CHAPTER IV
RESULTS AND DISCUSSION

1. Formation and Structures of Liquid Crystals from Surfactants and Lecithin

Most surfactants as well as lecithin formed liquid crystalline phases under the conditions used in this study. However, systems consisting of Brij®721, Arlamol®E, and water, as well as those of Arlatone®2121, Arlamol®E, and water, in the ratios used did not result in liquid crystalline phases within 7 days of storage. Partial phase diagrams of the liquid crystalline systems are displayed in Figure 17-21.

In this study, formation of liquid crystals from surfactants was conducted by melting method at 80^0C except for the systems formed from sodium dodecyl sulfate, decanol and water. To prevent hydrolysis of sodium dodecyl sulfate at elevated temperatures (Berger and Hiltrop, 1996) and evaporation of decanol from the systems, the preparations were prepared at room temperature. Formation of liquid crystals from lecithin was prepared by dissolving lecithin in chloroform and hydrating with water at 45^0C because melting method could not disperse lecithin in the form of molecular dispersion. When lecithin was studied in the form of liposomes, multilamellar liposomes (MLV) were prepared because MLV allows much more reproducible calorimetric measurements than small unilamellar liposomes (SUV) and features a higher transition co-operativity than both SUV and large unilamellar liposome (LUV) (Sainz et al., 1993). Therefore, MLV are more suitable for studying the effects of additives on the bilayer by DSC technique due to the higher co-operativity of the structure will allow sharper peaks to occur in the thermograms.

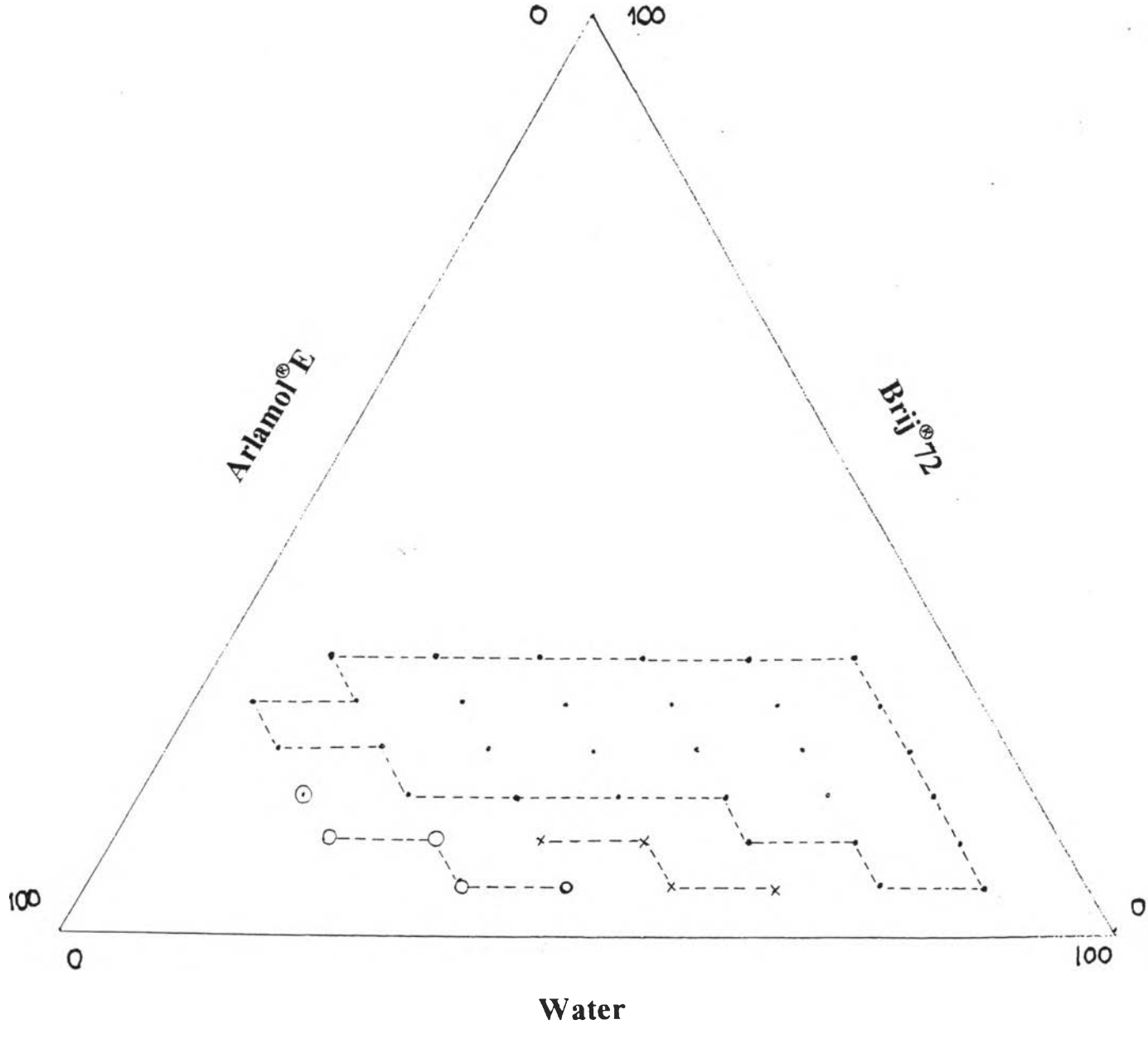


Figure 17. Ternary Phase Diagram from the System of Brij®72:Arlamol®E: Water: (•) Found or Partly Found, 1 Phase; (⊙) Found, 2 Separated Phases; (x) Not Found, 1 Phase; (o) Not Found, 2 Separated Phases.

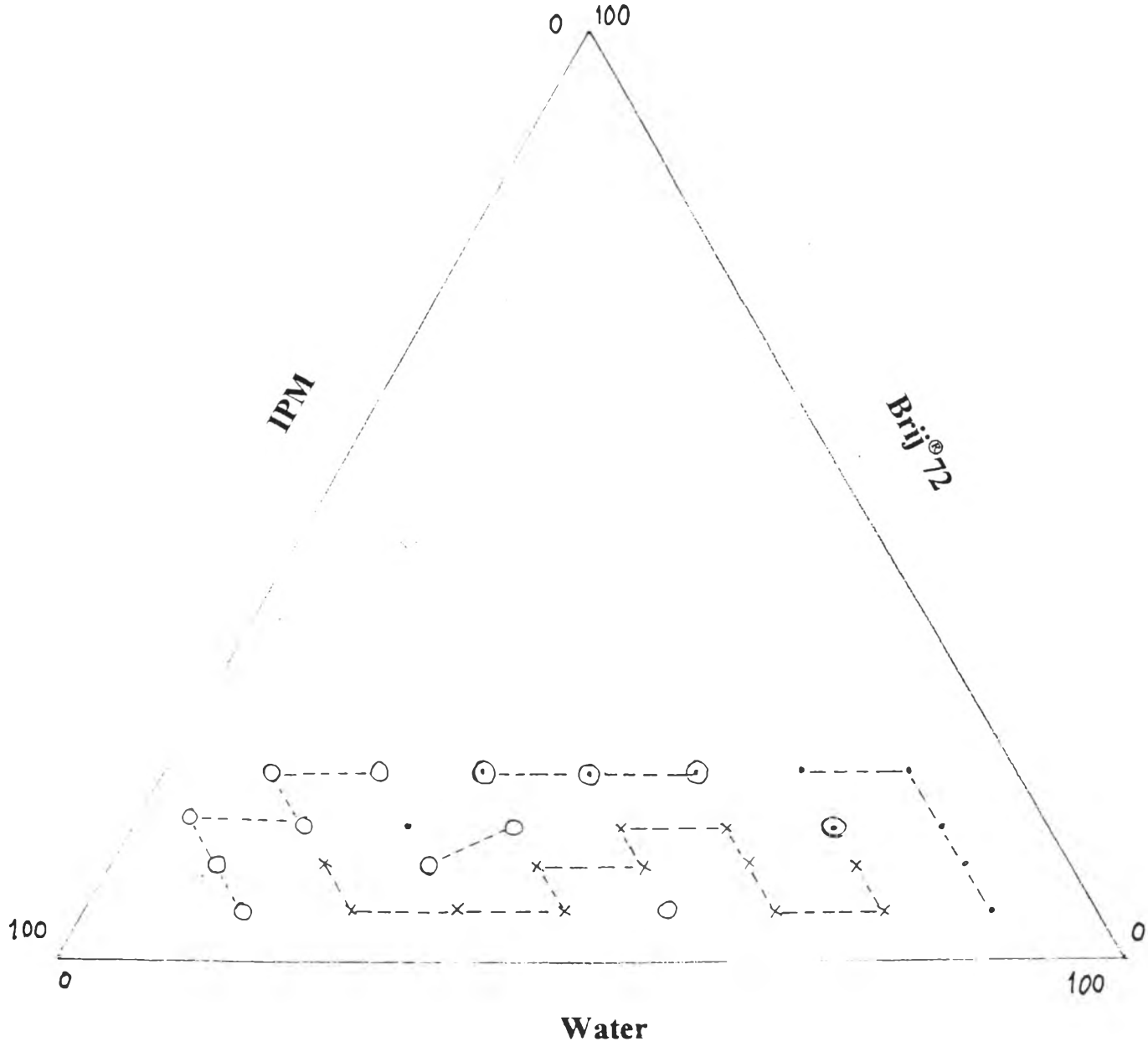


Figure 18. Ternary Phase Diagram from the System of Brij®72:IPM:Water: (•) Found or Partly Found, 1 Phase; (⊙) Found, 2 Separated Phases; (x) Not Found, 1 Phase; (o) Not Found, 2 Separated Phases.

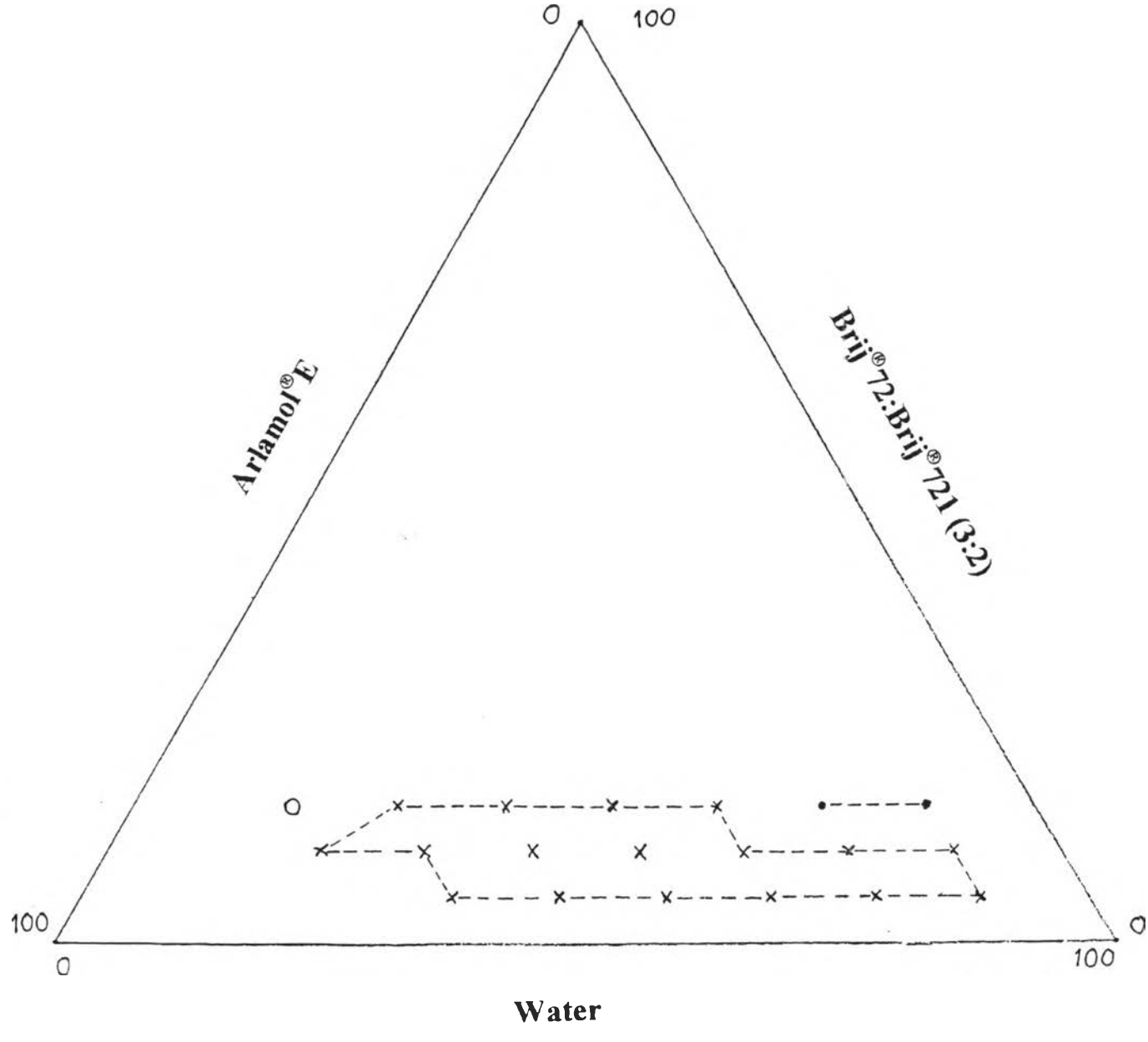


Figure 19. Ternary Phase Diagram from the System of Brij®72:Brij®721 (3:2): Arlamol®E:Water: (•) Found or Partly Found, 1 Phase; (◎) Found, 2 Separated Phases; (x) Not Found, 1 Phase; (o) Not Found, 2 Separated Phases.

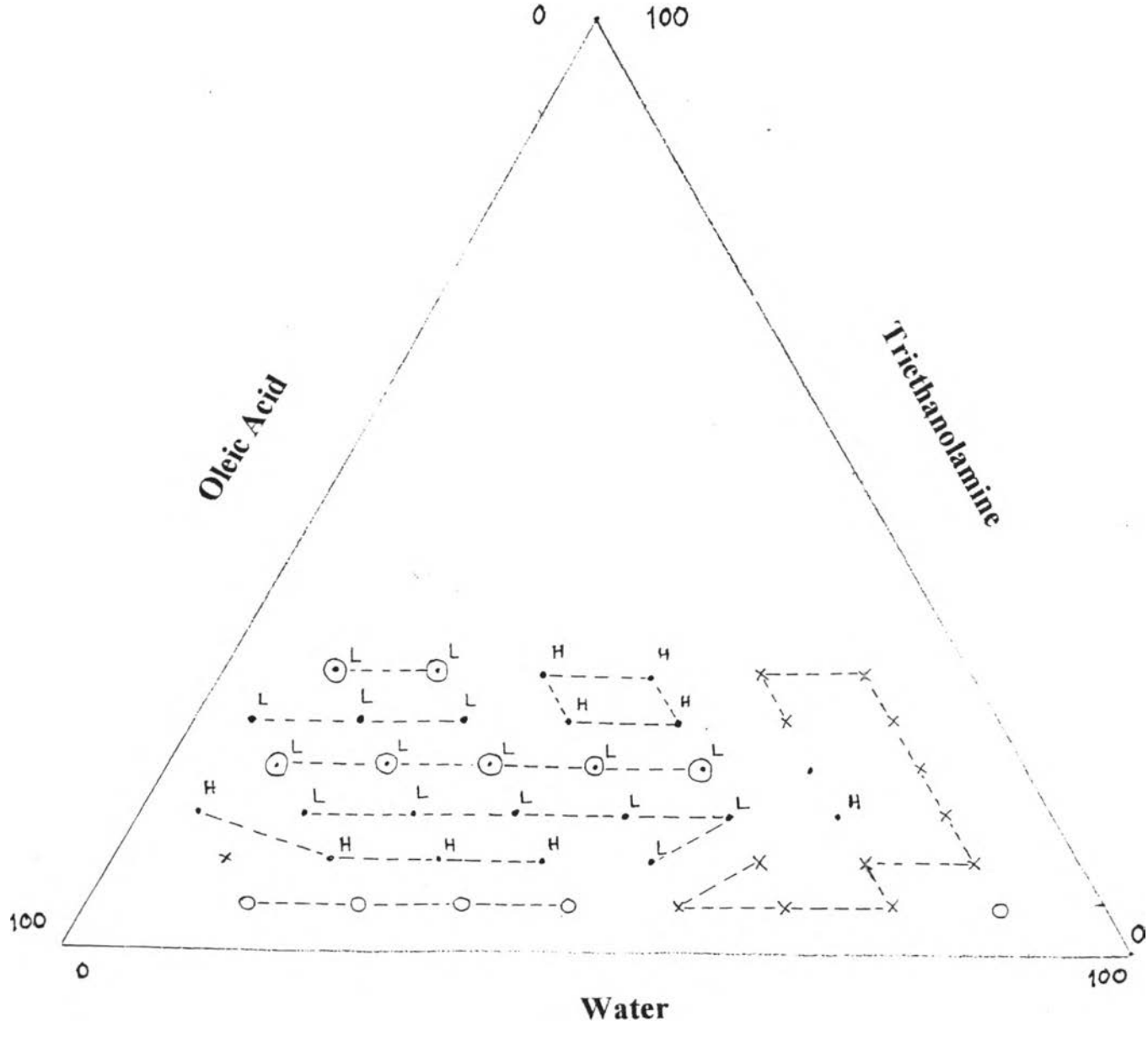


Figure 20. Ternary Phase Diagram from the System of Triethanolamine:Oleic Acid:Water: (•) Found or Partly Found, 1 Phase; (⊙) Found, 2 Separated Phases; (x) Not Found, 1 Phase; (o) Not Found, 2 Separated Phases; (L) Lamellar Phase; (H) Hexagonal Phase.

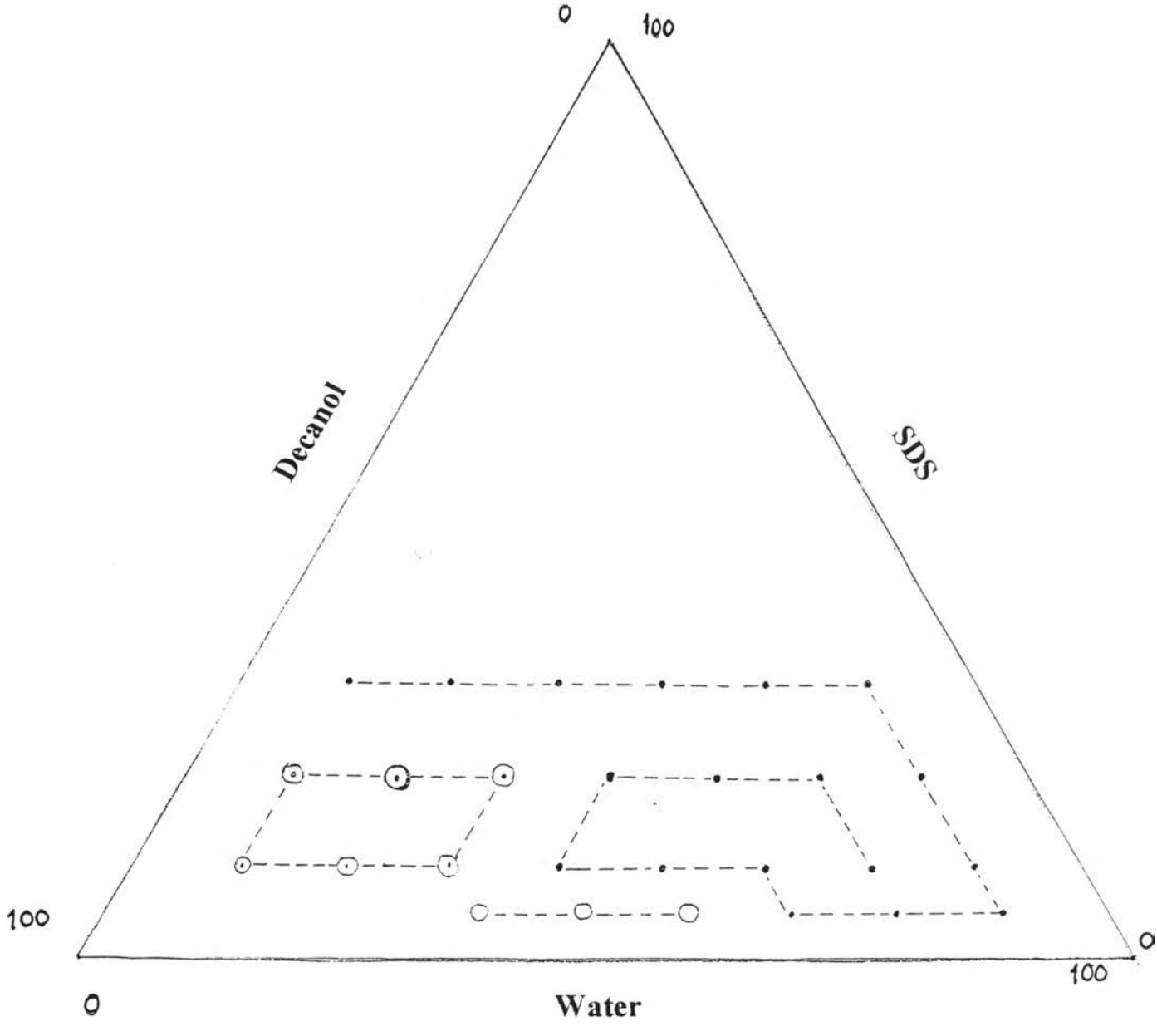


Figure 21. Ternary Phase Diagram from the System of SDS:Decanol:Water: (•) Found or Partly Found, 1 Phase; (◎) Found, 2 Separated Phases; (x) Not Found, 1 Phase; (o) Not Found, 2 Separated Phases.

2. Characterization of the liquid crystalline systems

2.1 Polarized Light Microscopy

The formation and the structure of various liquid crystals were identified by polarized light microscopy according to the classification established by Rosevear (1954), Ibrahim et al. (1992), and Suhaimi et al. (1994). Detailed descriptions of the formation and the structure of each system in this study are in Appendix A. Liquid crystals from lecithin, with water content of more than 60%, had a lamellar liquid crystalline structure (Figure 22). The Brij®72 and sodium dodecyl sulfate systems showed only lamellar liquid crystalline structures (Figure 23 and Figure 24) whereas the triethanolamine oleate systems showed both hexagonal and lamellar liquid crystalline structures (Figure 25) in the concentration range studied. The Brij®72 and water formed lamellar liquid crystalline structure in Brij®72 concentration of 10-40 %w/w. When an oil, such as Arlamol®E or isopropyl myristate (IPM), was added to the binary mixture of Brij®72 and water, a lamellar liquid crystalline structures were still evident at various ratios of the components. Figure 17 and 18 represent the ternary phase diagrams for Brij®72:Arlamol®E:water and Brij®72:IPM:water, respectively. The results showed that Brij®72:Arlamol®E:water gave the lamellar liquid crystalline structure in the wide range of ratios. When the oil was changed from Arlamol®E to isopropyl myristate, the lamellar liquid crystalline structure occurred in a narrower range. In pharmaceuticals and cosmetics, combination between two or more emulsifiers or the presence of a co-surfactant tends to increase emulsion stability (Friberg and Jansson, cited in Tyle and Frank, 1990). Hence, the effect of combining

Figure 22. Photomicrograph from Polarized Light Microscopy Showing the Lamellar Structure of Lecithin:Water (40:60).

