary phase diagram for the system of IPM / AOT / water. Microemulsion in region ■ and microemulsion gel in region ●. The right, left and top angles of the triangle were 100% water, surfactant and oil, respectively.

Table 4-4 Ratio of IPM : AOT : water that could form microemulsion and microemulsion gel

No.	Ratio of IPM:AOT:water (%)	Found	No.	Ratio of IPM:AOT:water (%)	Found	No.	Ratio of IPM:AOT:water (%)	Found
A2	10:80:10	ME gel	A21	30:60:10	ME	A37	50:30:20	ME
A3	10:70:20	ME	A22	30:50:20	ME	A42	60:30:10	ME
A4	10:60:30	ME	A23	30:40:30	ME	A43	60:20:20	ME
A12	20:70:10	ME	A29	40:50:10	ME	A47	70:20:10	ME
A13	20:60:20	ME	A30	40:40:20	ME			
A14	20:50:30	ME	A36	50:40:10	ME			

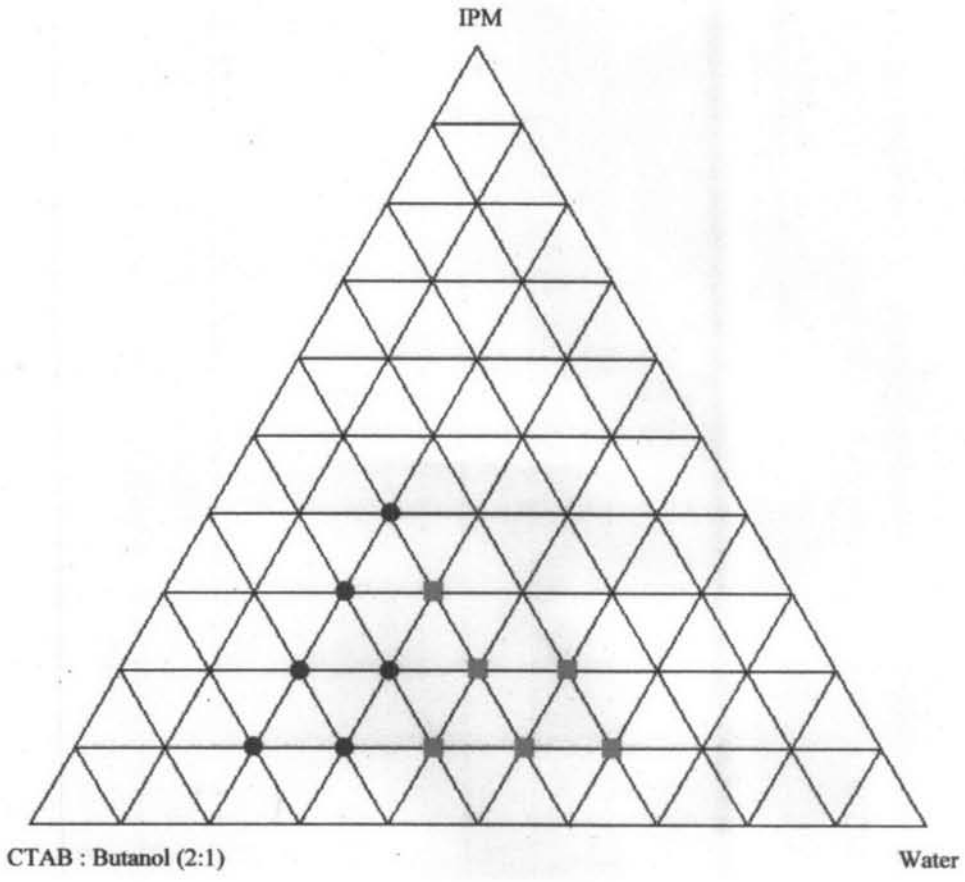

Figure 4-4 Pseudo-ternary phase diagram from the system of IPM / CTAB : butanol(2:1) / water. Microemulsion in region ■ and microemulsion gel in region ● The right, left and top angles of the triangle were 100% water, surfactant and oil, respectively.

Table 4-5 Ratio of IPM : CTAB and butanol (2:1): water that could form microemulsion and microemulsion gel

No.	Ratio of IPM : surfactant* :water (%)	Found	No.	Ratio of IPM : surfactant* :water (%)	Found	No.	Ratio of IPM : surfactant* :water (%)	Found
A3	10:70:20	ME gel	A7	10:30:60	ME	A16	20:30:50	ME
A4	10:60:30	ME gel	A13	20:60:20	ME gel	A22	30:50:20	ME gel
A5	10:50:40	ME	A14	20:50:30	ME gel	A23	30:40:30	ME
A6	10:40:50	ME	A15	20:40:40	ME	A30	40:40:20	ME gel

* surfactant and co-surfactant in ratio 2:1 of CTAB : n-butanol

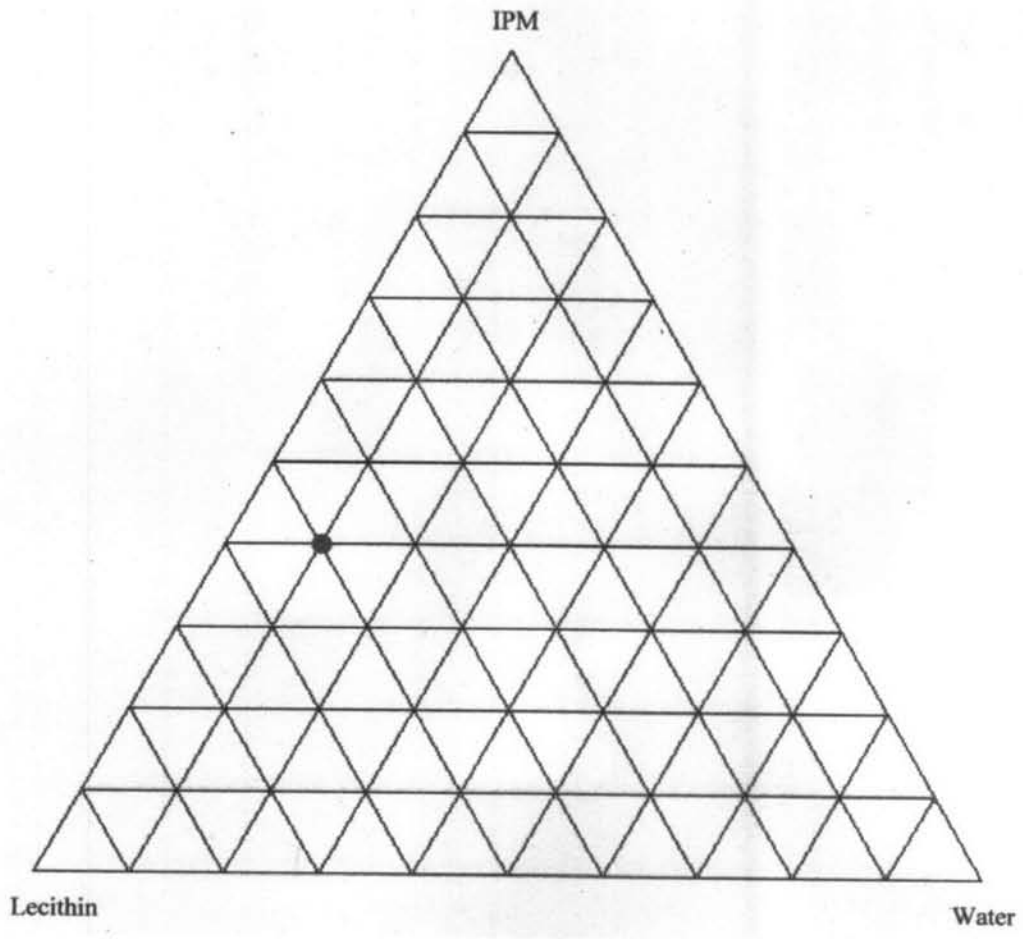

Figure 4-5 Pseudo-ternary phase diagram from the system of IPM / lecithin / water. Liquid crytal shown in region●. The right, left and top angles of the triangle were 100% water, surfactant and oil, respectively.

Table 4-6 Ratio of IPM : lecithin : water that could form liquid crytal

No.	Ratio of : IPM : lecithin : water (%)	Found
A29	40:50:10	liquid crytal

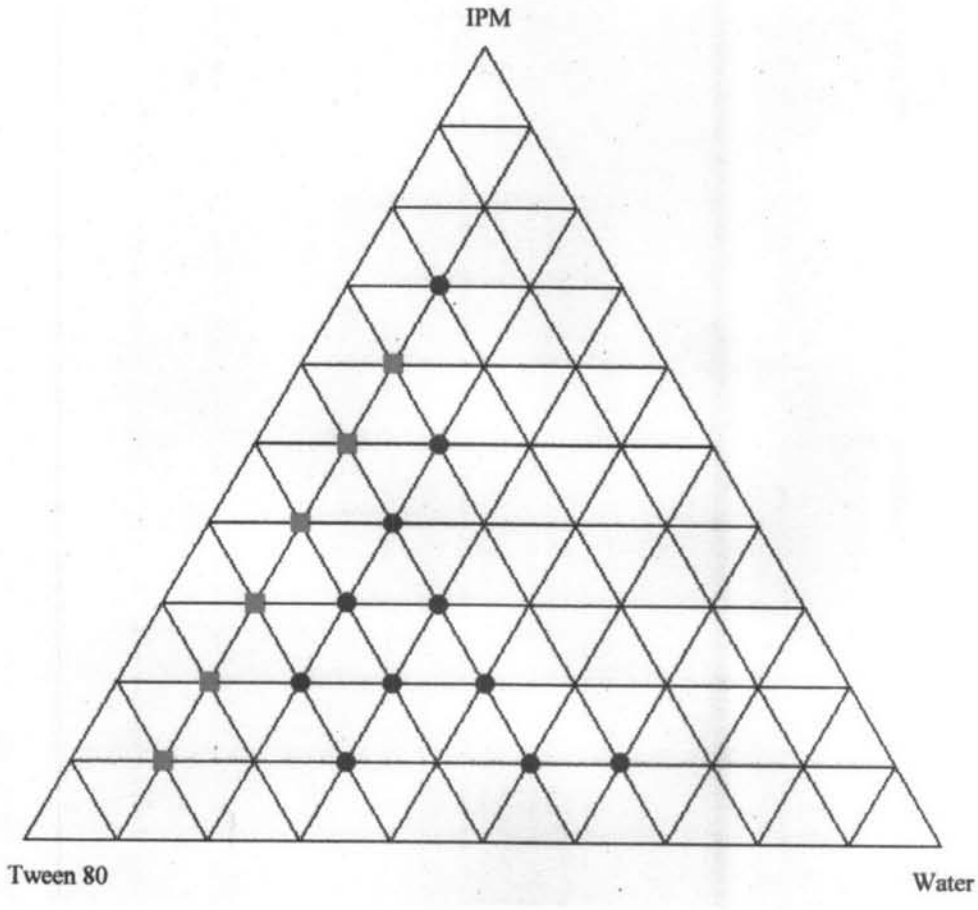

Figure 4-6 Pseudo-ternary phase diagram from the system of IPM / Tween 80 / water. Microemulsion in region ■ and microemulsion gel in region ● .The right, left and top angles of the triangle were 100% water, surfactant and oil, respectively.

