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

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Abstract

The phase behaviour of quaternary systems composed of lecithin/isopropyl myristate/water/and one of seven short-chain alcohols at various surfactant/cosurfactant mixing ratios (K_m) has been investigated by the construction of phase diagrams. A commercially available soybean lecithin (namely, Epikuron 170, phosphatidylcholine purity 68–72%) was used in the study. Phase diagrams showed the area of existence of a stable isotropic region along the surfactant/oil axis (i.e., reverse microemulsion area; L_2) in all systems regardless of the K_m. The existence of a second water-rich isotropic region (i.e., normal microemulsion area; L_1) was, however, seen to be very dependent upon the K_m and occurred in only a few instances. This second isotropic region, L_1, always occurred in conjunction with a liquid crystalline domain, although in some cases, particularly at the lower K_m, a liquid crystalline region was seen to occur without the presence of an L_1 phase. Comparison of the results with those obtained using higher purity lecithins (greater than 92% phosphatidylcholine content) indicated that while the phase diagrams were qualitatively similar, significant differences occurred at oil levels below 50%. It was found that the existence of an L_1 and LC region and the extent of the L_2 region were dependent upon both the purity of surfactant and the surfactant/cosurfactant mixing ratios (K_m).

Key words: Microemulsions; Soybean lecithin; Lecithin purity; Phase diagram; Phase behavior; Cosurfactant

1. Introduction

Microemulsions are generally defined as isotropic, transparent, thermodynamically stable mixtures of at least three components; water, oil, and a surfactant; usually in combination with a cosurfactant, typically a short chain alcohol.

As a consequence of their unusual thermodynamic properties, microemulsions are of considerable industrial importance; they have found practical applications in tertiary oil recovery (Bansal and Shah, 1977), as media for chemical and enzyme catalyzed reactions (Fendler, 1980;

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Holmberg and Osterberg, 1990; Robinson, 1990; Rees and Robinson, 1991; Xenakis et al., 1991) and as liquid membrane carrier agents (Tonder and Xenakis, 1982). So far microemulsions have not been exploited for pharmaceutical purposes. Yet, from a pharmaceutical formulation point of view, they offer many potential benefits; e.g., oil-in-water microemulsions may be used as a carrier for drugs which exhibit a poor aqueous solubility (Malcolmson, 1992; Malcolmson and Lawrence, 1993), while water-in-oil microemulsions may have use as sustained release intramuscular preparations (Gasco et al., 1990). Unfortunately, most work to date studying microemulsions has utilised oils, surfactants and cosurfactants unsuitable for pharmaceutical purposes. In order to make these systems pharmaceutically acceptable, it is necessary to formulate such systems using non-toxic, safe ingredients. It was thus decided to investigate the preparation of microemulsions using lecithin as surfactant, isopropyl myristate as oil and, because lecithin will not form microemulsions without the aid of a cosurfactant (Shinoda et al., 1991), a number of short-chain alcohols as cosurfactants.

In the first part of this study (Aboofazeli and Lawrence, 1993), we reported the pseudo-ternary phase diagrams of water/isopropyl myristate/lecithin (either the soybean lecithin Epikuron 200; phosphatidylcholine purity 94%, or the egg lecithin Ovothin 200; phosphatidylcholine purity 92%) systems using a wide range of short-chain alcohols as cosurfactant. These alcohols were used 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. Although differences were observed in the phase diagrams of the two lecithins, these discrepancies were, except in a few instances, minimal and not thought to be significant.

In the second part of our investigation, the same experiments have been performed using a less pure phospholipid, namely, Epikuron 170 (soybean lecithin, phosphatidylcholine purity 68–72%) so as to examine the influence of lecithin purity on phase behaviour. It was decided to use this grade lecithin for two reasons; first, the prohibitive cost of the higher purity lecithins and secondly, the fact that E170 has previously been used as a pharmaceutical adjuvant.

2. Materials and methods

2.1. Materials

The commercially available soybean lecithin, Epikuron 170 (E170), supplied by Lucas Meyer Co. (Germany) was used as received. Isopropyl myristate (IPM), *tert*-butanol and *sec*-butanol were obtained from Sigma Chemical Co. (Dorset, U.K.). Isobutanol was purchased from Fluka Chemicals Ltd (Glossop, U.K.) and *n*-pentanol from Aldrich Chemical Co. Ltd (Dorset, U.K.). *n*-Butanol was supplied by BDH Ltd (Poole, U.K.). *n*-Propanol and isopropanol were from FSA Laboratory Supplies (Loughborough, U.K.). All reagents were of the highest purity available and were used as received. Triple-distilled water from a well-seasoned, all-glass still was used throughout the study.

2.2. Construction of pseudo-ternary phase diagrams

Phase diagrams were constructed by the titration with triple-distilled water of a series of three-component mixtures (IPM/lecithin/cosurfactant) at room temperature. The course of each titration was monitored through cross polaroids in order to determine the boundaries of any microemulsion and birefringent liquid crystalline domains. When a sample exhibited birefringence, the titration was continued so as to determine the endpoint of the liquid crystalline area and establish the presence (or absence) of a second isotropic region. No attempt was made to identify in detail any other regions of the phase diagram.

