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Investigations into the formation and characterization of phospholipid microemulsions.
I. Pseudo-ternary phase diagrams of systems containing water-lecithin-alcohol-isopropyl myristate

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Summary

Pseudo-ternary phase diagrams have been constructed for systems comprising of water-lecithin-alcohol-isopropyl myristate. Two types of lecithin were used in this study, namely soybean (Epikuron 200) and egg lecithin (Ovothin 200). Seven short chain alcohols (i.e., *n*-propanol, isopropanol, *n*-butanol, *sec*-butanol, isobutanol, *tert*-butanol and *n*-pentanol) were investigated as cosurfactants. In each system studied, a large monophasic, isotropic, non-birefringent area was seen to occur along the surfactant/oil axis; while at low oil concentrations, a second isotropic, non-birefringent area, usually associated with a liquid crystalline phase, was observed in many systems. Both isotropic regions were stable at room temperature at least for 3 months. Although no significant difference was observed between the phase diagrams produced by the two types of lecithin, the extent of the isotropic regions was dependent upon both the nature of the cosurfactant and lecithin/cosurfactant mixing ratio (K_m).

Introduction

Microemulsions were first described by Hoar and Schulman (1943). They are homogeneous, transparent, thermodynamically stable dispersions of water and oil, stabilized by a relatively large amount of surfactant, frequently in combination with a cosurfactant; typically a short chain alcohol.

As a consequence of their many potential advantages (e.g., their clarity, high stability and ease of preparation), interest has rapidly grown in the use of microemulsions as pharmaceutical drug delivery systems. However, because of a lack of pharmaceutically acceptable systems, microemulsions have not yet been comercially exploited. Indeed, while an extensive amount of data has been collected for microemulsion systems containing alkanes and related oils (Baker et al., 1984a,b; Fletcher et al., 1984; Venable et al., 1986; Aveyard et al., 1989), few studies have been reported on systems in which pharmaceutically acceptable oils, such as isopropyl myristate or

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soybean oil, are employed (Fubini et al., 1988, 1989; Gallarate et al., 1988; Ktistis, 1990; Malcolmson and Lawrence, 1993; Trotta et al., 1989a). Likewise, little information has been published about the preparation of microemulsions stabilized by surfactants, such as phospholipids, suitable for drug delivery (Gasco et al., 1987, 1988, 1989; Gallarate et al., 1988; Evans et al., 1990).

Since phosphatidylcholines (lecithins) are naturally occurring non-toxic, biocompatible surfactants, the preparation of lecithin-based microemulsions is of considerable pharmaceutical interest. However, since lecithin has a strong tendency to form liquid crystalline structures, particularly in water phase (Bergenstahl and Fontell, 1983), a cosurfactant is necessary in order to produce lecithin-based microemulsions.

In the present paper, we report the pseudo-ternary phase diagrams of water-isopropyl myristate-lecithin systems prepared using a range of short chain alcohols at several surfactant/cosurfactant mixing ratios (K_m) of 1:1, 1.5:1, 1.77:1, 1.94:1, in order to determine the effect of the nature and concentration of the alcohol on the formulation of phospholipid microemulsions. A preliminary report of this study has been previously published (Aboofazeli and Lawrence, 1991).

Materials and Methods

Materials

Two comercially available lecithins, soybean and egg lecithin (Epikuron 200, E200; and Ovothin 200, O200; respectively), were supplied by Lucas Meyer Co. (West Germany) and used without further purification. Table 1 shows the fatty acid composition and purity of each lecithin.

Isopropyl myristate (IPM) and *tert*-butanol were purchased from Sigma Chemical Co. (Dorset, U.K.). Isobutanol was obtained from Fluka Chemicals Ltd (Glossop, U.K.) and *n*-pentanol from Aldrich Chemical Co. (Dorset, U.K.). *n*-Butanol and *sec*-butanol were purchased from BDH Ltd (Poole, U.K.), *n*-propanol and isopropanol were from FSA Laboratory Supplies (Loughborough, U.K.). Triple distilled water from a well seasoned all-glass system was used throughout the study.

TABLE 1

Compositions of soybean and egg lecithins

	Purity [a] (%)	% w/w fatty acid			
		Palmitic and stearic	Oleic	Linoleic	Linolenic
E200 (soybean)	95	16–20	8–12	62–66	6–8
O200 (egg)	92	10–33	28–32	13–17	–

[a] %w/w phosphatidylcholine.

Construction of pseudo-ternary phase diagrams

Appropriate amounts of lecithin, IPM and cosurfactant were weighed into screw-capped vials and the samples stirred until a clear solution was obtained. Phase diagrams were constructed by titrating these samples with aliquots of triple distilled water and stirring for a sufficiently long time to attain equilibrium. After equilibrium was reached, the mixtures were checked both visually for clarity, and through cross polarizers for the absence of a liquid crystalline (LC) phase. All mixtures produced optically clear, non-birefringent solutions (termed L_2 on the phase diagrams) at low water concentrations. When a sample exhibited birefringence, the titration was continued in order to determine the extent of the LC area and in an effort to establish the presence (or absence) of a second clear, non-birefringent region (termed L_1). All manipulations were performed at room temperature.

In order to show the variation of lecithin-alcohol-water-IPM on a phase diagram, the top apex of the triangle represents the lecithin/alcohol at a particular K_m, while the other apices represent oil and water concentrations. It should also be mentioned that due to the difficulties in establishing the extent of the liquid crystalline regions, the dashed lines represent boundaries that are not accurately determined.

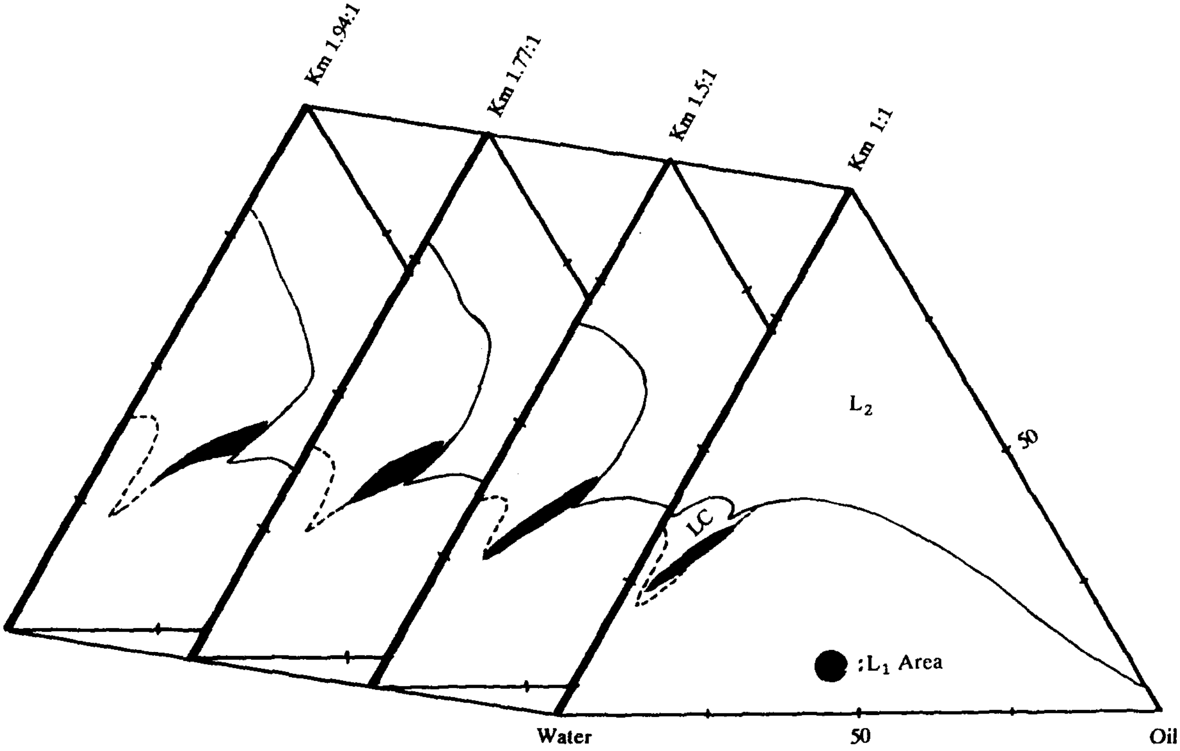

Fig. 1. Phase diagrams of the quaternary systems containing water/E200/*n*-propanol at different K_m values.