Table 4-7 Ratio of IPM : Tween 80 : water that could form microemulsion and microemulsion gel

No.	Ratio of IPM :Tween 80 :water (%)	Found	No.	Ratio of IPM :Tween 80 :water (%)	Found	No.	Ratio of IPM :Tween 80 :water (%)	Found
A2	10:80:10	ME gel	A14	20:50:30	ME	A30	40:40:20	ME
A4	10:60:30	ME	A15	20:40:40	ME	A36	50:40:10	ME gel
A6	10:40:50	ME	A21	30:60:10	ME gel	A37	50:30:20	ME
A7	10:30:60	ME	A22	30:50:20	ME	A42	60:30:10	ME gel
A12	20:70:10	ME gel	A23	30:40:30	ME	A47	70:20:10	ME gel
A13	20:60:20	ME	A29	40:50:10	ME gel			

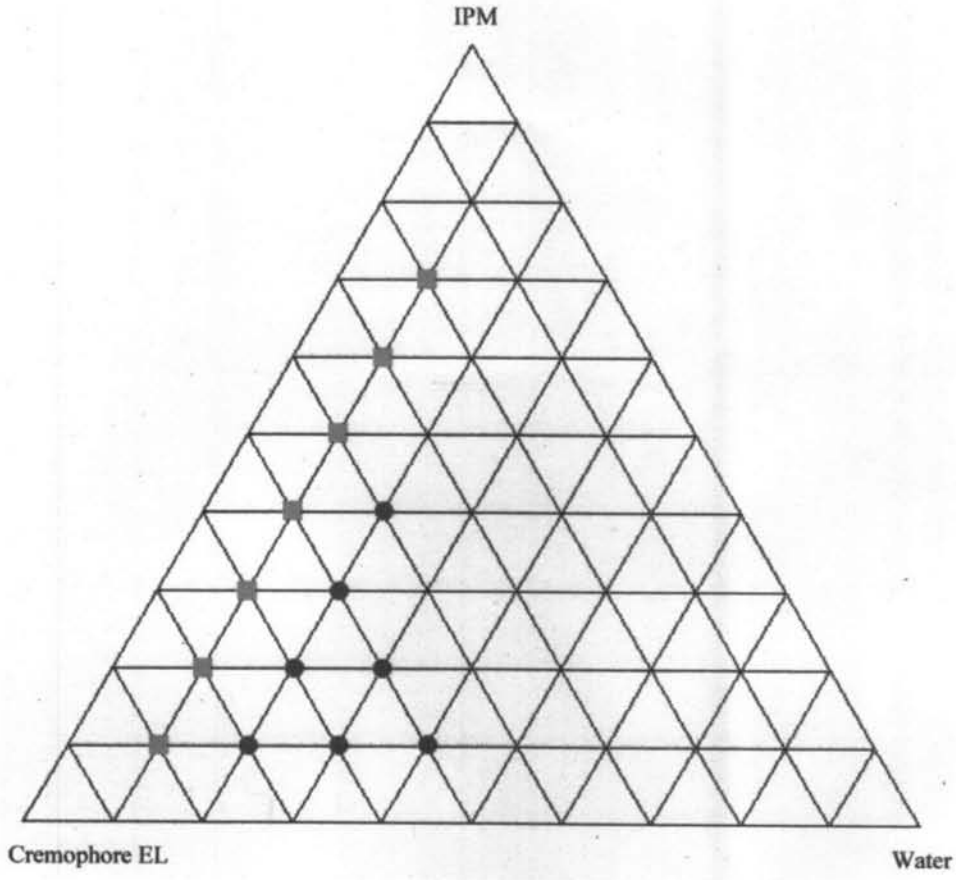

Figure 4-7 Pseudo-ternary phase diagram from the system of IPM / Cremophore EL / Water. Microemulsion in region ■ and microemulsion gel in region●. The right, left and top angles of the triangle were 100% water, surfactant and oil, respectively.

Table 4-8 Ratio of IPM : Cremophore EL : water that could form microemulsion and microemulsion gel

No.	Ratio of IPM :Cremophore EL :water (%)	Found	No.	Ratio of IPM :Cremophore EL :water (%)	Found	No.	Ratio of IPM :Cremophore EL :water (%)	Found
A2	10:80:10	ME	A13	20:60:20	ME gel	A30	40:40:20	ME gel
A3	10:70:20	ME gel	A14	20:50:30	ME gel	A36	50:40:10	ME
A4	10:60:30	ME gel	A21	30:60:10	ME	A42	60:30:10	ME
A5	10:50:40	ME gel	A22	30:50:20	ME gel	A47	70:20:10	ME
A12	20:20:10	ME	A29	40:50:10	ME			

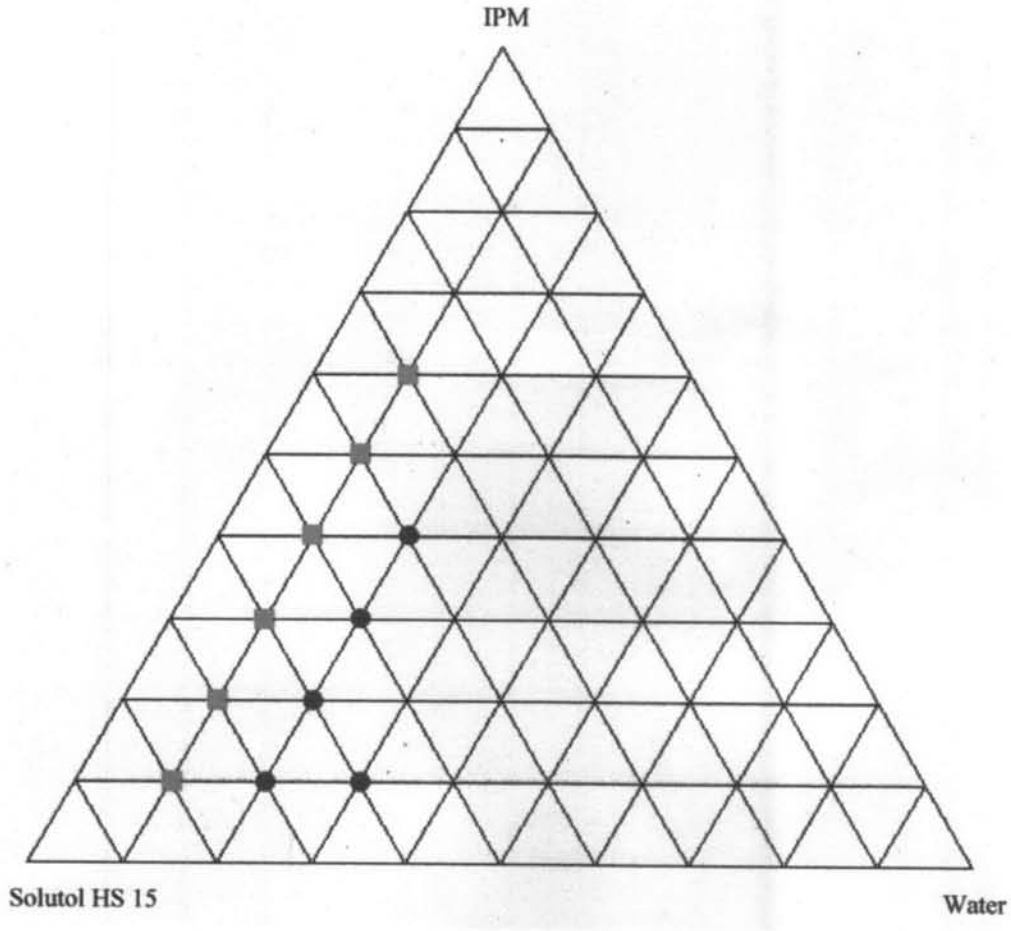

Figure 4-8 Pheudo-ternary phase diagram from the system of IPM / Solutol HS 15 / Water. Microemulsion in region ■ and microemulsion gel in region ●. The right, left and top angles of the triangle were 100% water, surfactant and oil, respectively.

Table 4-9 Ratio of IPM : Solutol HS 15 : water that could form microemulsion and microemulsion gel

No.	Ratio of IPM :Solutol HS 15 :water (%)	Found	No.	Ratio of IPM :Solutol HS 15 :water (%)	Found	No.	Ratio of IPM :Solutol HS 15 :water (%)	Found
A2	10:80:10	ME	A13	20:60:20	ME gel	A30	40:40:20	ME gel
A3	10:70:20	ME gel	A21	30:60:10	ME	A36	50:40:10	ME
A4	10:60:30	ME gel	A22	30:50:20	ME gel	A42	60:30:10	ME
A12	20:70:10	ME	A29	40:50:10	ME			

2.4.2 Drug incorporation

After preparation of the pseudo-ternary phase diagram and the "clear" region was observed, the stable systems, which was not separated into two phases overnight after preparation, were found in the systems of AOT, CTAB, Lecithin and Tween 80. These systems were selected for investigation of the maximum drug loading. The composition of each system are shown in Table 4-4.

Table 4-10 Composition of microemulsions for investigation of the maximum drug loading

Formulation	Composition (%)					Maximum loading amount (%)
	Surfactant	Water	IPM	Surfactant	Cosurfactant (n-butanol)	
L1	AOT	17	41	42	-	3
L2	CTAB	20	40	27	13	5
L3	Lecithin	15	40	35	10	2
L4	Tween 80	57	8	35	-	>15

The results showed that the amount of GS HCl loading in Tween 80 microemulsion system was the maximum, where the amount of GS HCl loading in AOT microemulsion system was the minimum. When considering the ratio of composition in those microemulsion systems, it was shown that the amount of GS HCl loading were veried according to the ratio of the water phase.

It has been reported that, the amount of drug which can be incorporated into microemulsion depends on both the microemulsion structure and the properties of the drug (Malmsten, 2002). GS HCl is soluble in water, dissociates and becomes charged. It behaves largely as a salt, and might therefore has a significant impact on the formation of microemulsion by ionic surfactant, whereas those formed by nonionic surfactants are less sensitivity to the presence of drug. Accordingly the drug loading amount in Tween 80 microemulsion are the most.