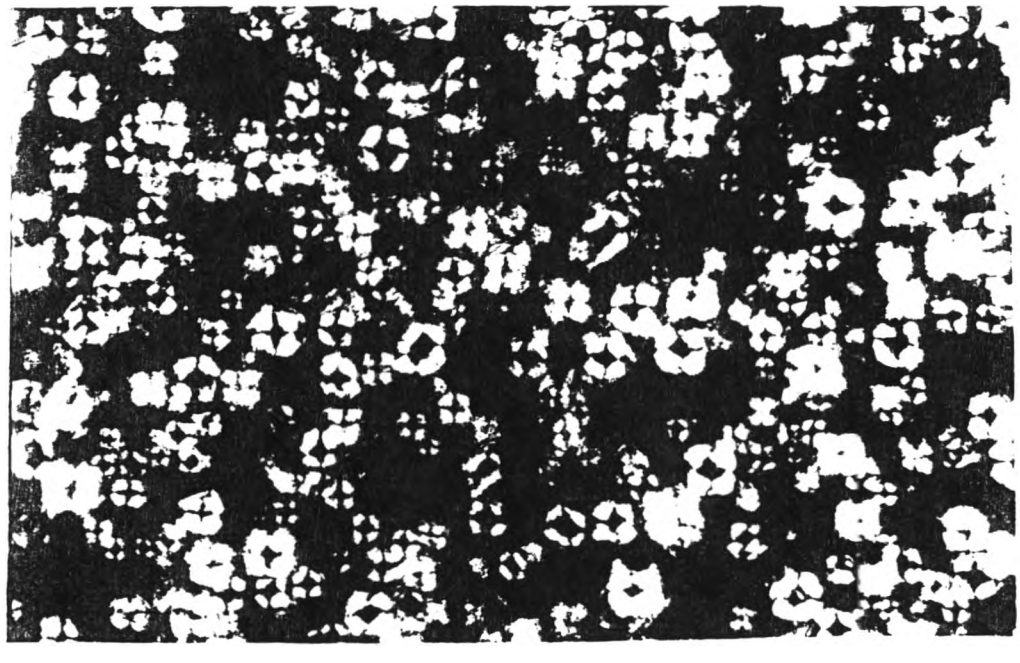
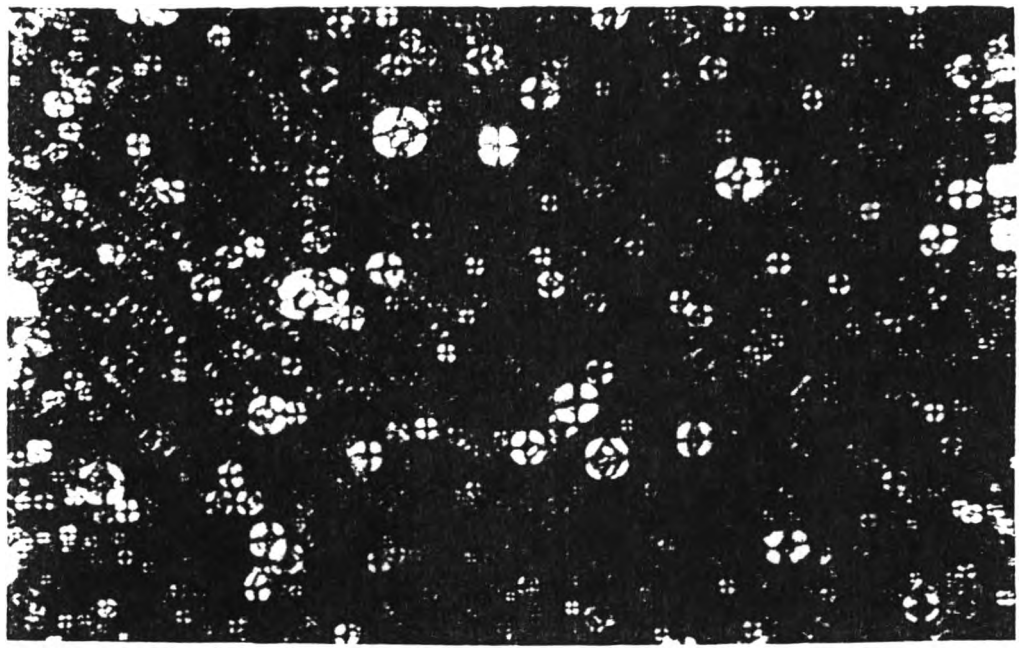
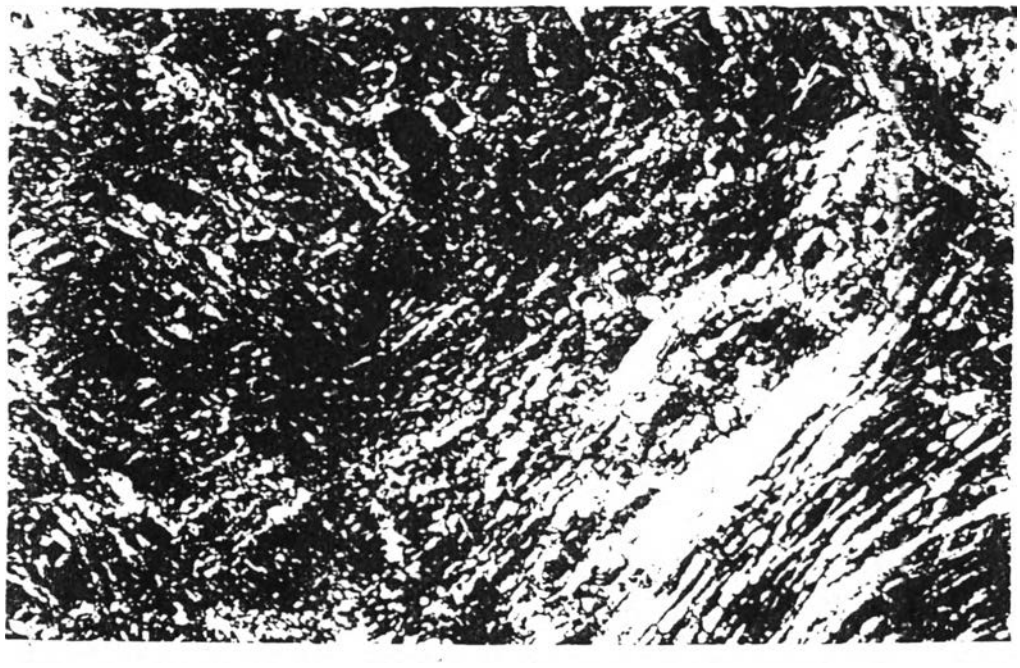


Figure 23. Photomicrograph from Polarized Light Microscopy Showing the Lamellar Structure of Brij®72:Arlamol®E:Water (15:10:75).



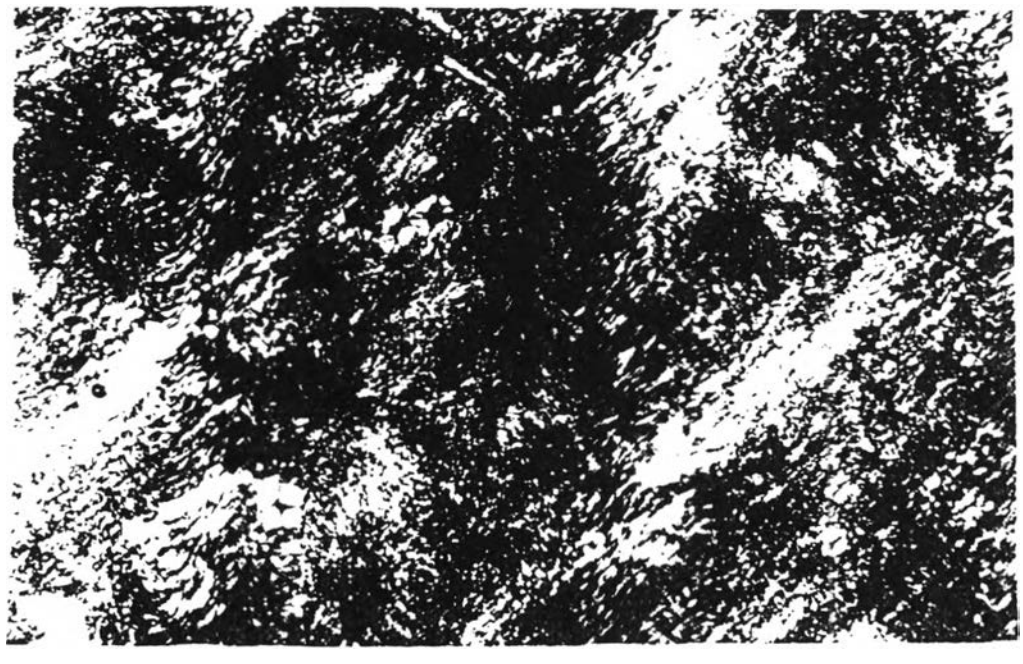
(a)



(b)

Figure 24. Photomicrographs from Polarized Light Microscopy Showing the Lamellar Structure of SDS:Decanol:Water (a) 5:10:85 (b) 20:30:50.

(a)



(b)

Figure 25. Photographs from Polarized Light Microscopy showing the structure of Triethanolamine:Oleic Acid:Water (a) 10:50:40, hexagonal (b) 15:50:35, lamellar.

effect of two surfactants on the formation and structures of liquid crystals between two surfactants was also studied by using Brij®72 :Brij®721 in the ratio of 3:2. The results in Appendix A (Table A.3) show that the combination of Brij®72 and Brij®721 caused the occurrence of lamellar liquid crystals only at the ratio of the surfactant:oil:water of 15:10:75 in the concentration range studied. No difference in the stability of lamellar liquid crystals was evident under a polarized light microscope when the system with Brij®721 was compared with the system combining Brij®72 and Brij®721 when the same total surfactant concentration was used. This suggests that combining Brij®72 with Brij®721 might have interferred with the alignment of the surfactants at the interface and made the liquid crystal formation less probable.

In some systems of liquid crystalline phase, the instability of liquid crystals occurred, e.g., in the liquid crystalline system composed of triethanolamine, oleic acid and water in the ratio of 15:60:25. In this system, lamellar liquid crystals occurred after 7 days of preparation but disappeared after 2 months when observed under a polarized light microscope. In some systems, the structure of liquid crystal changed, e.g., the liquid crystalline system composed of triethanolamine, oleic acid, and water in the ratio of 25:50:25 and that of sodium dodecyl sulfate, decanol and water in the ratio of 10:20:70. The appearances of liquid crystalline texture changed from the pattern similar to that shown in Figure 26 to the pattern similar to that shown in Figure 27 and from the pattern similar to that shown in Figure 24a to the pattern similar to that shown in Figure 24b, respectively. In addition, the physical appearance also changed, e.g., the liquid crystalline system composed of sodium dodecyl sulfate, decanol, and water in the ratio of 10:30:60 changed from a homogeneous gel to two separated phases, but liquid crystal formation still existed when observed under a polarized light

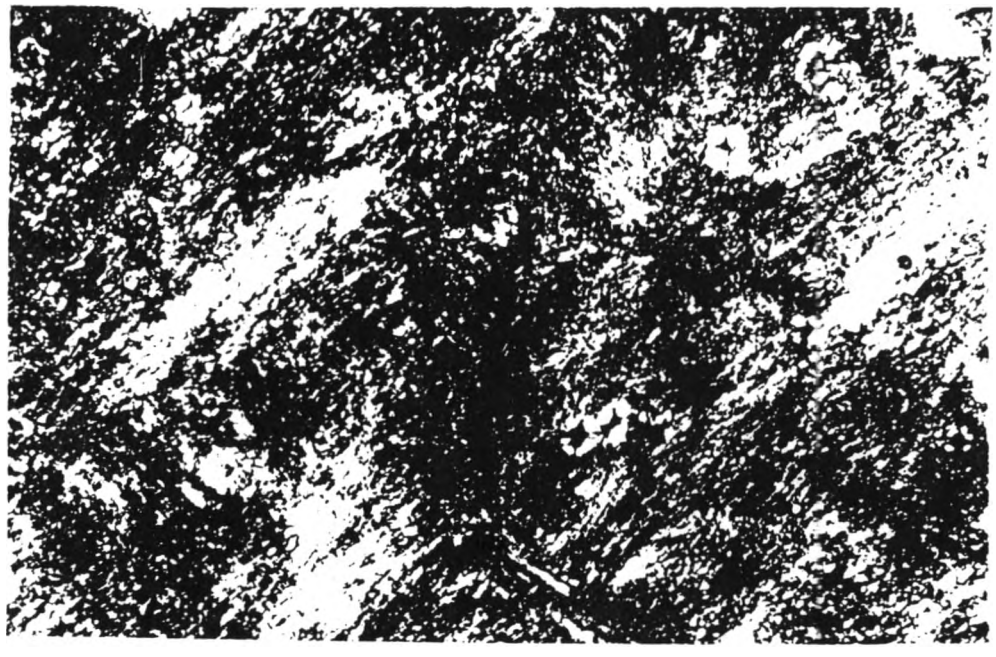


Figure 26. Photomicrograph from Polarized Light Microscopy Showing the Lamellar Structure of Triethanolamine:Oleic Acid:Water (15:50:35).

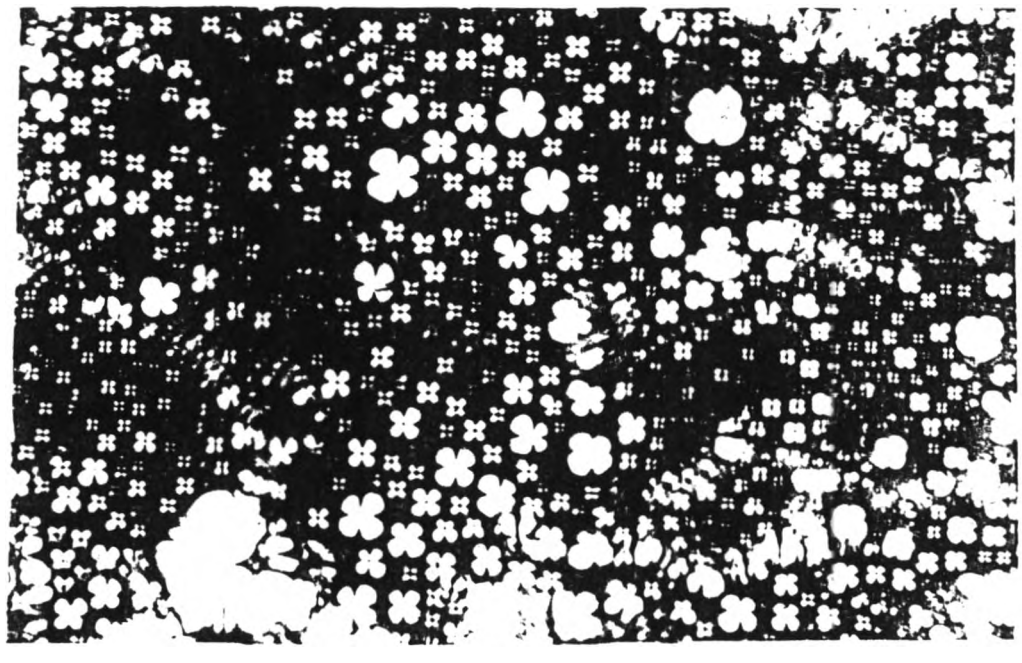


Figure 27. Photomicrograph from Polarized Light Microscopy Showing the Lamellar Structure of SDS:Decanol:Water (30:40:30).

microscope. Appendix B shows photomicrographs of representatives of the liquid crystals viewed between cross polarizers at room temperature. From the results of polarized light microscopy, the formation and structures of lyotropic liquid crystalline phases depended on the characteristic of the amphiphilic compound, the other component in the system, the ratio of the components, and time.

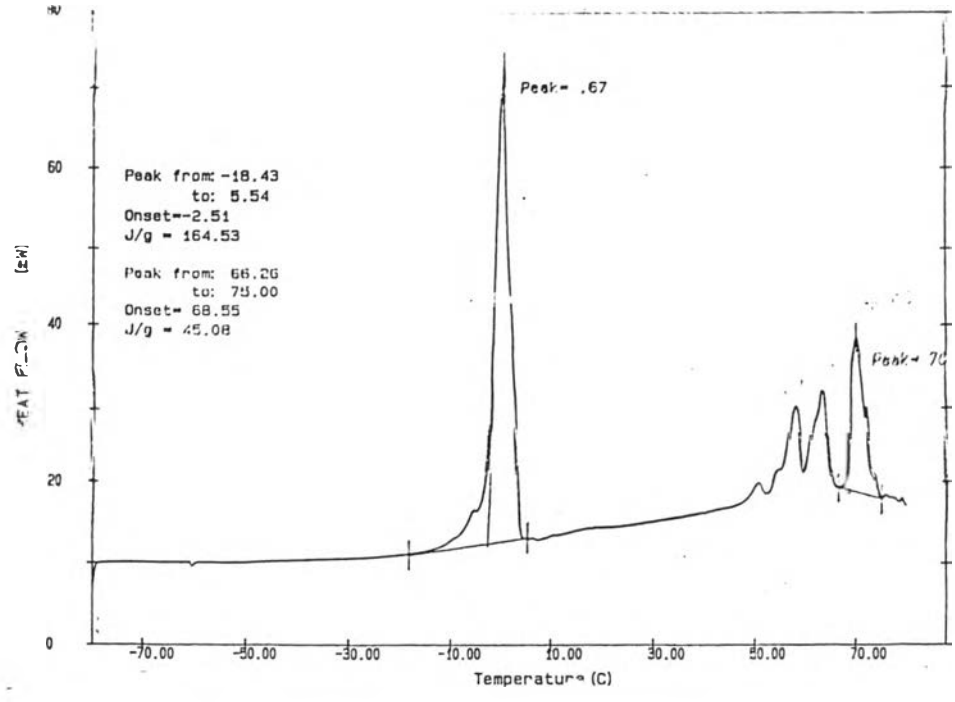
2.2 Differential Scanning Calorimetry (DSC)

Surfactants and lecithin can exist in both gel and liquid crystalline states. Though some of the surfactants, e.g., Brij®72 and sodium dodecyl sulfate, are solid at room temperature, the systems prepared were all in the liquid crystalline state. This was verified by DSC since the transition temperatures of all systems were below room temperature.

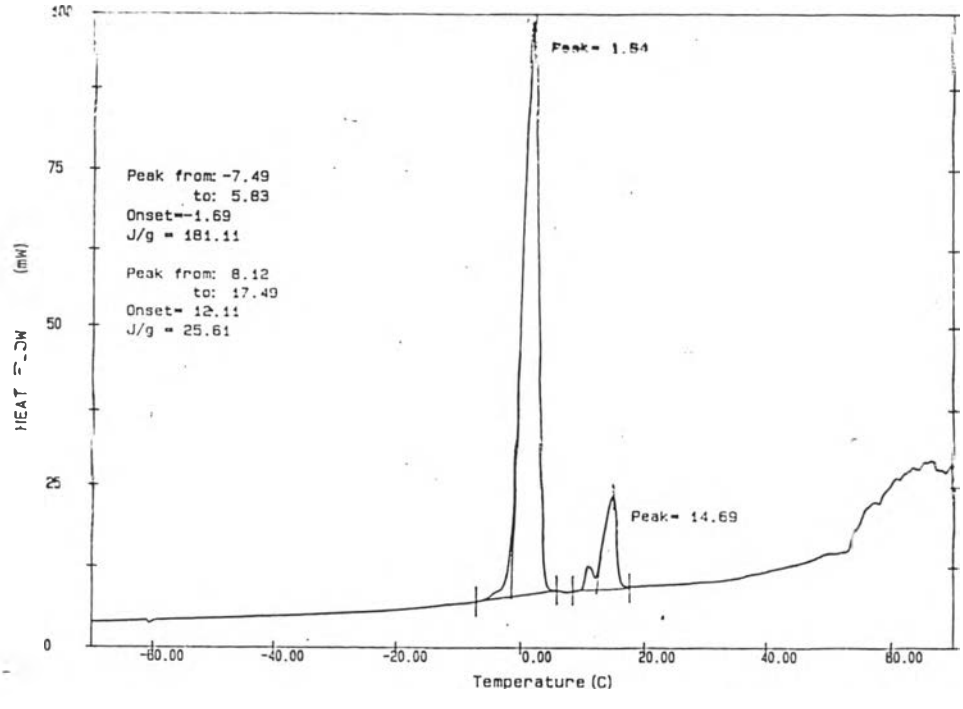
DSC experiments were performed on the systems that displayed good stability after 2 months from preliminary studies with polarized light microscopy under the step 2.1 of the methods in Chapter II. The chosen systems either showed different liquid crystalline structures with minimal concentrations of sufactants or showed the same liquid crystalline structure with different component ratios. The liquid crystalline structures selected were closed lamellar liquid crystalline (see Figure 22, 23, 24a), planar lamellar liquid crystalline (see Figure 24b, 25b), and hexagonal (see Figure 25a). Systems that formed closed lamellar liquid crystals were Brij®72:water (40:60), Brij®72:Arlamol®E:water (15:10:75), sodium dodecyl sulfate (SDS):decanol (D): Water (W) (5:10:85), lecithin:water (40:60), and liposomes. Those displayed the planar lamellar liquid crystalline structure were triethanolamine (T):oleic acid (O):

Water (W) (15:50:35) and SDS:D:W (20:30:50). The hexagonal structures were the systems of T:O:W (10:50:40) and T:O:W (25:30:45).

From the DSC thermograms, the transition temperature of the liquid crystalline system composed of Brij®72:water (40:60) did not differ from Brij®72:Arlamol®E: water (15:10:75) (Figure 28). This suggests that the majority of the surfactant molecules were in the similar phase in the two systems. However, in the system with Arlamol®E, there appeared another smaller peak at 14.69°C. Hence, Arlamol®E introduced another phase into the system. Since T_m of pure Arlamol®E (Figure 29) and Brij®72 are not in the vicinity of the new peak, this peak was not from separation of pure Arlamol®E or Brij®72 from the system. On the other hand, this peak might be from a new phase composed of surfactant molecules at the interface of the oil droplets which were in a different environment than the majority of surfactant molecules in the bilayer structure. The main transition of the system should be attributed by molecules of the surfactant forming liquid crystal bilayers surrounding the oil droplets. Since the main transition temperature of the system did not shift in the presence of Arlamol®E, there was no evidence that Arlamol®E participated significantly in the surfactant bilayer structure on a molecular basis. However, from the structural point of view, Arlamol®E is also an amphiphile, the chemical nomenclature of which is polyoxypropylene-15-stearyl ether; it should be able to intermix with Brij®72 molecules, at least partly. If that had happened, the extent of the intermixing was not large enough to cause any significantly effects on transition of the system. For oils without an amphiphilic structure, such as liquid paraffin, this possibility could be ruled out (Mueller-Goymann and Frank, 1986).



(a)



(b)

Figure 28. DSC Thermograms of the Liquid Crystalline Systems Composed of (a) Brij®72:Water (40:60) (b) Brij®72:Arlamol®E:Water (15:10:75).

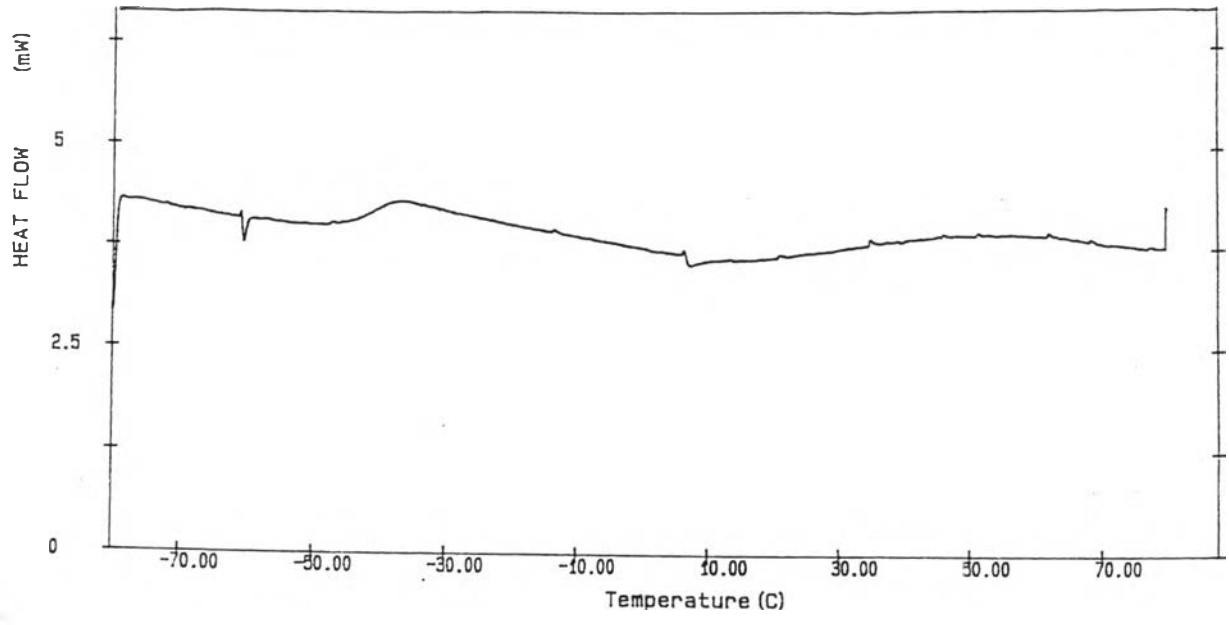
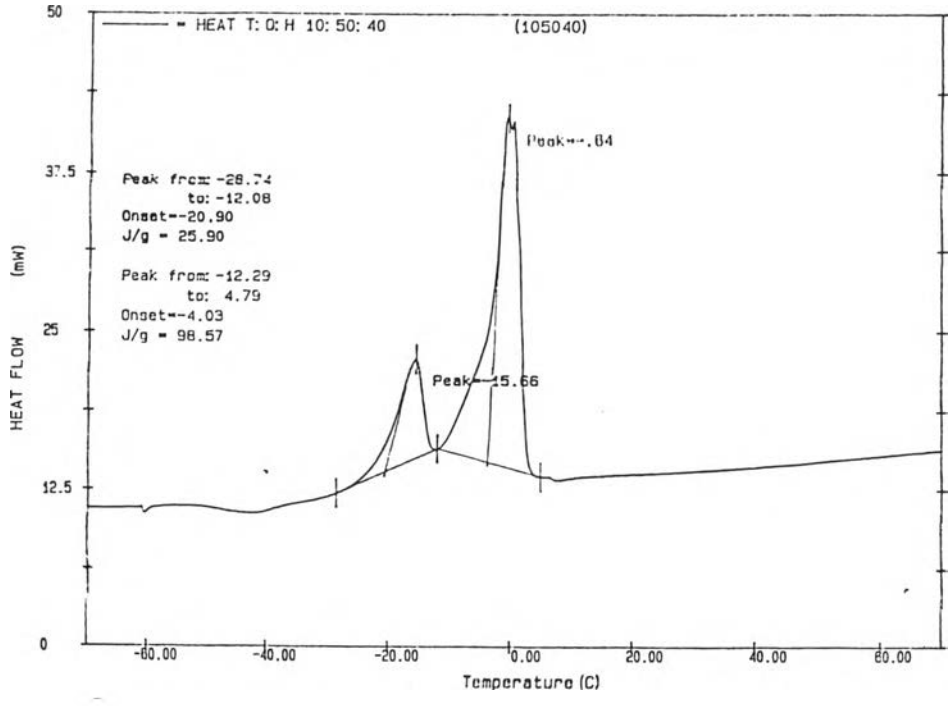
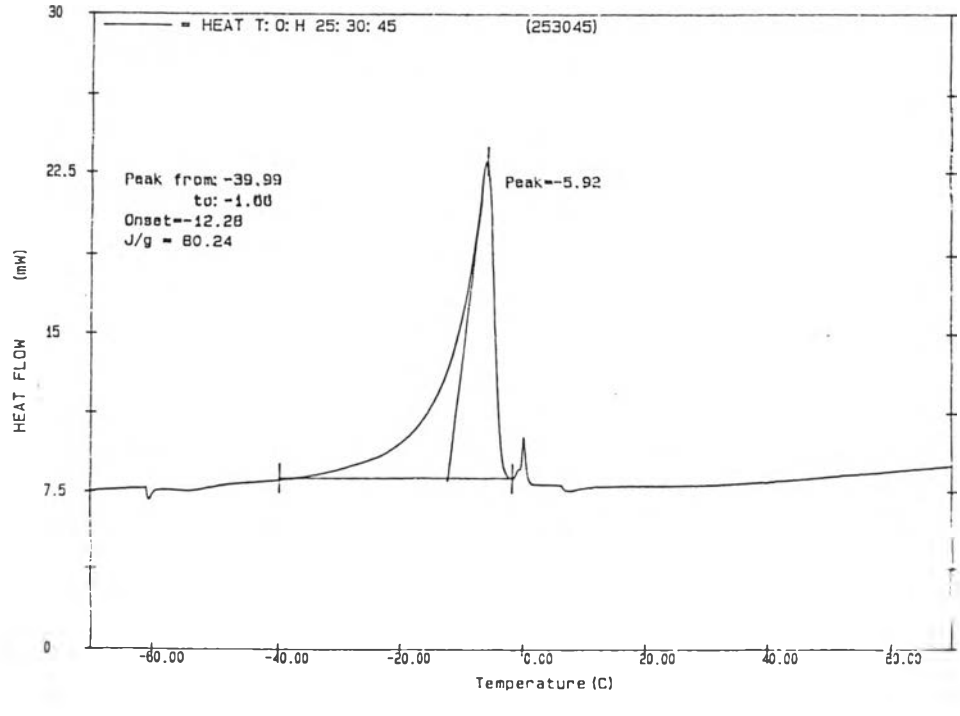


Figure 29. DSC Thermogram of Pure Arlamol®E.