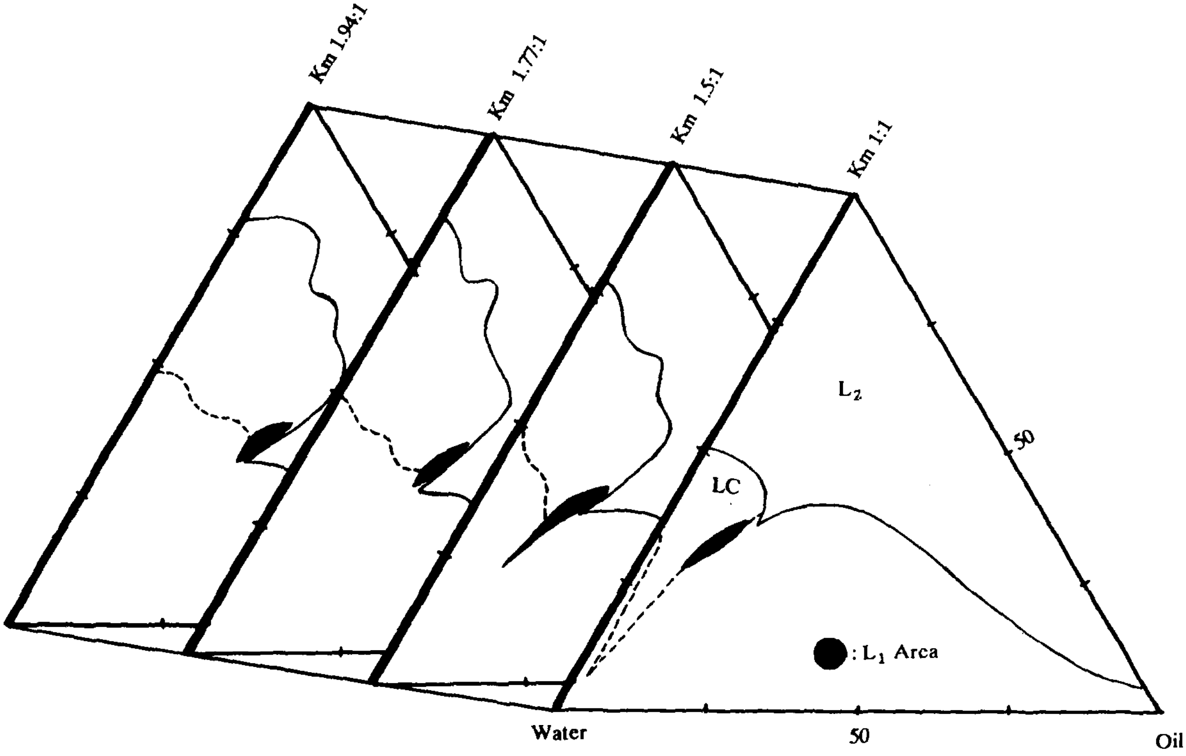

Fig. 2. Phase diagrams of the quaternary systems containing water/E200/isopropanol at different K_m values.

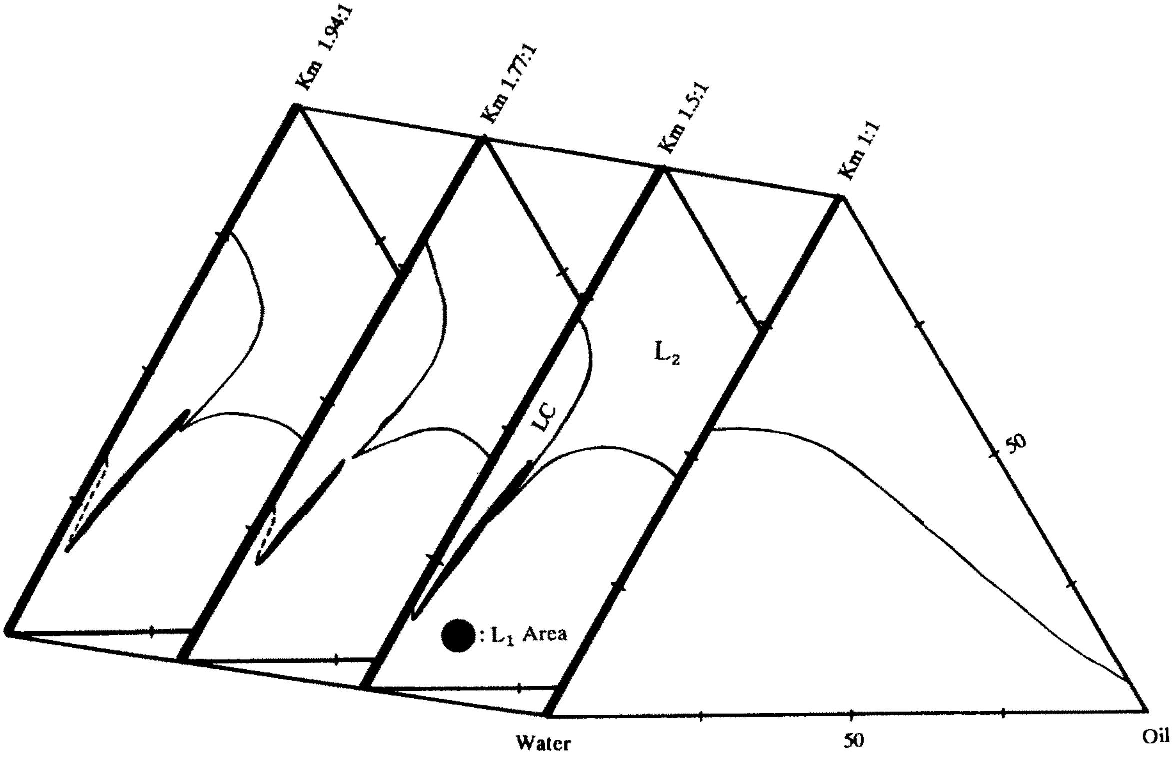

Fig. 3. Phase diagrams of the quaternary systems containing water/E200/*n*-butanol at different K_m values.