In addition, GS HCl microemulsion of different surfactants exhibited a different appearance, as shown in Figure 4-9. In the systems of AOT and CTAB system, they exhibited no color, whereas Tween 80 and lecithin systems exhibited a yellow microemulsion. The color of the microemulsion result from the color of its surfactant.

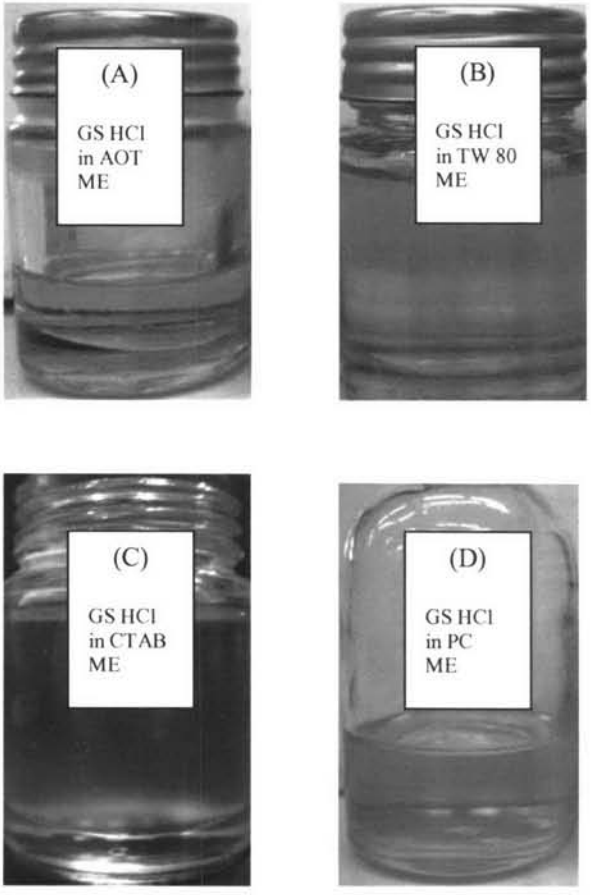

Figure 4-9 Glucosamine hydrochloride microemulsion prepared with various surfactants (A) AOT microemulsion (B) Tween 80 microemulsion (C) CTAB microemulsion (D) lecithin (PC)microemulsion

From the results that are described above, the surfactants and ratio of compositions were selected for preparation microemulsions to study physical properties and *in vitro* permeation. An antioxidant, BHT , was added in lecithin and Tween 80 formulations for improving their stability. The glucosamine hydrochloride microemulsions composition are showed in Table 4-10.

Table 4-11 Glucosamine hydrochloride microemulsion composition for investigation of physical properties and *in vitro* permeation studies

Formulation	surfactant	Composition (%)					
		Glucosamine	water	IPM	Surfactant	Cosurfactant (n-butanol)	Antioxidant (BHT)
F7	AOT	2	15	41	42		
F8	CTAB	2	18	40	27	13	
F9	Lecithin	0.5	12.9	41.5	35	10	0.1
F10	Lecithin	2	12.9	40	35	10	0.1
F11	Tween80	0.5	56.4	8	35		0.1
F12	Tween80	2	54.9	8	35		0.1
F13	Tween80	2	14.9	33	50		0.1
F14	Tween80	2	57.9	3	37		0.1
F15	Tween80	10	46.9	8	35		0.1

2.4.3 Evaluation of microemulsion dosage form

2.4.3.1 Physical properties

Table 4-12 Physical properties of glucosamine hydrochloride microemulsion

formulation	Surfactant	GS HCl (%)	Physical properties		
			Color / clarity	Viscosity (cP)[a]	pH
F7	AOT	2	no/transparent	Below 100	4.96
F8	CTAB	2	no/transparent	Below 100	3.47
F9	Lecithin	0.5	yellow/transparent	Below 100	5.50
F10	Lecithin	2	yellow/transparent	Below 100	5.51
F11	Tween 80	0.5	yellow/transparent	920.6	3.84
F12	Tween 80	2	yellow/transparent	933.4	3.96
F13	Tween 80	2	yellow/transparent	1,084.2	3.27
F14	Tween 80 (ME Gel)	2	yellow/transparent	2,640.2	3.57
F15	Tween 80	10	yellow/transparent	930.6	3.99

[a] measured with spindle number 63 and 64 at ambient temperature.

Table 4-13 The changing of physical appearance of glucosamine microemulsion preparation after 3, 7 and 30 days

Formulation	Surfactant	Physical appearance changing		
		3 days	7 days	30 days
F7	AOT	No change	Phase separating and precipitation	Phase separating and precipitation, brown precipitate
F8	CTAB	Phase separating	Phase separating and precipitation	Phase separating and precipitation, brown precipitate
F10	Lecithin	No change	No change	Little brown color
F12	Tween 80	No change	No change	No change

The changing of physical appearance of those microemulsion were phase separating, precipitation and browning in color. GS HCl microemulsion prepared from Tween 80 showed better stability than the other systems, as shown in Table 4-8. The changing in color to brown color was not only found in microemulsion dosage form, but also found in other dosage forms, solution, hydroalcoholic solution and gel, so the browning color is significant stability problem of GS HCl dosage form.

The browning color is caused by a reaction of carbonyl-amino groups that found in the substance such as aldehydes, ketone and reducing sugar with amine, amino acid, peptides and proteins. Machanism of browning in sugar-amine systems occur in three stages.

1. Initial stage, caused by (A) sugar-amine condensation and (B) Amadori rearrangement, exhibited no color changing.
2. Intermediate stage, caused by (C) sugar dehydration and (D) sugar fragmentation, (E) Amino acid degradation, exhibited no color changing or yellowing.
3. Final stage, caused by (F) adol condensation and (G) aldehyde-amine polymenzation to formation of heterocyclic nitrogen polymers and copolymers as shown in Figure 4-10.

From the sugar-amine reaction as described above, the GS HCl dosage forms were possibly influenced by many factor such as chemical structure of GS HCl, having sugar and amine in the structure, water in the dosage form and CO_2 in the environment. The changing to brown color after storage of these GS HCL preparations were exhibited and were found to develop more quickly in GS HCl solution than the other dosage form.

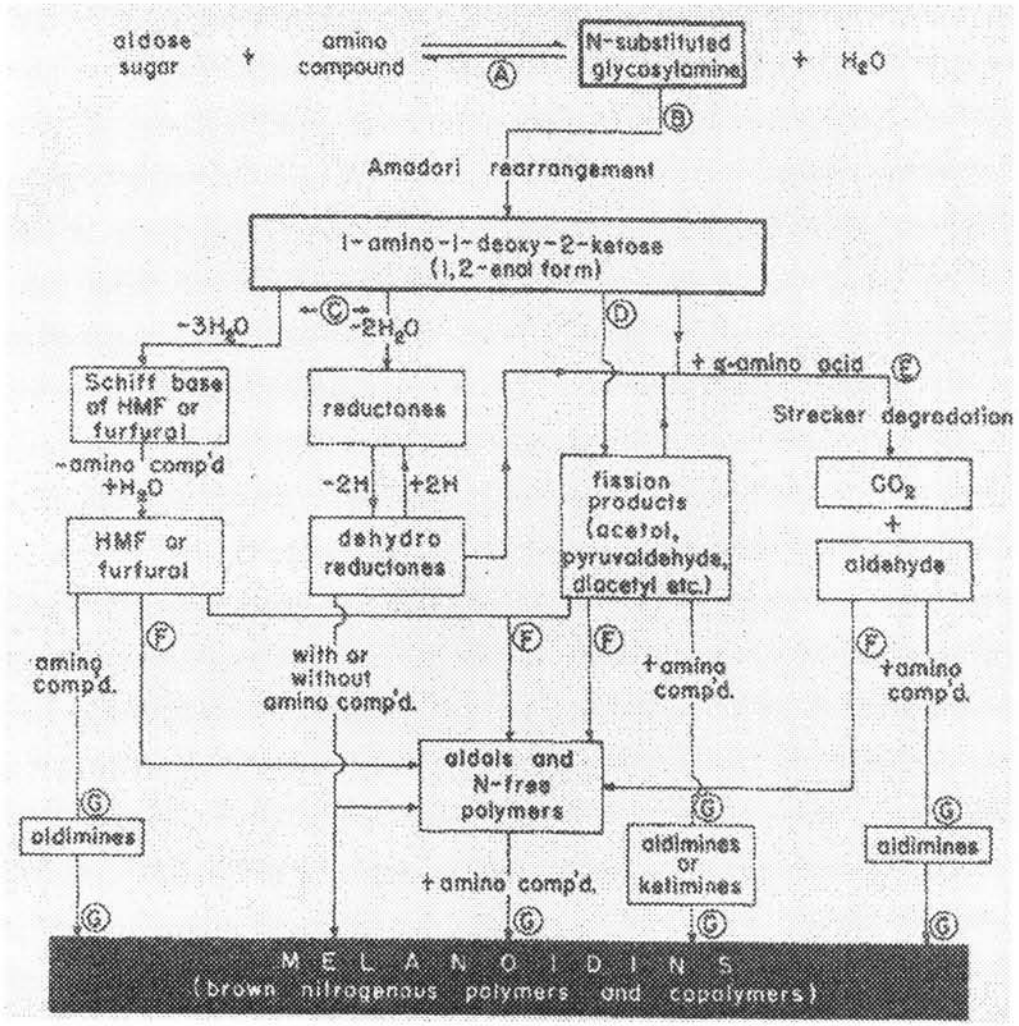

Figure 4-10 The browning in sugar-amine system (Obtained from Hodge, 1953)

2.4.3.2 Determination type of microemulsion

When adding 1% of the color dye, Brilliant blue, in the GS HCl microemulsions after preparation, the results are shown in Figure 4.10 and 4.11. The dye color dissolved in GS HCl microemulsion, that were prepared from CTAB (formulation F8), Tween 80 (formulation F12). Thus the microstructure of these system was oil in water (o/w). And in GS HCl microemulsion that were prepared from AOT (formulation F7) Tween 80 (formulation F13) and lecithin (formulation F10), it was found that the dye color could not dissolve in these systems, and the microstructure of those microemulsion was assumed to be water in oil microemulsion (w/o).