Triethanolamine:oleic acid:water systems gave both planar lamellar and hexagonal structures (see Figure 25). These systems had totally different transition behaviors, regardless of the structure formed. That is, the hexagonal phase formed by different ratios of components gave different transition patterns (Figure 30). Since triethanolamine and oleic acid can interact to produce triethanolamine oleate, a known surfactant, the ratio of triethanolamine and oleic acid in the system might play a major role in determining the microscopic structure of the liquid crystalline phase. It is probable that in the excess of oleic acid , the molecules might penetrate into the lipophilic region of the liquid crystalline structure and affect the structure of liquid crystals. This might be the cause of the difference in transition pattern of the above two systems of triethanolamine:oleic acid:water, even though the texture of the liquid crystals were the same.



(a)



(b)

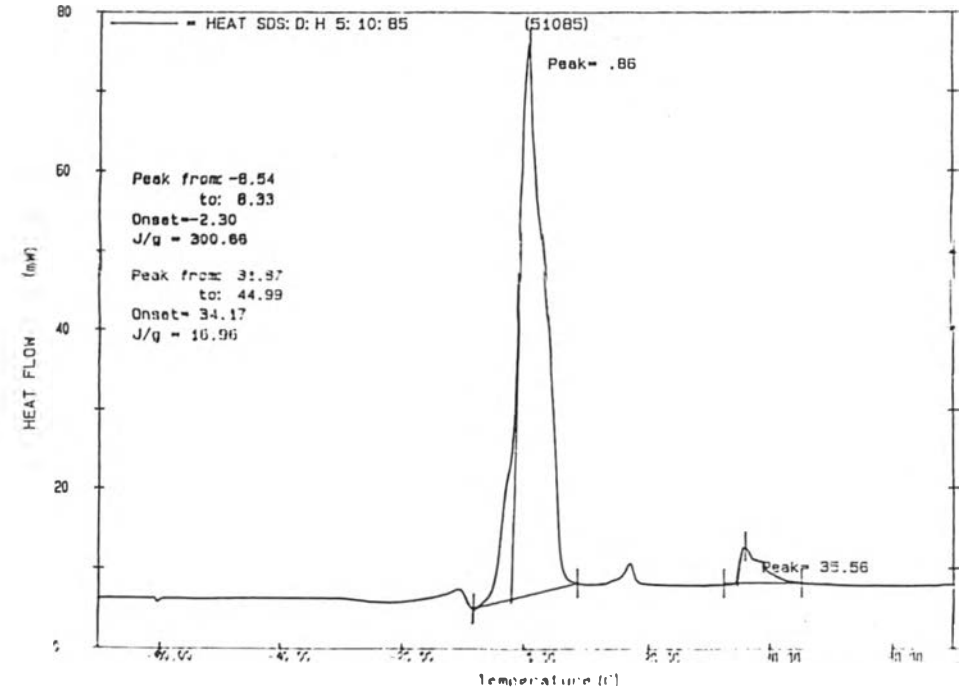
Figure 30. DSC Thermograms of the Hexagonal Liquid Crystalline Systems Composed of Triethanolamine:Oleic acid: and Water (a) 10:50:40 (b) 25:30:45.

The system from SDS:decanol:water selected for DSC investigation were of closed lamellar and planar lamellar textures (see Figure 24). The main transitions of the two systems were at the same temperature, but the difference in peak width was evident (Figure 31). The system with SDS:decanol:water (20:30:50) displayed a slightly broader peak when compared with the system with the component ratio of 5:10:85. The broader peak means less co-operativity of the molecules constituting the structure (Gennis, 1989). An exothermic peak was present in the former system where SDS content was high. This might be the evidence of recrystallization of the surfactant, which was not revealed by polarized light microscopy probably because the crystals formed were too small, both in size and in quantity.

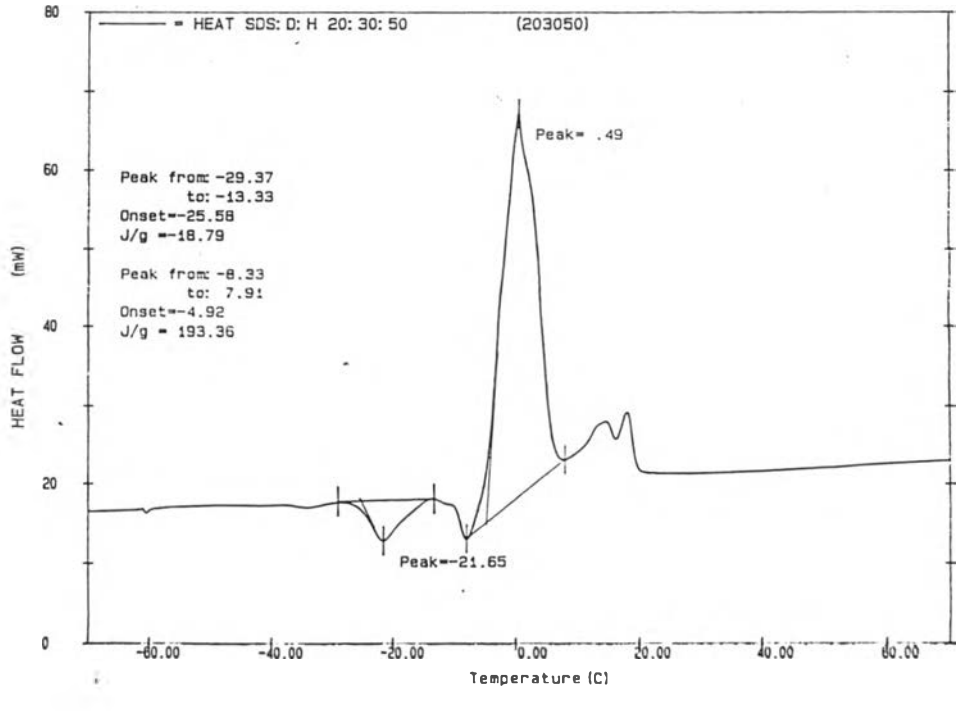
Liquid crystalline systems from lecithin had the same characteristics in terms of transition temperature and peak width (Figure 32). However, ΔH of the liposomal system was much higher than the system composed of lecithin:water (40:60). This suggests the higher co-operativity of the molecules in the liposomal system (Gennis, 1989). Figure 22 shows inhomogeneity in the texture of lecithin:water (40:60) that might be responsible for less co-operativity of the molecules constituting the structure. The photograph of liposomes was not available for comparison due to the minute size of the vesicles.

3. Effects of Additives on the Formation and Structures of Liquid Crystalline Systems

The summary of effects of additives on the structure and other properties of the liquid crystalline systems are shown in Tables 3-6. Thermograms of each liquid crystalline system with additives are displayed in Appendix E.

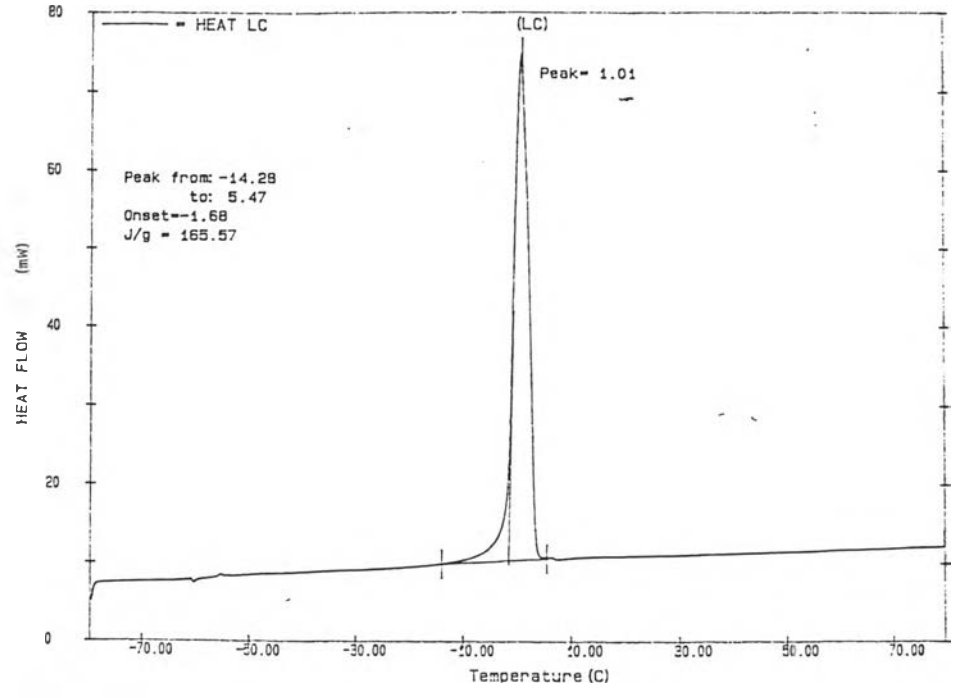


(a)

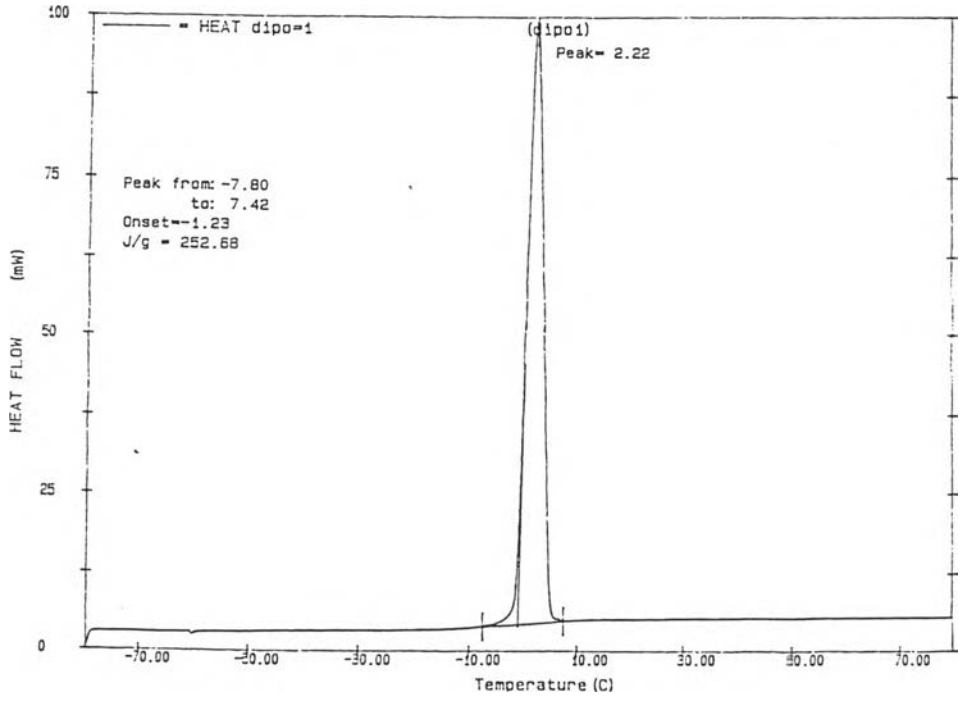


(b)

Figure 31. DSC Thermograms of the Liquid Crystalline Systems Composed of SDS:Decanol:and Water (a) 5:10:85 (b) 20:30:50.



(a)



(b)

Figure 32. DSC Thermograms of the Liquid Crystalline Systems Composed of Lecithin:Water (a) Emulsion (40:60) (b) Liposomal System.

Table 3. Effects of Trehalose

System	Structure without additive	Structure with additive	% of additive	T_m	Co-operativity	Exothermic peak
Brij®72:Arlamol®E:water (15:10:75)	closed lamellar	closed lamellar	3	unchanged	increased	no
triethanolamine:oleic acid:water (15:50:35)	planar lamellar	planar lamellar	10	unchanged	increased	no
triethanolamine:oleic acid:water (25:30:45)	hexagonal	disappeared	30	-	-	-
sodium dodecyl sulfate (SDS):decanol:water (20:30:50)	planar lamellar	planar lamellar	20	decreased	decreased	yes
lecithin:water	closed lamellar	closed lamellar	20	decreased	decreased	no
liposomal system	closed lamellar	closed lamellar	1	unchanged	unchanged	no

Table 4. Effects of 10% urea

System	Structure without additive	Structure with additive	T_m	Co-operativity	Exothermic peak
Brij®72:Arlamol®E:water (15:10:75)	closed lamellar	closed lamellar	decreased	increased	no
triethanolamine:oleic acid:water (10:50:40)	hexagonal	planar lamellar	decreased	increased	yes
triethanolamine:oleic acid:water (15:50:35)	planar lamellar	planar lamellar	decreased	increased	yes
triethanolamine:oleic acid:water (25:30:45)	hexagonal	hexagonal	decreased	decreased	no
SDS:decanol:water (20:30:50)	planar lamellar	planar lamellar	decreased	decreased	no
lecithin:water	closed lamellar	closed lamellar	decreased	decreased	no
liposomal system	closed lamellar	closed lamellar	unchanged	decreased	no

Table 5. Effects of Sodium Chloride

System	Structure without additive	Structure with additive	% of additive	T_m	Co-operativity	Exothermic peak
Brij®72:Arlamol®E:water (15:10:75)	closed lamellar	closed lamellar	6	decreased	decreased	no
triethanolamine:oleic acid:water (15:50:35)	planar lamellar	planar lamellar	1	unchanged	unchanged	no
triethanolamine:oleic acid:water (25:30:45)	hexagonal	hexagonal	1	unchanged	increased	yes
SDS:decanol:water (20:30:50)	planar lamellar	planar lamellar	1	unchanged	decreased	yes
lecithin:water	closed lamellar	closed lamellar	9	decreased	decreased	no

Table 6. Effects of α-Tocopherol

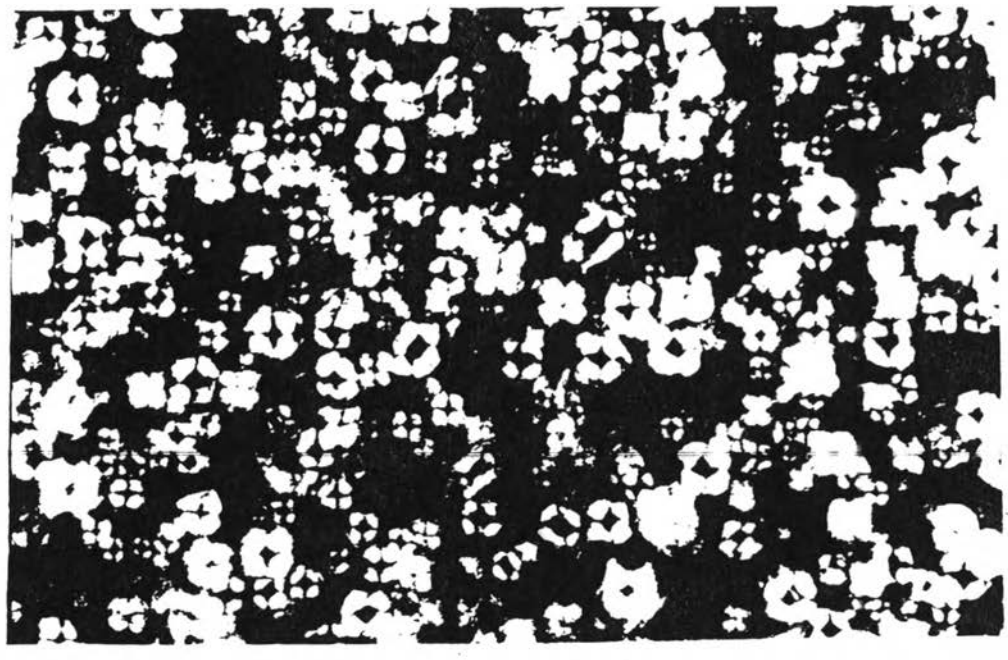
System	Structure without additive	Structure with additive	%of additive	T_m	Co-operativity	Exothermic peak
Brij®72:Arlamol®E:water (15:10:75)	closed lamellar	closed lamellar	3	unchanged	increased	no
triethanolamine:oleic acid:water (15:50:35)	planar lamellar	hexagonal	15	unchanged	increased	yes
triethanolamine:oleic acid:water (25:30:45)	hexagonal	planar lamellar	10	unchanged	unchanged	no
SDS:decanol:water (20:30:50)	planar lamellar	planar lamellar	5	unchanged	decreased	yes
lecithin:water	closed lamellar	closed lamellar	5	unchanged	unchanged	no
liposomal system	closed lamellar	closed lamellar	1	unchanged	unchanged	no

3.1 Effects of Trehalose

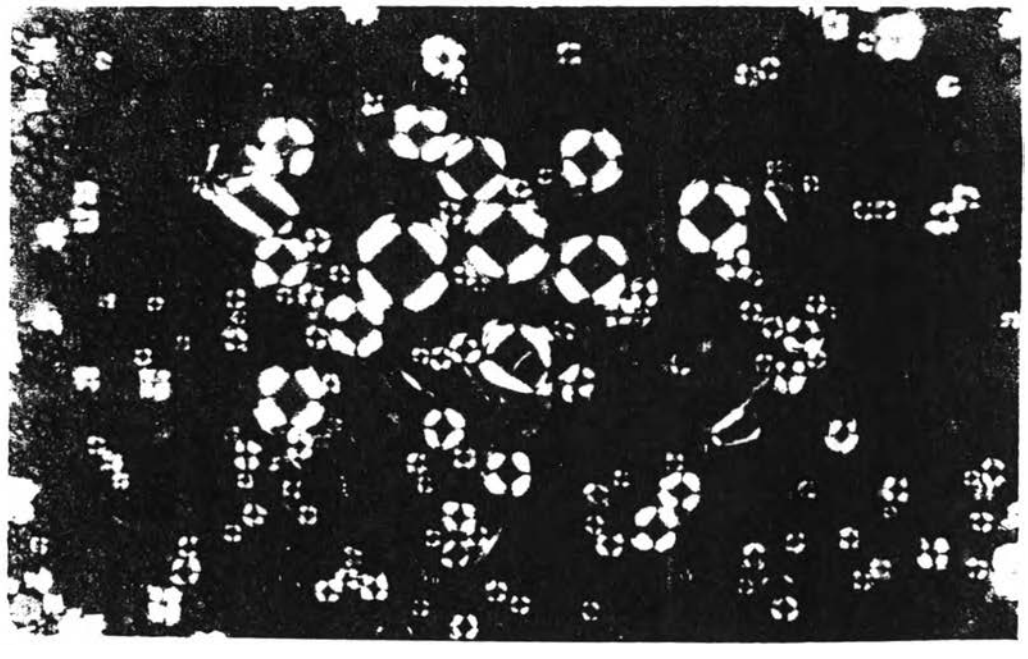
Water soluble trehalose is used primarily as a cryoprotective agent. It has drawn much attention as a promising cryoprotectant for liposomes during lyophilization process for commercial production of the delivery system. Crow et al. (1986) found that trehalose provided satisfactory cryoprotection for liposomes in their laboratory experiments. When trehalose was added into liquid crystalline systems, it did not induce a change in the structure of liquid crystals both from surfactants and lecithin until its concentration had reached a certain value. Above the maximal tolerable concentration, the structure of liquid crystals disappeared, either partially or completely depending on the structure-forming surfactant. For example, in the Brij®72 system, the liquid crystalline structure disappeared partially when 5% trehalose was added (Figure 33). The gross physical appearance of the preparation also changed when the concentration of trehalose reached a certain level made, possibly by the removal of water from the sytem due to high aqueous solubility of the additive.

Even when it did not prohibit formation of liquid crystalline structure, addition of trehalose into liquid crystalline systems could cause some changes in the characteristics of the system though the changes could not be detected by polarized light microscopy. DSC thermograms, however, could detect changes in the transition temperature, the peak width, and the enthalpy of these systems.

The presence of 20% trehalose in the liquid crystalline system composed of lecithin and water in the ratio of 40:60 resulted in an increase in the peak width and a decrease in the transition temperature and ΔH (Figure 34). This suggests that trehalose might have an influence on the behavior of the phospholipid bilayer by increasing the

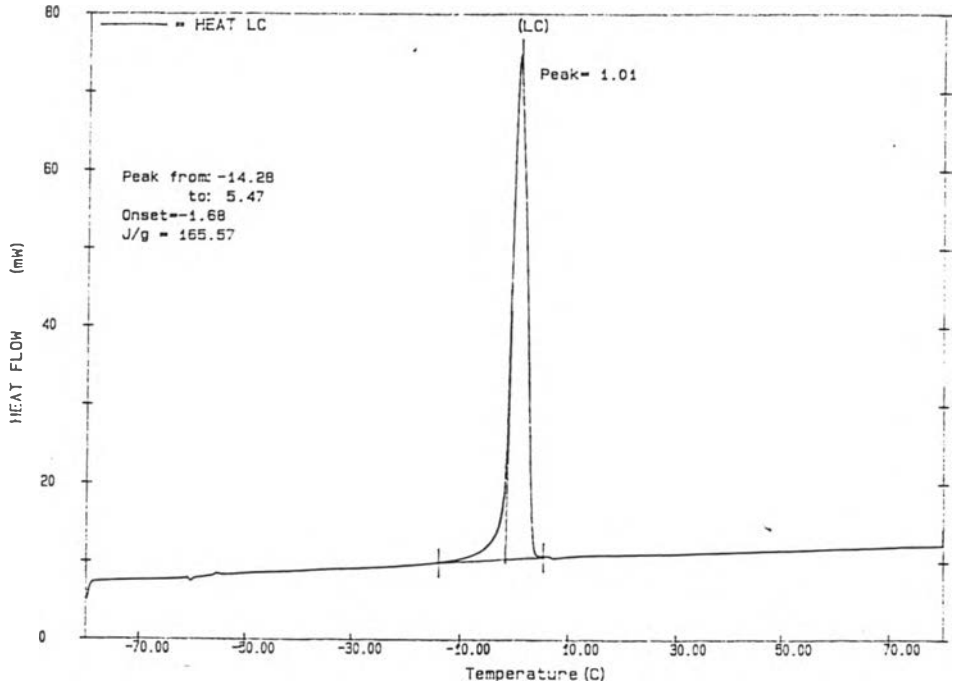


(a)

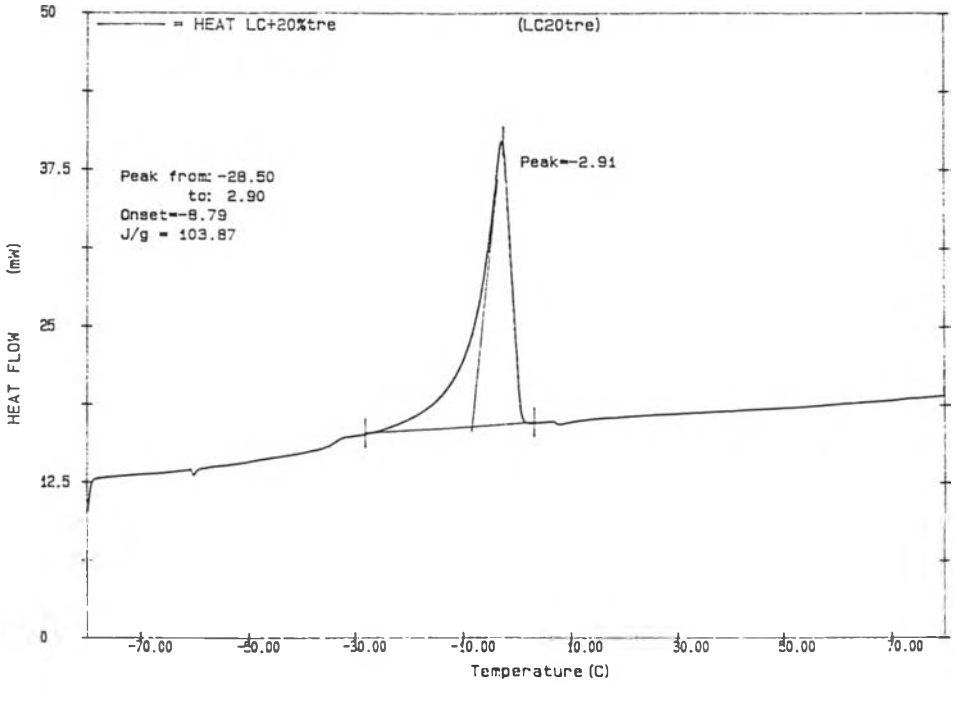


(b)

Figure 33. Photomicrographs from Polarized Light Microscopy Showing the Lamellar Structure of Brij®72:Arlamol®E:Water (a) Without Trehalose (b) With 5% Trehalose.