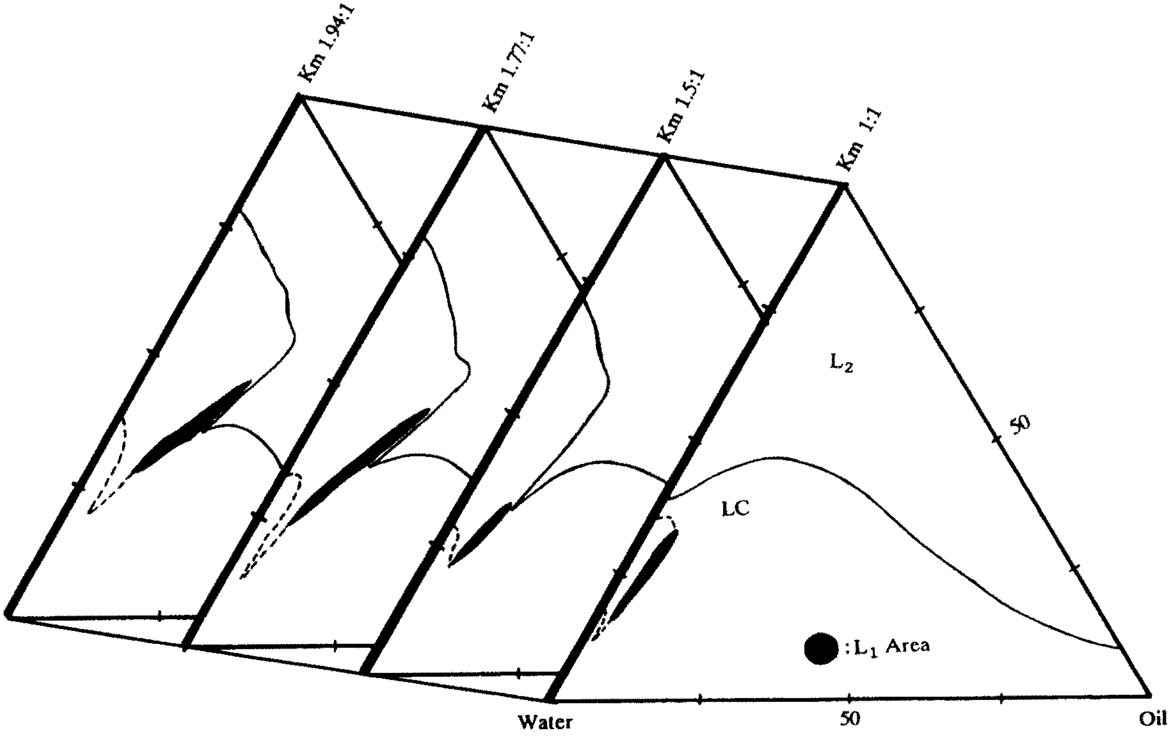

Fig. 4. Phase diagrams of the quaternary systems containing water/E200/*sec*-butanol at different K_m values.

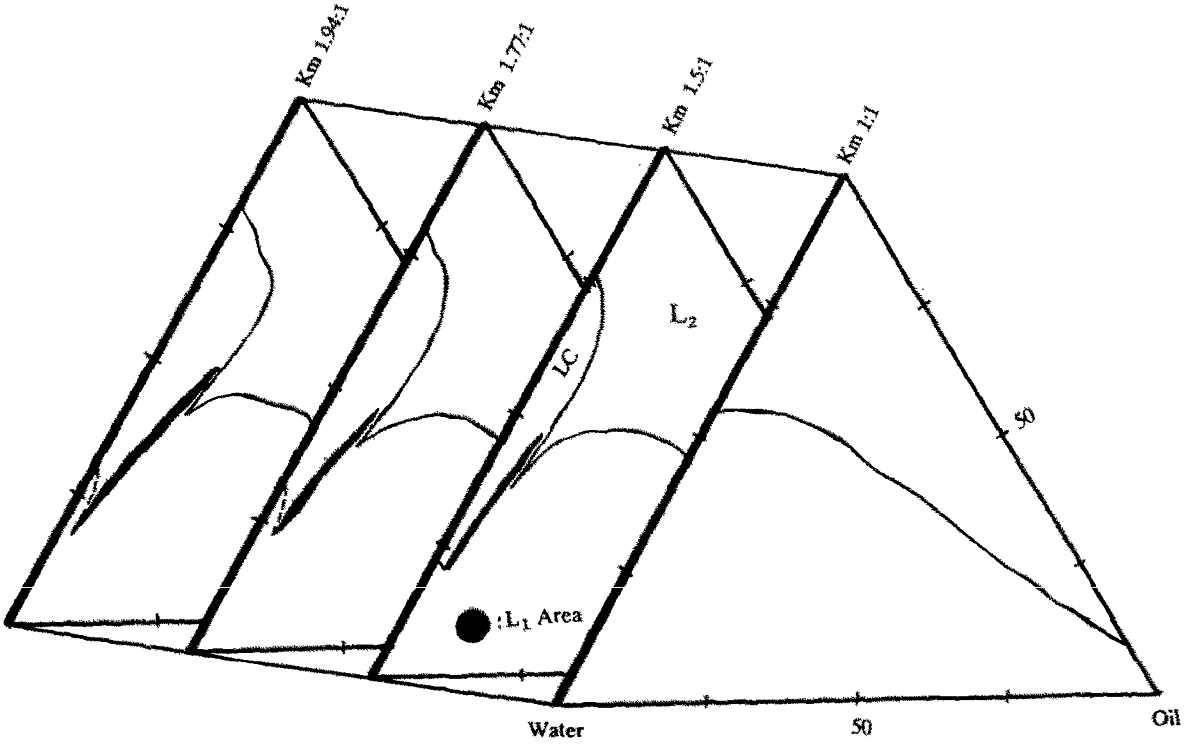

Fig. 5. Phase diagrams of the quaternary systems containing water/E200/isobutanol at different K_m values.

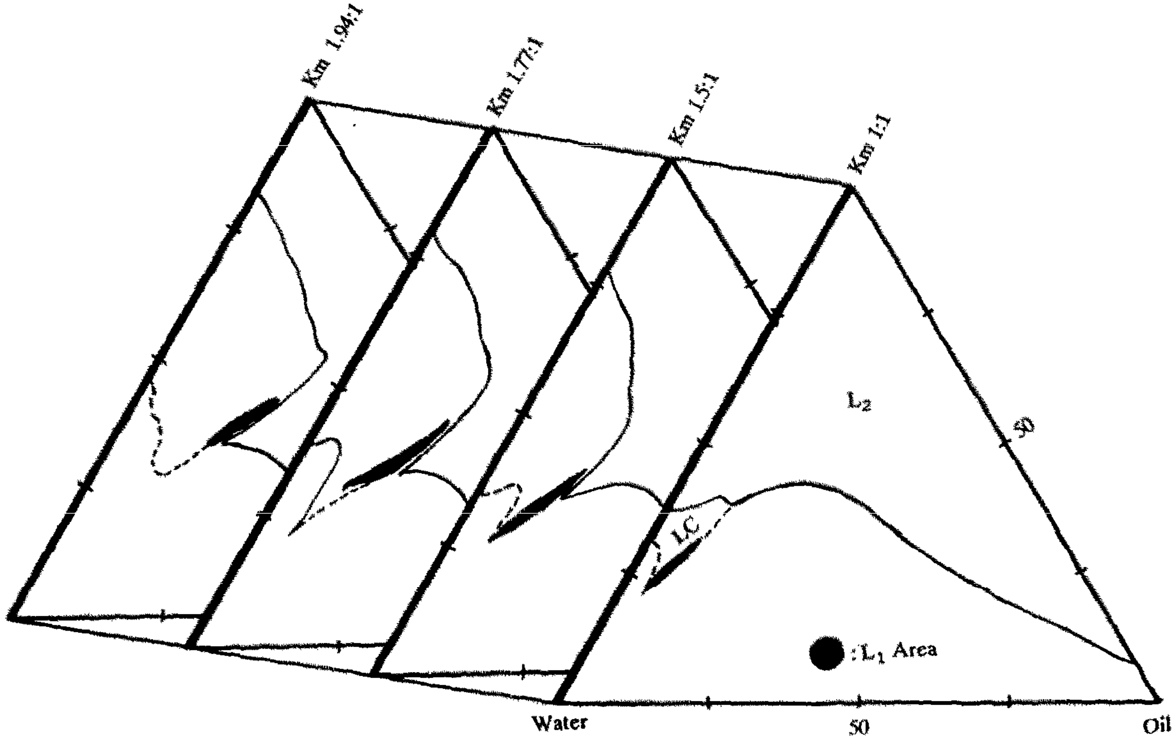

Fig. 6. Phase diagrams of the quaternary systems containing water/E200/*tert*-butanol at different K_m values.