The phase behaviour of the systems was mapped on phase diagrams with the top apex representing the lecithin/cosurfactant at a particular K_m and the other apices representing oil and water. The L_1 and L_2 regions were respectively identified as low oil and low water, transparent, isotropic microemulsions. The liquid crys-

talline (LC) area was defined here as that in which all samples showed birefringence under polarized light. Both isotropic regions identified on the phase diagram were stable for at least three months at room temperature.

3. Results

Figs. 1–7 show the pseudo-ternary phase diagrams for the system E170/IPM/water/alcohol with the seven cosurfactants at different K_m values. It should be mentioned that the phase diagrams are overlaid because no significant difference in the phase behaviour was observed for all systems at high oil concentrations. Also, because of the difficulties in determining the endpoint of the LC regions, dashed lines show boundaries that are not accurately determined.

3.1. Isotropic L_2 region

As can be seen when using E170 (Fig. 1–7), all systems showed a large isotropic area along the surfactant/oil axis, namely, L_2 (reverse aggregates). It is clearly obvious that the amount of water solubilized in the L_2 area is dependent upon both the nature of the cosurfactant and K_m. Increasing the K_m frequently leads to a reduction in the extent of the L_2 region due to the presence of a liquid crystalline area in the oil poor part of the phase diagram. The existence of this LC region leads to the appearance of a peak in the amount of water solubilized. Not every alcohol at each K_m, however, exhibited an LC region; for example both *n*-butanol and isobutanol did not produce an LC region at a K_m of 1:1, while *n*-pentanol did not exhibit an LC region at any K_m tested. In these systems, the maximum amount of water incorporated occurred

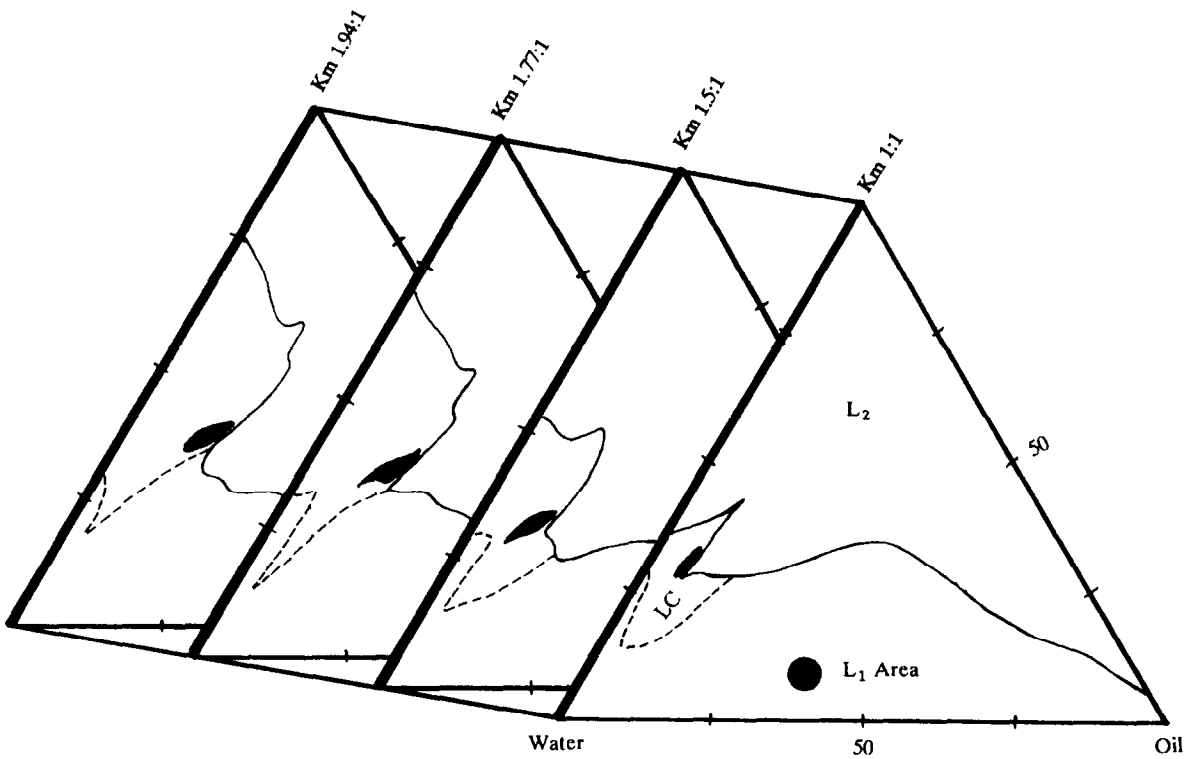

Fig. 1. Phase diagrams of quaternary systems containing E170/IPM/water/*n*-propanol at different K_m.

at very low oil concentrations. Table 1 gives the maximum amount of water solubilized and the corresponding surfactant/oil ratio for each alcohol at each K_m tested.