(a)



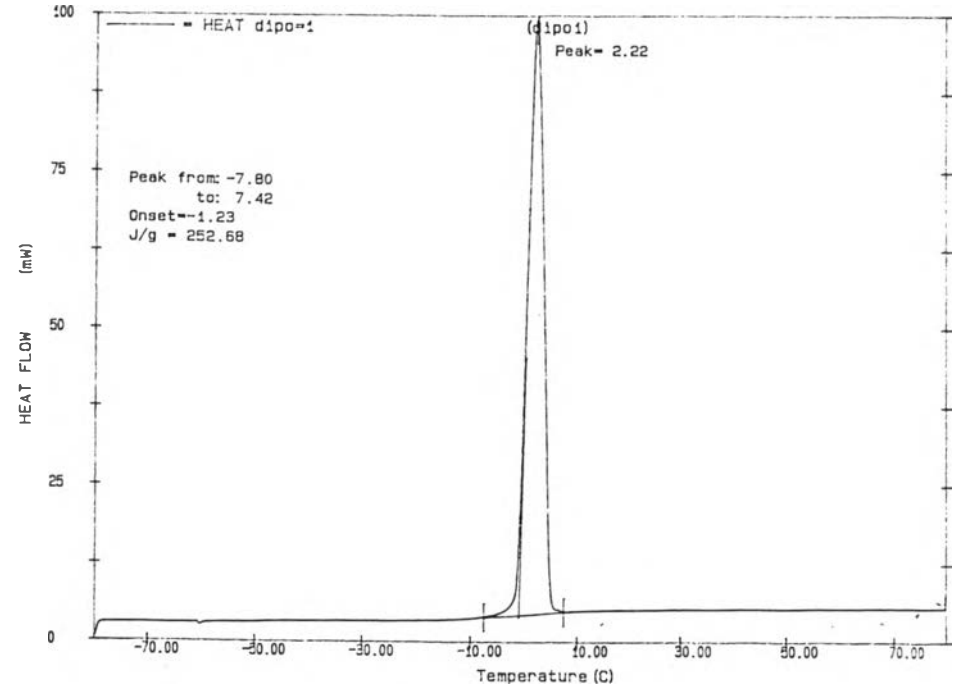
(b)

Figure 34. DSC Thermograms of the Liquid Crystalline Systems composed of Lecithin:Water (40:60) (a) Without Trehalose (b) With 20%Trehalose.

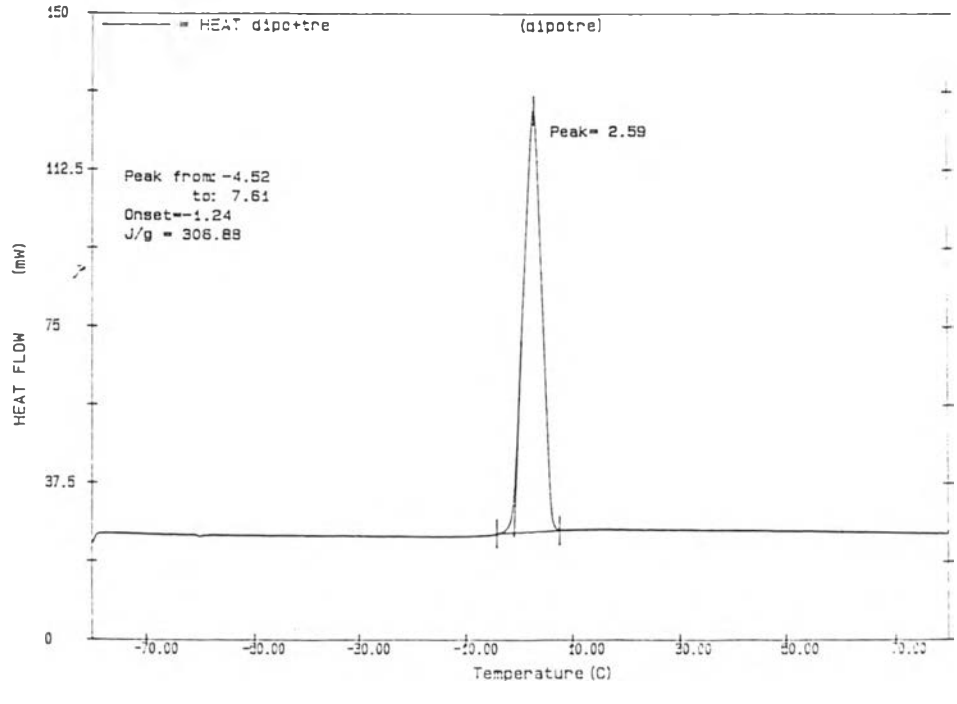
distance between the headgroups of adjacent phospholipid molecules, so the acyl chains of the bilayer phospholipid had more space to move around. The bilayer then became more fluidized, resulting in a lower transition temperature. This effect was not seen with liposomes (Figure 35), probably because of the lower trehalose concentration incorporated into the liposomal structure due to the high aqueous solubility of the sugar. Alternatively, the method of hydration may influence the interaction between trehalose and the headgroup of lecithin. The latter is more probable since trehalose increased ΔH of liposomes, implying a greater co-operativity of the molecules: an opposite effect of what was seen with the other phospholipid system. This result agrees with the higher degree of homogeneity of the liposomal systems.

DSC analysis showed that the system of triethanolamine:oleic acid:water (15:50:35) consisted of at least two populations of liquid crystals (Figure 36) as also revealed by polarized light microscopy (see Figure 25b.). When trehalose was added to the system, the thermogram showed more co-operativity of the system as seen from the increase in ΔH and the merging tendency of the two peaks (Figure 37). The similar effect could also be seen in the system of Brij®72:Arlamol E:water (15:10:75) (Figure 38).

Trehalose might also inhibit recrystallization of SDS in the system of SDS:decanol:water (20:30:50) (Figure 39), probably by interacting with the headgroup of the surfactant.



(a)



(b)

Figure 35. DSC Thermograms of the Liposomal Systems (a) Without Trehalose (b) With 5% Trehalose.

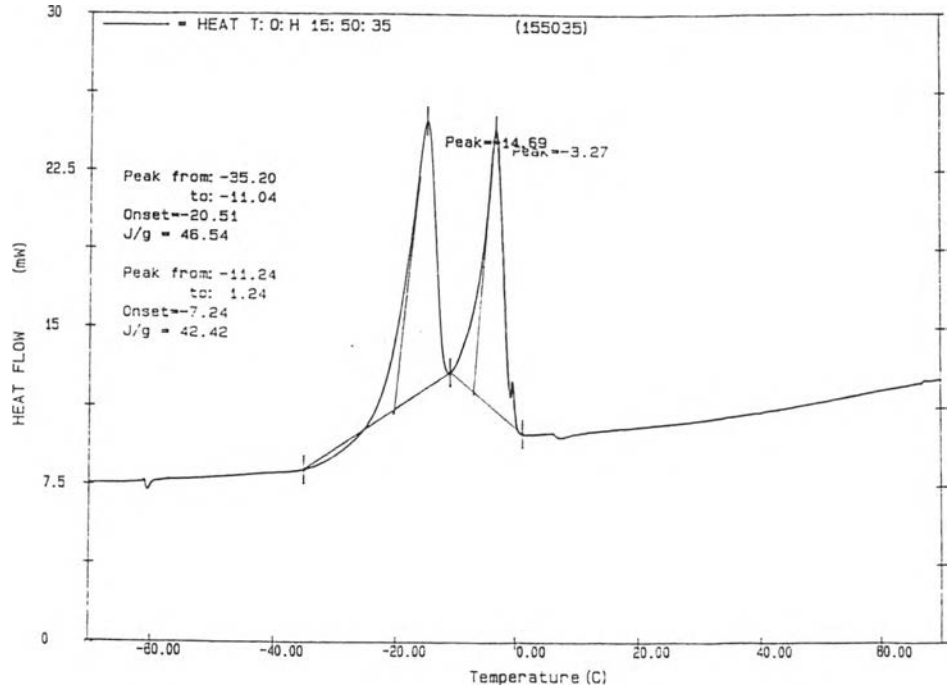


Figure 36. DSC Thermogram of the Liquid Crystalline System Composed of Trithanolamine:Oleic Acid:Water (15:50:35).

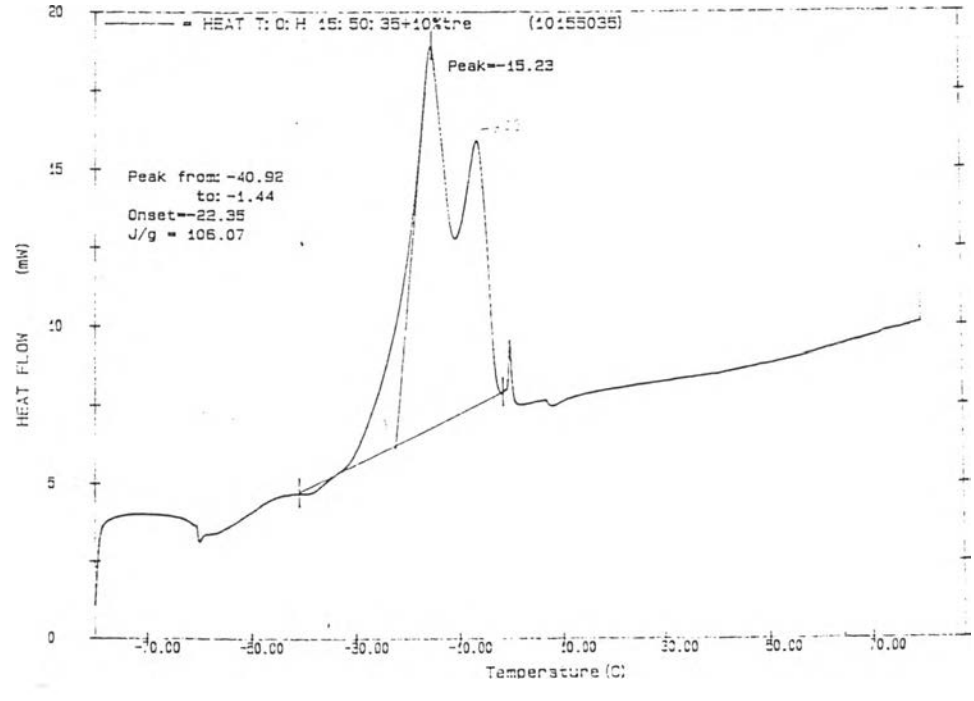
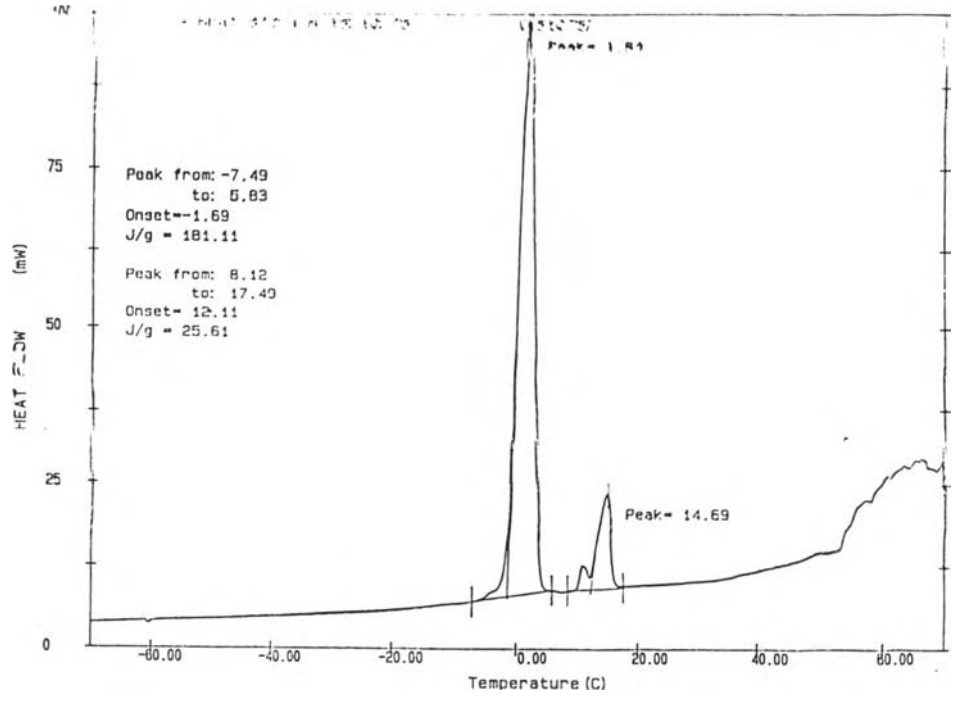
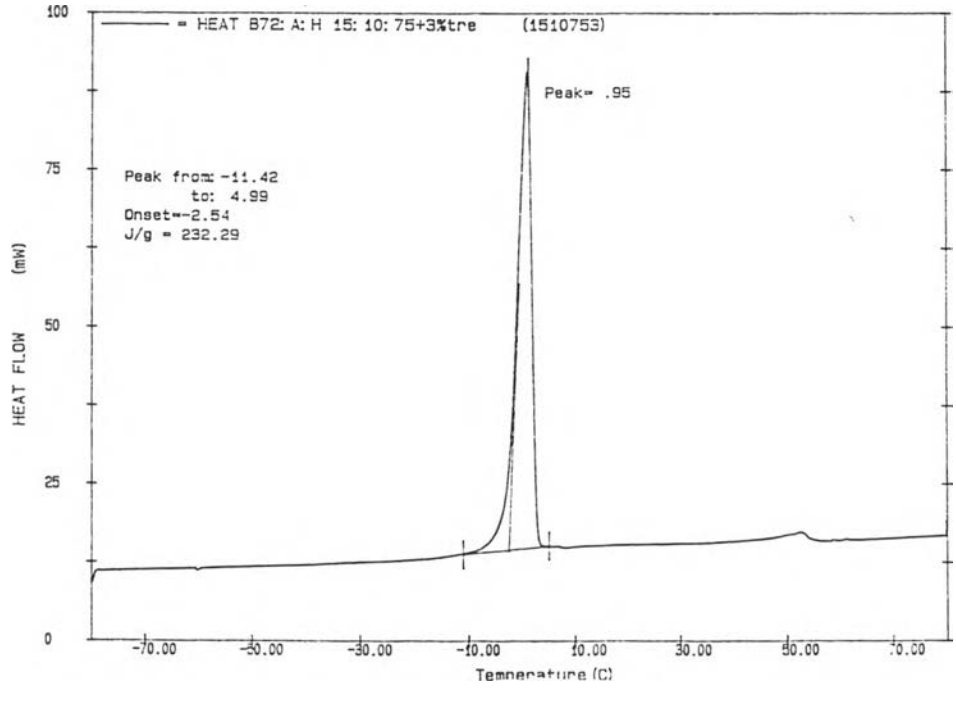


Figure 37. DSC Thermogram of the Liquid Crystalline System Composed of Trithanolamine:Oleic Acid:Water (15:50:35) With 10% Trehalose.

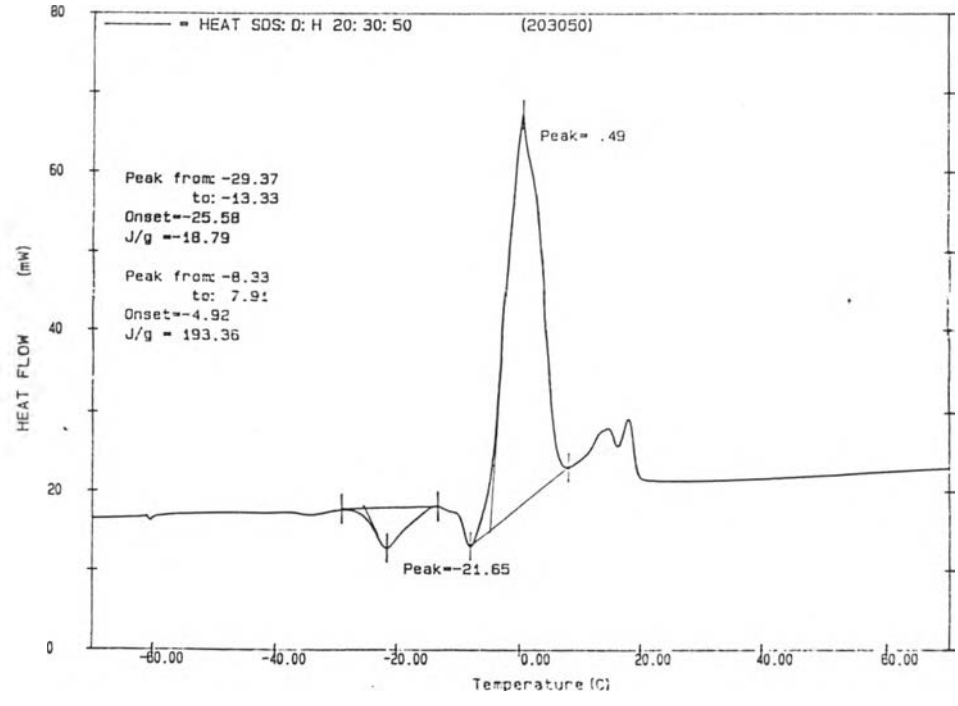


(a)

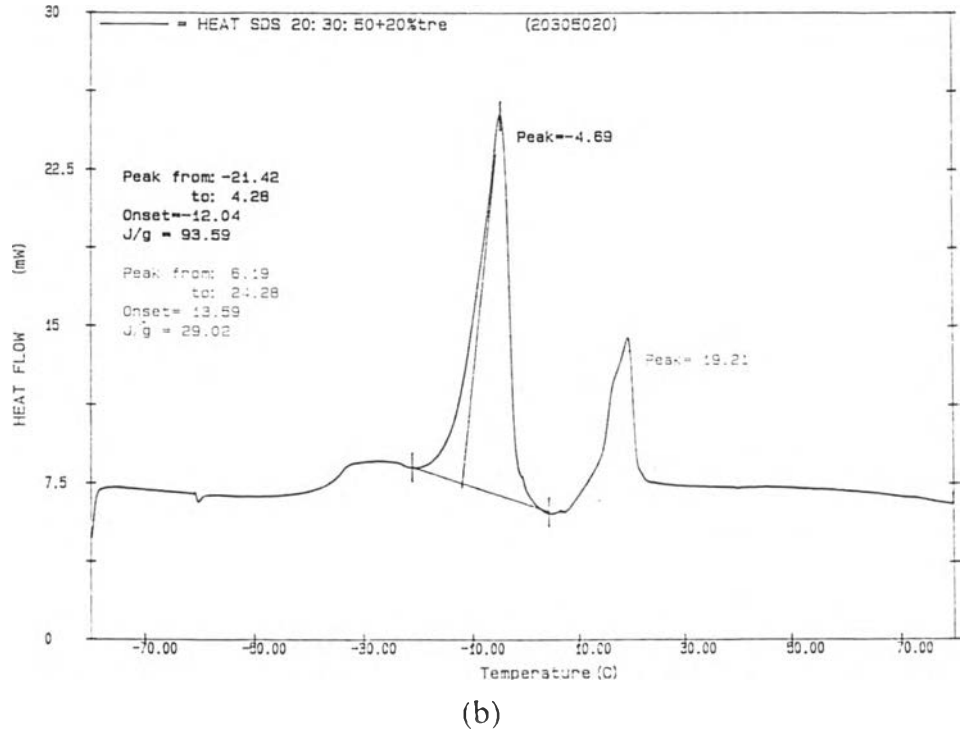


(b)

Figure 38. DSC Thermograms of the Liquid Crystalline Systems Composed of Brij®72:Arlamol®E:Water (15:10:75) (a) Without Trehalose (b) With 3% Trehalose.



(a)

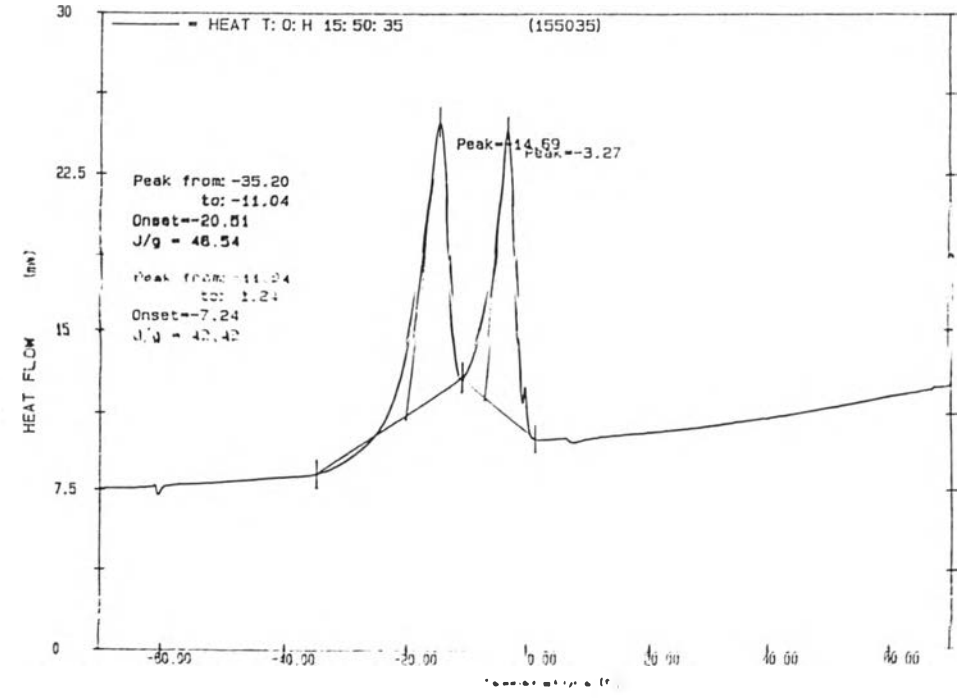


(b)

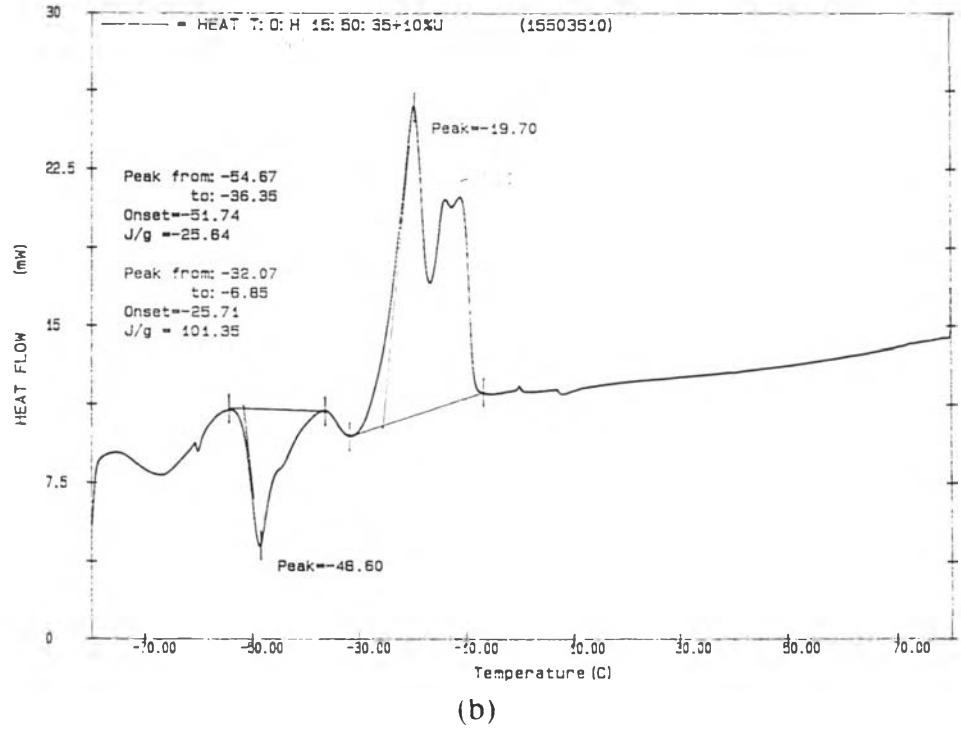
Figure 39. DSC Thermograms of the Liquid Crystalline Systems Composed of SDS:Decanol:Water (20:30:50) (a) Without Trehalose (b) With 20% Trehalose.

3.2 Effects of Urea

When 10% urea was added to the liquid crystalline systems, a phase change occurred in the liquid crystalline structure from a hexagonal structure to a planar lamellar structure in the system composed of triethanolamine, oleic acid, and water in the ratio of 10:50:40 % w/w. The underlying mechanism could be water removal from the hexagonal structure induced by the high concentration of urea. When the bulk water was excluded from the structure, the surfactant molecules came closer together, giving a chance of lyotropic phase reversion. The packing rearrangement of liquid crystals in other systems studied was not evident by polarized light microscopy when urea was added to the system. The effects of urea on phase behavior of the liquid crystalline system was also investigated by DSC. The results showed that the gel to liquid crystalline transition temperatures were decreased in all systems. This suggests that the distance between the headgroups of surfactants and lecithin increased due to increasing hydration of the headgroups by urea; therefore, the acyl chains had more space to mobilize resulting in the lower transition temperature. Urea might also affect the interlamellar space and water content of the bilayer. However, this speculation could not be verified since the appropriate technique, i.e., small angle X-ray diffraction, was not accessible. Urea also had the same effect as trehalose in increasing co-operativity of some systems as evident by DSC thermograms (Figure 40). However, with the liposomal system, urea induced incomplete annealing of liposomal vesicles as observed by optical microscopy. This may be responsible for the second peak shown in DSC thermograms of liposomal systems with and without urea (Figure 41). This reduction in system co-operativity was also observed with lecithin in the form of emulsion with lamellar structure (Figure 42).