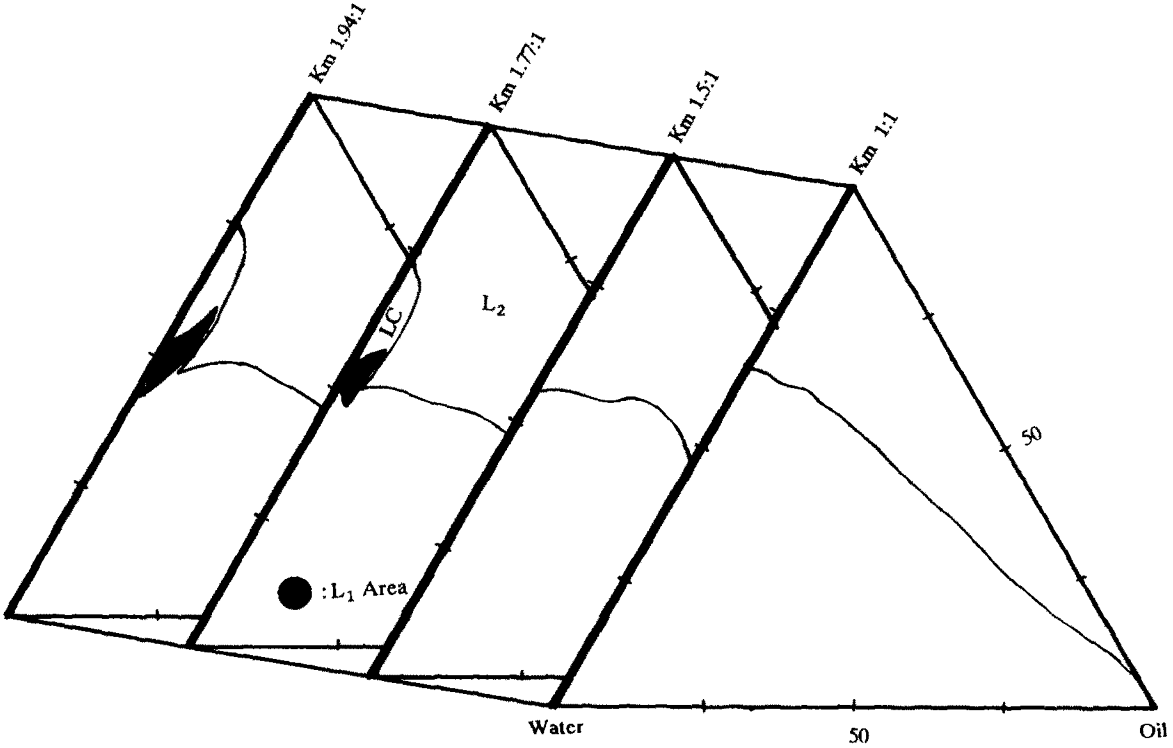

Fig. 7. Phase diagrams of the quaternary systems containing water/E200/n-pentanol at different K_m values.

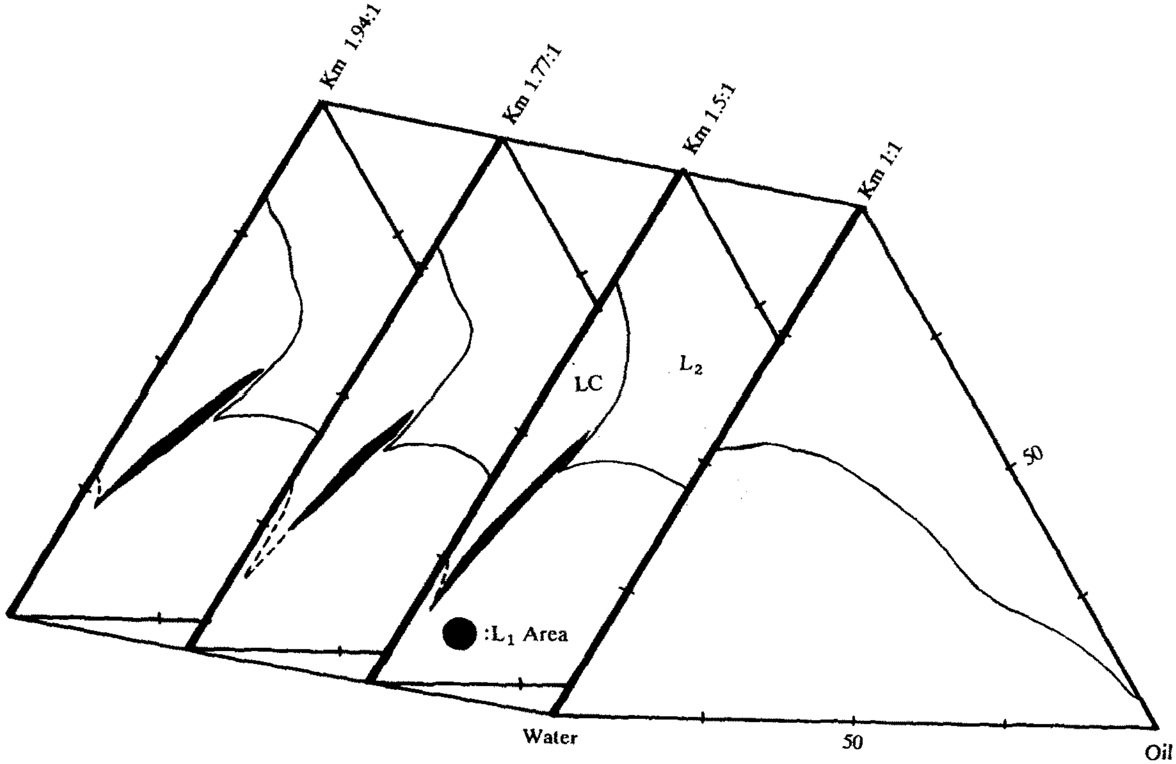

Fig. 8. Phase diagrams of the quaternary systems containing water/O200/n-butanol at different K_m values.

Results

The pseudo-ternary phase diagrams of systems containing E200 with four different mixing ratios (K_m) are shown in Figs 1–7. The phase diagrams are overlaid because the phase behaviour for all systems did not change much at high oil concentrations. It should be noted that, since in most systems there was little difference between phase behaviour observed with E200 and O200 and in order to avoid overcrowding of this paper, only the phase diagrams for E200 systems are presented. Where appropriate, any differences observed between the phase diagrams of the two types of lecithin will be mentioned in the text. For comparative purposes, the phase diagrams for the O200 systems in the presence of *n*-butanol and *sec*-butanol are also reported (Figs 8 and 9).

Isotropic L_2 region

As can be seen, in all phase diagrams, irrespective of the alcohol and the K_m, the L_2 region extends over a very wide area and covers a whole range of surfactant concentrations. However, both the nature of cosurfactant and K_m exert a significant influence on the extent of the L_2 region. At a K_m of 1:1, the L_2 region extends over a wide area of each of the phase diagrams allowing a wide range of water concentrations to be solubilized. In some systems, this region is associated with a liquid crystalline phase in the oil poor part of the phase diagram. At K_m greater than 1:1, the existence of a liquid crystalline phase in the surfactant rich, oil poor, part of the phase diagram considerably reduces the extent and alters the appearance of the L_2 region which now exhibits an apparent maximum in the amount of water solubilized. As the K_m increases, the extent of the liquid crystalline region increases and the maximum solubilization peak moves towards the middle of the phase diagram. As a consequence, the surfactant/oil ratio which can solubilize the maximum amount of water decreases as the K_m increases. This ratio has its lowest value at the highest K_m studied.