3.2. Isotropic L_1 region

In some systems, at low oil concentrations, another isotropic area (L_1; normal aggregates) was observed. In contrast to the L_2 region, the L_1 region extends over a very limited area in the high water content part of the phase diagrams and always occurred next to an LC region. Although in some cases, generally at low K_m, an LC region was seen in the absence of an L_1 area; for example, *n*-butanol at K_m of 1.5:1 and 1.77:1, *sec*-butanol at K_m of 1:1 and 1.5:1, *tert*-butanol at K_m of 1:1, and isobutanol at K_m of 1.5:1 all exhibited LC regions in the absence of an L_1 domain. At higher K_m, however, each alcohol that produces an L_1 area does so in conjunction with an LC region.

Table 1 gives the approximate amount of oil solubilized together with the corresponding range of the total surfactant concentrations required for each of the L_1 regions. In general, the L_1 region is capable of solubilizing only a small amount of IPM, generally less than 15%. The nature of cosurfactant and K_m have a great influence on the existence of this area; for example, *n*-butanol at K_m of 1.94:1, *sec*-butanol and isobutanol at K_m of 1.77:1 and 1.94:1, and *tert*-butanol at all K_m (except 1:1) are able to produce the L_1 area, while *n*-pentanol cannot produce this region at any of K_m examined in this study.

3.3. Comparison between different grade lecithins

As mentioned earlier there was little difference seen in the ternary phase diagrams obtained

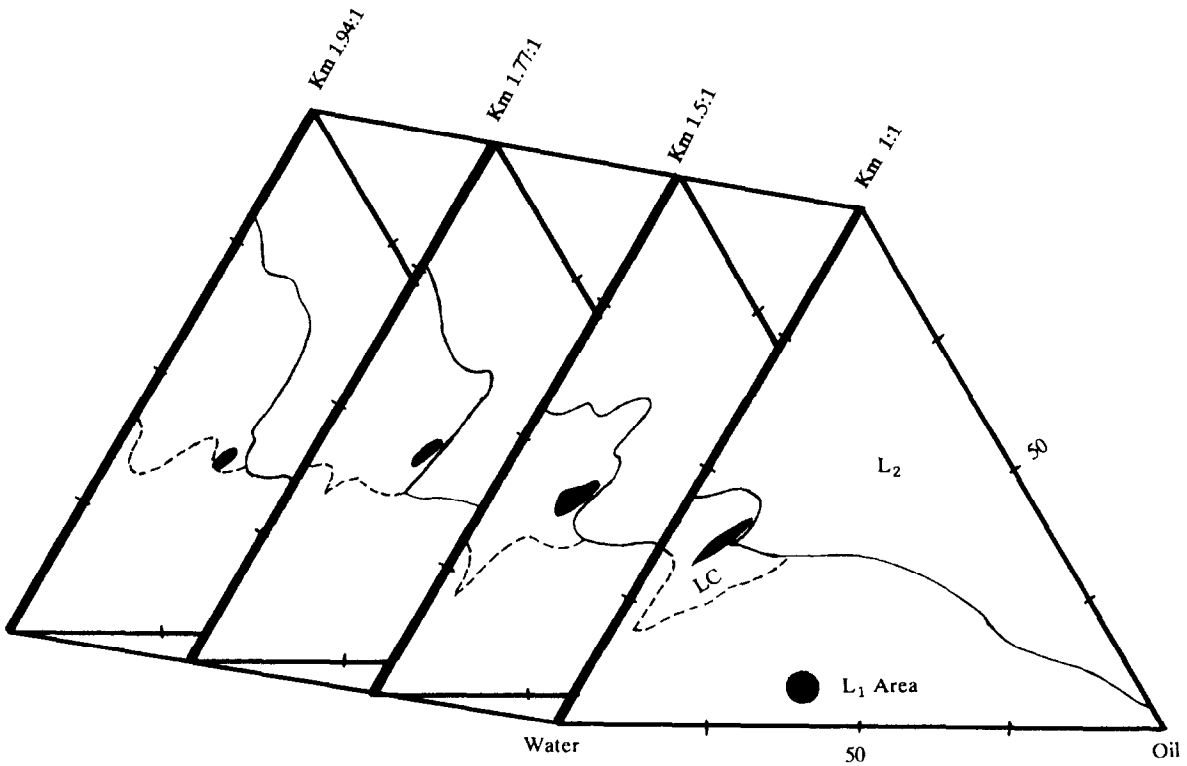

Fig. 2. Phase diagrams of quaternary systems containing E170/IPM/water/isopropanol at different K_m.

when using Epikuron 200 (E200) or Ovothin 200 (O200) (Aboofazeli and Lawrence, 1993).

It should be noted, however, that in a limited study examining the use of *n*-butanol as cosurfactant, Attwood et al. (1992) observed slight differences between these two grades of lecithin, particularly at low oil concentrations. These differences were attributed to the nature of the fatty acid impurities in the two types of lecithin.

