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Investigations into the formation and characterization of phospholipid microemulsions. IV. Pseudo-ternary phase diagrams of systems containing water-lecithin-alcohol and oil; the influence of oil

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Abstract

Phase studies have been performed for quaternary systems composed of egg lecithin, cosurfactant, water and oil. The lecithin used was the commercially available egg lecithin Ovothin 200 (which comprises ≥ 92% phosphatidylcholine). The cosurfactants employed were propanol and butanol, and these were used at lecithin/cosurfactant mixing ratios (K_m) of 1:1 and 1.94:1 (weight basis). Six polar oils were investigated, including the alkanoic acids, octanoic and oleic, their corresponding ethyl esters and the medium and long chain triglycerides, Miglyol 812 and soybean oil. All oils, irrespective of the alcohol and the K_m used, gave rise to systems that produced a stable isotropic region along the surfactant/oil axis (designated as a reverse microemulsion system). In addition, the systems incorporating propanol at both K_m and butanol at a K_m of 1.94:1, generally gave rise to a liquid crystalline region and, in some cases, a second isotropic non-birefingent area (designated as a normal microemulsion system). The phase behaviour observed was largely dependent upon the alcohol and K_m used and the size and the polarity of the oil present.

Keywords: Microemulsion; Egg lecithin; Phase diagram; Phase behavior; Oil

1. Introduction

Microemulsions are homogeneous, transparent, thermodynamically stable dispersions of water and oil, stabilized by surfactant, usually in conjunction with a cosurfactant, typcially a short chain alcohol. As pharmaceutical drug delivery systems microemulsions have many advantages, not the least being their clarity, high stability and ease of preparation. Although microemulsions have been extensively examined from a physicochemcial point of view (Langevin, 1986) most of the systems invesitigated are inappropriate for pharmaceutical purposes, mainly because of the ingredients used. By far the majority of work to

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date has involved the use of ionic surfactants, alcohol cosurfactants and oils such as hexane and benzene, all of which are unsuitable for the purposes of pharmaceutical formulation. In our research we have embarked on a study to formulate lecithin-based microemulsions suitable for the purposes of drug delivery. To date we have investigated the effect of lecithin purity (Aboofazeli and Lawrence, 1994) and the nature of the cosurfactant (Aboofazeli and Lawrence, 1993; Aboofazeli et al., 1994). In the present study the aim has been to examine the influence of a range of polar oils on the formation of lecithin-based microemulsions. Six polar oils have been considered including the alkanoic acids, octanoic and oleic acid, their corresponding ethyl esters and the medium and short chain triglycerides, Miglyol 812 and soybean oil.

2. Material and methods

2.1. Materials

Ovothin 200 (O200, egg lecithin, phosphatidylcholine content greater than 92%) was obtained from Lucas Meyer (Germany). Soybean oil (SBO), ethyl oleate (EOL), oleic acid (OLA), ethyl octanoate (EOC), octanoic acid (OCA) and butoxyethanol were supplied by Sigma Chemical Co. (UK). *n*-Butanol, *n*-propanol and triethylene glycol mono-*n*-butyl ether were purchased from Fluka Chemicals Ltd (UK). Diethylene glycol mono-*n*-butyl ether was supplied by Aldrich Chemical Co. (UK). Miglyol 812 (MIG) was obtained from Hüls AG (Germany). 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 the phase diagrams

Phase diagrams were constructed at room temperature (approx. 21°C) as outlined in Aboofazeli and Lawrence (1993). At least 20 initial compositions were used. The resultant phase behaviour was mapped on pseudo-ternary phase diagrams. The top apex of the system represents the lecithin/cosurfactant mixing ratio and the other two apices represent oil and water. The regions L_1 and L_2, indicated on the phase diagrams at low oil and water content respectively were fluid transparent isotropic microemulsions which were stable for a sufficiently long time (i.e., at least 1 month) at room temperature (approx. 21°C). Birefringent liquid crystalline regions are marked LC. Unmarked areas indicate multiphase regions. Although the clear regions at low oil and water content were designated as L_1 and L_2, i.e., normal and reverse microemulsion regions, respectively, it must be recognised that more than one type of phase structure may be present in each of these regions. For example, systems composed of low water and high oil (designated as L_2 in this study) may variously contain reverse microemulsion droplets, weakly structured aggregates or even no structure, in which case the microemulsion is actually a cosolvent system.