Figs 10 and 11 illustrate the amount of solubi-

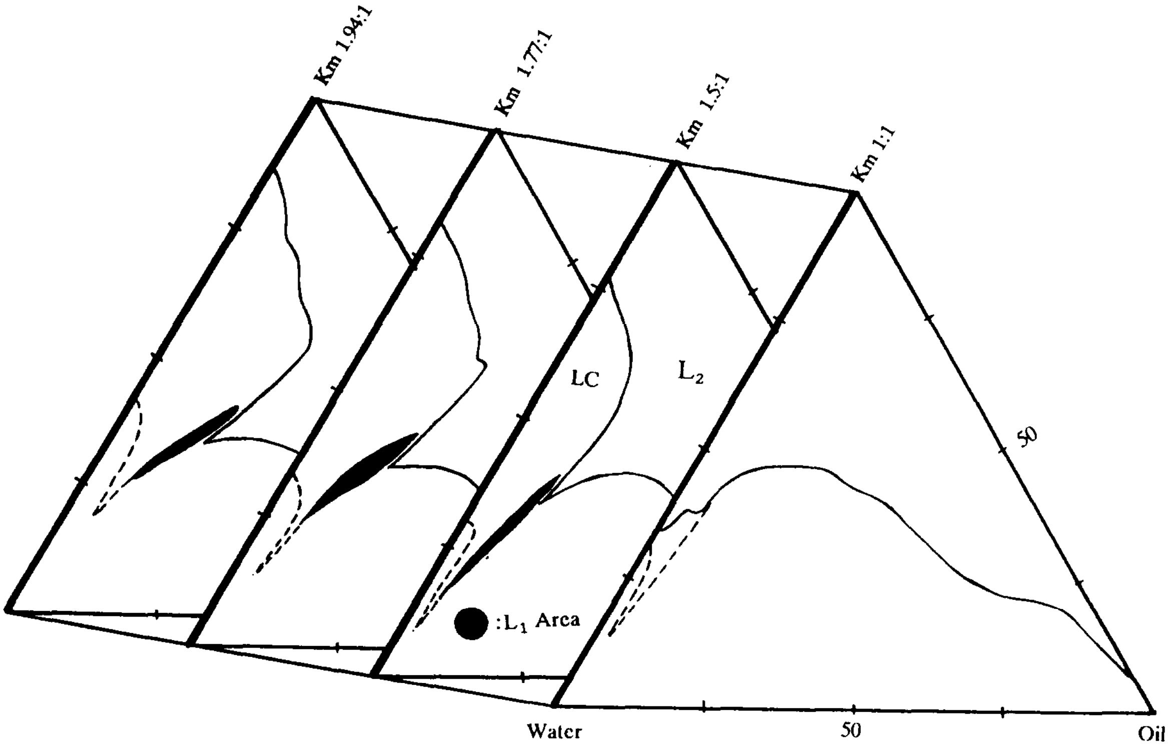

Fig. 9. Phase diagrams of the quaternary systems containing water/O200/*sec*-butanol at different K_m values.

lized water in the L_2 region as a function of total surfactant concentration for each lecithin/alcohol system at each K_m value. In all cases, the area under the curve represents the transparent (L_2) region. At a K_m of 1 : 1, the amount of water incorporated into the L_2 region increases with increasing the amount of total surfactant. At K_m greater than 1 : 1, a sharp maximum in the amount of water solubilized is observed. This is a consequence of the liquid crystalline phase. However, it should be noted that although the maximum solubilization peak is observed at K_m greater than 1 : 1, because of the presence of a LC phase, some systems with a K_m of 1 : 1 also show this maximum (Figs 10a, b and 11b).

In general, the following generalizations can be made about the systems examined:

(a) None of the systems are capable of solubilizing water with less than 5 wt% total surfactant concentrations. However, in some systems (e.g., E200 + *n*-butanol, K_m of 1 : 1), a minimum of 10 wt% total surfactant concentration is required for the formation of a L_2 region.

(b) In systems containing E200, at a given surfactant/cosurfactant concentration and above 20 wt% surfactant, the amount of water solubilized increases with increasing K_m. Similar results were obtained in systems containing O200 but only above total surfactant concentrations of 25 wt%. Below 20 wt% total surfactant levels for E200

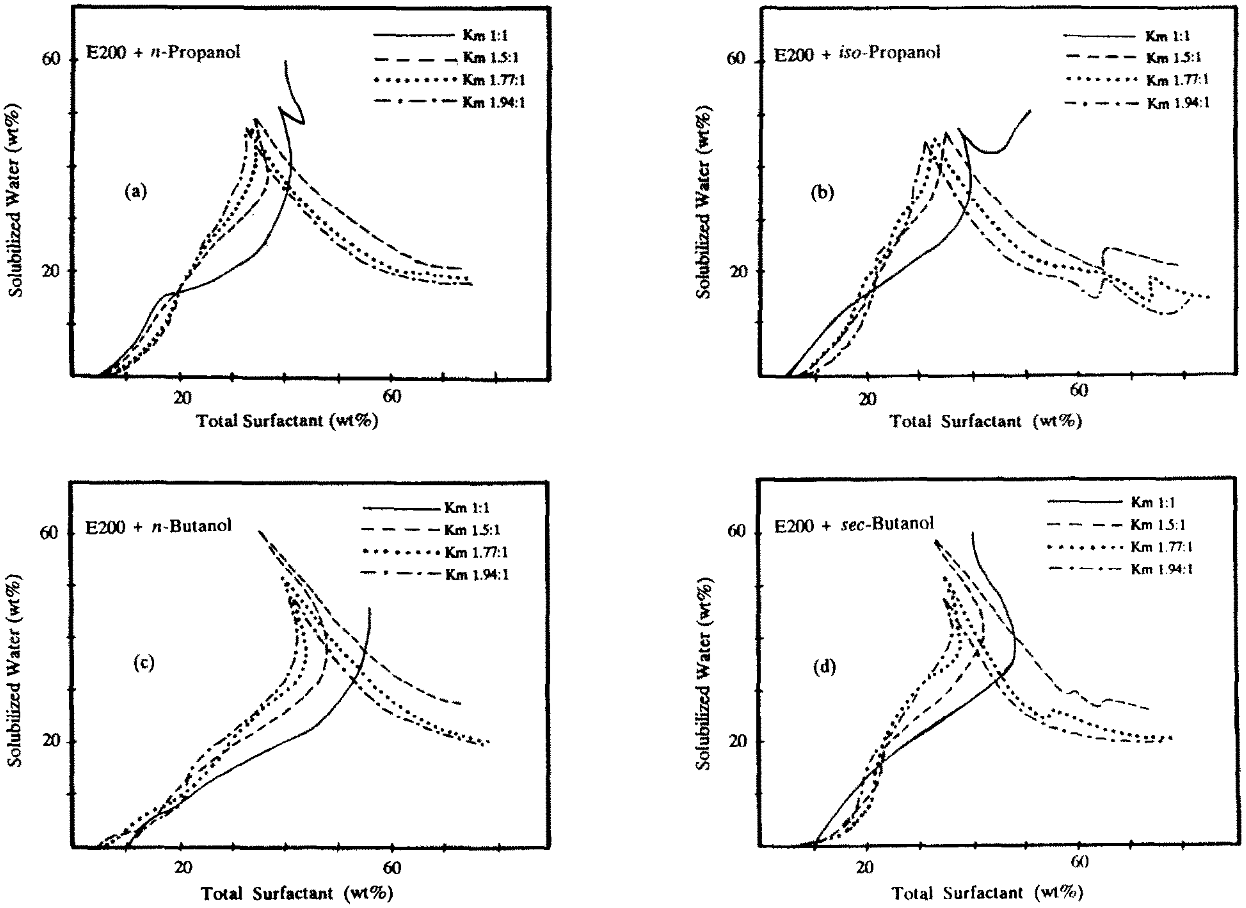

Fig. 10. Water solubilizing capacity of L_2 region for E200 systems as a function of total surfactant concentration.