Before pointing out the differences between the phase diagrams obtained in this study with E170 and the other grades of lecithin (E200 and O200), it is beneficial to mention the similarities. Regardless of the grade of lecithin used:

(1) The extent of the isotropic regions, L_1 and L_2, was dependent upon both the nature of the cosurfactant and the K_m.
(2) The L_2 region covers the whole range of surfactant/cosurfactant concentrations, generally allowing a wide range of water concentrations to be solubilized.
(3) When associated with an LC region in the oil poor part of the phase diagram, the L_2 area is reduced, resulting in the appearance of a maximum in the amount of water solubilized.
(4) As the K_m increases, the maximum solubilization peak moves towards the middle of the phase diagram. In other words, at higher values of K_m studied, the surfactant/oil ratio which can solubilize the maximum amount of water has its lowest value.
(5) The surfactant/oil ratio required for maximum water 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.
(6) Some systems, particularly those with surfactant/oil ratios close to but less than that required for the formation of an LC phase, pro-

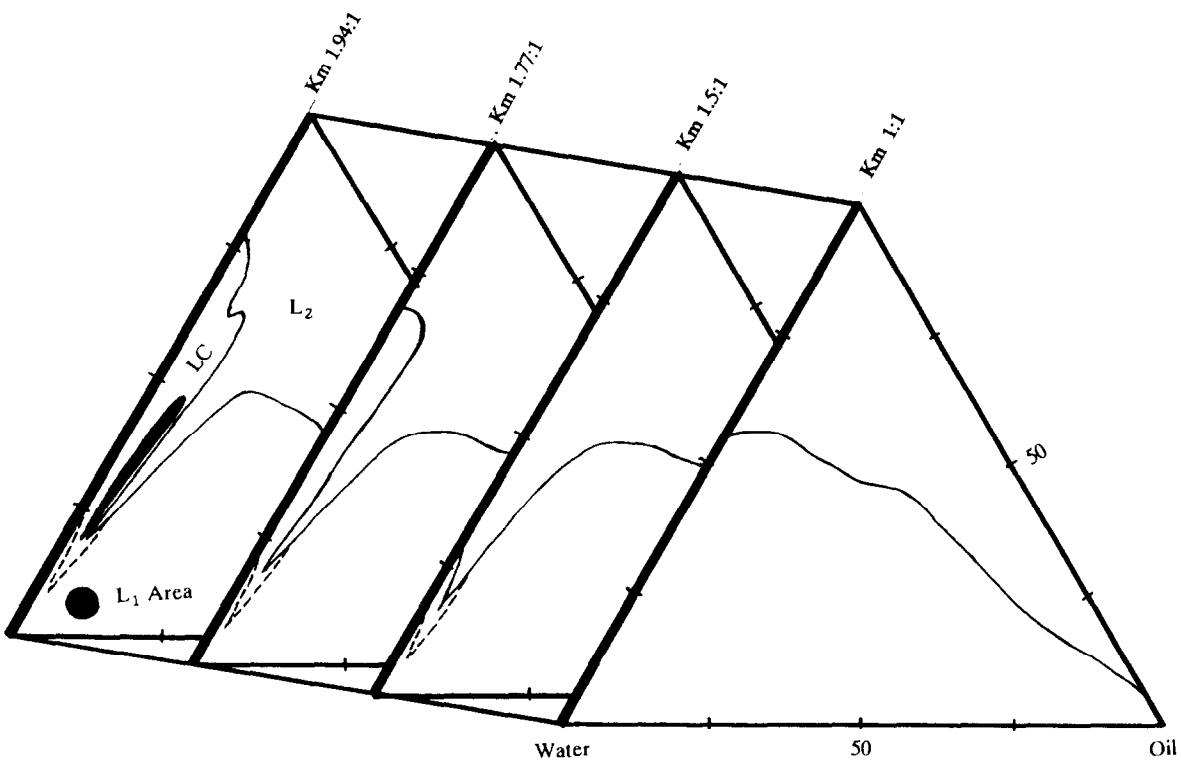

Fig. 3. Phase diagrams of quaternary systems containing E170/IPM/water/*n*-butanol at different K_m.

duced very viscous L_2 samples at high water content.

(7) In all systems, the L_1 region extends over a very limited area in the water-rich part of the phase diagrams and is able to solubilize only a small amount of IPM; the position and the extent of this region are dependent on the nature of the cosurfactant and K_m.

Although there are similarities in the phase diagrams produced by the various grades of lecithin, there are a number of significant differences. These discrepancies are most noticeable in systems that produce either an LC or LC and L_1 regions at oil concentrations below 50% w/w; in systems containing greater than 50% w/w oil, no significant difference in phase behaviour was observed. In comparison to E200 and O200, E170 produces: (1) a larger L_2 area with *n*-butanol, *sec*-butanol and isobutanol, which generally extends further into the water rich region of the phase diagram; (2) an LC region that also frequently extends further into the water rich area; (3) in some systems, an LC area without the presence of an associated L_1 region; frequently the LC region or L_2 area encompasses the compositions producing an L_1 microemulsion with the higher purity lecithins; (4) an L_1 area which solubilizes a smaller range of oil concentrations, over approximately the same range of surfactant concentration as the purer lecithins.