2.3. Determination of the relative polarity of the oils

The relative polarity of the oils was determined after the method of Kahlweit et al. (1983) by simply observing the partitioning of a range of cosurfactants, namely, C_4OH and C_4E_j ($j = 1$–3) between the various oils and water at room temperature (approx. 21°C). The cosurfactants (25 wt%) were added to equal masses of oil and water, and the equilibrium phase behaviour noted by determining the relative volumes of the resultant phases. Multiphase behaviour was described by four categories, namely, $\underline{2}$, $\bar{2}$, $\rlap{-}2$, or 3 (Knickerbocker et al., 1982). The systems designated $\underline{2}$ were two-phase systems in which the mass fraction of the lower aqueous phase was greatest (in these systems the cosurfactant resides mostly in the lower aqueous phase). The designation $\bar{2}$ describes a two-phase system in which the mass fraction of the upper phase was largest (in this case the cosurfactant resides primarily in the upper oil phase). The $\rlap{-}2$ indicates a two-phase system in which the mass fractions of the upper and lower phases were approximately equal (here the cosurfactant partitions equally between both phases). The 3 denotes a three-phase system in which most of the cosurfactant resided in the middle phase. Note that in all instances the density of the oils investigated was less than that of water.

2.4. Determination of mutual solubility

Mutual solubility of the various oils and water was determined by equilibrating at room temperature (approx. 21°C) an equal mass of oil and water. After equilibration was achieved (assumed to be 24 h) the mutually saturated phases were separated and their refractive index determined by means of an Abbe 60/ED precision refractometer illuminated with a sodium lamp (D line, 589.6 nm). As refractive index is an additive property, the solubility of the oils in water and vice versa was estimated with reference to the refractive index of pure water and oil established under the same conditions.

3. Results

The pseudo-ternary phase diagrams of the systems investigated are shown in Fig. 1–4. In a number of instances, because the phase diagrams obtained with the different oils are virtually superimposable, only representative diagrams are shown. Furthermore, certain of the diagrams are overlaid at low water and high oil content because the phase behaviour did not change significantly in this region.

3.1. Butanol-K_m 1:1

All oils behaved in a similar manner in that they all produced systems which at low water contents, gave a large clear (L_2) region extending over all possible ratios of surfactant and oil. None of the oils examined produced systems which exhibited either an LC region or a second clear (L_1) area. Intrestingly, although the extent of the L_2 area was only very slightly dependent upon the oil used, the oils could be divided into two groups, with the members of each group exhibiting virtually identical phase diagrams. The first group consisted of MIG and the ethyl esters, EOL and EOC, while the second group contained SBO and the alkanoic acids, OLA and OCA. For this reason Fig. 1 shows the phase diagrams obtained for the systems containing the oils EOL and OLA. As can be seen from Fig. 1 the second group of oils gave rise to a slightly smaller L_2 region.

3.2. Butanol-K_m 1.94:1

The phase diagrams obtained for these systems are given in Fig. 2. Again all oils produced systems which exhibited an L_2 region that covered