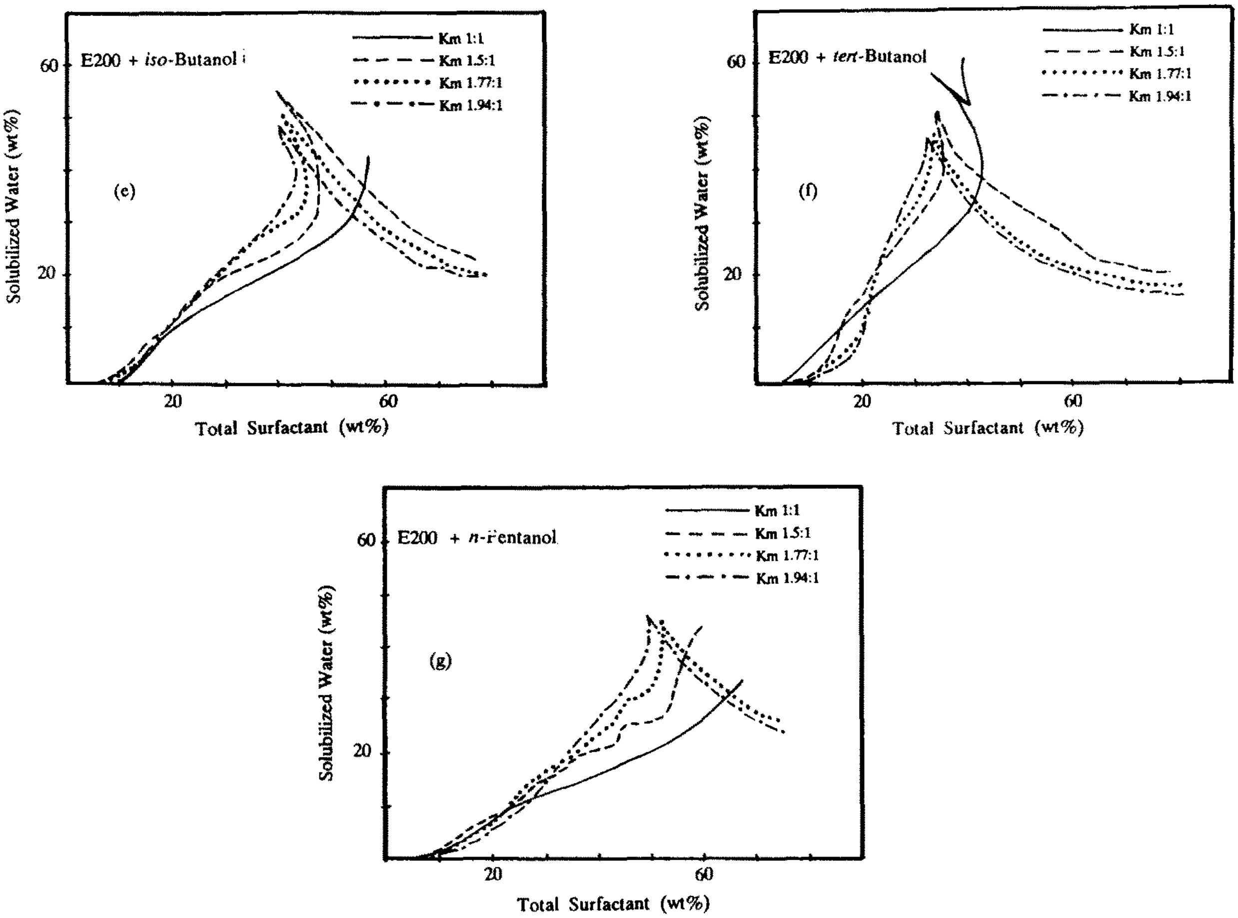

Fig. 10 (continued).

(and 25 wt% for O200), there is a less pronounced difference in solubilization capacity between the different mixing ratios. These observations show the dependence of the water solubilizing capacity on the K_m of each system.

(c) The surfactant/oil ratio required for the maximum solubilization of water generally decreases as the K_m increases, regardless of the alcohol and lecithin used.

(d) At K_m greater than 1:1, the total surfactant concentration which can solubilize maximum amount of water, irrespective of the kind of lecithin, is least in the presence of isopropanol, *n*-propanol and *tert*-butanol and greatest in the presence of *n*-pentanol. In other words, the surfactant/oil ratio required for maximum solubilization has its minimum value in systems containing isopropanol, *n*-propanol and *tert*-butanol whereas the maximum value is obtained when *n*-pentanol is present.

(e) In O200 systems the water solubilizing capacity of the system, below 20 wt% surfactant/cosurfactant content, increases slowly. Within a narrow range of total surfactant concentration (20–25 or 25–30 wt%), a sharp increase in the solubilizing capacity is observed, particularly at K_m greater than 1:1. This sharp increase also occurs in E200 systems containing *sec*-butanol and *tert*-butanol, especially at K_m of 1.77:1 and 1.94:1 (Fig. 10d and f).

Isotropic L_1 area

This region extends over a very limited area in the water rich part of the phase diagrams and is capable of solubilizing only a small amount of IPM. The position and extent of this area is dependent upon the nature of cosurfactant and K_m.

As shown in Figs 3, 5 and 7, *n*-butanol and isobutanol at K_m of 1 : 1, and *n*-pentanol at K_m of 1 : 1 and 1.5 : 1, are not capable of producing the L_1 area with E200. In O200 systems, *n*-butanol, *sec*-butanol, isobutanol and *n*-pentanol at a K_m of 1 : 1 are not able to produce the L_1 region.

Generally, with an increase in the K_m, the L_1 area moves to the middle of the phase diagrams, i.e., to higher oil contents. Likewise, the range of surfactant/cosurfactant concentrations over which the isotropic solutions are stabilized changes. For example, in E200 systems, the amount of oil incorporated into the L_1 area in the presence of isobutanol increases from between 1–5 wt% (at a K_m of 1.5 : 1 and a total surfactant concentration of between 19 and 48 wt%) to 2–11 wt% (at a K_m of 1.94 : 1 and the range of 19–50 wt% of total surfactant concentration). In order to solubilize the same amount of oil (namely, 1–5 wt%) in the presence of *n*-pentanol, the range of total surfactant concentration required is 45–61 wt% at a K_m of 1.94 : 1. In O200/isobutanol system, at a K_m of 1.5 : 1, 16–48 wt% total surfactant is required in order to solubilize 1–8 wt% oil, while at a K_m of 1.94 : 1, 4–12 wt% oil is incorporated into the L_1 area at