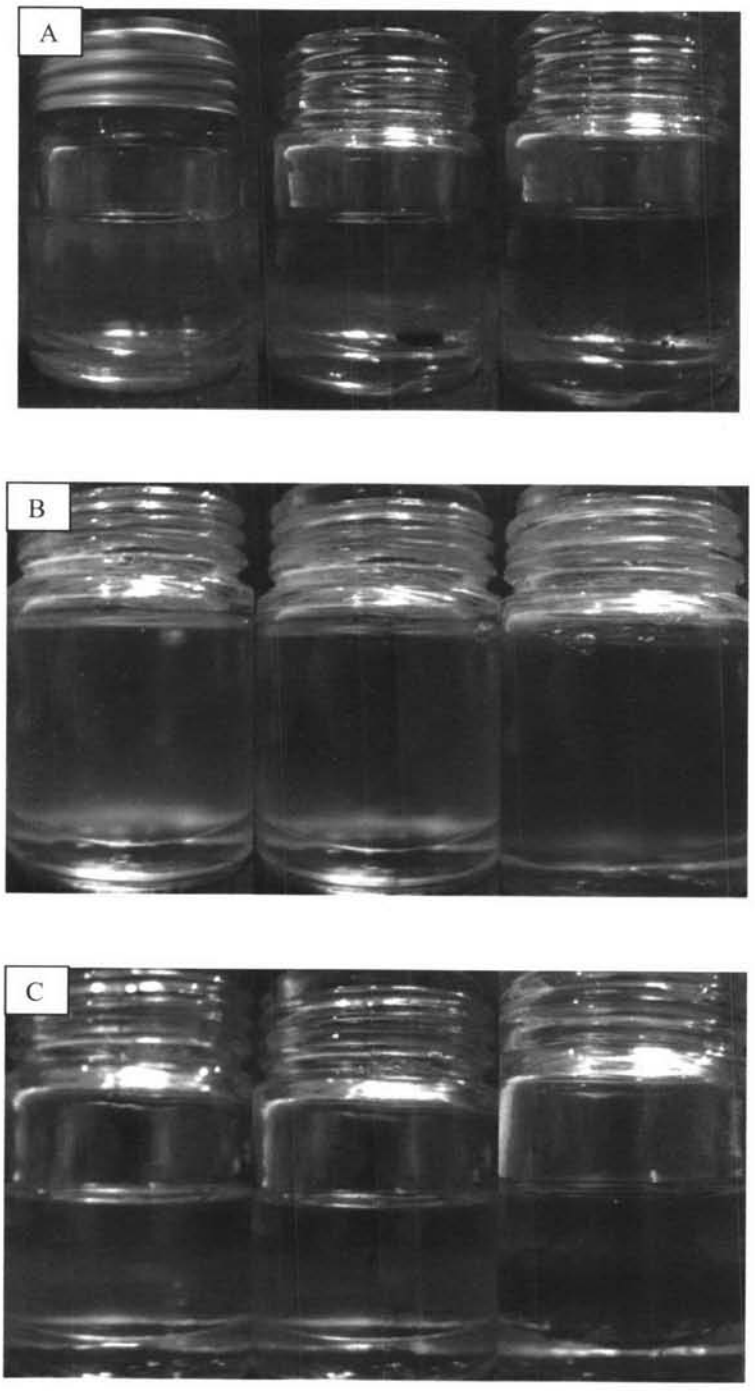

Figure 4-11 Photograph of dissolving of 1%Brilliant blue in glucosamine hydrochloride microemulsion prepared from various surfactant (A) in AOT microemulsion (B) in CTAB microemulsion (C) in lecithin microemulsion

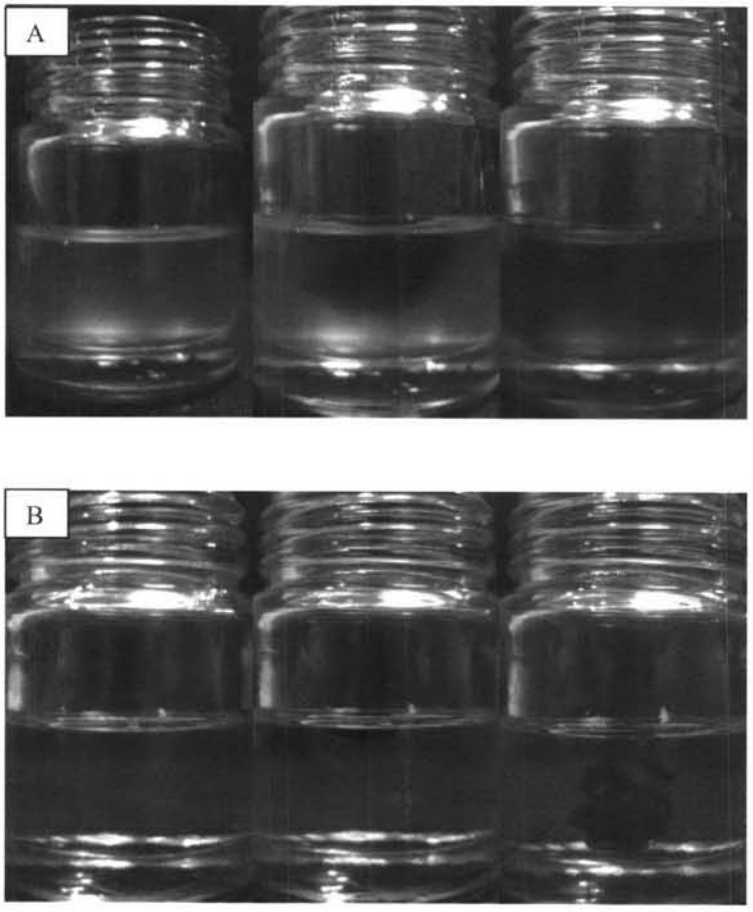

Figure 4-12 Photograph of dissolving of 1%Brilliant blue in glucosamine hydrochloride microemulsion prepared from (A) Tween 80 (o/w) microemulsion (B) Tween 80 (w/o) microemulsion

Polarized light microscopy

When the samples of GS HCl microemulsions was examined by polarized light microscope, almost all the samples were not visible in polarized light exhibited not birefringence, shows that the samples were optically isotropic and not a liquid crystalline.

Transmission electron microscopy

The results from transmission electron microscopy of the GS HCl in microemulsions prepared from various surfactants, AOT, CTAB, lecithin and

Tween 80, are shown in Figure 4-13 - 4-16. The morphology of those GS HCl microemulsions appeared to be spherical structure and having 10-100 nm in diameter confirming that microemulsion is the transparent because of the droplet size in the dispersed phase is very small, usually below 140 nm in diameter (Peltola et al., 2002).

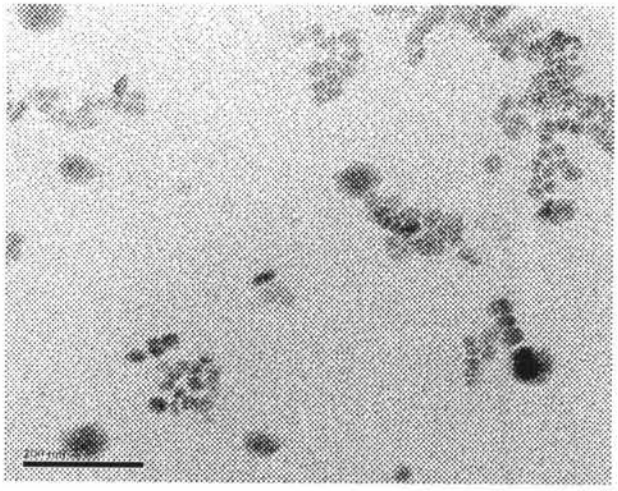

Figure 4-13 Transmission electron micrograph showing the size and morphology of glucosamine hydrochloride in AOT microemulsion

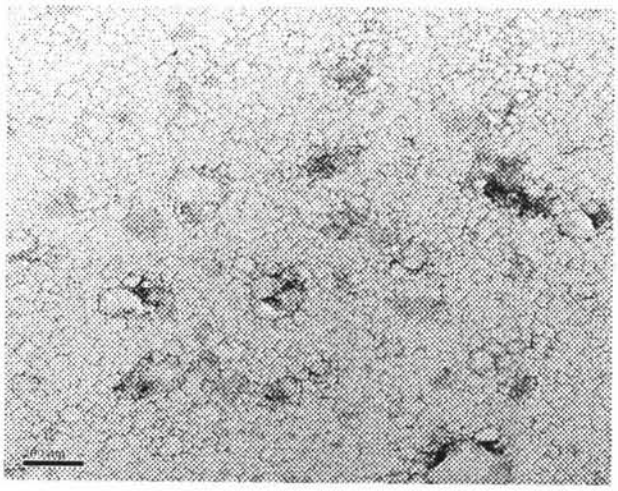

Figure 4-14 Transmission electron micrograph showing the size and morphology of glucosamine hydrochloride in CTAB microemulsion

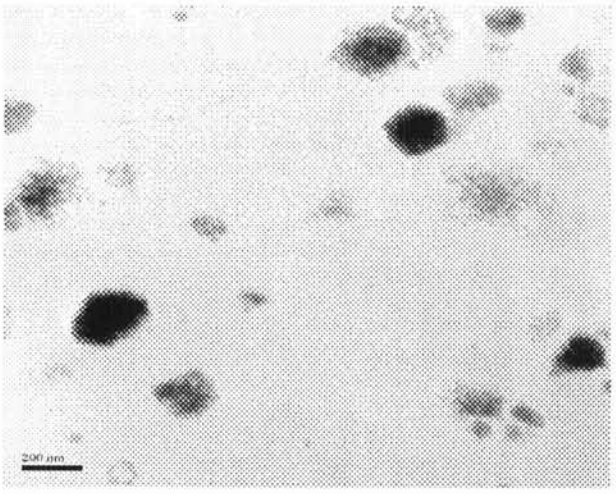

Figure 4-15 Transmission electron micrograph showing the size and morphology of glucosamine hydrochloride in lecithin microemulsion

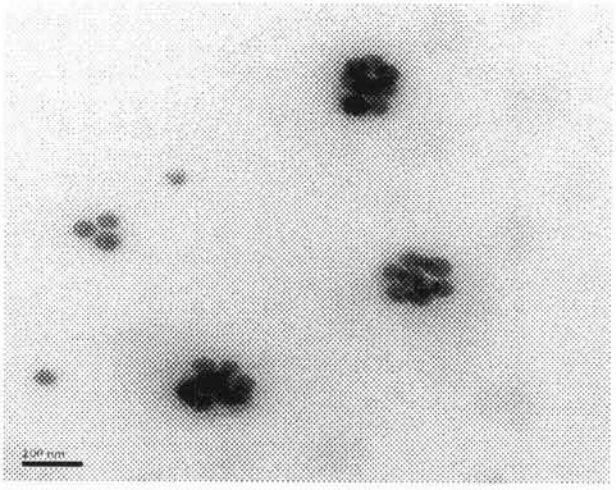

Figure 4-16 Transmission electron micrograph showing the size and morphology of glucosamine hydrochloride in Tween 80 microemulsion

3. Permeation through pig ear skin

3.1 High-performance liquid chromatographic technique for drug analysis

The quantitative of GS HCl was analyzed by HPLC technique by Pre-column with PITC derivatization. The analysis method validation parameter such as specificity, linearity, precision and accuracy complied to the specification of the European Agency for the Evaluation of Medicinal Products *Human Medicines Evaluation Unit.* The validation results are given in APPENDIX A.