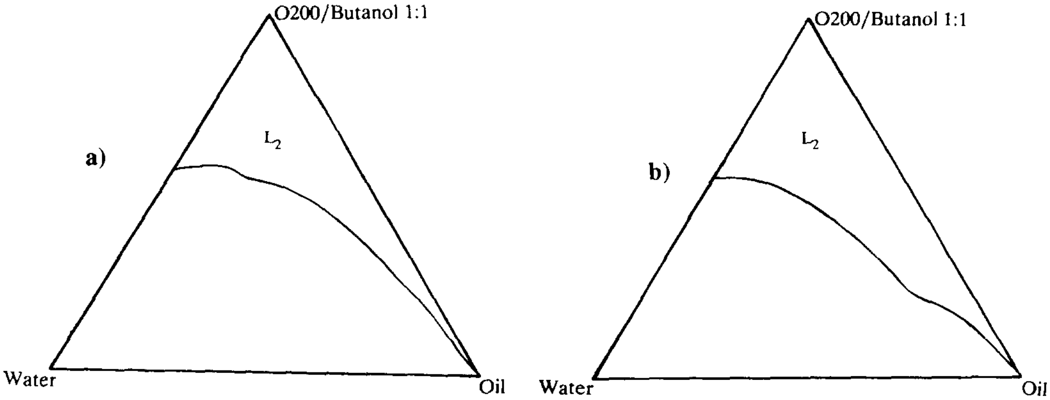

Fig. 1. Phase diagrams at room temperature (21°C) of the quaternary systems containing oil/water/O200/butanol and a K_m of 1:1; (a) oleic acid, (b) ethyl oleate.

all possible ratios of surfactant and oil. In addition, all oils gave rise to systems that exhibited an LC region. Furthermore, with the exception of the systems containing MIG and SBO, all other systems yielded an L_1 region. The differences between the extents of the various regions seen with the different oils were more marked at this K_m. For example, EOL produced a system which showed a slightly more extensive LC region than that prepared with OLA, EOC and OCA. In each of these cases, however, the LC region produced covered a reasonably wide range of water and surfactant concentrations, although the LC seen with the system containing OCA was very narrow, incorporating only a very limited amount of oil. In addition, the L_1 area observed with system containing OCA was also very much smaller than that seen when using the other non-triglyceride oils. Furthermore, there was a trend in the position of the L_1 region (see also propanol at both K_m) in that the alkanoic acid containing systems incorporated slightly less oil than those exhibited by their corresponding ethyl ester.