Interestingly, in systems which produced only an L_2 domain, no difference in the extent of this region was observed with lecithin type.

4. Discussion

The previous study (Aboofazeli and Lawrence, 1993) examined the ability of two grades of lecithins to produce balanced microemulsions, that is microemulsions formed over a wide range of oil, water and surfactant compositions, in the presence of a range of short-chain alcohols. Both

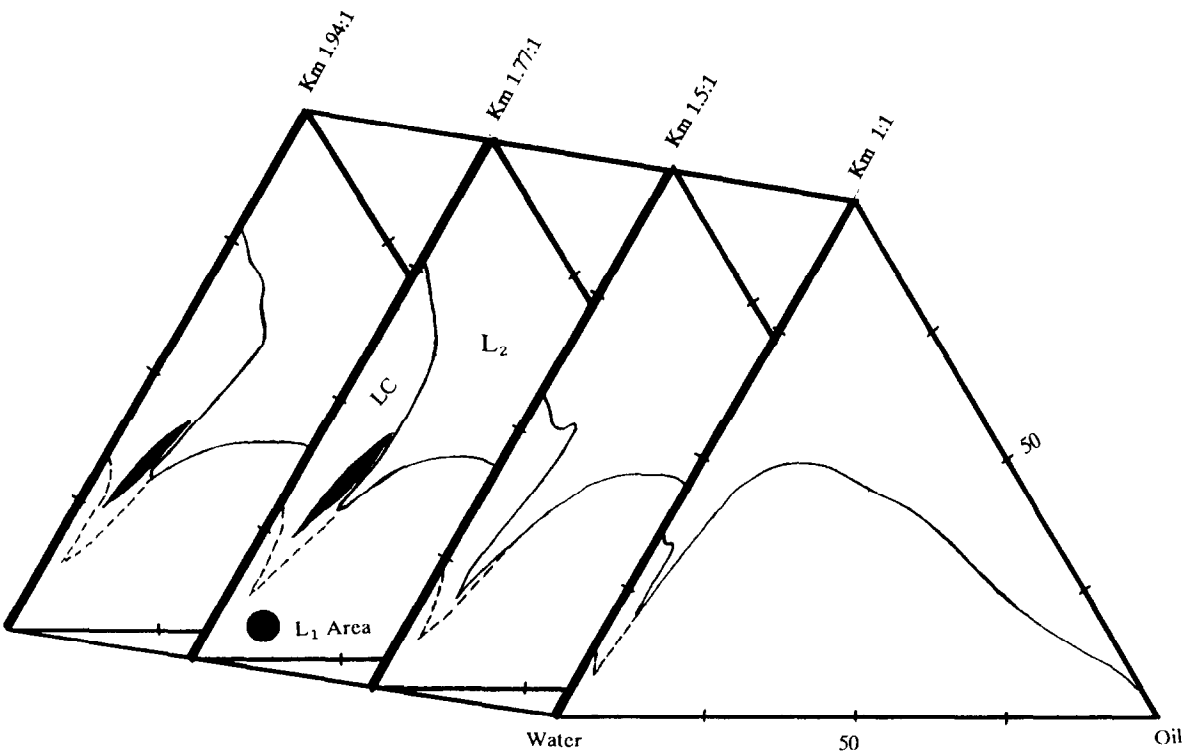

Fig. 4. Phase diagrams of quaternary systems containing E170/IPM/water/*sec*-butanol at different K_m.

Table 1
Water and oil solubilizing capacity of E170 / IPM / water / alcohol systems

Cosurfactant	K_m	s/o [a]	% w/w water [b]	% w/w oil [c]	% w/w surfactant [d]
n-Propanol	1:1	4:1	65	6–7 [f]	29–34
	1.5:1	2.5:1	57	6–12	29–36
	1.77:1	2.3:1	50	10–16 [f]	34–42
	1.94:1	1.9:1	55	10–15	33–40
Isopropanol	1:1	3:1	55	7–12 [f]	32–41
	1.5:1	2:1	52	11–16 [f]	36–42
	1.77:1	1.9:1	48	17–19	40–44
	1.94:1	1.5:1	44	17–19	34–45
n-Butanol	1:1	19:1 [e]	41	[g]	[g]
	1.5:1	9:1	80	[g]	[g]
	1.77:1	9:1	78	[g]	[g]
	1.94:1	7.3:1	76	2–5	20–47
sec-Butanol	1:1	6.7:1	73	[g]	[g]
	1.5:1	4:1	76	[g]	[g]
	1.77:1	3.3:1	60	4–11 [f]	25–45
	1.94:1	3.3:1	60	4–10 [f]	24–41
Isobutanol	1:1	19:1 [e]	39	[g]	[g]
	1.5:1	9:1	80	[g]	[g]
	1.77:1	7:1	77	2–6 [f]	18–50
	1.94:1	5.7:1	55	2–8 [f]	18–54
tert-Butanol	1:1	4.5:1	64	[g]	[g]
	1.5:1	3:1	57	7–11 [f]	31–38
	1.77:1	2.3:1	51	8–14 [f]	32–42
	1.94:1	2.3:1	47	13–16	38–43
n-Pentanol	1:1	19:1 [e]	29	[g]	[g]
	1.5:1	19.1 [e]	38	[g]	[g]
	1.77:1	19:1 [e]	49	[g]	[g]
	1.94:1	19:1 [e]	56	[g]	[g]