3.2 Glucosamine hydrochloride permeation profiles in the skin

The *in vitro* permeation study was selected as a tool for determining the most suitable system for delivery GS HCl through pig skin. The one of important factors of the *in vitro* permeation experiment, is the sink condition of reception medium in receptor compartment. The ideal receptor phase provides an accurate simulation of the condition pertaining to *in vitro* permeation of the test compound.
A general rule, the concentration of the permeant in the receptor medium should not be allowed to exceed approximately 10% of saturation solubility (Brain, Walters and Watkinson, 2002).

In this study, the PBS 7.4 was used as receptor medium, the maximum amount of GS HCl permeant for sink condition calculated are shown in Table 4-12.

Table 4-14 The maximum amount of glucosamine hydrochloride permeant for sink condition

GS HCl solubility in PBS 7.4 (mg/ml)	98.07
Approximate volume of receptor compartment (ml)	14
Saturated amount of GS HCl (μg) in receptor medium	1.37×10^{6}
10% of saturated amount of GS HCl for sink condition (μg)	$< 1.37\times10^{5}$

In the *in vitro* permeation study of GS HCl, the calculated amount of permeant had not been over than the maximum amount of GS HCl permeant permitted for sink condition, being not more than 1.37×10^5 µg. It confirmed that the experiment was carried out under sink condition.

The *in vitro* permeation study of GS HCl formulation system across pig ear skin was conducted by franz-diffusion cell. After the sample was collected at 0, 3, 6, 12, 18 and 24 hours, the amount of permeated GS HCl was then calculated and the permeation profile of those preparation are plotted as shown in Figure 4-17 – 4-25.

Effect of drug concentration in solution dosage form

In GS HCl solution dosage form, which used water as the solvent, the drug concentrations were varied at 0.5, 2 and 10%. The percent cumulative drug permeation of GS HCl at 24 hours was 31.82, 34.04 and 39.74%, respectively as shown in Figure 4-17. From the results, it was shown that increasing drug concentration had only little effect on drug permeation.

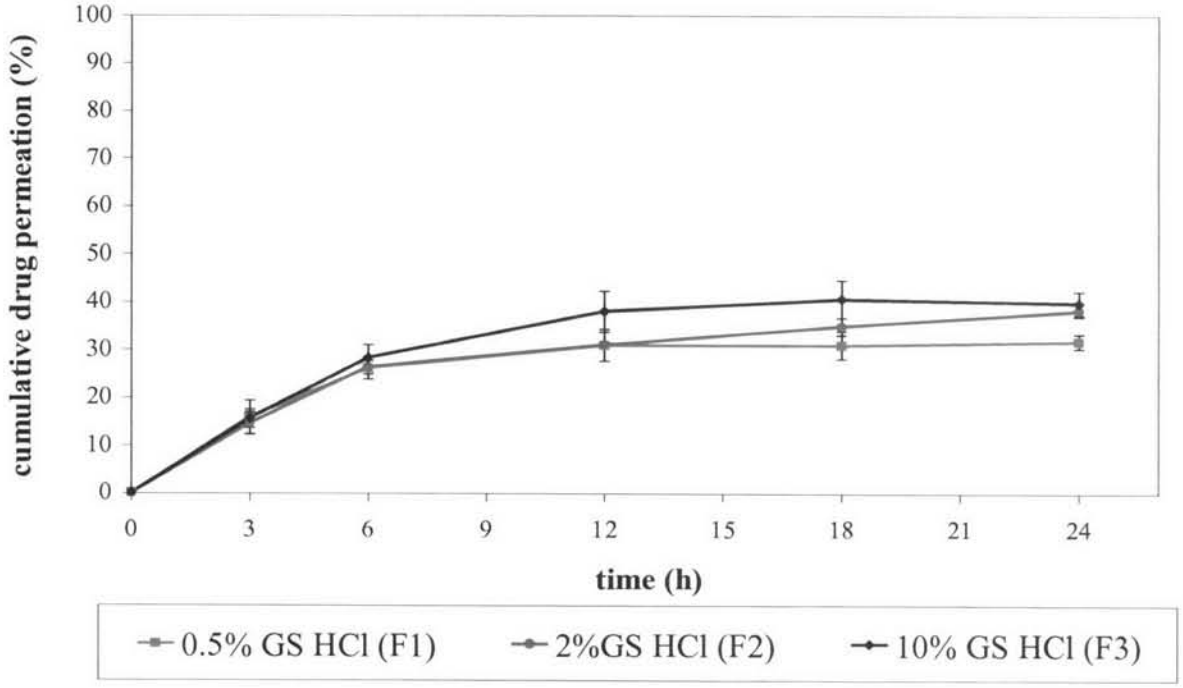

Figure 4-17 Permeation profiles of GS HCl solution, concentration 0.5, 2 and 10 w/w across pig-ear skin at 32°c (n=6)

Effect of ethanol concentration

In 2% GS HCl hydroalcoholic solution, using water and ethanol as binary mixture solvent, the effect of ethanol amount on drug permeation are shown in Figure 4-18. The result showed that, after 24 hours the permeation of GS HCl in hydroalcoholic solution containing 10% ethanol was 38.05%, which was higher than both in hydroalcoholic solution containing 60% ethanol (23.90%) and solution dosage form (34.04%). Thus, this showed that at high amount of water with low concentration of ethanol, a permeation enhancer could enhance the permeation of drug through skin but when concentration of ethanol was increased, the permeation of drug was dropped, probably caused by the high concentration ethanol which could denature proteins on the skin membrane. It has been reported that not only 95% ethanol but also in the range of approximately 70 % ethanol causes dehydration of tissue or even denaturation of protein on the skin which reduces ionic transport to the receptor medium (Sznitowska, 1996).

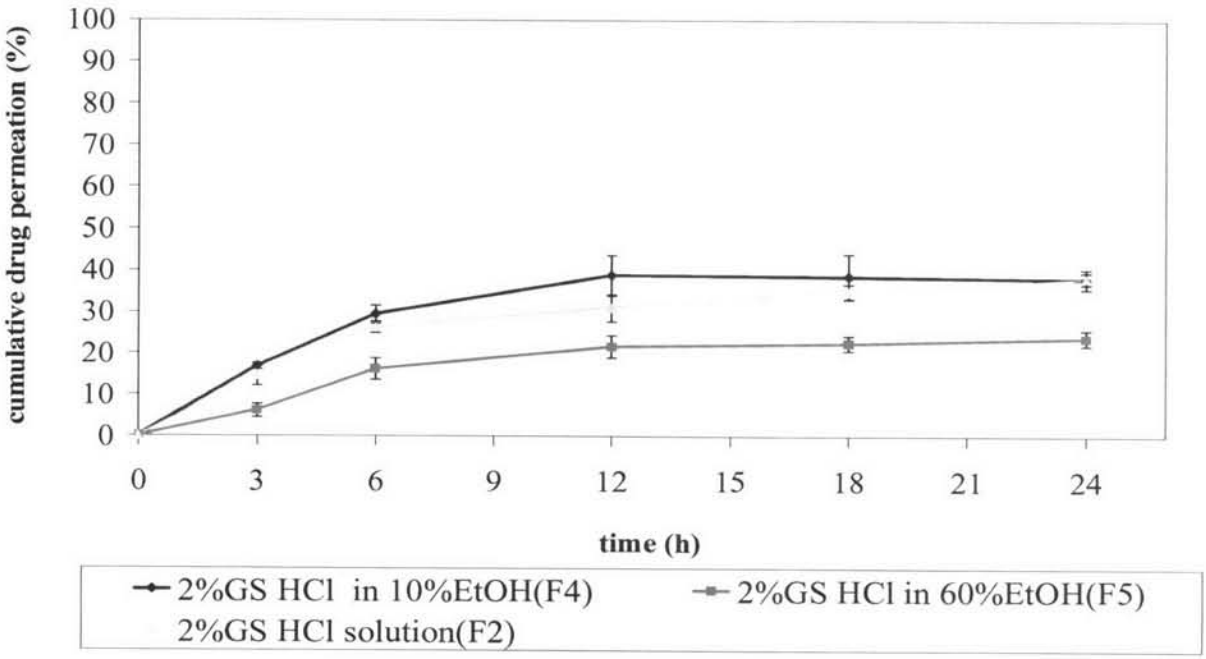

Figure 4-18 Permeation profiles of 2% GS HCl solution and 2% GS HCl hydroalcoholic solution in the ratio of ethanol to water 10:90 % w/w and 60:40 % w/w across pig-ear skin at 32°c (n=6)

Effect of HPMC gel

Many commercial products of glucosamine are available in gel preparation because gel dosage form is water washable and is more favorable in patients. In this study GS HCl was prepared using HPMC as a gel forming. When the 2% GS HCl permeation at 24 hours from HPMC gel dosage form was compared with that form GS HCl solution as a control group, the cumulative release of GS HCl were 27.63% and 37.08%, respectively as shows in Figure 4-19. From this results, it exhibited the fact that gel is not the most potential dosage form to enhance the permeability of GS HCl. The effect of high water content in the formulation on permeability was hindered by hydrophilic nature of the polymer, the hydrophilic drug would prefer to be trapped in the hydrophilic network. However, from the physical stability results of dosage forms in this study, gel exhibited the most stable dosage form in relative to the other dosage forms.