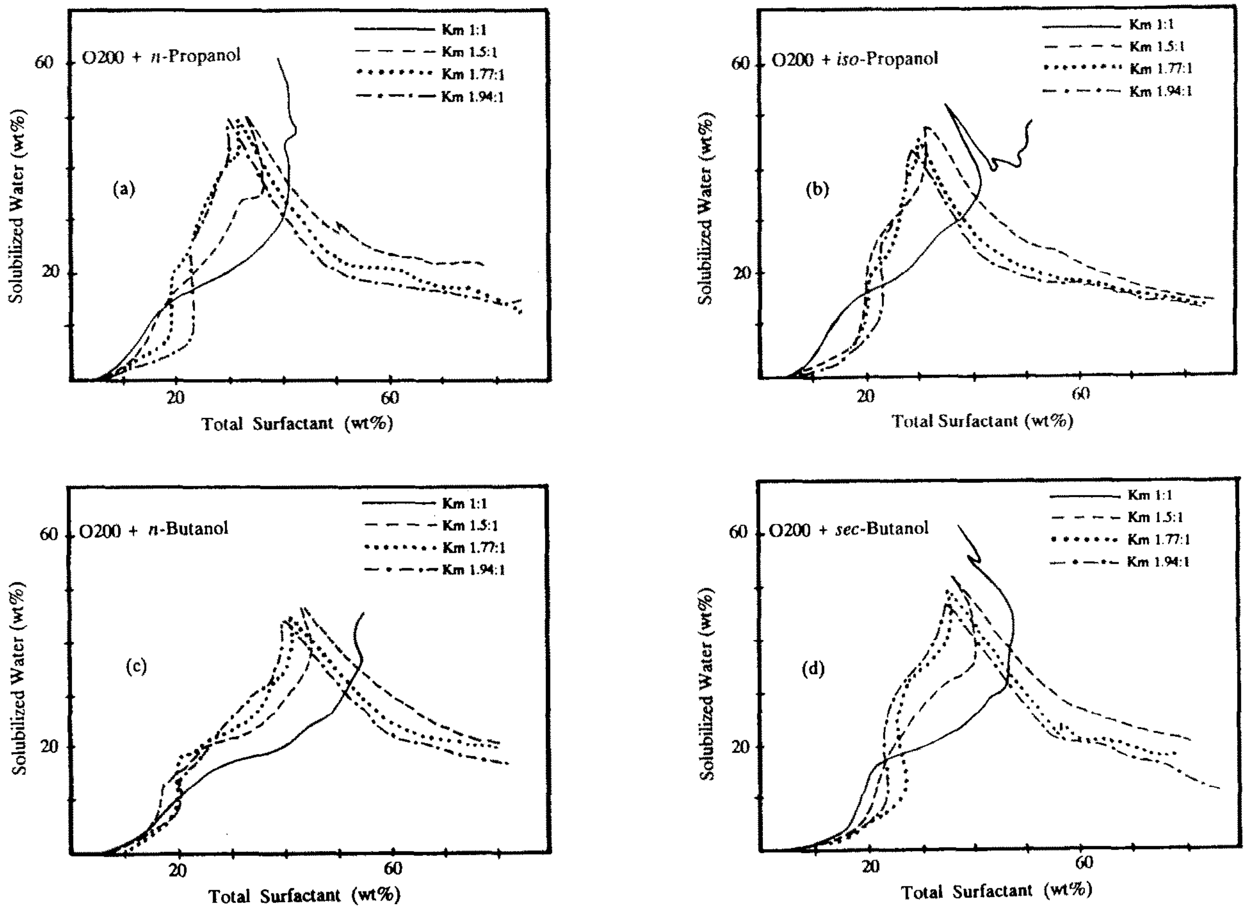

Fig. 11. Water solubilizing capacity of L_2 region for O200 systems as a function of total surfactant concentration.

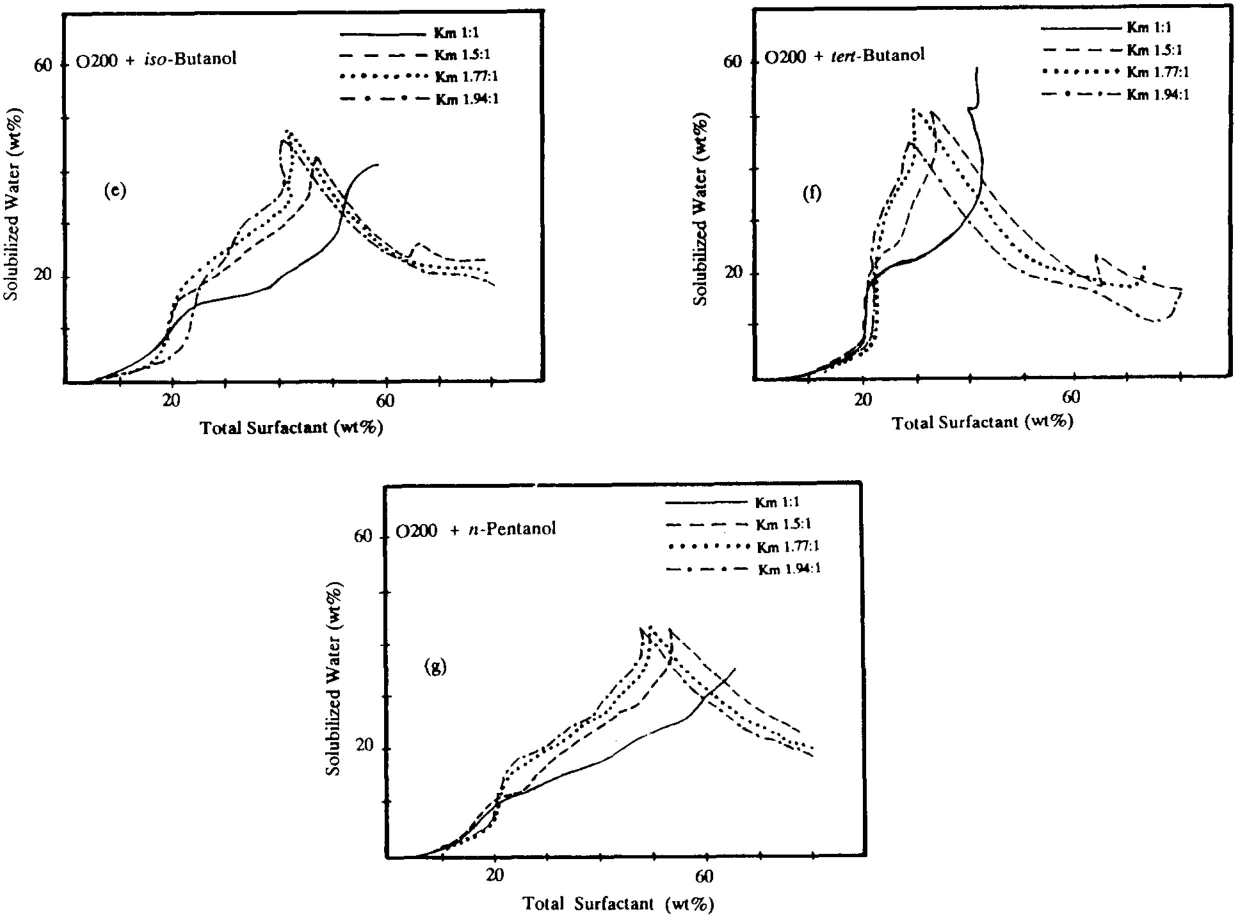

Fig. 11 (continued).

24–47 wt% total surfactant levels. Table 2 indicates the approximate range of solubilized oil and corresponding range of total surfactant concentration for each L_1 system at different K_m values. It should be mentioned that in some systems, streaming birefringence was observed in water rich part of the L_1 area.

Discussion

These observations may be explained with reference to the influence of various cosurfactants on the spontaneous curvature of the lecithin molecules at hydrocarbon-water interface. The differences between the alcohols may be explained by their different alkyl chain lengths and volumes, their cross sectional areas and their different solubilities in water.