[a] Surfactant/oil ratio capable of solubilizing maximum amount of water; [b] maximum amount of water solubilized in L_2 area; [c] approximate range of oil solubilized in L_1 area; [d] total surfactant concentration; [e] the greatest ratio examined; [f] streaming birefringence may be observed at high water content; [g] no L_1 region was observed.

types of lecithin contained at least 92% phosphatidylcholine, the difference between the soybean and egg lecithin being the nature of the component fatty acids (Table 2). Unfortunately, phosphatidylcholine is slightly too lipophilic a molecule to form balanced microemulsions when used as the sole surfactant (Shinoda et al., 1991). Its high critical packing parameter (CPP), approx. 0.8 (Cornell et al., 1986), means it tends to form lamellar phases or bilayers (Israelachvili et al., 1976). It is therefore necessary to adjust (reduce) its CPP and its spontaneous curvature in order to form microemulsions (Shinoda et al., 1991). This is usually achieved by the addition of a cosurfactant, frequently a short-chain alcohol. The cosurfactant can act by either interchelating between surfactant molecules at the oil/water interface and/or by decreasing the hydrophilicity of the aqueous phase. In the production of a balanced lecithin microemulsion, the cosurfactant has an additional role in that it can also act to reduce the tendency of lecithin to form a highly rigid film (Binks et al., 1989), thus allowing the interfacial film to take up the different curvatures required to form balanced microemulsions (De Gennes and Taupin, 1982).

Although in the previous study changing the nature of the alcohol used significantly altered

Table 2
Compositions of soybean and egg lecithins

Lecithin	% w/w PC [a]	Lyso-PC [b]	Other PL [c]	% w/w of total fatty acid			
				Palmitic and stearic	Oleic	Linoleic	Linolenic
Epikuron 200 (soybean)	> 94	max. 3	max. 1	16–20	8–12	62–66	6–8
Epikuron 170 (soybean)	> 68	max.4	20–25 [d]	16–20	8–12	62–66	6–8
Ovothin 200 (egg)	> 92	max. 3	max. 2	39–47	28–32	13–17	–

[a] Phosphatidylcholine; [b] lysophosphatidylcholine; [c] phospholipid; [d] 10–13% phosphatidylethanolamine, 10–12% other phospholipids and glycolipids.

the phase diagrams obtained, there was very little difference seen between the types of lecithin investigated even at the higher K_m It is at the higher K_m and in the oil-deficient part of the phase diagram that it would be anticipated there would be discrepancies between lecithins. This is because at higher K_m, there is less alcohol available to alter the CPP of the lecithin, and consequently the nature of the component fatty acids of the lecithin would be expected to become important in determining the effective CPP. From these studies, it is obvious that the nature and concentration of alcohol cosurfactant present were more important in determining phase behaviour than the type of lecithin.