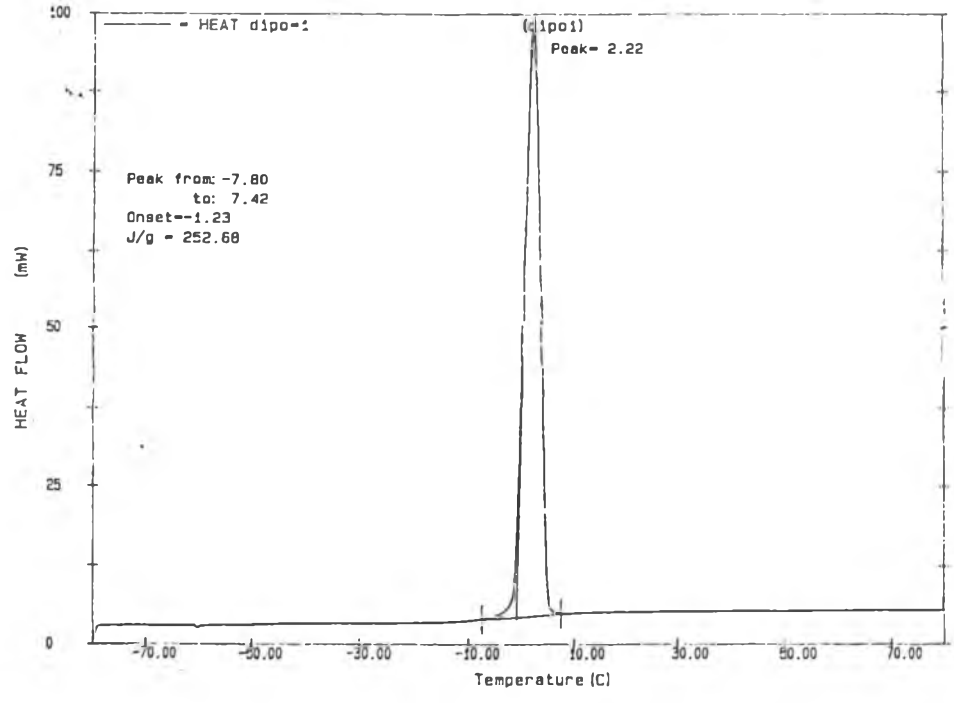


(a)

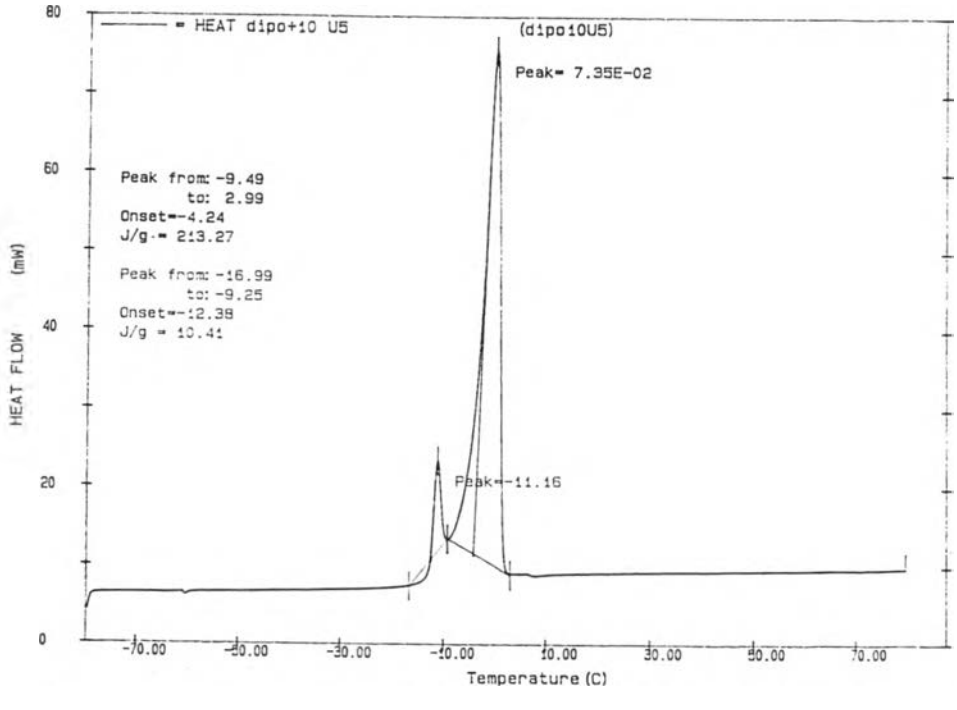


(b)

Figure 40. DSC Thermograms of the Liquid Crystalline Systems Composed of Triethanolamine:Oleic Acid:Water (15:50:35) (a) Without Urea (b) With 10% Urea.

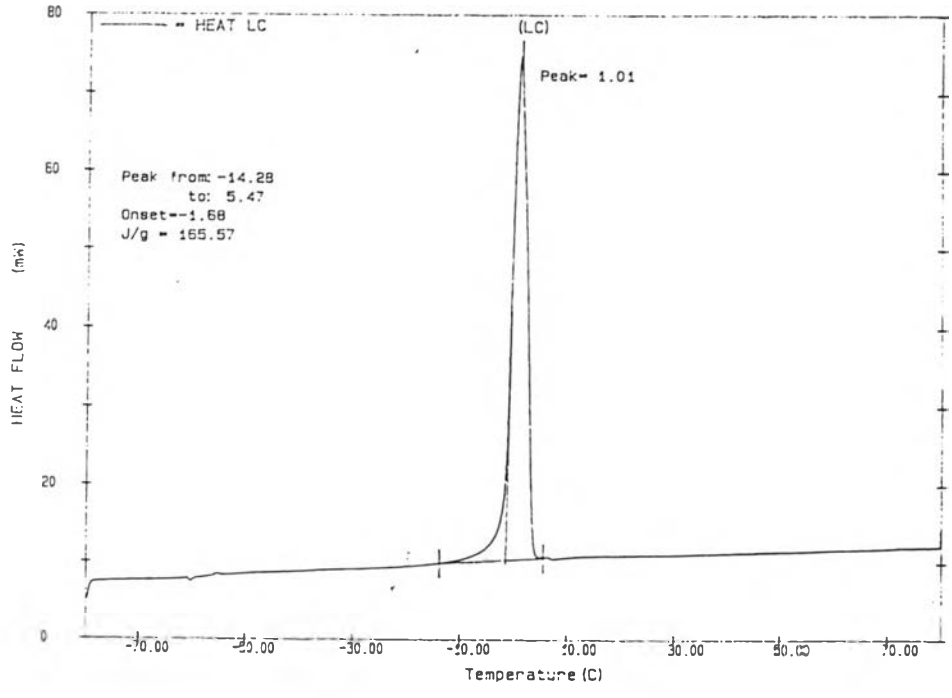


(a)

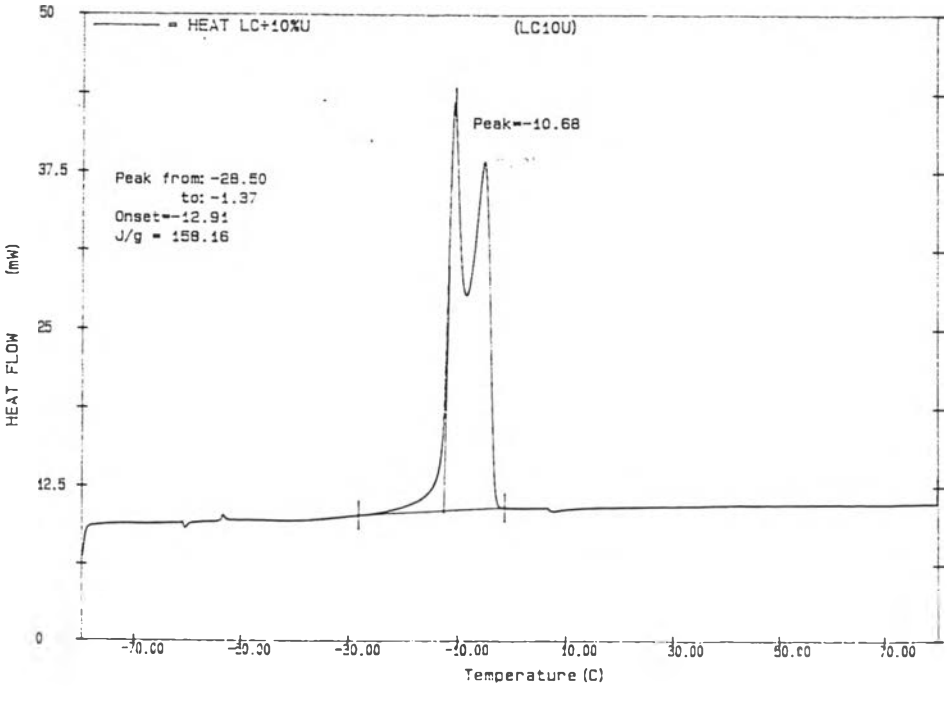


(b)

Figure 41. DSC Thermograms of the Liposomal Systems (a) Without Urea (b) With 10% Urea.

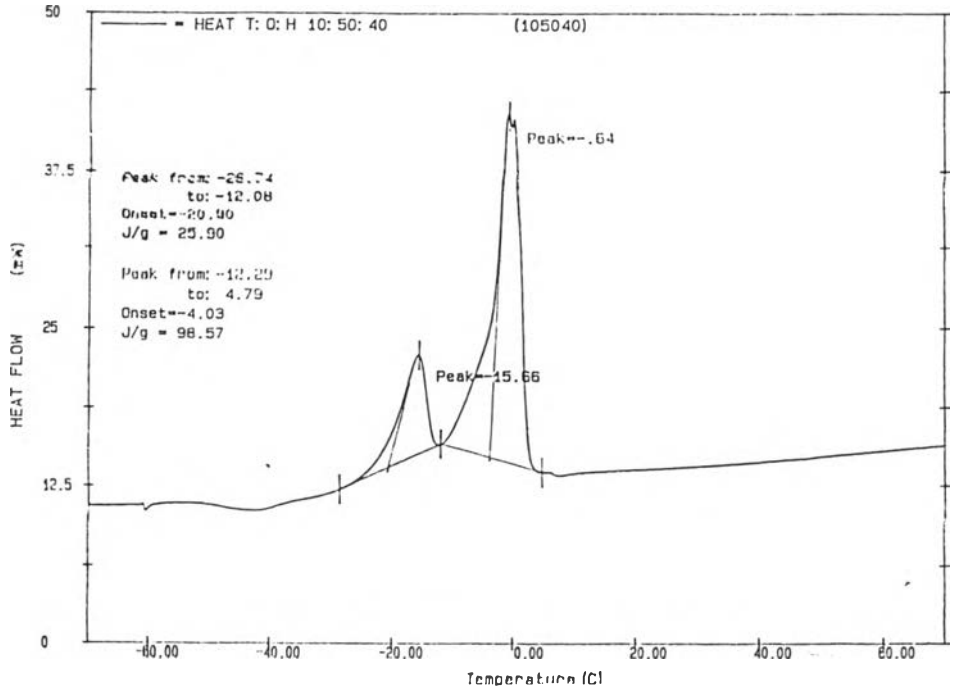


(a)

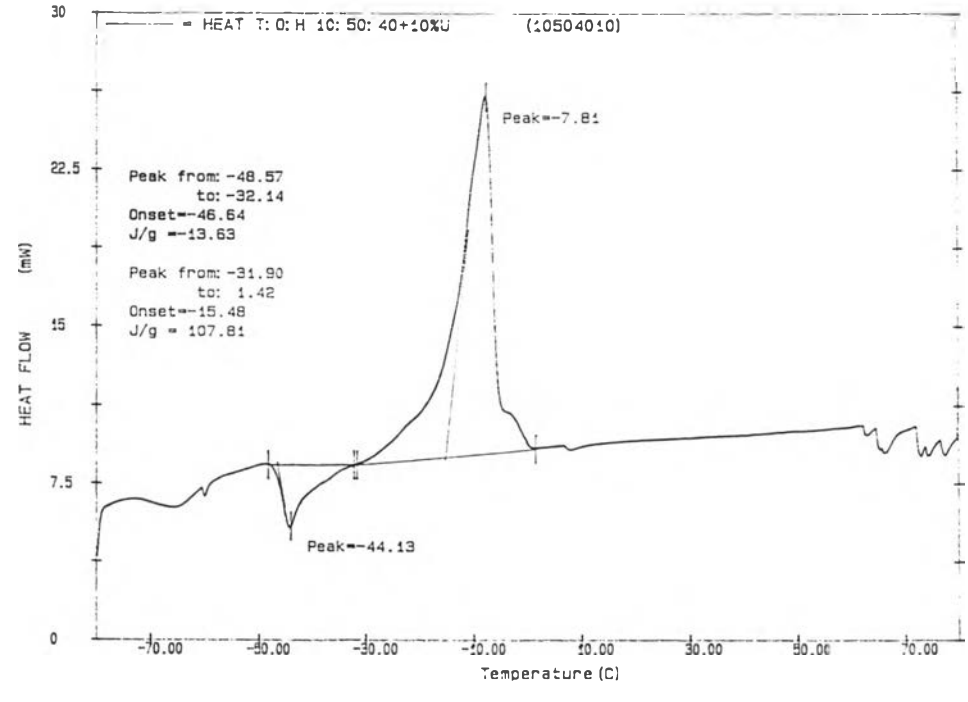


(b)

Figure 42. DSC Thermograms of the Liquid Crystalline Systems Composed of Lecithin:Water (40:60) (a) Without Urea (b) With 10% Urea.



(a)

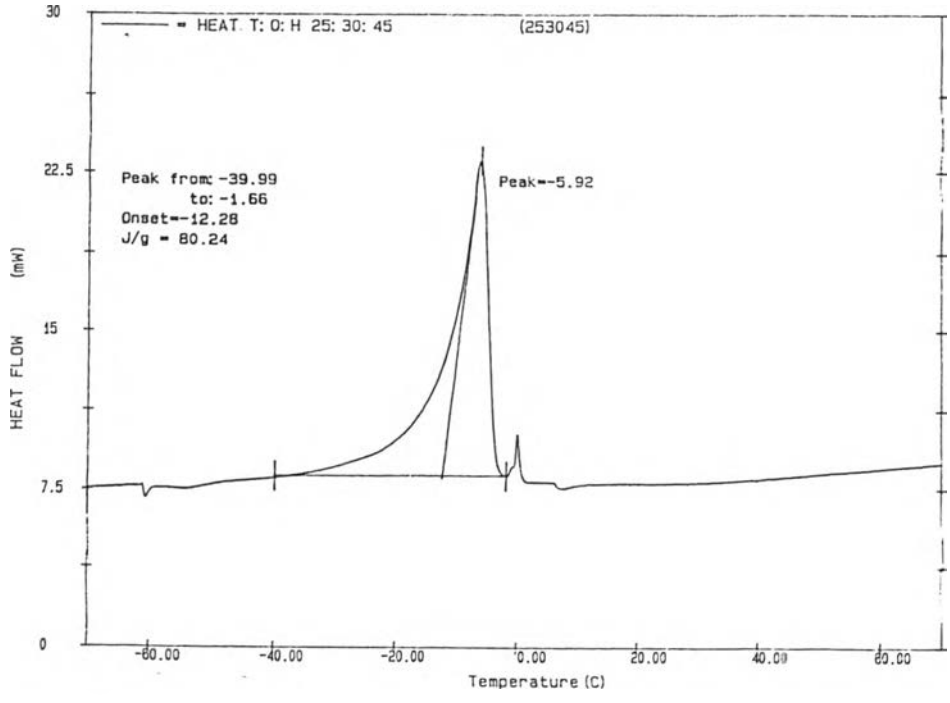


(b)

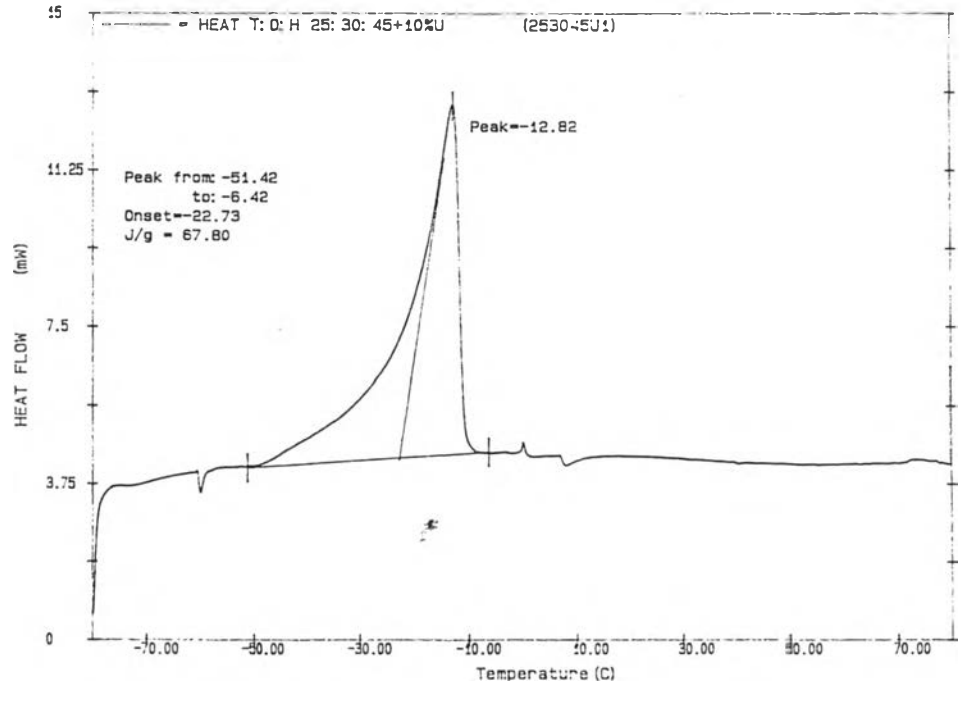
Figure 43. DSC Thermograms of the Liquid Crystalline Systems Composed of Triethanolamine:Oleic Acid:Water (10:50:40) (a) Without Urea (b) With 10% Urea.

In triethanolamine:oleic acid:water systems, effects of urea depended on the ratio of the components in the system. In systems with excess oleic acid (triethanolamine:oleic acid:water 10:50:40 and 15:50:35, equivalent to molar ratios of triethanolamine:oleic acid of 0.39 and 0.55, respectively), urea induced an exothermic peak which might be corresponding to recrystallization of oleic acid (Figure 43 and see Figure 40). Urea also increased co-operativity of the main transition of these systems. In the first system, urea caused a phase change from hexagonal structure (see Figure 25a) to planar lamellar structure (similar to Figure 25b). This phase change agrees well with the increase in co-operativity of the system. The phase change from hexagonal structure to lamellar structure could be explained with the same reasoning as the effect of urea on transition temperature. As the headgroups were more hydrated, the acyl chains were more far apart, and the interaction between the hydrocarbon chains that kept the molecules in the hexagonal structure was reduced. The curvature of the hexagonal structure became more flattened. Finally the radius of curvature became too great for the hexagonal structure to exist, and the phase became lamellar. For the triethanolamine:oleic acid:water (25:30:45) system with excess triethanolamine content (molar ratio of triethanolamine:oleic acid of 1.64), urea decreased co-operativity of the system as can be seen from the broader peak width and a decrease in ΔH of DSC thermogram in Figure 44.

For the SDS: decanol:water (20:30:50) system of planar bilayer, urea imposed the same effect as trehalose. That is, the recrystallization peak disappeared, and a new endothermic peak was evident (Figure 45). In this case, urea might induced hydration of the recrystallized surfactant molecules, and the hydrated SDS could form a binary liquid crystalline structure (Myer, 1997) which gave a new endothermic peak in the thermogram.

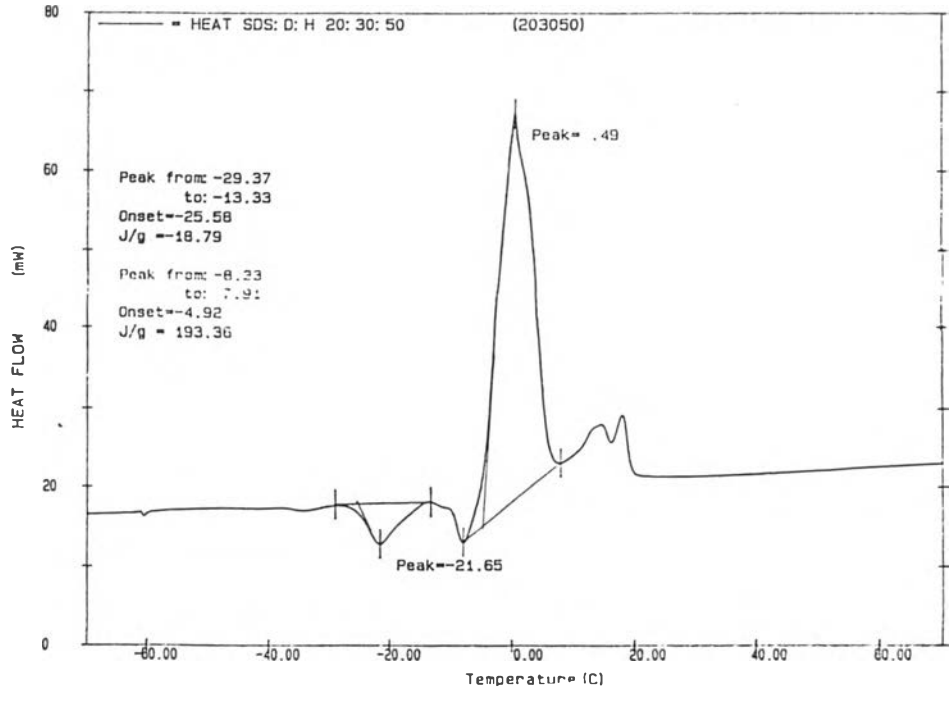


(a)

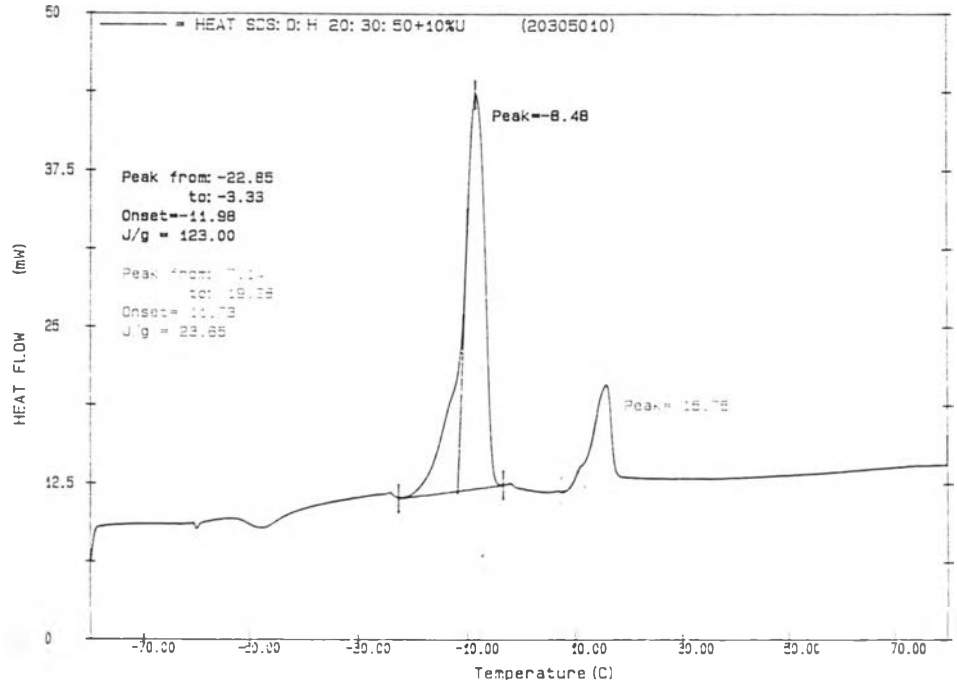


(b)

Figure 44. DSC Thermograms of the Liquid Crystalline Systems Composed of Triethanolamine:Oleic Acid:Water (25:30:45) (a) Without Urea (b) With 10% Urea.



(a)



(b)

Figure 45. DSC Thermograms of the Liquid Crystalline Systems Composed of SDS:Decanol:Water (20:30:50) (a) Without Urea (b) With 10% Urea.

3.3 Effects of Sodium Chloride

Sodium chloride is widely used in a variety of pharmaceutical formulations. Its primary use is to produce isotonic solutions. Sodium chloride is also an additive used to control drug release from microcapsules (Tirkkonen and Paronen, 1992). It has also been used to control micelle size (Richard, 1975; McDonald and Richardson, 1981) and to adjust the viscosity of dispersions by altering the ionic character of the formulation (Okor, 1993).

The effect of sodium chloride on ionic liquid crystal-forming surfactants was very large compared to that on nonionic surfactants. In systems consisting of triethanolamine and SDS, the liquid crystalline structures were totally abolished at sodium chloride concentration of more than 1%. This suggests low tolerance to ionic additives of ionic surfactants. Increasing ionic strength of the system due to addition of sodium chloride might be responsible for the inability of the surfactant molecules to arrange into liquid crystalline structures since the headgroup could not display strong ionic properties at high ionic strength. On the other hand, nonionic surfactants were not affected except at very high concentrations. The nonionic ones did not suffer from this effect probably because they have no charged surface, and there would rather be some other explanations, such as the salting-out effect, for the effects of the ionic additives at very high concentrations which needs to be further investigated.

Sodium chloride, like trehalose, did not induced a change in the liquid crystalline structure. However, in the system composed of Brij®72, Arlamol®E, and water in the ratio of 15:10:75% w/w, high concentrations of sodium chloride (6% or more by weight) induced clustering of liquid crystal droplets by salting out (Figure 46).

Sodium chloride affected thermal behavior of the liquid crystalline systems. It increased co-operativity of the transition in the system of triethanolamine:oleic acid:water (25:30:45) and induced an exothermic peak as seen from the DSC thermograms (Figure 47). Since the system was excess in triethanolamine (triethanolamine:oleic acid ratio of 1.64), it is speculated that the exothermic peak was from recrystallization of excess triethanolamine. As a result of this recrystallization process, the rest of the system was composed only of triethanolamine oleate and water resulting in better co-operativity of the system since the molecules were all alike. Thus, the main transition peak was sharper, and ΔH of the transition increased.