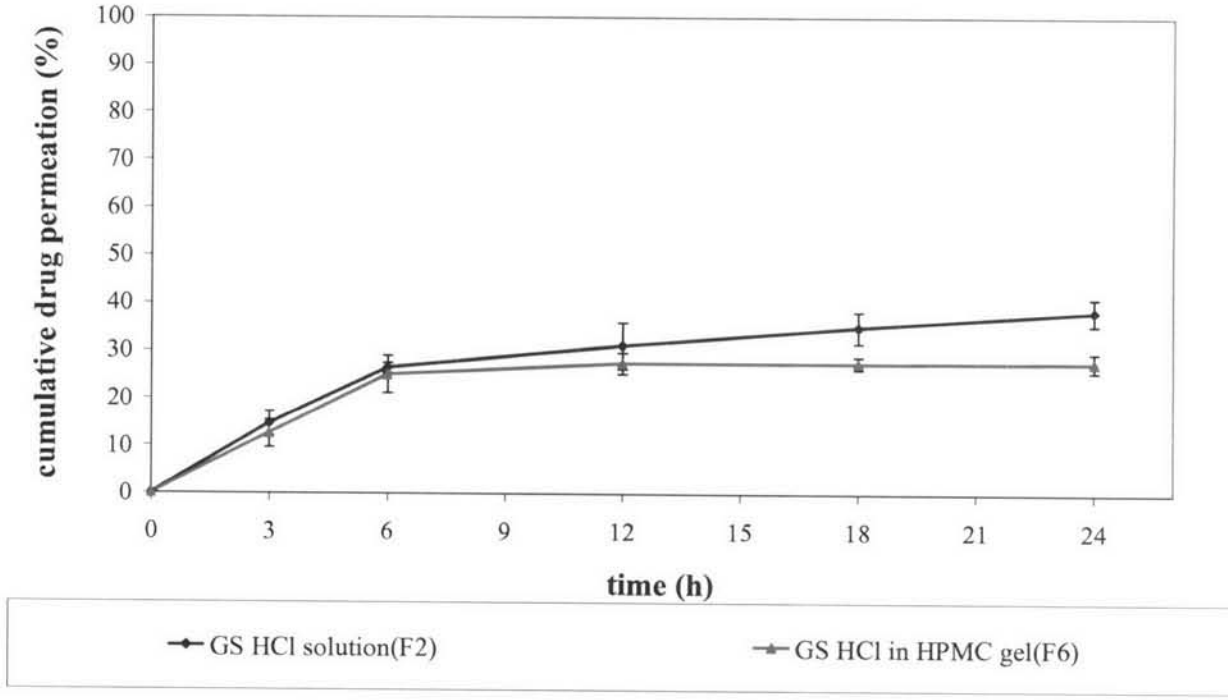

Figure 4-19 Permeation profiles of 2% GS HCl solution and GS HCl in HPMC gel across pig-ear skin at 32°c (n=6)

Effect of surfactant type

The effect of surfactant type was studied by preparation 2%GS HCl microemulsion using various surfactants. The percent cumulative permeation at 24 hours of GS HCl in AOT, CTAB, lecithin and Tween 80 microemulsions were 19.87%, 20.89%, 0.83% and 47.78%, respectively as shown in Figure 4-20. The GS HCl in Tween 80 microemulsion showed the highest GS HCl permeation. Thus the types of surfactant had a significant effect on GS HCl permeation. The ionic surfactant groups showed the minimum effect in improving permeation of GS HCl.

Due to the fact that microemulsion formation depends on the appropriate ratio of three components, oil, water and surfactant, the amount of these microemulsion components could not kept constant in the same ratio. When water amount in each preparation was considered, the maximum water amount was found in Tween 80 formulation. This confirmed that the amount of GS HCl permeation followed to the water amount in microemulsion system.

In addition, when Tween 80 microemulsion and lecithin microemulsion, which had the same amount of surfactant were compared, they exhibited the different in drug permeation. When AOT and CTAB microemulsion which had different ratios of the surfactant in the formulation, they were found to obtain almost the same pattern of drug permeation. Therefore, one factor that may affect the permeability of GS HCl through skin was the type of surfactant.

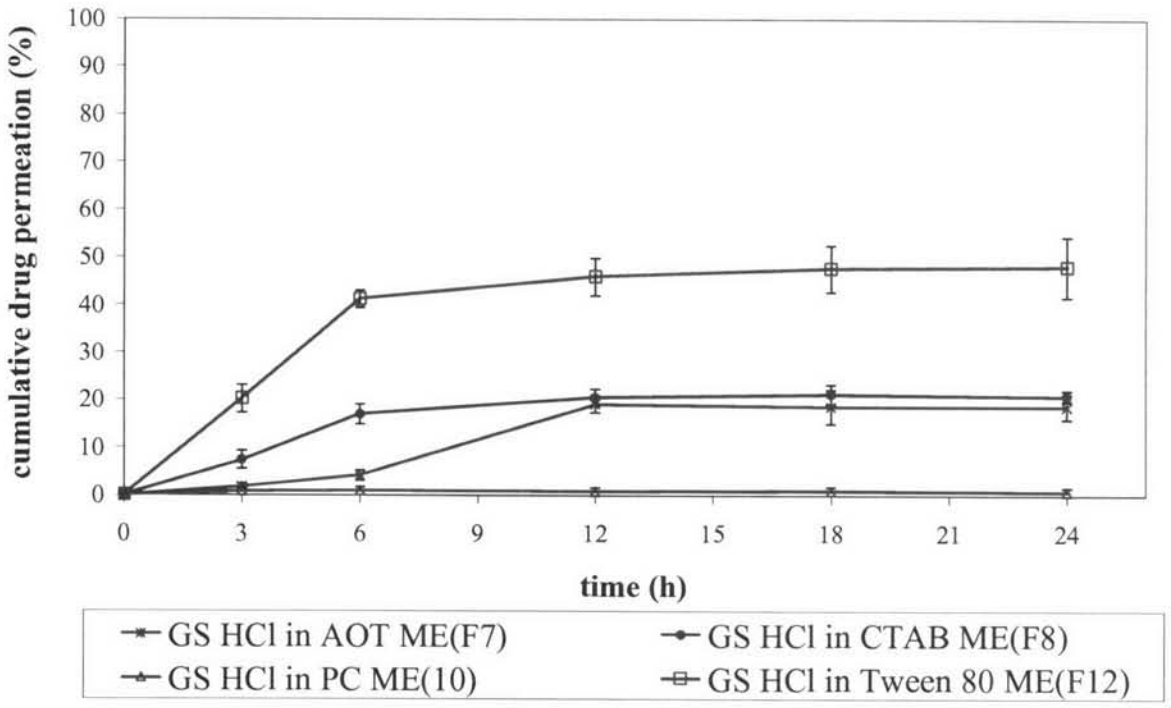

Figure 4-20 Permeation profiles of 2% GS HCl in microemulsion, prepared form AOT, CTAB, Lecithin and Tween 80 across pig-ear skin at 32°c (n=6)

Effect of drug concentration in Tween 80 microemulsion system

The effect of drug concentration in Tween 80 microemulsion dosage form, showed a similar trend as found in the solution dosage form. The increased amount of GS HCl in preparation had a little effect on the permeation amount of drug through skin. The percent cumulative permeation are shown in Figure 4-21.

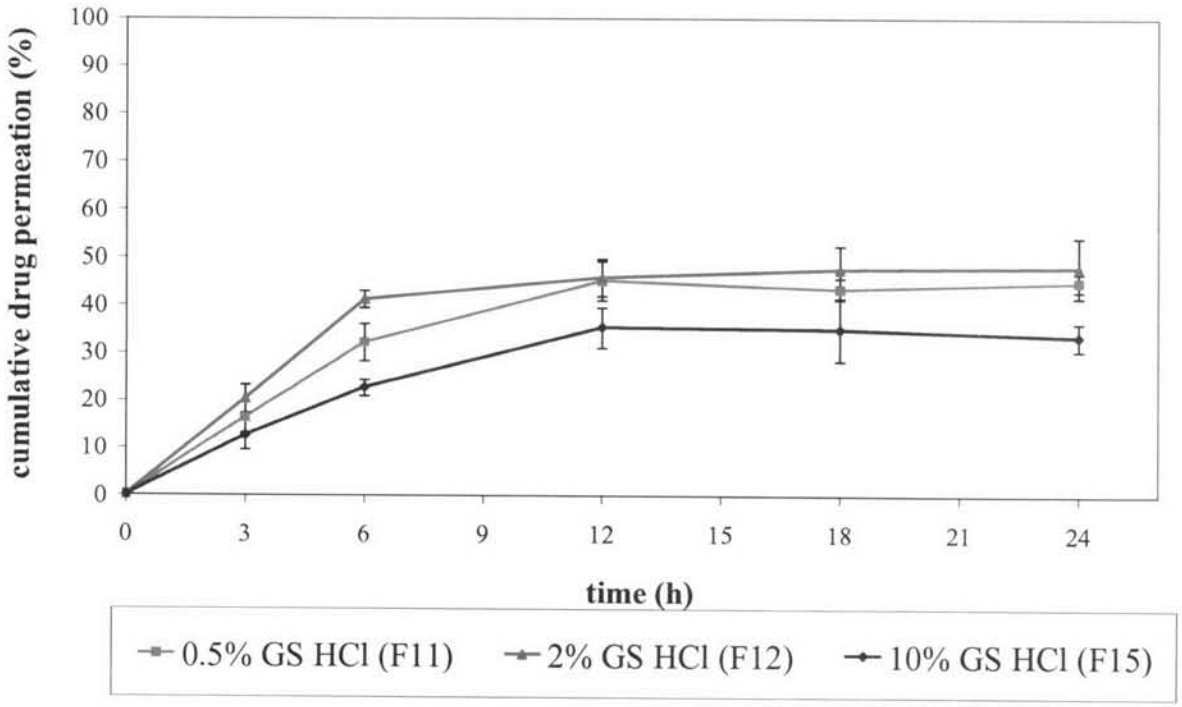

Figure 4-21 Permeation profiles of GS HCl in Tween 80 microemulsion, concentration 0.5, 2 and 10% w/w across pig-ear skin at 32°c (n=6)

Effect of the viscosity of Tween 8o microemulsion system

The effect of the viscosity of microemulsion system was studied by comparing the percent cumulative amount of the 2% GS HCl in Tween 80 microemulsion, viscosity 1,084.2 cP, and microemulsion gel, viscosity 2,640.2 cP. The results showed that the permeability of GS HCl in Tween 80 microemulsion dosage form was 45.84% and that in Tween 80 microemulsion gel was 47.78% at 24 hours as shows in Figure 4-22. This showed that the viscosity of dosage form did not affect on the permeability of GS HCl.