In the present study, the same range of alco-

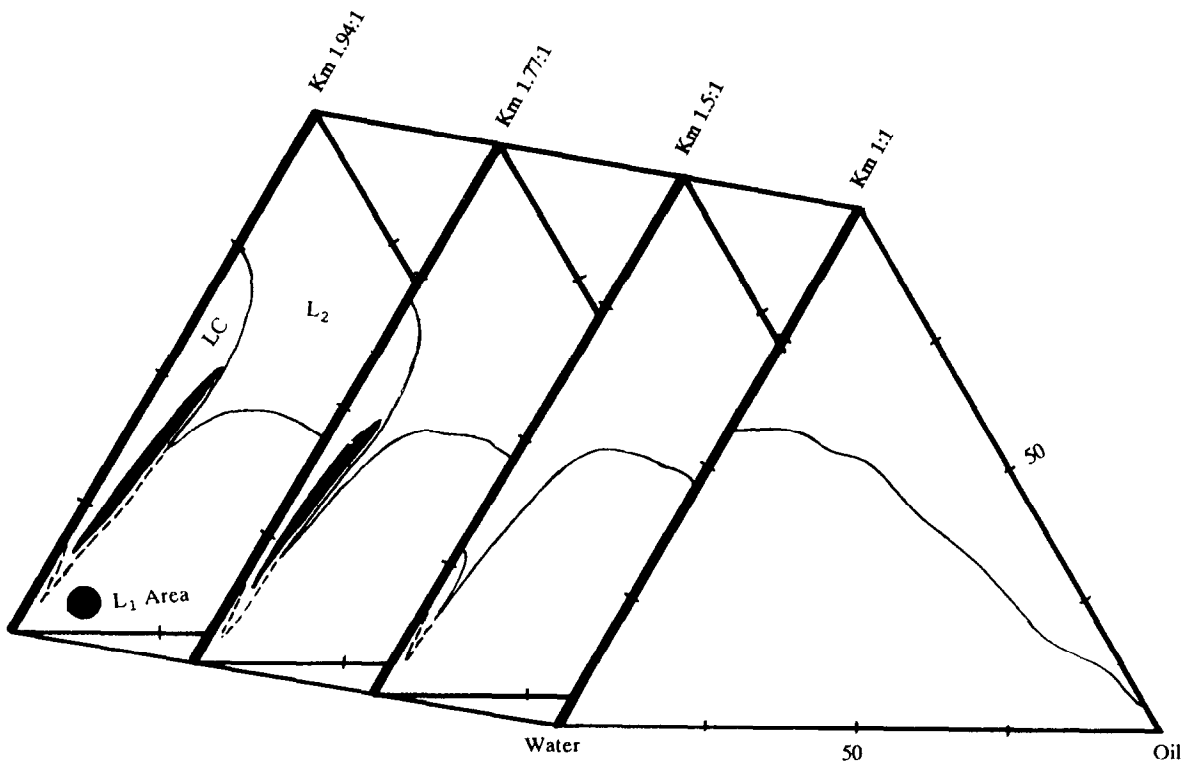

Fig. 5. Phase diagrams of quaternary systems containing E170/IPM/water/isobutanol at different K_m.

hols were used but this time in conjunction with a less pure grade of lecithin (E170) (Table 2). While the results followed the same trend as those obtained previously in that the more water soluble alcohols such as *n*-propanol were most effective in producing balanced microemulsions, there were significant changes observed in the phase diagrams particularily in the oil-poor part of the phase diagram.

The main difference in composition between E200 and O200 and E170 is that the latter contains not only phosphatidylcholine (68–72%) but also phosphatidylethanolamine (10–13%) together with other phospholipids and glycolipids (10–12%). Although these molecules are generally considered to be surfactants in their own right, it is not unreasonable in the present system to treat them as cosurfactants, as they will exert an effect on the CPP. Indeed, Shinoda et al., (1991) suggested that it would be possible to decrease the CPP by the partial substitution of lecithin with hydrophilic surfactants such as lysolecithin, phosphatidylinositol, a charged phosphatidylcholine analogue, or a hydrophilic nonionic surfactant. Such cosurfactants would not be expected to alter the hydrophilicty of the aqueous phase, but to exert their influence only on the interfacial layer. In addition, as a result of the long-chain fatty acids present in these cosurfactants, they would not be expected to be as effective as the short chain alcohols in reducing the tendency of lecithin (phosphatidylcholine) to form rigid interfacial films.

Each of the non-phosphatidylcholine components present in E170 will influence the effective CPP of the lecithin in a different manner. For example, the presence of phosphatidylethanolamine composed of unsaturated fatty acids would be expected, due to its tendency to form reverse micelles, to increase the effective CPP (Isrealachvili et al., 1980). In contrast, glycolipids such as the gangliosides GM_1 and GM_2 are classified