The phase diagrams produced by the SBO and

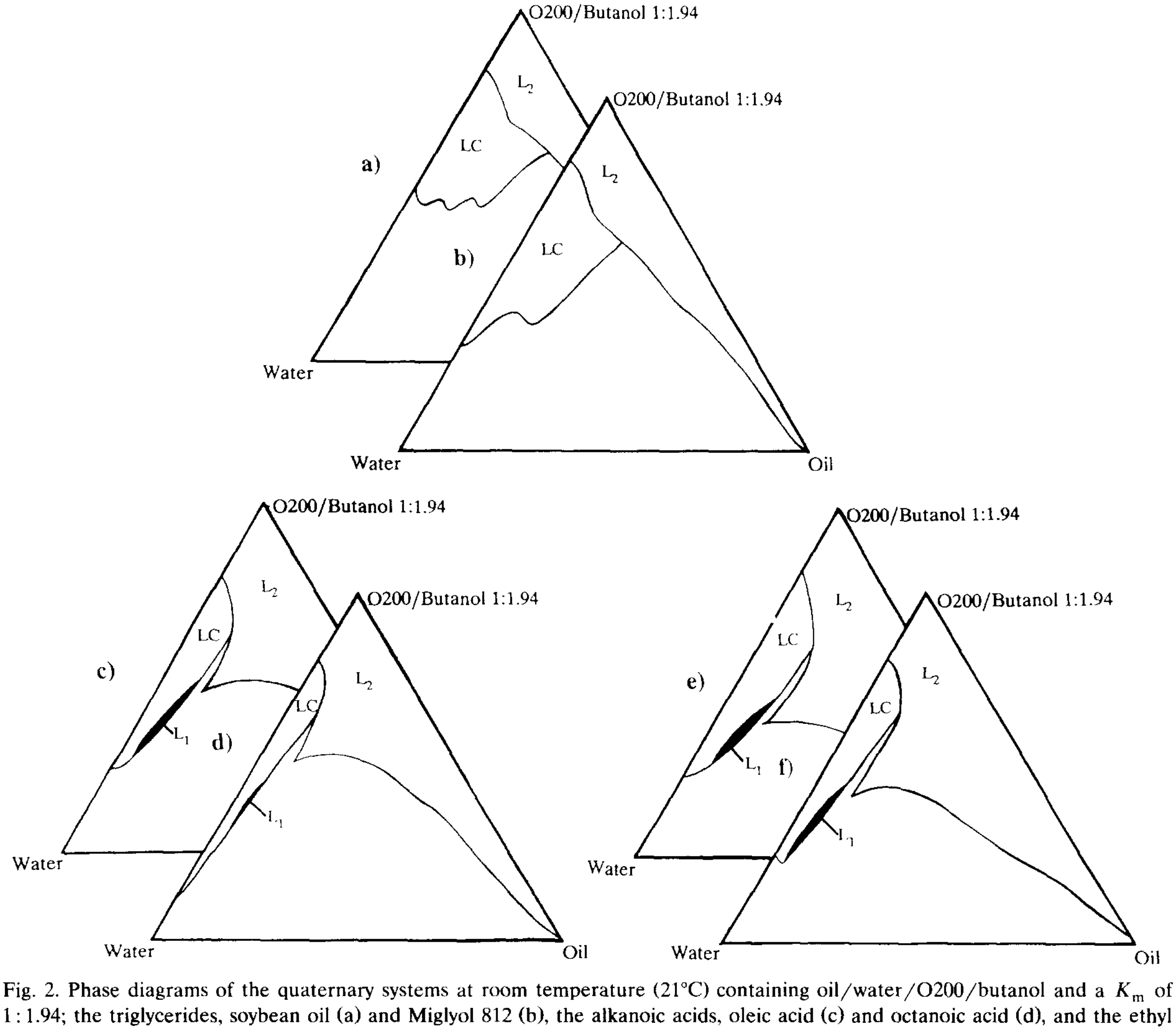

Fig. 2. Phase diagrams of the quaternary systems at room temperature (21°C) containing oil/water/O200/butanol and a K_m of 1 : 1.94; the triglycerides, soybean oil (a) and Miglyol 812 (b), the alkanoic acids, oleic acid (c) and octanoic acid (d), and the ethyl esters of alkanoic acids, ethyl oleate (e) and ethyl octanoate (f).

MIG containing systems were very different from those produced by the other oils. The L_2 regions produced by MIG and SBO were very narrow and could only take up a maximum of about 20 wt% water, compared with a maximum of about 45 wt% achieved when using EOC as the oil. In contrast the LC region produced by the systems containing SBO and MIG covered a wider range of oil concentrations than seen with the non-triglyceride oils, although they did not extend to as high a water concentration.

3.3. Propanol-K_m 1 : 1

Fig. 3 shows the phase diagrams obtained with propanol at this K_m. As before all oils produced an L_2 region covering all the range of possible surfactant-oil combinations. Systems containing EOL, OLA and EOC also exhibited an LC region together with an L_1 area. In these cases the LC and L_1 regions produced were very small. Unusually the system incorporating OCA produced only L_2 and L_1 regions. The L_1 region produced by this system could incorporate an extremely limited range of oil concentrations. As with butanol at a K_m of 1.94 : 1 there was evidence of a trend in the position of the L_1 region, in that the systems produced by the alkanoic acids incorporated slightly less oil than those exhibited by their corresponding ethyl ester. The mixtures containing MIG produced an extensive L_2 region to-