Sodium chloride decreased transition temperatures of the systems with Brij®72: Arlamol®E:water (15:10:75) (Figure 48) and lecithin:water (40:60) (Figure 49). This effect could not be explained by a simple mechanism of either increased ionic strength or salting out. These two mechanisms should have resulted in increasing transition temperature of the system as reported by Powell et al. (1994). Sodium chloride also reduced co-operativity in the systems of lecithin:water (40:60) (see Figure 49) and SDS:decanol:water (20:30:50) (Figure 50). The mechanism underlying this effect is not clear.

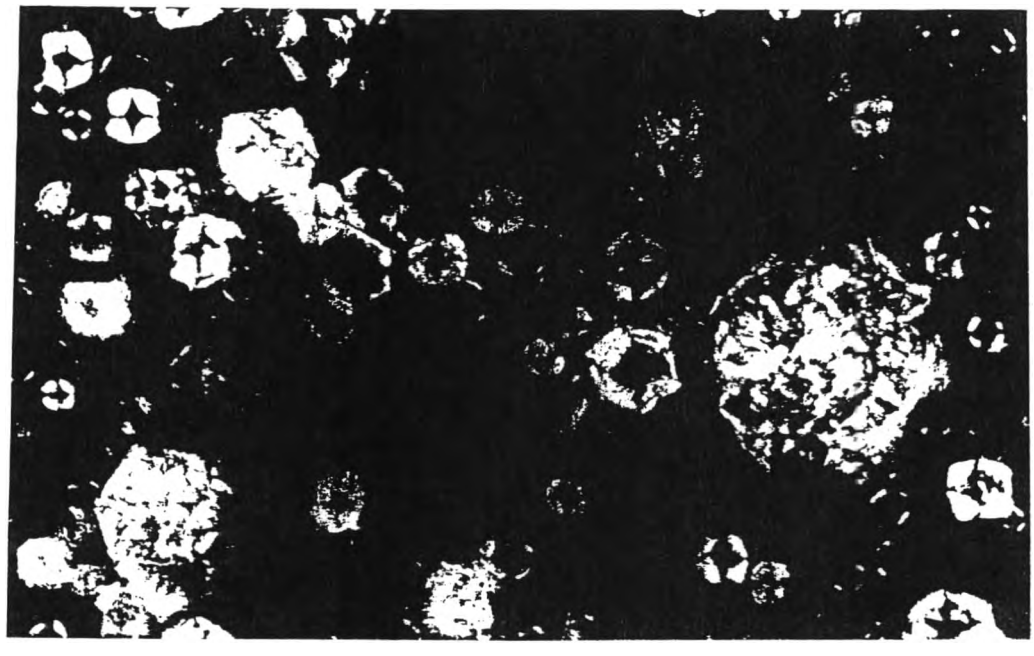
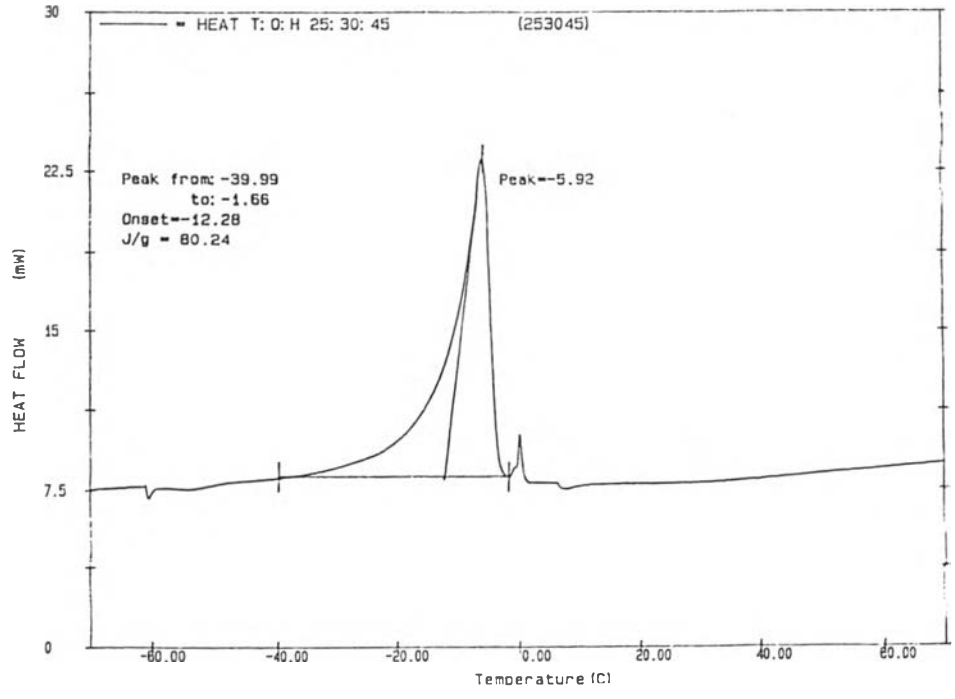
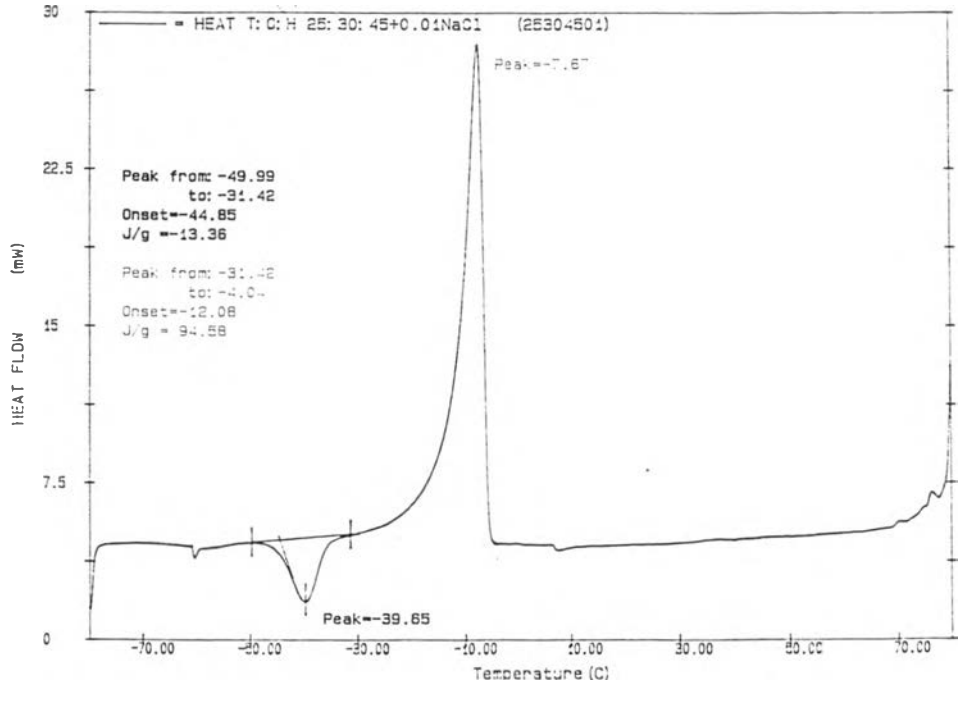


Figure 46. Photomicrograph from Polarized Light Microscopy Showing the Lamellar Structure of Brij®72:Arlamol®E:Water (15:10:75) With 6% Sodium Chloride.

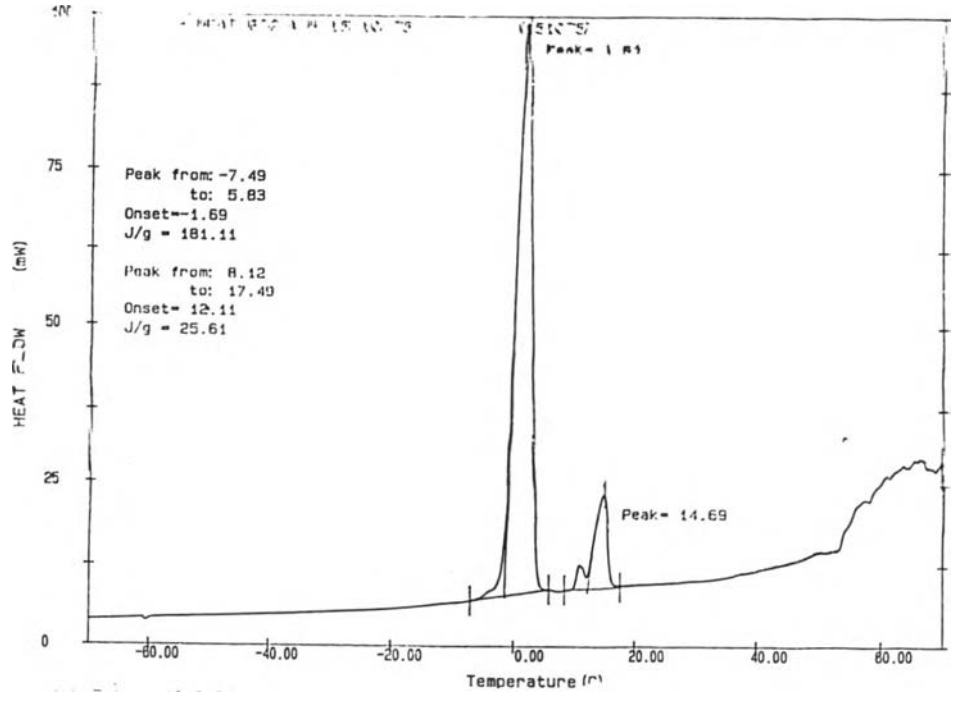


(a)

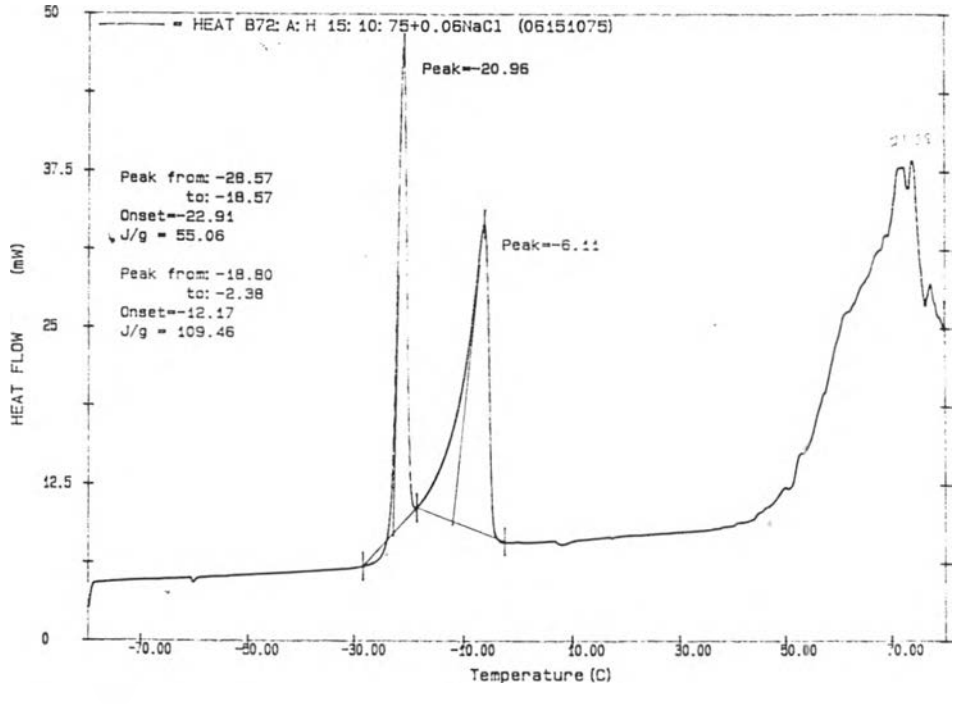


(b)

Figure 47. DSC Thermograms of the Liquid Crystalline Systems Composed of Triethanolamine:Oleic Acid:Water (25:30:45) (a) Without Sodium Chloride (b) With 1% Sodium Chloride.

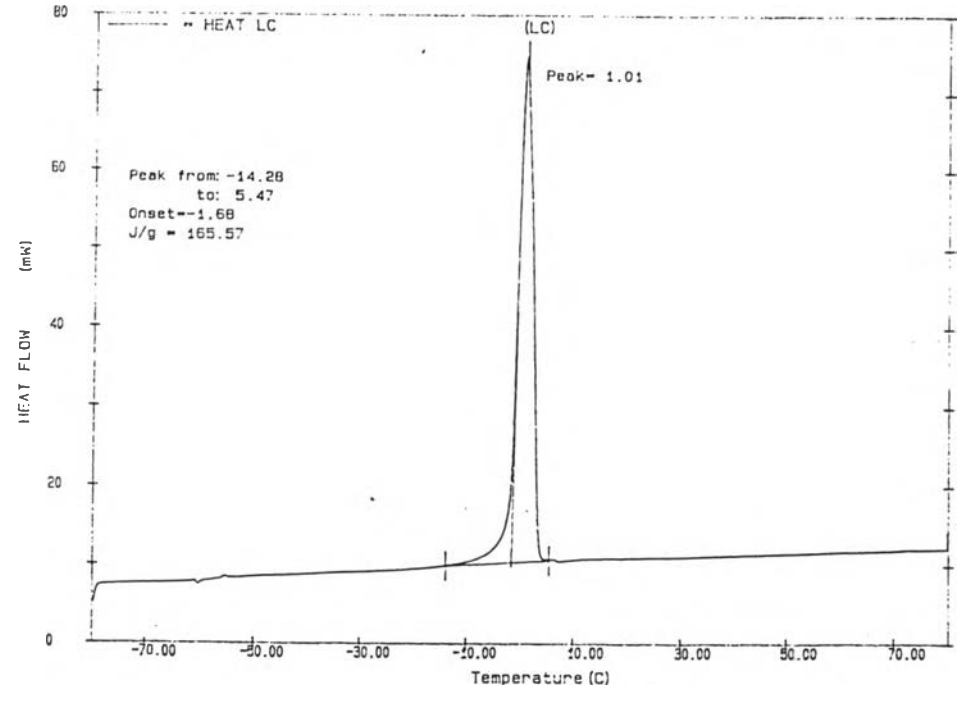


(a)

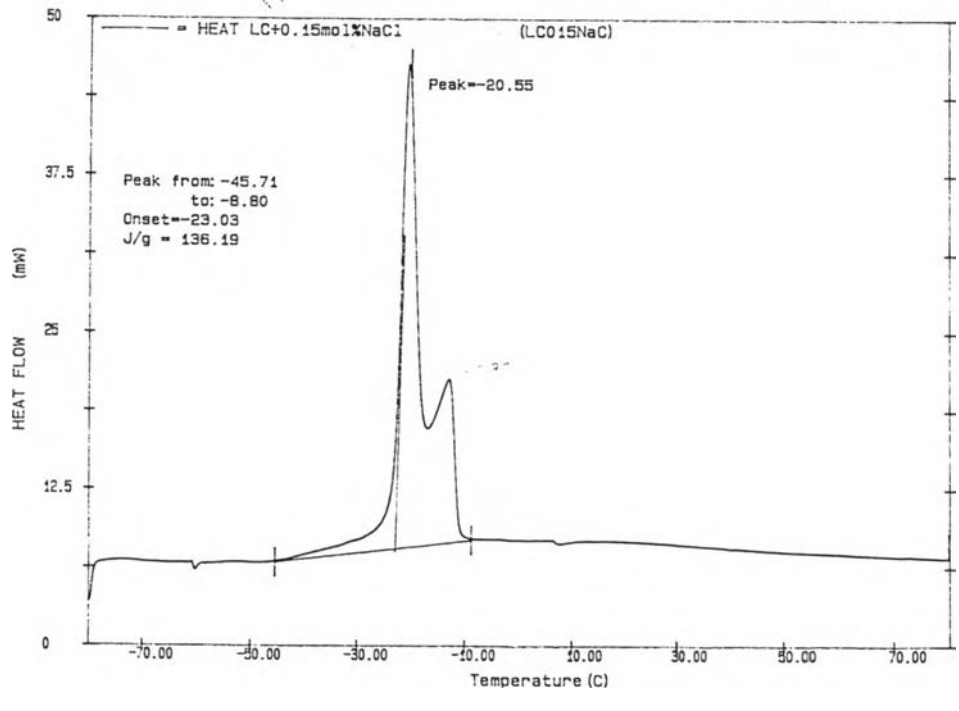


(b)

Figure 48. DSC Thermograms of the Liquid Crystalline Systems Composed of Brij®72:Arlamol®E:Water (15:10:75) (a) Without Sodium Chloride (b) With 6% Sodium Chloride.

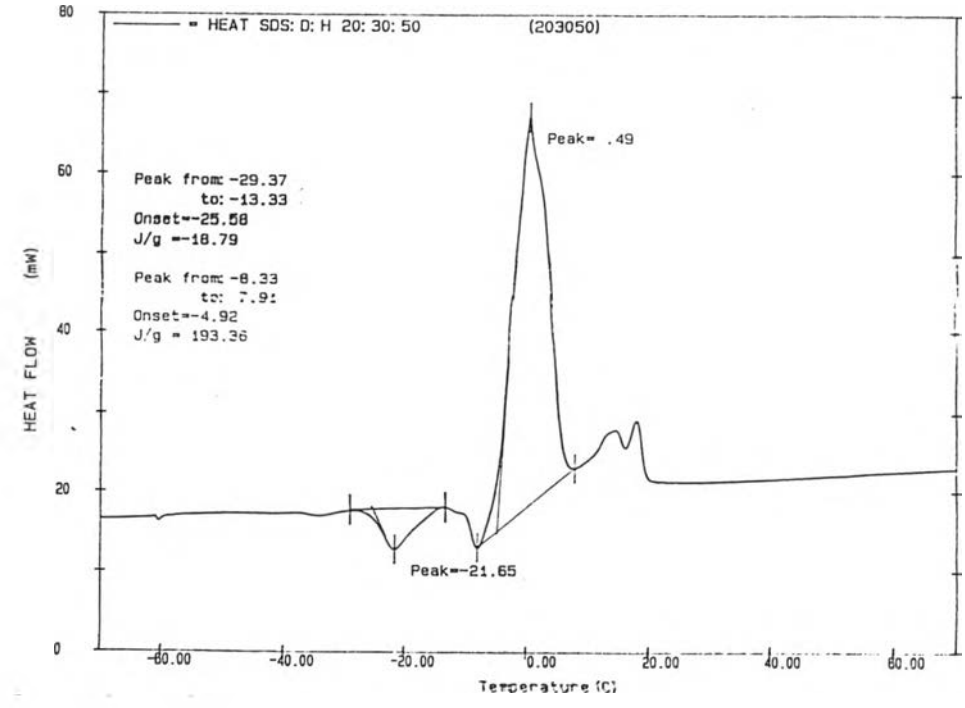


(a)

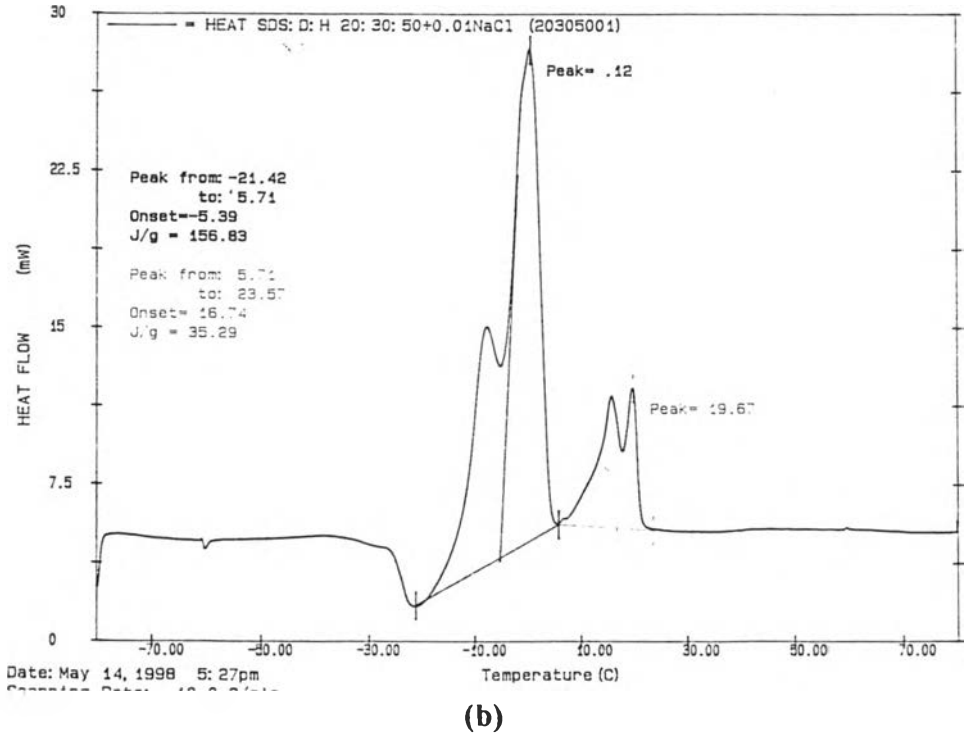


(b)

Figure 49. DSC Thermograms of the Liquid Crystalline Systems Composed of Lecithin:Water (40:60) (a) Without Sodium Chloride (b) With 9% Sodium Chloride.



(a)



(b)

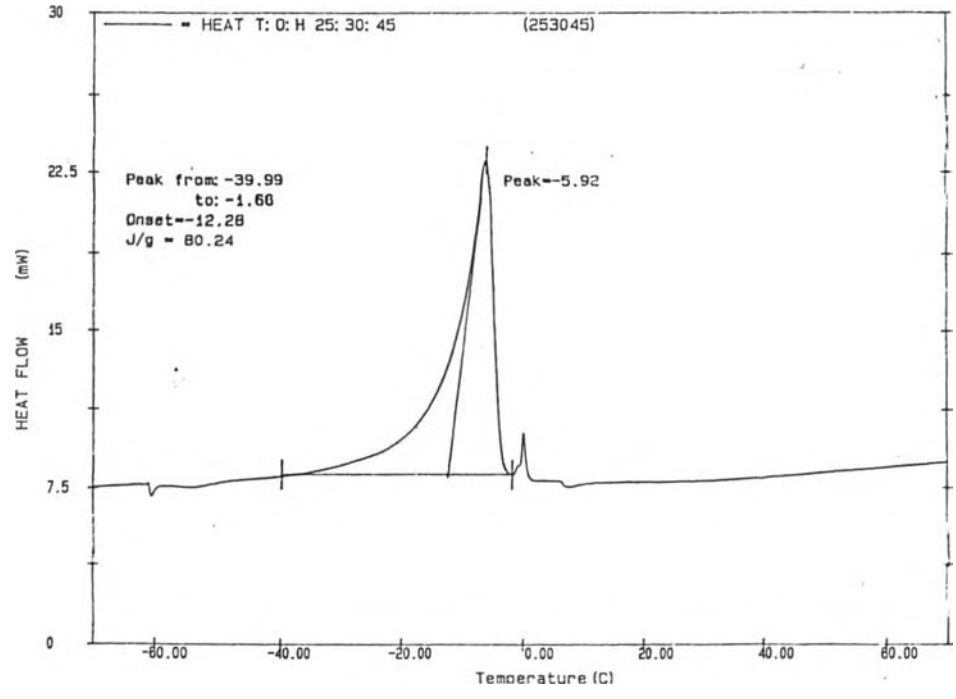
Figure 50. DSC Thermograms of the Liquid Crystalline Systems Composed of SDS:Decanol:Water (20:30:50) (a) Without Sodium Chloride (b) With 1% Sodium Chloride.

3.4 Effects of α-Tocopherol

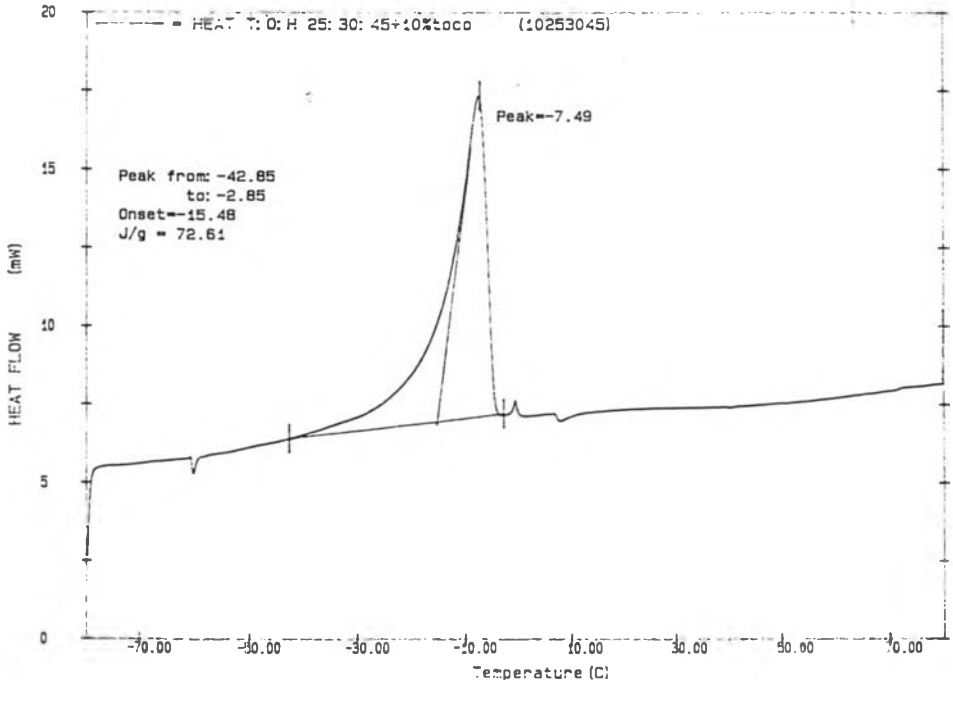
α-Tocopherol, a lipophilic antioxidant, is unlike all other additives used in this study. Since it is lipohilic, it can be included in the structure of liquid crystals whereas all other additives will be restricted to the water phase of the system. Hence, it participates in directing the liquid crystalline structure of the system. When α-tocopherol was added to a ternary system of triethanolamine:oleic acid:water, the system became a quaternary system. The resultant liquid crystalline phase could be totally different from the ternary system. For example, when the components were changed from triethanolamine:oleic acid:water (25:30:45) to the same components plus 10% α-tocopherol, the liquid crystals found were in the form of planar lamellar structure instead of the hexagonal structure found with the ternary system. The change in the opposite direction from a hexagonal structure to a planar lamellar structure was also seen (see Table 6).