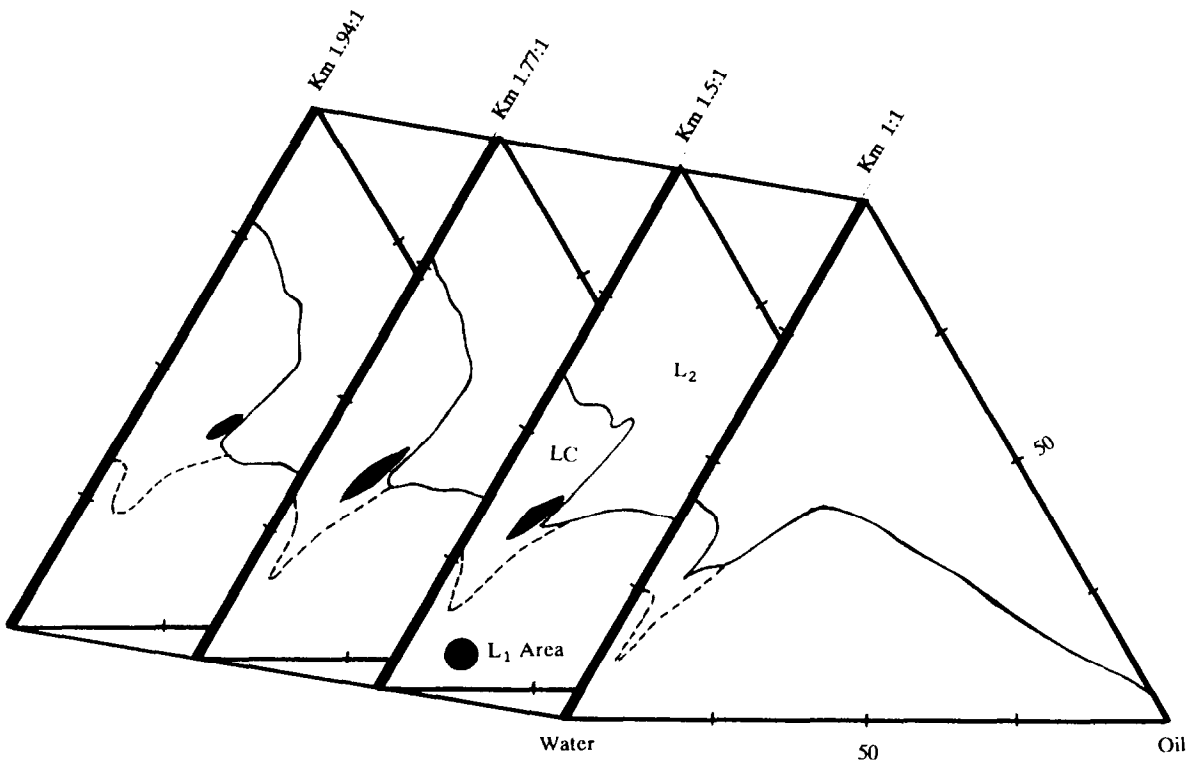

Fig. 6. Phase diagrams of quaternary systems containing E170/IPM/water/*tert*-butanol at different K_m.

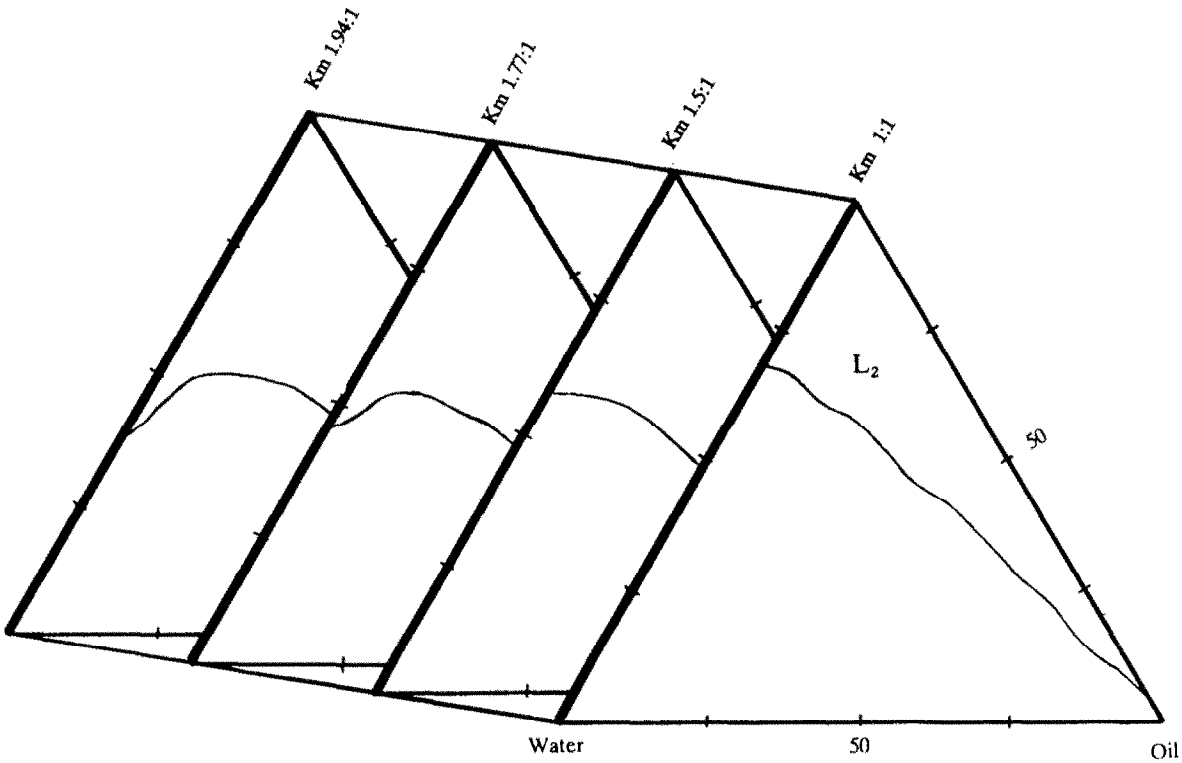

Fig. 7. Phase diagrams of quaternary systems containing E170/IPM/water/*n*-pentanol at different K_m.

as hydrophilic surfactants in that they aggregate to form normal micelles (Corti et al., 1991) and would thus be expected to reduce the effective CPP. Unfortunately, while it is possible to predict the influence of each component on the resulting CPP, it is impossible to predict the actual CPP of the resulting mixture.

In comparison with E200 and O200, the main difference observed in some systems when using E170 is the extension of the L_2 area towards the water-rich part of the phase diagram, together with the trend of producing an LC region at higher water contents. This suggests that the E170 is more effective at promoting the formation of balanced microemulsions, probably due to a reduced effective CPP.

It is, however, important to note that the differences between grades of lecithin are considerably less than the influence of changing the alcohol cosurfactant. Therefore, it is not unreasonable to use E170 in pharmaceutical formulation in place of either E200 or O200.

5. Acknowledgement

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