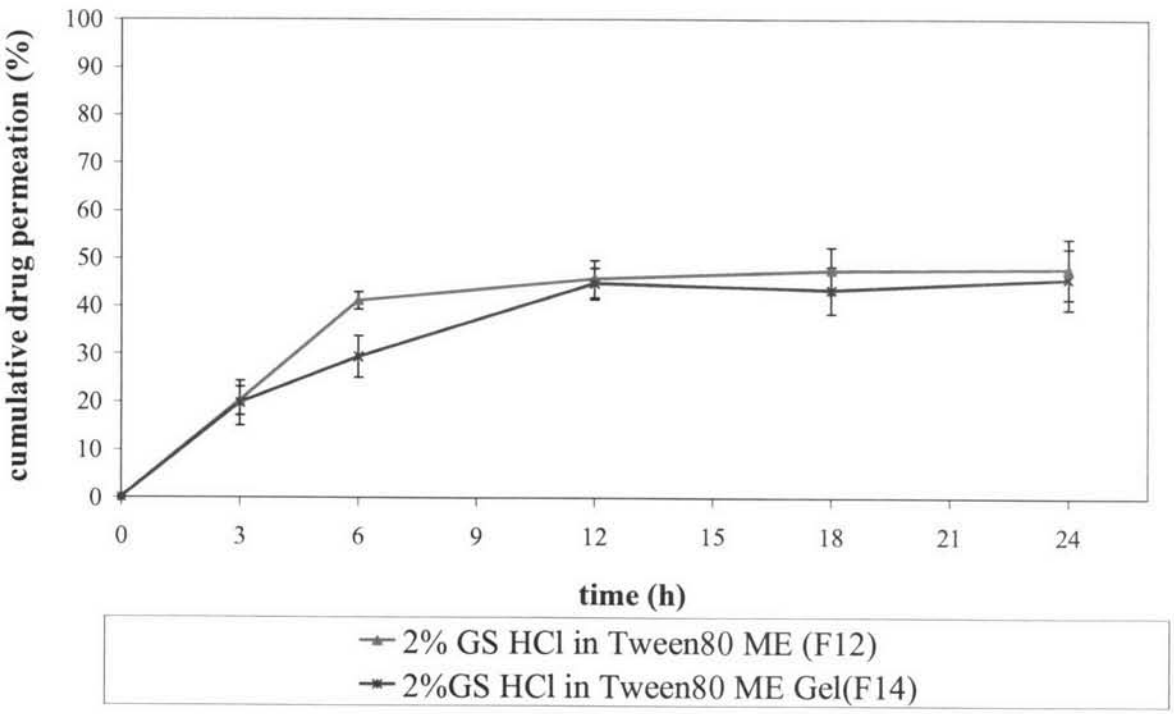

Figure 4-22 Permeation profiles of 2% GS HCl in Tween 80 microemulsion, dosage form microemulsion and microemulsion gel across pig-ear skin at 32°c (n=6)

Effect of Tween 80 microemulsion structure type

The effect of the structure of microemulsion system were studied by comparing the percent cumulative amount of GS HCl in (o/w) Tween 80 microemulsion and in (w/o) Tween 80 microemulsion. It was found the permeability of GS HCl in (o/w) Tween 80 microemulsion dosage form was 47.78% and that in (w/o) Tween 80 microemulsion was 25.12% at 24 hours as shows in Figure 4-23. Thus, the structure of microemulsion dosage form could affect on drug permeation. In addition, type of microemulsion structure that is suitable for derivery GS HCL across the skin was oil in water because the ability to solubilisation of GS HCl in the o/w microemulsion system is more than that in the w/o microemulsion system.

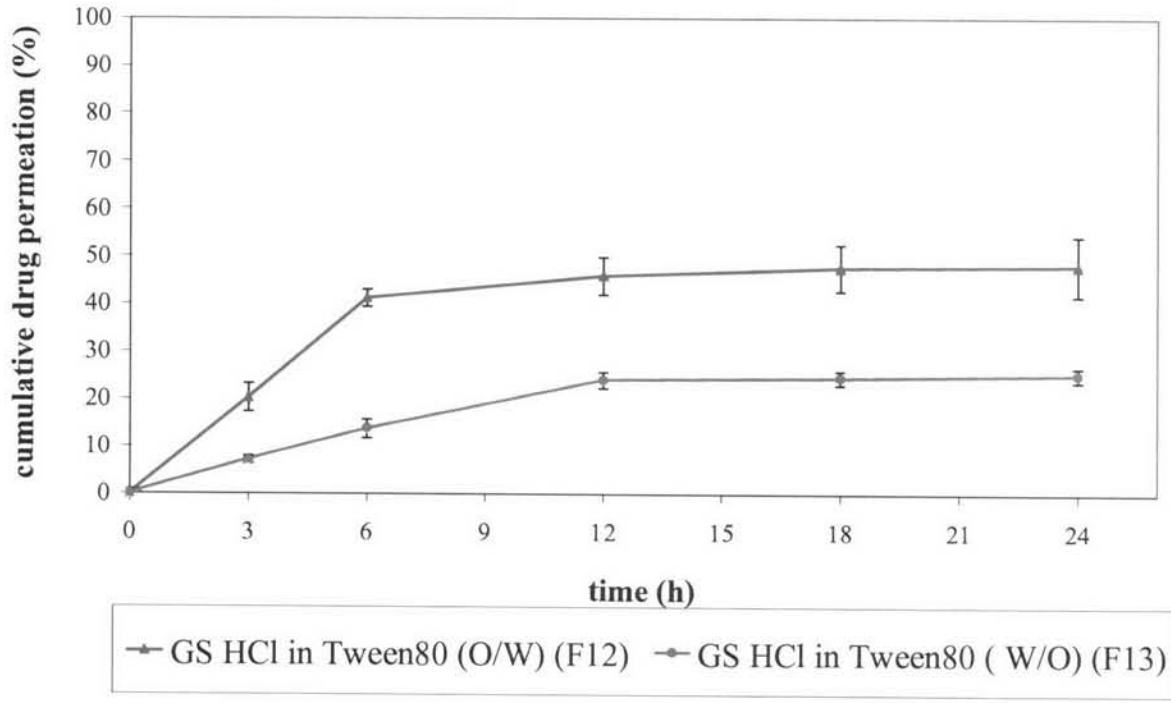

Figure 4-23 Permeation profiles of 2% GS HCl in Tween 80 microemulsion (o/w) and 2% GS HCl in Tween 80 microemulsion (w/o) across pig-ear skin at 32°c (n=6)

Effect of the microemulsion system

The effect of microemulsion system were studied by comparing microemulsion dosage form with the micelle system of water and Tween 80 (in this sytem, oil were substituted by water). The permeability of drug in system of Tween 80, water and isopropyl myristate, that rearranges microemulsion structure was more than that in the micelle system of water and Tween 80. The percent cumulative release at 24 hours of GS HCl in Tween 80 microemulsion was 47.78%, which was higher than that in the micelle system, 31.46%, as shown in Figure 4-24, The results showed that the appropriate microemulsion system could enhance the permeation of GS HCl through skin rather than micelle system of water and surfactant. For the very water soluble drug such as GS HCl, the potential to solubilize drug of surfactant does not influence the permeation of drug. However the micelle system could lower

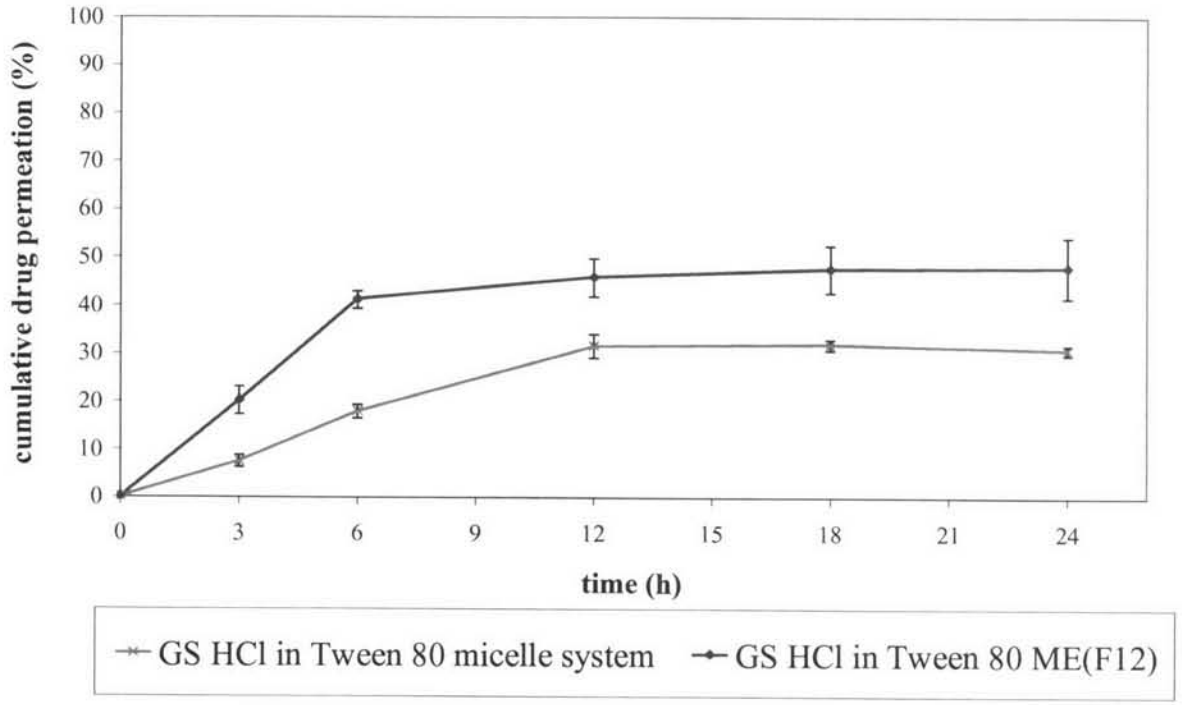

Figure 4-24 Permeation profiles of 2% GS HCl in Tween 80 microemulsion and 2% GS HCl in the micelle system of Tween 80 and water across pig-ear skin at 32°c (n=6)

Effect of dosage form

When the percent cumulative permeation of GS HCl in various dosage forms were compared, it was found that the permeability of GS HCl was the most when it was formulated to be Tween 80 (o/w) microemulsion, followed by the hydroalcoholic solution contain 10% ethanol, water solution, the micelle system of Tween 80, HPMC gel, CTAB microemulsion, AOT microemulsion and lecithin microemulsion, as shown in Figure 4-25.