Lecithin is not capable of producing isotropic solutions of water and oil without an alcohol cosurfactant because of being slightly too lipophilic (Shinoda et al., 1991). This is because although lecithin is lipophobic due to its heavily hydrated zwitterionic head groups, it is a strongly hydrophobic molecule due to its long hydrocarbon chain. Shinoda et al. (1991) have stated that in order to form lecithin microemulsions over a range of oil-water concentrations (i.e., balanced systems), the hydrophilic-lipophilic balance of lecithin should be increased by decreasing the hydrophilicity of the polar solvent. Alcohols have

TABLE 2

Oil solubilizing capacity of L_1 area

Alcohol	K_m	E200		O200	
		% w/w oil	% w/w surfactant [b]	% w/w oil	% w/w surfactant [b]
n-Propanol	1:1	4–13 [a]	23–38	7–13	27–38
	1.5:1	6–17 [a]	25–41	12–20 [a]	29–42
	1.77:1	10–20 [a]	30–41	14–21	33–40
	1.94:1	10–22	30–41	19–23	35–40
Isopropanol	1:1	7–12	28–37	10–12 [a]	30–37
	1.5:1	10–19	23–39	16–21	34–39
	1.77:1	20–25 [a]	37–42	21–27 [a]	36–41
	1.94:1	22–27 [a]	37–40	25–31 [a]	30–38
n-Butanol	1:1	–	–	–	–
	1.5:1	1–5	15–45	2–11	16–54
	1.77:1	2–7	19–40	5–12	28–49
	1.94:1	2–9	19–44	3–16	22–49
sec-Butanol	1:1	3–6	17–32	–	–
	1.5:1	4–8	24–34	4–12 [a]	20–41
	1.77:1	6–18	25–46	6–13 [a]	25–43
	1.94:1	7–19	29–45	8–18	25–41
Isobutanol	1:1	–	–	–	–
	1.5:1	1–5	19–48	1–8	16–48
	1.77:1	2–8	17–47	2–11	19–51
	1.94:1	2–11	19–50	4–12	24–47
tert-Butanol	1:1	4–8	23–33	6–11 [a]	26–37
	1.5:1	6–15 [a]	24–40	11–17 [a]	30–40
	1.77:1	10–21	30–43	12–23 [a]	32–42
	1.94:1	12–23 [a]	35–43	16–31 [a]	29–42
n-Pentanol	1:1	–	–	–	–
	1.5:1	–	–	1–3	53–61
	1.77:1	1–3	48–59	1–7	46–59
	1.94:1	1–5	45–61	1–8	40–58

[a] Streaming birefringence may be observed at high water content.

[b] Total surfactant.

an important role in decreasing the hydrophilicity of the polar solvent. Therefore, the more polar (water soluble) alcohols are better at forming balanced microemulsions.

In addition, Osipow (1963) showed that for microemulsion to be formed, the interfacial film should not be too condensed, otherwise the appropriate curvature required for droplet formation would not be obtained. Indeed, lecithin is known to form highly rigid film (Binks et al., 1989). The addition of a short chain alcohol will act to reduce the rigidity of the condensed film formed by lecithin allowing the formation of aggregates.

It is well established that the nature of the surfactant aggregate is governed by the ratio v/a_0l_c, where v, a_o and l_c are the hydrocarbon volume, the optimum head group area and the hydrocarbon tail length, respectively, of a surfactant molecule (Israelachvili et al., 1976). This ratio defines the spontaneous curvature of a particular surfactant. A v/a_0l_c value higher than 1 causes the spontaneous formation of reverse aggregates, whereas a lower value (less than 0.5) indicates the presence of normal aggregates. For phospholipids, the large hydrophobic core (v) relative to the head group (a_0) gives a large value of v/a_0l_c ratio of about 0.8 (Cornell et al., 1986), indicating that the formation of lamellar phases are favoured. In a surfactant/cosurfactant/oil/water system, the structure of aggregates will be affected by the interactions between the surfactant head groups (an effect on a_0) and by any penetration of oil into the hydrophobic tail region and the presence of any cosurfactant in the interfacial region (an effect on v and/or a_0).

It is expected that, depending upon the chain length of the oil molecules, penetration of the oil into the surfactant chains will increase the effective hydrocarbon chain volume of the surfactant molecules and thus, increase the value of v/a_0l_c (Chen et al., 1986). Consequently, in the systems investigated, while IPM is a relatively large molecule, the possibility of some penetration into the surfactant chains should not be discounted.

Shinoda and co-workers (1991) have stated that short chain alcohols increase both the hydrophobic volume and the area per head group (a_0) of lecithin molecules at the interface and thus, affect the spontaneous curvature of the surfactant molecules. It is also thought that alcohols are capable of swelling the chain volume allowing substantial oil uptake (Chen et al., 1986).

The distribution of the alcohols between the aqueous phase, the hydrocarbon phase and the interface, is dependent upon their partition coefficients (Trotta et al., 1989b). The more hydrophilic alcohols (e.g., *n*-propanol) would be expected to be distributed primarily between the aqueous and the interfacial layer, whereas the more hydrophobic alcohols (e.g., *n*-pentanol) would be expected to be distributed mainly between the hydrocarbon phase and the interfacial layer.

n-Pentanol has less solubility in water compared to the other alcohols used in this study and thus is expected to be partitioned mainly in the oil phase and the hydrocarbon parts of the surfactant layer. Consequently, the extent of the L_2 area, irrespective of the K_m, in the presence of *n*-pentanol is less than that when the other alcohols are present (Figs 10 and 11). However, an increase in the total surfactant concentration causes an increase in the capacity of the system to solubilize water. At K_m greater than 1:1, the surfactant/oil ratio required for the formation of the LC phase in the presence of *n*-pentanol is more than that in the presence of the other alcohols. *n*-Pentanol may increase the chain volume of the surfactant molecules (due to its chain length and possibly increased penetration of oil). This, in turn, decreases the water solubilizing capacity of the system.

sec-Butanol, *n*-butanol and isobutanol are intermediate in solubility in water. Thus, they are expected to be partitioned between the aqueous phase and the interfacial layer. Although the capacity for water solubilization increases in the presence of these alcohols (possibly due in part to the increased flexibility of the interface), the amount of total surfactant (or surfactant/oil ratio) required for the formation of the LC phase is less than that obtained for systems containing *n*-pentanol. This is probably due to the increased number of surfactant and cosurfactant molecules at the interface and/or to the increased oil penetration into the surfactant chains.

tert-Butanol, *n*-propanol and isopropanol are partitioned mainly in the aqueous phase and partially in the polar parts of the surfactant layer. Therefore, they produce high balanced systems and increase the solubilizing capacity. In the presence of these alcohols, the LC structures are formed with lower surfactant/oil ratios. It is possible that the large cross sectional area of tert-butanol may promote the formation of the LC phase.

In general, the number of cosurfactant molecules positioned at the interface will be reduced as the K_m increases. Therefore, increased rigidity of the interface causes the extension of the LC phase.

The low capacity of normal aggregates for solubilizing oil may be explained by considering this fact that due to the large hydrocarbon chains of phospholipid molecules, the aggregate is not capable of solubilizing a large amount of oil.

As described in Materials and Methods, the stock solutions were prepared by mixing surfactant, cosurfactant and oil. On addition of water, clear solutions containing reverse aggregates were initially formed. Depending upon the alcohol and K_m, further addition of water induced the deformation of the aggregates causing either the appearance of turbidity or the presence of a liquid crystalline region. In many of the systems exhibiting turbidity, this was not permanent and after a short-term storage the seperation of layers was observed. The increasing amount of water led to the formation of macroemulsion, especially at the water rich part of the phase diagrams. When LC region was encountered, further dilution with water frequently resulted in the transition of the LC phase to the L_1 phase. These changes may be explained with reference to the change in the curvature of interfacial film. As the water content increases in a fluid system containing reverse aggregates, the interfacial area also increases, thus decreasing the total surfactant concentration at the interface resulting in an increase of the interfacial tension. As more water is added, a critical point is reached at which the interfacial area decreases and reverse aggregates convert to LC structure. Upon further addition of water, inversion to a fluid system containing normal aggregates occurs.

Although no significant difference was observed in the phase behaviour between E200 and O200, a possible explanation for the small changes may be the difference in the fatty acid and phosphatidylcholine contents of the two lecithins (Table 1).

Among different alcohols examined in this study, *sec*-butanol, *n*-butanol and isobutanol have been found to have the optimum properties such that they allow a wide range of surfactant concentrations over which the isotropic solutions are stabilized as well as producing isotropic solutions with relatively high solubilizing capacity.

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