The effect of α-tocopherol on thermal behavior of the system composed of triethanolamine, oleic acid and water in the ratio of 25:30:45 was further investigated by DSC. The presence of α-tocopherol in the new system resulted in only a slight decrease in the transition temperature from -5.92 ^{0}C to -7.49 ^{0}C and in ΔH from 80.24 J/g to 72.61 J/g (Figure 51). A previous study by Quinn (1995) also shows the same effect in decreasing the transition temperature and ΔH when α-tocopherol was added to a liposomal system.

From these experiments, the results showed that the lyotropic liquid crystals accommodated different solutes to varying degrees. The presence of a solute dissolved in the liquid crystalline system could influence the formation and the structures of



(a)



(b)

Figure 51. DSC Thermograms of the Liquid Crystalline Systems Composed of Triethanolamine:Oleic Acid:Water(25:30:45) (a) Without α-Tocopherol (b) With 10% α-Tocopherol.

liquid crystals as well as the gross physical appearance when the solute concentration reached a certain level. These changes could influence the drug solubilizing ability of the system or affect the potential of the systems to control drug release which should be further investigated.

4. Assessment of the Possibilities of Using Liquid Crystalline Systems as Drug Delivery Systems

4.1 Ability to Solubilize Propylthiouracil (PTU) and Triamcinolone Acetonide (TA)

The saturation of each drug in the liquid crystalline systems was studied by varying concentration of the drug in each system. The saturation solubility of each drug was determined by polarizing microscopy. The highest drug concentration at which drug crystals were not observed after 3 days was considered to represent the saturation solubility of the drug in that system. The results of these experiments are shown in Appendix C. The liquid crystalline systems from Brij®72:Arlamol®E:water and SDS:decanol:water could hold a maximum of 0.2% PTU. Maximal saturation concentration of PTU in the systems of triethanolamine:oleic acid:water was 1.1%. Maximal concentration obtained with lecithin systems was 0.5%. The results showed that the solubility of PTU in liquid crystalline systems was more than the solubility of PTU in water (0.15%) and in a typical model of o/w emulsion. The reference aqueous solubility used was experimentally determined in this study since the quality of water and temperature can affect the solubility. The method and the results of the solubility

study are shown in Appendix F. PTU is an amphiphile. 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 the liquid crystalline system and water. Therefore, PTU solubility in the liquid crystalline systems was expected to increase by being incorporated into the structure of liquid crystals. The PTU-containing liquid crystalline systems were investigated by DSC (Appendix E). The results showed that addition of PTU at its saturation concentration did not cause any significant changes in the DSC thermograms.

In the case of liquid crystalline system in the form of liposomes, PTU was added into phospholipid film. When water was added to hydrate the thin film of lecithin to form liposome, most PTU partitioned into the water phase resulting in low concentration of the drug in the liposomal bilayer (Appendix C, Table C.2-C.5). To reduce the partitioning of the drug into the aqueous phase, the film was hydrated with a solution of 0.8 mg/ml of PTU in water. The amount of PTU in the pellet retrieved from 1 ml liposomal suspension increased from 496.33 µg to 1,256.67 µg. The concentration of 0.8 mg/ml of PTU in water was the maximal concentration of PTU which no recrystallization was observed when the solution was stored in the refrigerator for more than 48 hours. This was crucial since lecithin is not stable at room temperature, and liposomal preparations were to be kept refrigerated. This suggests that incorporation of drugs with the same solubility properties as PTU into liposomal bilayers for sustained release or for other reasons requires existence of the drugs in the water phase in order to force the drugs to remain in the bilayer.

As for TA, the liquid crystalline systems studied could not accommodate any significant amounts of the drug. Less than 0.1% could be solubilized in these systems. This is probably due to the large inflexible structure of the drug. The molecule of the

drug is composed of rigid rings, and the drug has a high molecular weight of 434.49 (Appendix F). Since the drug is hydrophobic, it would have to stay in the hydrophobic region of the liquid crystalline structure. But because of the size and rigidity of the molecule, the steric hindrance would become a problem since the presence of TA molecules in the liquid crystalline structure would destroy the hydrophobic interaction between the surfactant molecules. In a commercial preparation, Aristocort®, TA also exist in solid crystals at the concentration of 0.1% as observed under a polarized light microscopy. Thus, further studies with TA were not continued.

4.2 Water Evaporation Rates from Liquid Crystalline systems

The results from this study showed that water evaporation rates from all liquid crystalline systems including the nonionic cream base were different from bulk water (Appendix G). Profiles of percent cumulative water loss normalized to the total water content in the preparation versus time are displayed in Figure 52. Raw data with means and standard deviations (SD) are displayed in Appendix G. Low evaporation rate means that the water can be retained in the preparation for a longer period of time, which is especially useful when the preparation is aimed at percutaneous delivery. When the cumulative water loss from bulk water was plotted as a function of time, the water evaporation rate was constant, indicating a zero-order evaporation kinetics (Figure 53). Figure 52 shows that the system composed of triethanolamine, oleic acid and water at the ratio of 25:30:45 displayed highest evaporation rate though the amount of initial water of this system was close to those of the other systems. This system also had the highest viscosity. Furthermore, the triethanolamine systems still had higher

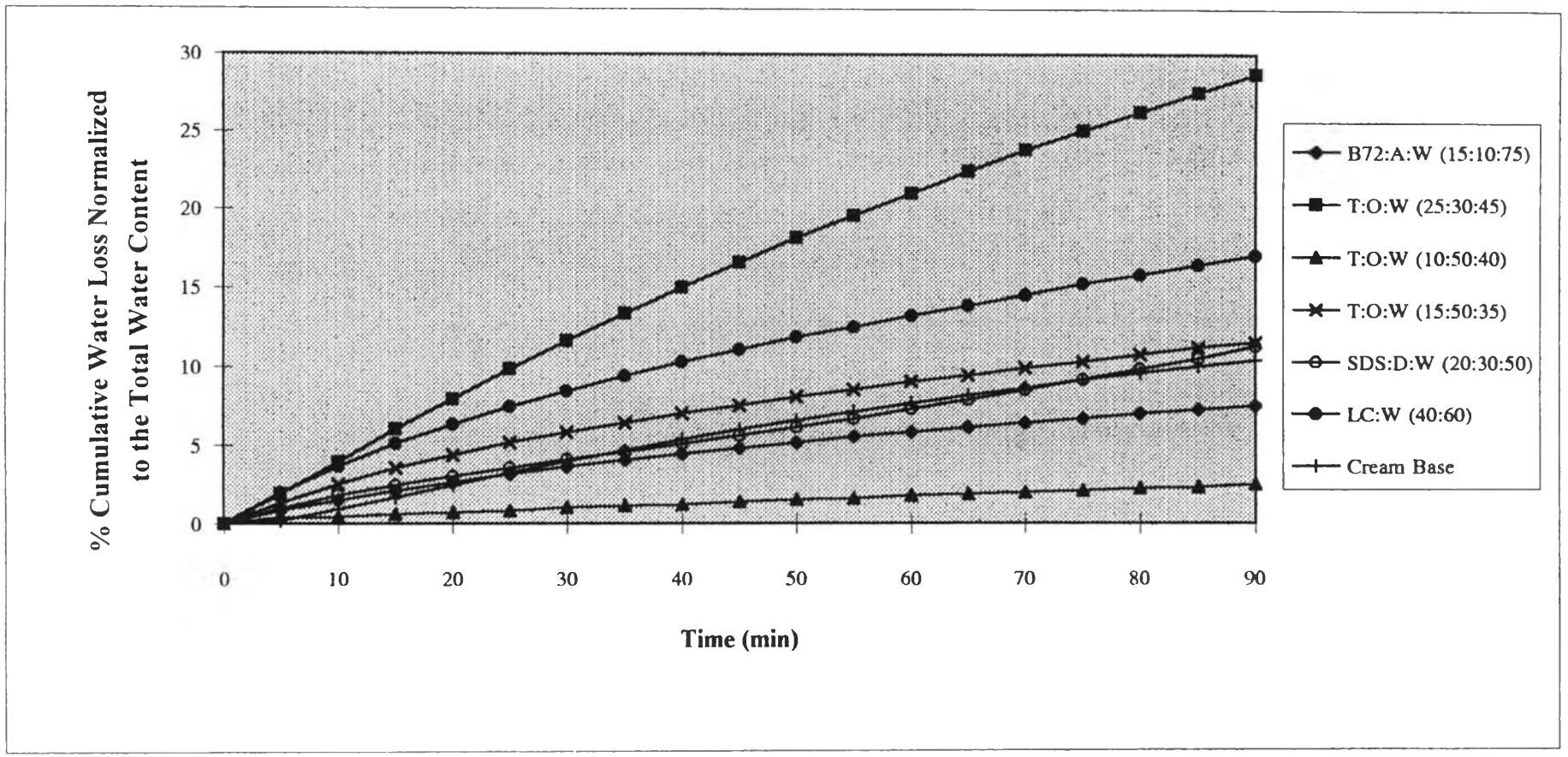


Figure 52 Percent Cumulative Water Loss Normalized to the Total Water Content of the Liquid Crystalline Systems (mean of triplicate runs) Versus Time. Standard deviations are displayed in Appendix G.

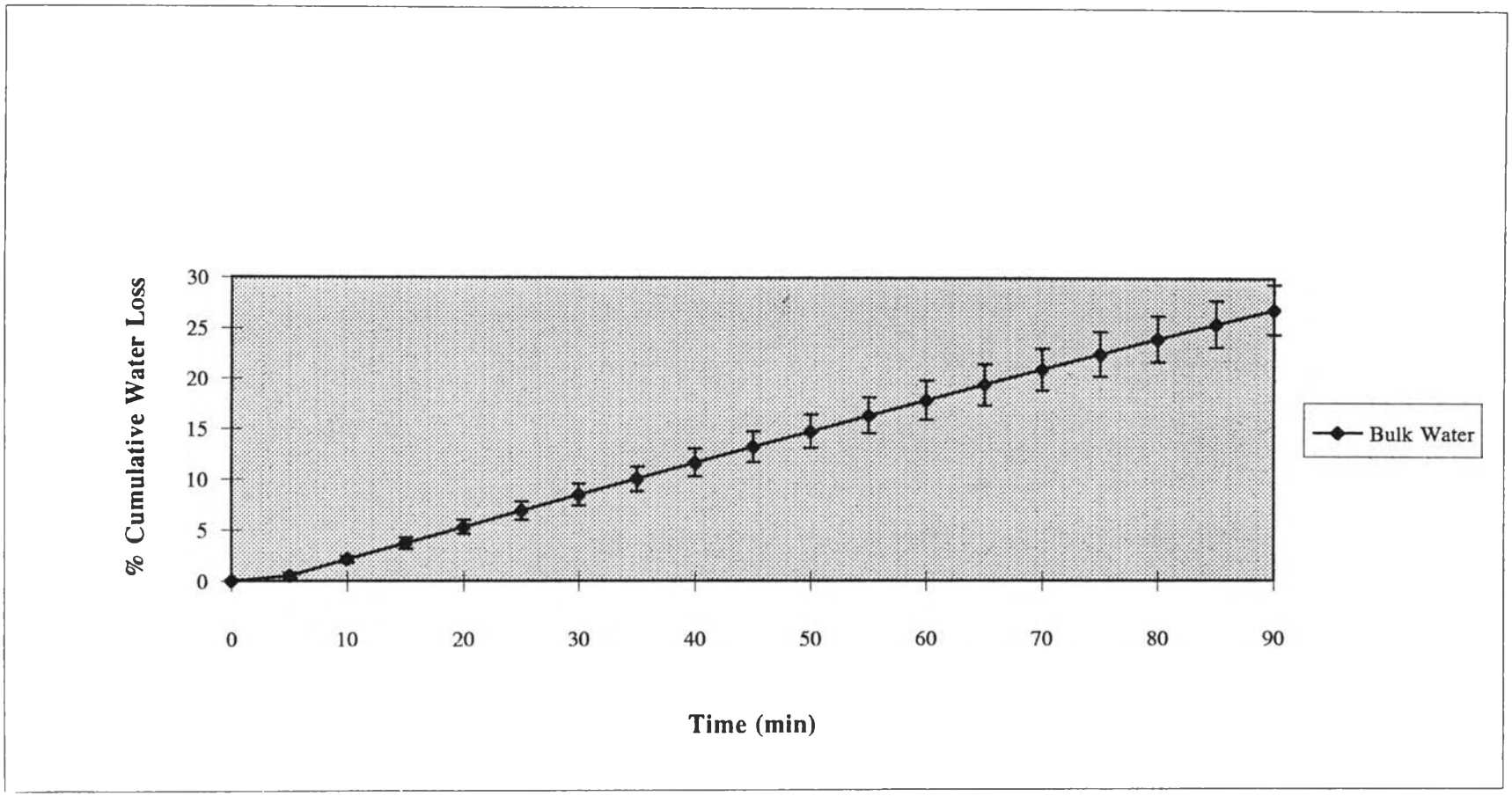


Figure 53. Percent Cumulative Water Loss (mean±SD) from Bulk Water as a Function of Time.

evaporation rate when compared to a system with a volatile component (for example, the SDS system with decanol). These results showed that the structure of liquid crystals, rather than the viscosity of the preparation, controlled water evaporation from the systems. In addition, the above results also imply that water evaporation rate was not controlled solely by the phase of liquid crystals present. The systems with triethanolamine:oleic acid:water in the ratios of 25:30:45 and of 10:50:40 had totally different capability to retain water although the two systems displayed the same hexagonal structure (Figure 54). Though liquid crystalline structures were expected to retain water for a much longer period of time due to the tightly bound water molecules at the headgroups of the structure, the setup of this experiment did not allow such evaluation. The water loss seen in this study should be from the unbound portion of water, i.e., the bulk water. If the effect of bound water is tc be studied, an experimental setup using thin films must be used so that all bulk water can be removed within a short period of time (Friberg and Kayali, 1988).

4.3 Drug Release from Liquid Crystalline Systems

Validation of analytical method for quantitative determination of PTU release from lyotropic liquid crystalline systems, a nonionic cream base, and an aqueous solution by UV spectrophotometry was conducted to verify that the method used was sufficiently accurate and precise. The validation methods and the data are shown in Appendix H. The results showed that UV method was sufficiently accurate and precise. However, some of the liquid crystalline systems slowly dissolved into the release medium and slightly contributed to the background absorbance of the medium. Therefore, controls of these systems (triethanolamine:oleic acid:water and SDS:

(a)

(b)

Figure 54. Photomicrographs from Polarized Light Microscopy Showing the Hexagonal Structures of Triethanolamine:Oleic Acid:Water (a) 10:50:40 (b) 25:30:45.

decanol:water) were also conducted using the systems without PTU. The absorbance read from the control was deducted from the absorbance of the sample for each and every time point. The PTU spectra were also monitored periodically throughout the experiment for every system including control. Linearity of the analysis was satisfactory; linear regression analysis yielded a correlation coefficient (R^2) of 0.9999. The representative calibration curve of the standard solution is shown in Figure H.4 of Appendix H. These results indicated that UV spectrophotometry was acceptable for quantitative analysis of PTU for the purposes of this study.

A number of experimental methods have been developed in order to characterize the release profiles of drugs from colloidal disperse systems (Washington, 1990). These experiments include membrane diffusion method, sampling and separation method, in situ method, and continuous flow method as described in Chapter II. As mentioned earlier, the sampling and separation method and the in situ method require filtration of the dispersed phase which are not very practical. Continuous flow method requires highly sophisticated instruments. Thus, the membrane diffusion method using Franz diffusion cells was selected to evaluate the in vitro release of PTU from liquid crystalline systems in this present study. This method can control surface area for drug diffusion from the donor to the receptor compartment. In order to maintain sink condition where the concentration of drug in the release medium is less than 10% of the saturation concentration of the drug in the medium (Burrows et al., 1994), a large portion of the receptor fluid (10 ml) was removed from the diffusion cell at appropriate time intervals, and the fresh medium was replaced. The total recovery of the drug into the sink should be checked in order to account for any losses. If the release profile does not reach 100% at 'infinite' times, then the possibility of experimental errors due to drug loss should be investigated. PTU

did not encounter this problem under the experimental conditions used. This could be evident from the experiment with PTU in the aqueous solution where the cumulative amount of the released drug approached 100% at 24 hours (Figure 55). However, when the receptor fluid was water, the wavelength of maximal absorption of PTU shifted for about 15 nm (from 275.4 nm to 260 nm) in the system of triethanolamine:oleic acid:water as compared to the spectrum from the standard solution due to dissolution of either triethanolamine or triethanolamine oleate into the release medium. In such systems, pH of the receptor fluid was increased from 6.5 to 8-9 over 24 hours of the experiment. In order to control the pH, a buffer system was introduced. Since the pH of the water used in the study was 6.5, Sorensen phosphate buffer pH 6.5 was selected for the release medium. The selected buffer was capable to keep the pH at 6.5 throughout the experiment, and the same release medium was used for all liquid crystalline systems studied. The wavelength shift of triethanolamine systems in the buffered medium was reduced to only abount 5 nm (270.2 nm versus 275.4 nm). This wavelength shift might suggest a slight change in PTU environment. Since triethanolamine systems were erodible, and because PTU is an amphiphile, it is possible that released PTU molecules were in an aggregate form. However, the difference in absorbance at these two different wavelengths was negligible (Appendix J). Thus, the corresponding concentration was calculated based on the absorbance at 275.4 nm for all systems studied.

For the release study, the liquid crystalline systems were selected on the basis of the satisfactory gross physical appearance, the diversity in the liquid crystalline phases formed, and the variety of surfactants used. The drug concentration in each system was at its maximum in the system. These systems were as follows:

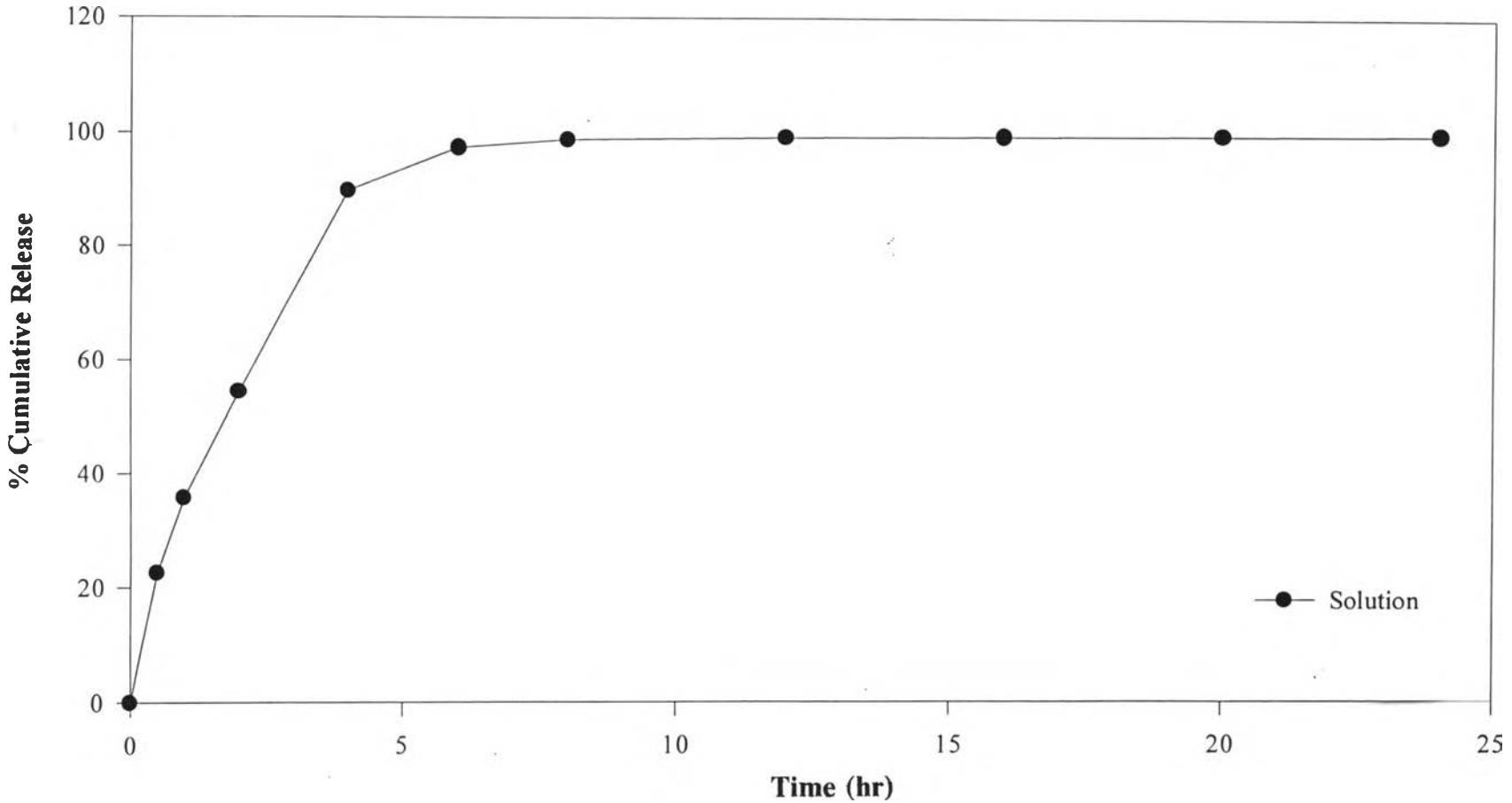


Figure 55. Release Profile of PTU from Aqueous Solution.

- Brij®72:Arlamol®E:water (15:10:75) with 0.2% PTU

- Triethanolamine:oleic acid:water (10:50:40 and 15:50:35) with 0.6% PTU

- Sodium dodecyl sulfate:decanol:water (20:30:50) with 0.2% PTU

- Lecithin:water (40:60) with 0.5% PTU

- Liposomal system hydrated with 0.8 mg/ml PTU solution

A nonionic cream base recommended in the Ministry of Public Health Hospital Formulary was also included in this study for comparison. This preparation also gave the lamellar liquid crystalline structure when observed under a polarized light microscope at 3 days after preparation (Figure 56) but the total concentration of surfactants used was much lower than in other systems. When PTU was added to the nonionic cream base, the crystals of the drug were found even at the concentration of 0.2% (Figure 57) whereas crystals were not found in other liquid crystalline systems. This shows that the saturation solution of PTU in the nonionic cream base was lower than 0.2%. Lowering the concentration of surfactant in the preparation might have limited the extent of liquid crystal formation and, thus, the ability of the system to accommodate PTU molecules. Besides, formation of liquid crystals in the nonionic cream base was slower than the systems with higher surfactant content. In other systems, liquid crystals were formed almost immediately after preparation. However, the process of preparation might also be responsible for the difference seen since the nonionic cream base was prepared according to the standard method for o/w emulsion (the "beaker" method). The influence of the process on liquid crystal formation was formerly reported (Eccleston, 1990).

The release profiles of PTU from liquid crystalline systems including the nonionic cream base are shown in Figure 58. The complete release data are in Appendix J. The diffusion profile of PTU from aqueous solution is shown in Figure 55

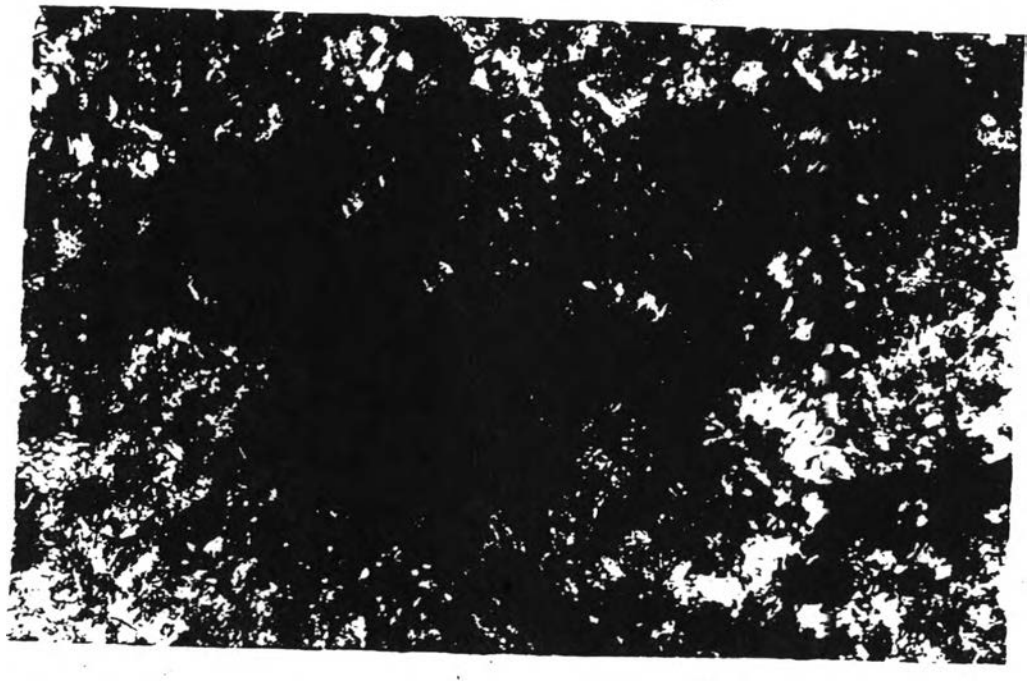


Figure 56. Photomicrograph from Polarized Light Microscopy Showing the Lamellar Structure of Nonionic Cream Base.

Figure 57. Photomicrograph from Polarized Light Microscopy Showing the PTU Crystals in Nonionic Cream Base With 0.2% PTU.