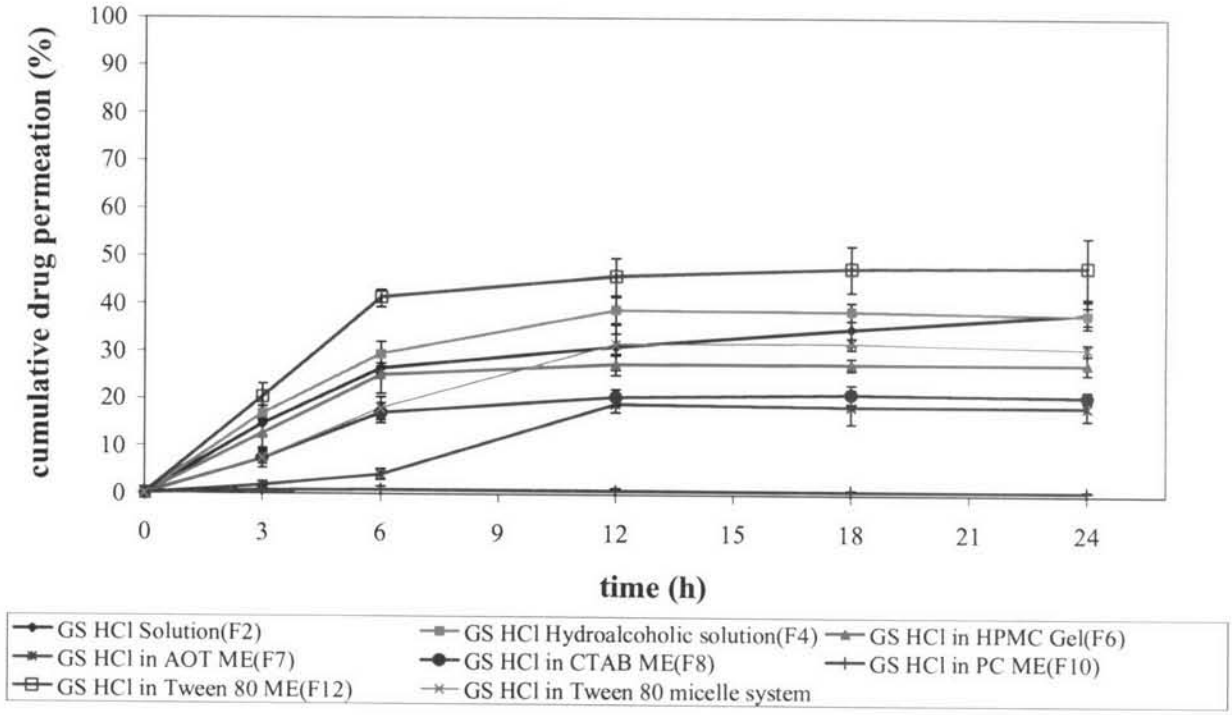

Figure 4-25 Permeation profiles of 2% glucosamine in various dosage form across pig-ear skin at 32°c (n=6)

Accordingly, the dosage forms that showed relatively high permeability of GS HCl were involved with high water content in the formulation. The permeation through pig ear skin of GS HCl depend on the water amount in formulation as reported for some compound such as glucose (Kleilgaard, 2002). In addition the ability to sulubilize GS HCl of the formulation were the important factor influencing permeation enhancement of this drug. When GS HCl chemical properties are considered, GS HCl is a hydrophilic drug with high water solubility and its chemical structure is amino-sugar, the possibly pathway of GS HCl is may be favorable to transappendageal route.

Transappendageal route is the pathway to transport drug via the sweat glands, hair follicles or the sebaceous glands (Cullander, 1991). These skin appendages, however, actually occupy only 0.1% of the total human skin surface. Therefore, it is now widely believed to be the principal pathway for the large molecular size molecule (Suhonen et al., 1999) and hydrophilic drugs (Mitragotri,

2003) such as glucosamine. There has been the study about the significance of transappendageal pathway on permeation of hydrophilic drug by using the skin devoid of hair follicle and sweat gland, such as an area behind the ear of guinea pig, the new born rat skin, which the appendages do not form in rat until 3-4 days after birth, compared with normal skin. It was found that the hydrophilic compound had significantly higher rates of permeation through the normal skin than through both the appendage-free skin model (Cullander, 1991).

When passive transports of hydrophilic compound through each route is considered. There was not direct experiment evidence in favor of transdermal pathway. For the paracellular pathway, the lipid lamellae surrounding the corneocytes form a continuous phase from the top of stratum corneum allowed only a small polar molecule and ions can transport through. In the important pathway for the hydrophilic drug, the transappendageal, in each route of this pathway, hair follicle and sebaceous glands have little evidence that suggests that hair follicle and sebaceous glands are pathway of passive absorption of ionized substance (Cullander, 1991) ; sweat glands must be the main route of transappendageal pathway as effects of the application of ionic substance such as antiperspirants, topical aqueous scopolamine hydrobromide to the surface of the skin could exhibit clinical effect (Stewart, Danto and Maddin, 1978).

The pore transport implies that the flow component are concentrated inside the keratinized wall of epidermal duct, that is also impermeable, if the duct wall does leaked, the transport is interrupted. The leaky duct wall can be caused by the high pressure than 250 mm Hg from long-term occlusion with a topical formulation resulting in such clogging of the ducts. If this occurs, the fluid is retained in the duct and pressure is incleased (Stewart, Danto and Maddin, 1978). The additional factor to consequences of pore transport is the physicochemical influences on pore. If the pH of the interior of the pore is different from that of the surface, which is probably the case, since the skin pH is about 4-5, while that of the dermis is near 7, then the charge of the permeant could be alternated as its passes through the skin and might cause it to precipitate. From the factor as describe above may be the reason why after 6 hours of the study the cumulative amounts of GS HCl permeant were not increased.

3.3 Determination of glucosamine hydrochloride permeability in the skin

Table 4-15 Permeability constant (k_p) and flux of glucosamine hydrochloride in various dosage form across pig ear skin

Formulation	Dosage form	Composition (%)					k_p (cm/s)	Flux ($\mu g/cm^2*h$)
		GS HCl	water	ethanol	HPMC 15cP	HPMC 5000cP		
F2	solution	2	98	-	-	-	$6.4x10^{-5}$	$0.32x10^3$
F4	Hydroalcoholic solution	2	88.2	9.8	-	-	$7.0x10^{-5}$	$0.27x10^3$
F6	gel	2	93	-	2	3	$6.0x10^{-5}$	$0.41x10^3$

Formulation	microemulsion (surfactant)	Composition (%)						k_p (cm/s)	Flux ($\mu g/cm^2*h$)
		GS HCl	water	IPM	Surfactant	butanol	BHT		
F7	AOT	2	15	41	42	-	-	$0.99x10^{-5}$	$0.10x10^3$
F8	CTAB	2	18	40	27	13	-	$3.8x10^{-5}$	$0.39x10^3$
F10	Lecithin	2	12.9	40	35	10	0.1	$0.21x10^{-5}$	$0.02x10^3$
F12	Tween80	2	54.9	8	35	-	0.1	$9.6x10^{-5}$	$0.46x10^3$
*Micelle system	Tween 80	2	59	-	39	-	-	$4.4x10^{-5}$	$1.0x10^3$

* the micelle system of Tween 80 and water system

From the determination of GS HCl permeability in the skin, the most suitable drug delivery system through skin for GS HCl was GS HCl in Tween 80 microemulsion (o/w) because of the higher permeability constant (k_p) and flux were $9.6x10^{-5}$ and $1.0x10^3$ as shown in Table 4-15.

3.4 Determination the effect of glucosamine hydrochloride microemulsion on the pig-ear skin membrane by light microscopy

The effects of GS HCl microemulsion on pig-ear skin was conducted under light microscopy (LM). The skin sample were divided into three groups: the freshy pig-ear skin, experimental and control groups, the control group is the skin that was treated with PBS 7.4 instead of microemulsion experiment. The control and experimental group were taken to franz-diffusion cells and the time of study was 24 hours. After that the skin sample were taken to prepare for viewing under the light microscope. The epidermis in semi-thin sections from those of sample were seen to have four layers and showed a normal appearance as shown in Figure 4-30.

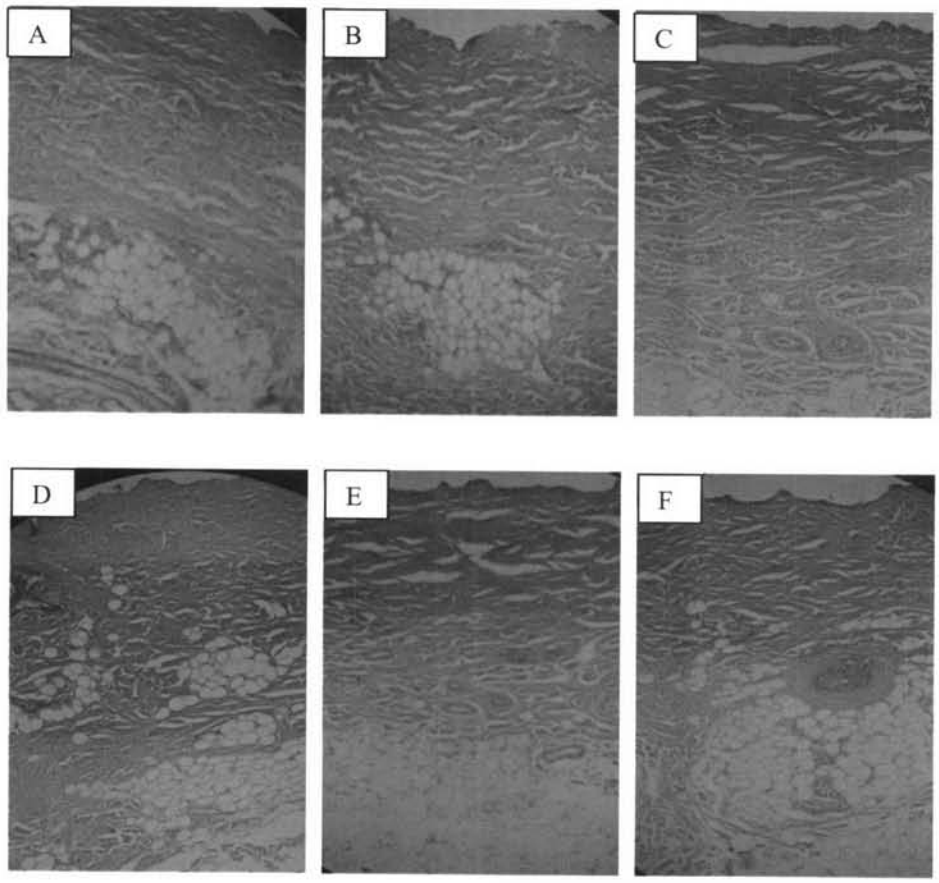

Figure 4-26 Light micrograph showing the ultrastructure of (A) pig-ear skin microemulsion-untreated group (B) pig-ear skin control group (C-F) pig-ear skin microemulsion-treated group ; prepared from AOT, CTAB, lecithin and Tween 80, respectively.