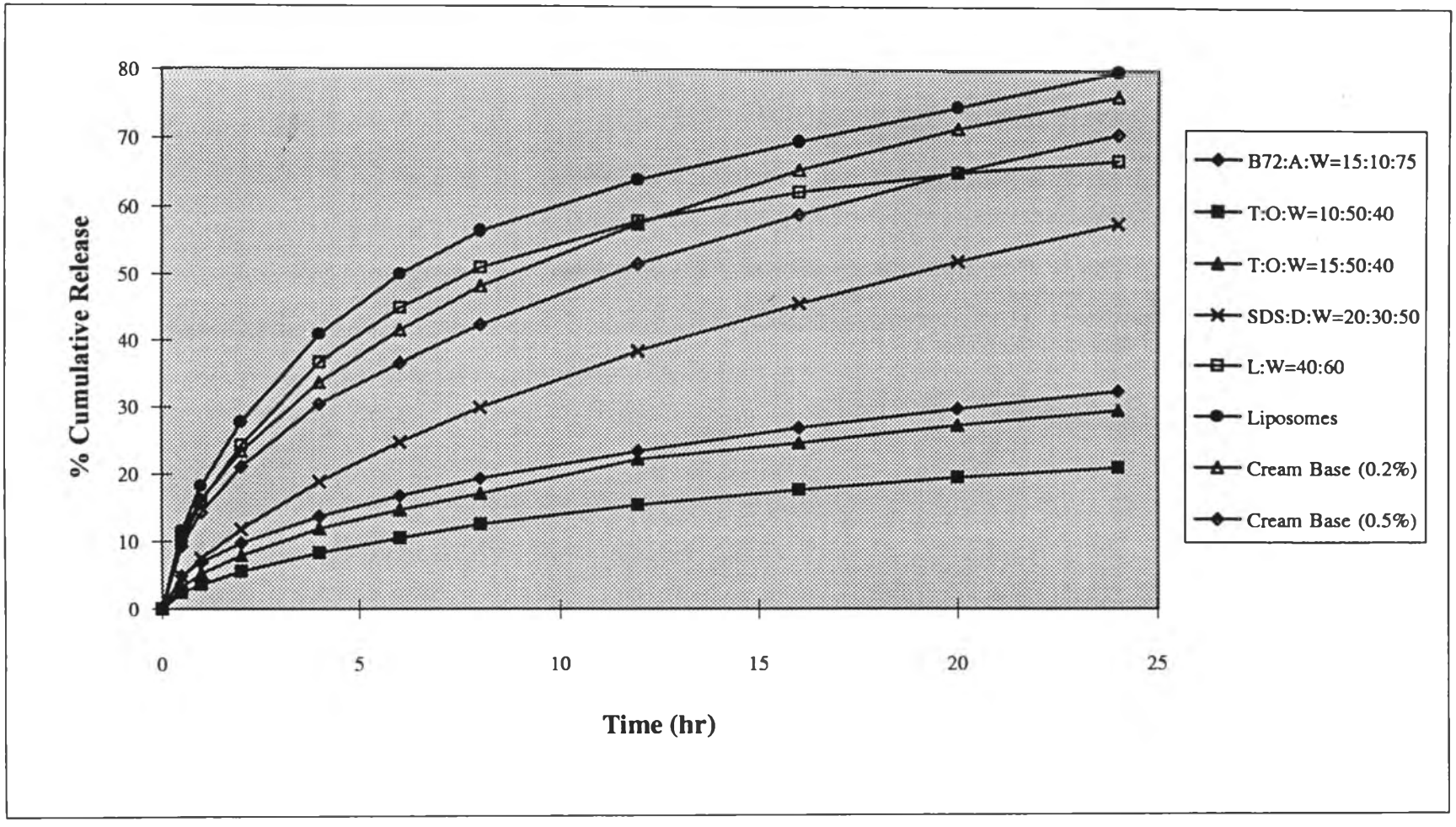


Figure 58. Release Profiles (mean of triplicate runs) of PTU from Liquid Crystalline Systems Including the Nonionic Cream Base. Standard deviations are displayed in Appendix J.

where diffusion was complete in about 8 hours. The release of PTU plateaued off at 66% of the drug content from the system of lecithin and water in the ratio of 40:60, while the release of PTU from the other systems including the nonionic cream base still continued at 24 hours of the experiment. After the experiment was terminated at 24 hours, the samples in the donor compartment were retrieved and observed under a polarized light microscope. The drug crystals were found in the liquid crystalline system of Brij®72. These crystals did not exist before the release experiment. When the retrieved samples were left at room temperature without protection from water evaporation, the crystals disappeared. This suggests that during the release experiment the composition of the system had been changed due to the water from the receptor compartment diffusing through the membrane. The system with higher water content might not be able to accommodate the same amount of PTU molecules, and the drug recrystallized from the system. When the water in the system was allowed to evaporate, the system returned to its original structure, and the drug redissolved into the system. Thus, water content was crucial in maintaining the structure and properties of liquid crystalline systems. Similar phenomenon was not observed with the other systems.

Several mathematical models have been used to describe the release of the drug. The release data obtained in this study were analyzed in order to describe the release characteristics with one of the following models: (i) the drug release was controlled by the diffusion of the drug through the matrix or the diffusion-controlled release (Higuchi, 1961; Burrows et al., 1994), (ii) the drug release followed first-order kinetics (Burrows et al., 1964), and (iii) the release followed zero-order kinetics. In diffusion-controlled release the cumulative amount of drug released per unit surface area of the system is directly proportional to the square root of time. When the

percentages of drug release in this experiment were plotted against the square root of time, the linear relationship was observed in the systems of Brij®72, triethanolamine, and nonionic cream base (Figure 59). In these systems, the drug release followed the square-root of time relationship, and the amount of drug present was greater than or close to the solubility of the drug in the vehicle. The model assumption of constant concentration of drug in the vehicle was hold in these systems. Crystals were evident in the systems of Brij®72 and nonionic cream base. Although the drug crystals were not found in the systems of triethanolamine after 24 hours of in vitro drug release studies, the system still followed a diffusion-controlled drug release mechanism because the amount of drug released in 24 hours was very low compared to the initial amount. Thus, the PTU concentrations in these systems were relatively constant during the period studied. For a system to follow first-order release, the log amount of drug remaining in the system must be directly proportional to the time. The system with SDS followed first-order kinetics with a rate constant of 0.015 and R^2 of 0.9929 (Figure 60). The other systems could not be fitted to diffusion-controlled, first-order kinetics or zero-order kinetics. More than one mechanisms might be responsible for the release of PTU from these systems. Most of release profiles from liquid crystalline systems reported in the literature were diffusion-controlled (Mueller-Goymann and Frank, 1986; Mueller-Goymann and Hamann, 1993; Geraghty et al., 1996; Chang and Bodmeier, 1997). Some liquid crystalline systems prepared from a monoglyceride were reported to give release profiles that followed a first-order kinetics (Burrows, Collett, and Attwood, 1994). However, even with the same liquid crystalline system, release profiles depend also on the drug incorporated into the system. Burrows et al. (1994) reported different release profiles from similar liquid crystalline systems with several drugs. In addition to the kinetics of drug release, the

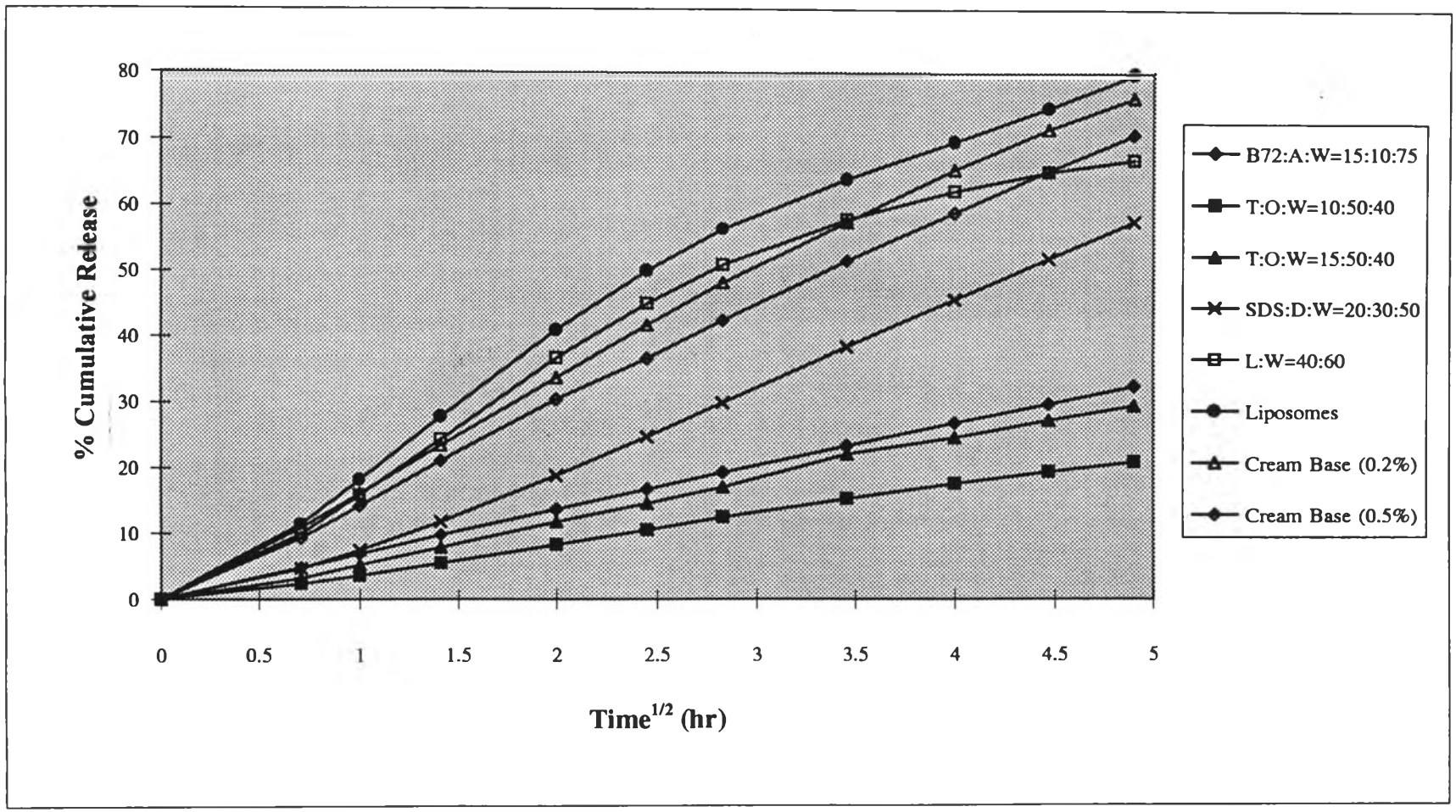


Figure 59. Percentage of Drug Release Against the Square Root of Time.

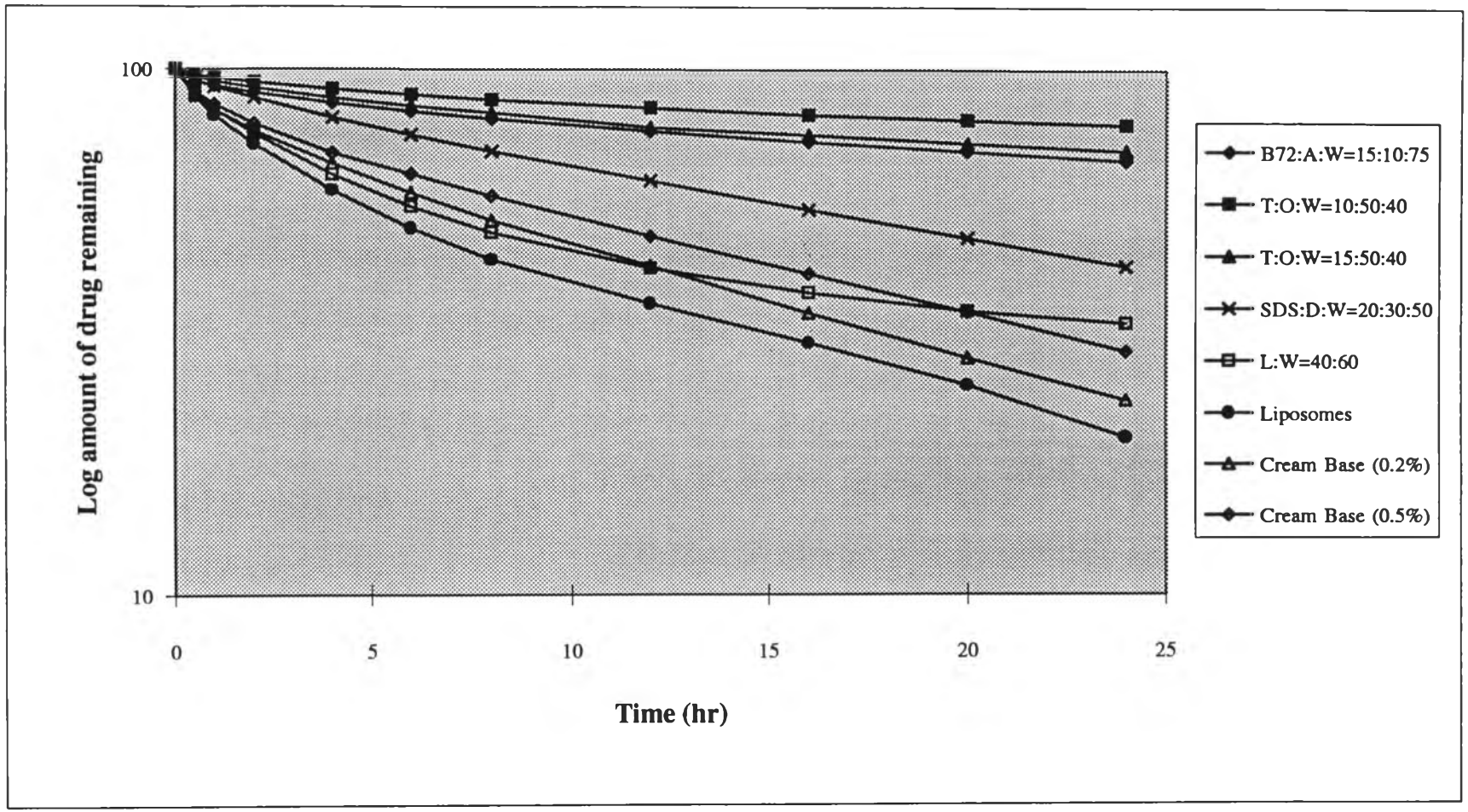


Figure 60. Log Amount of Drug Remaining in the Donor Compartment Versus Time.

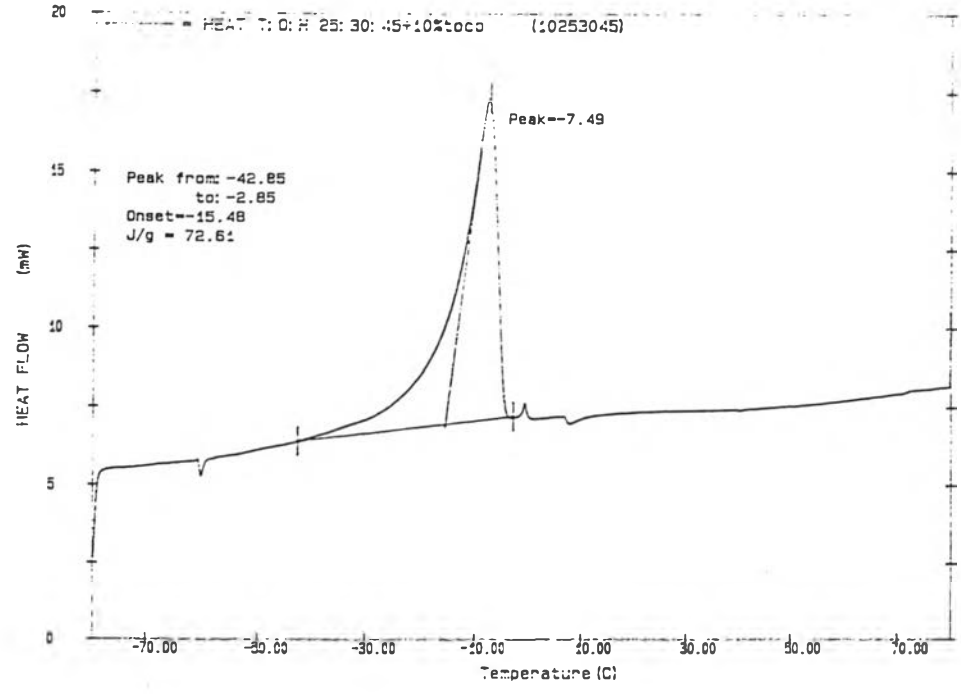
release rate also depends on several factors including drug loading, method of preparation, and chemical form of the drug incorporated (Mueller-Goymann and Frank, 1986). Hence, in order to use the liquid crystalline system to control drug release, several formulation factors as well as process variables have to be taken into account.

4.4 Stability of Liquid Crystalline Systems

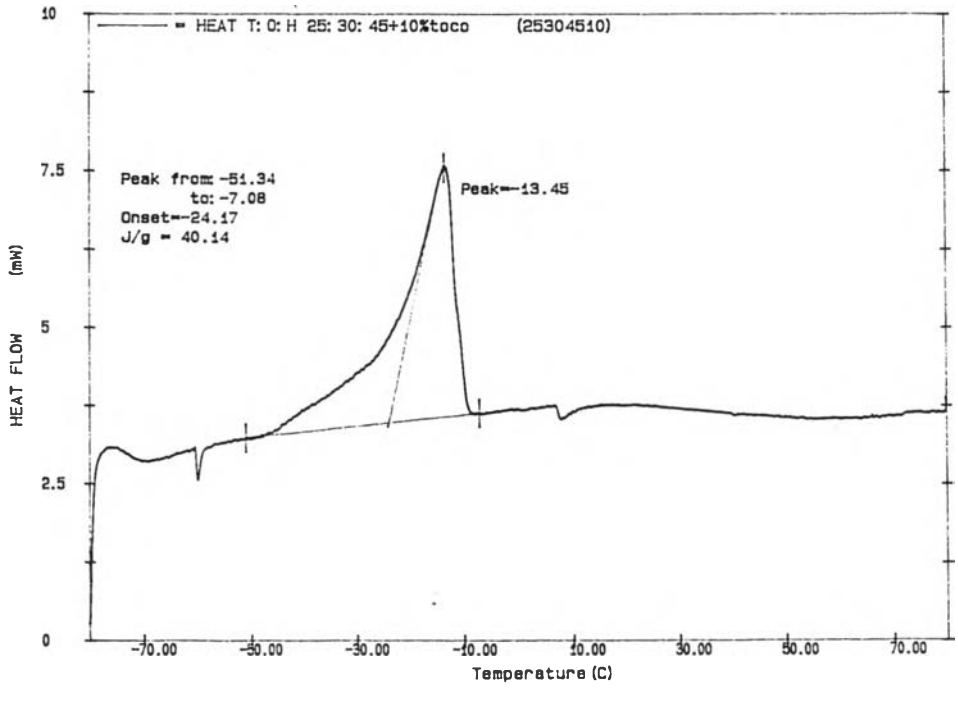
Stability evaluation of selected liquid crystalline systems was performed after 2 months of storage at room temperature for systems with surfactants and in a refrigerator for systems with lecithin including liposomal systems. The results at 2 months were compared with those observed at 3 days after preparation. Stability was evaluated from the gross physical appearance, the texture from polarized light microscopy, and the thermogram from DSC.

4.4.1 Liquid Crystalline Systems with Additives

The structure and physical appearance changed in some systems after 2 months of storage in these systems. For the triethanolamine:oleic acid:water (25:30: 45) system with 10% α-tocopherol, the structure changed from figure 54b to the pattern similar to that shown in Figure 27. The preparation became liquefied. In this system, the DSC thermogram (Figure 61) shows that T_m and ΔH decreased whereas peak width increased, suggesting less co-operativity in the structure. In the system of SDS: decanol:water (20:30:50) with 5% α-tocopherol, the preparation liquefied, and



(a)



(b)

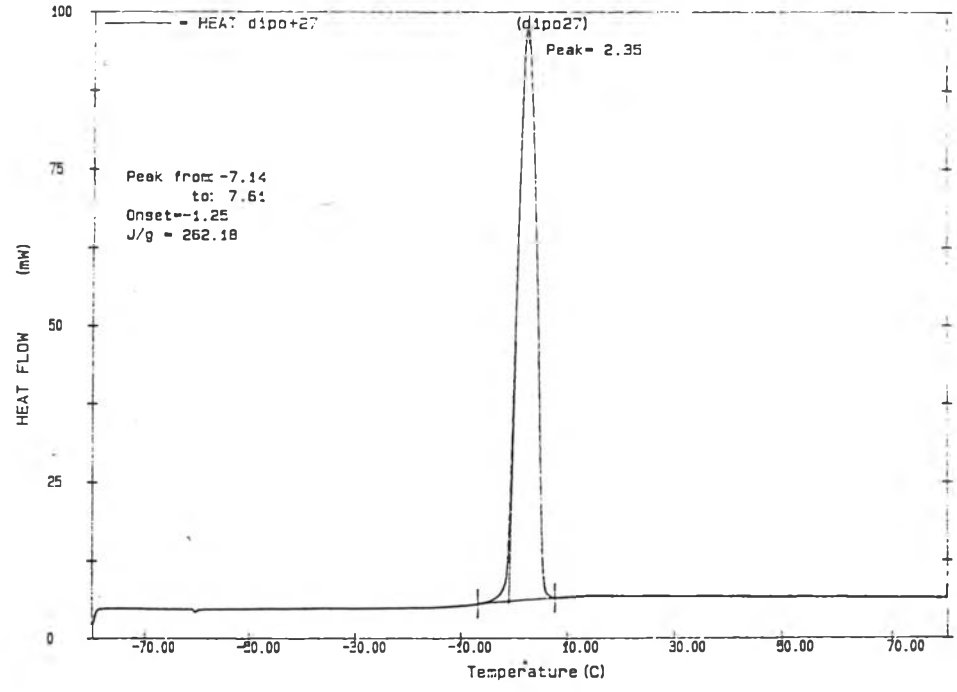
Figure 61. DSC Thermograms of the Liquid Crystalline Systems Composed of Triethanolamine:Oleic Acid:Water (25:30:45) With 10% α-Tocopherol (a) at 3 Days (b) at 2 Months.

coalescence of α-tocopherol took place. In some systems, there was no change under a polarized light microscope, but changes were observed with DSC. For example, in the liposomal system with 1% α-tocopherol, co-operativity of the system slightly increasesd as seen from the slightly decrease in peak width (Figure 62). Co-operativity of the systems tended to increase after 2 months in systems with urea. Some systems with trehalose displayed decreased co-operativity. Almost no change was observed in systems with sodium chloride. Other results from this study are shown in Appendix E.

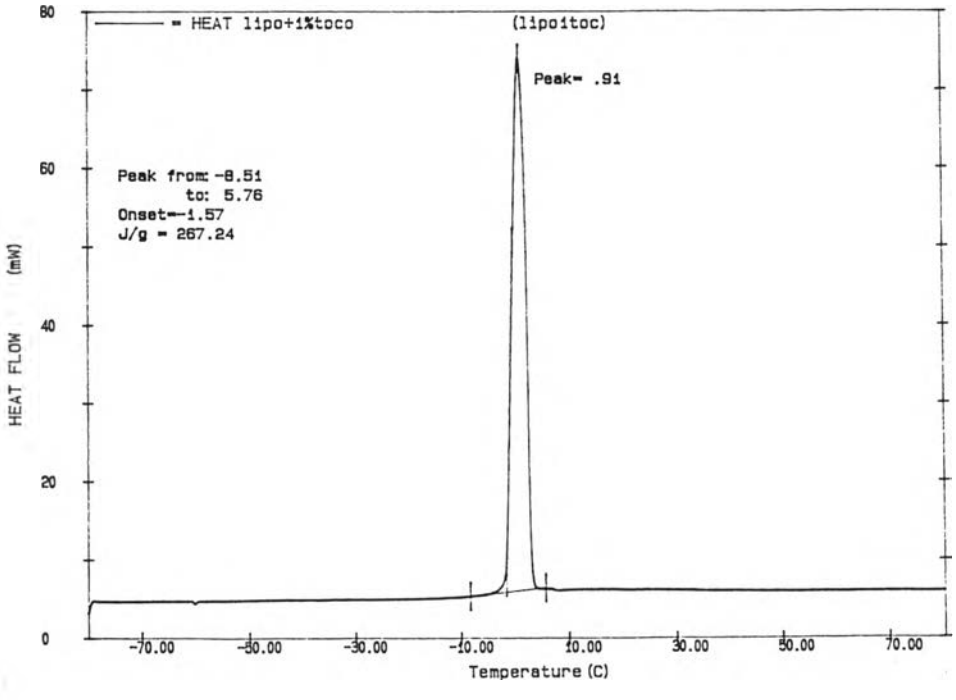
4.4.2 Liquid Crystalline Systems with PTU

After 2 months of storage, the saturation solubility of PTU in each liquid crystalline system was monitored by polarized light microscopy (Appendix C). Thermal behavior of the system was also monitored by DSC in the systems with no visible change in gross physical appearance and liquid crystalline textures. Results of this study are shown in Appendix E. Recrystallization of PTU occurred only in one system: abundant drug crystals were observed in the system of Brij®72:Arlamol®E: water (20:10:70). DSC revealed alteration in thermal behavior in some of the systems. For example, peak widths of the liposomal system and the system of SDS:decanol: water (5:10:85) decreased corresponding to increase co-operativity of these systems.

In conclusion, the additional substances, either additives or drugs, could influence stability of the liquid crystalline systems in terms of physical appearance, liquid crystalline texture, and molecular interactions within the liquid crystalline structure.



(a)



(b)

Figure 62. DSC Thermograms of the Liposomal System With 1% α-Tocopherol (a) at 3 Days (b) at 2 Months.