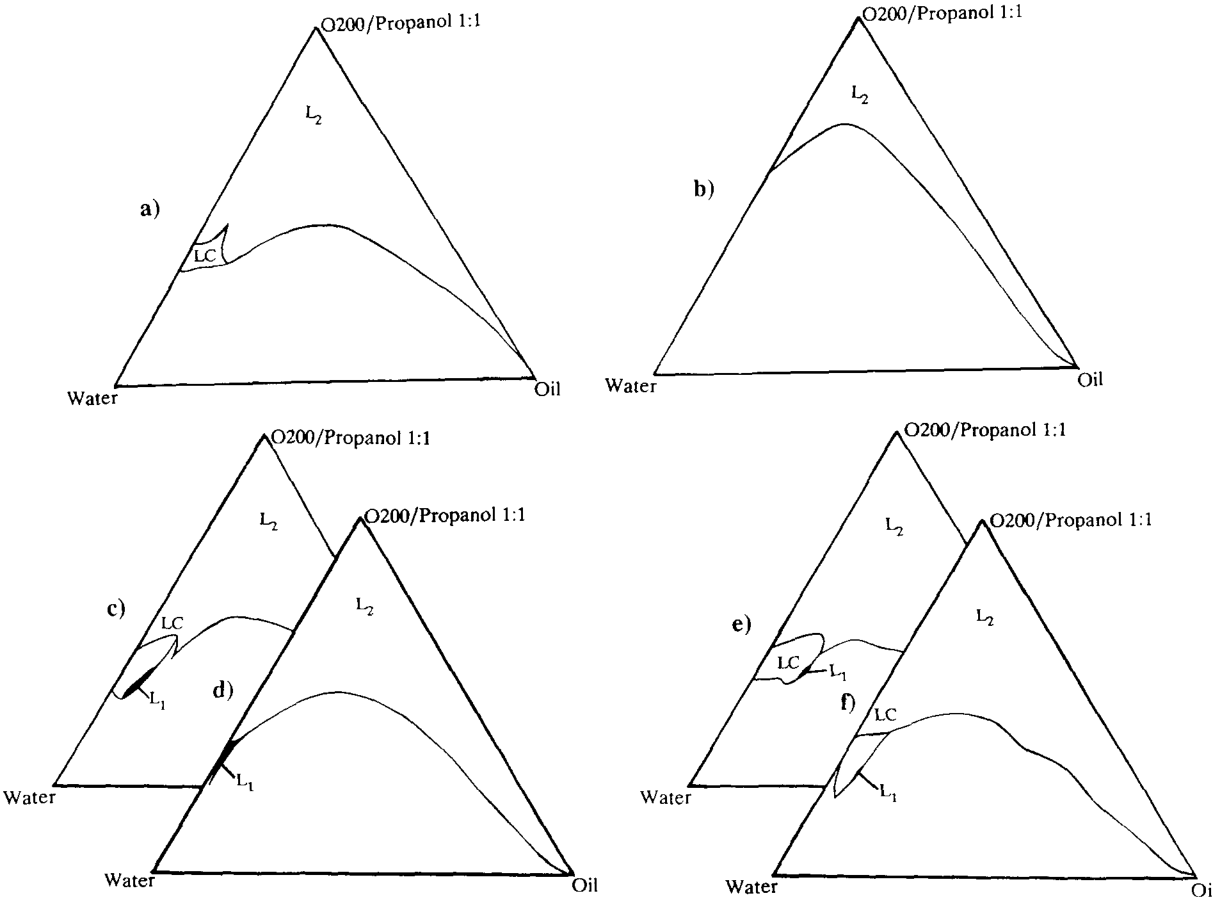

Fig. 3. Phase diagrams at room temperature (21°C) of the quaternary systems containing oil/water/O200/propanol and a K_m of 1 : 1; the triglycerides, soybean oil (a) and Miglyol 812 (b), the alkanoic acids, oleic acid (c) and octanoic acid (d), and the ethyl esters of alkanoic acids, ethyl oleate (e) and ethyl octanoate (f).

gether with a small LC area, while those incorporating SBO exhibited only a small L_2 region.

3.4. Propanol-K_m 1.94 : 1

These phase digrams obtained for these systems are given in Fig. 4. Again all the systems tested exhibited an L_2 region, the extent of which was dependent upon the oil studied. The systems containing the oils EOL, OLA and EOC also gave rise to phase diagrams containing both an LC and L_1 region. In contrast however the system containing OCA produced only an LC region. The extent of the LC was greatest in the system containing EOL and smallest in that containing OAC. With these systems the same trend was observed in the position of the L_1 region as seen with propanol at a K_m of 1 : 1 and butanol at a K_m of 1.94 : 1.

Neither of the triglyceride oils gave rise to phase diagrams that exhibited L_1 regions. MIG produced a narrow L_2 region exhibiting a maxim-

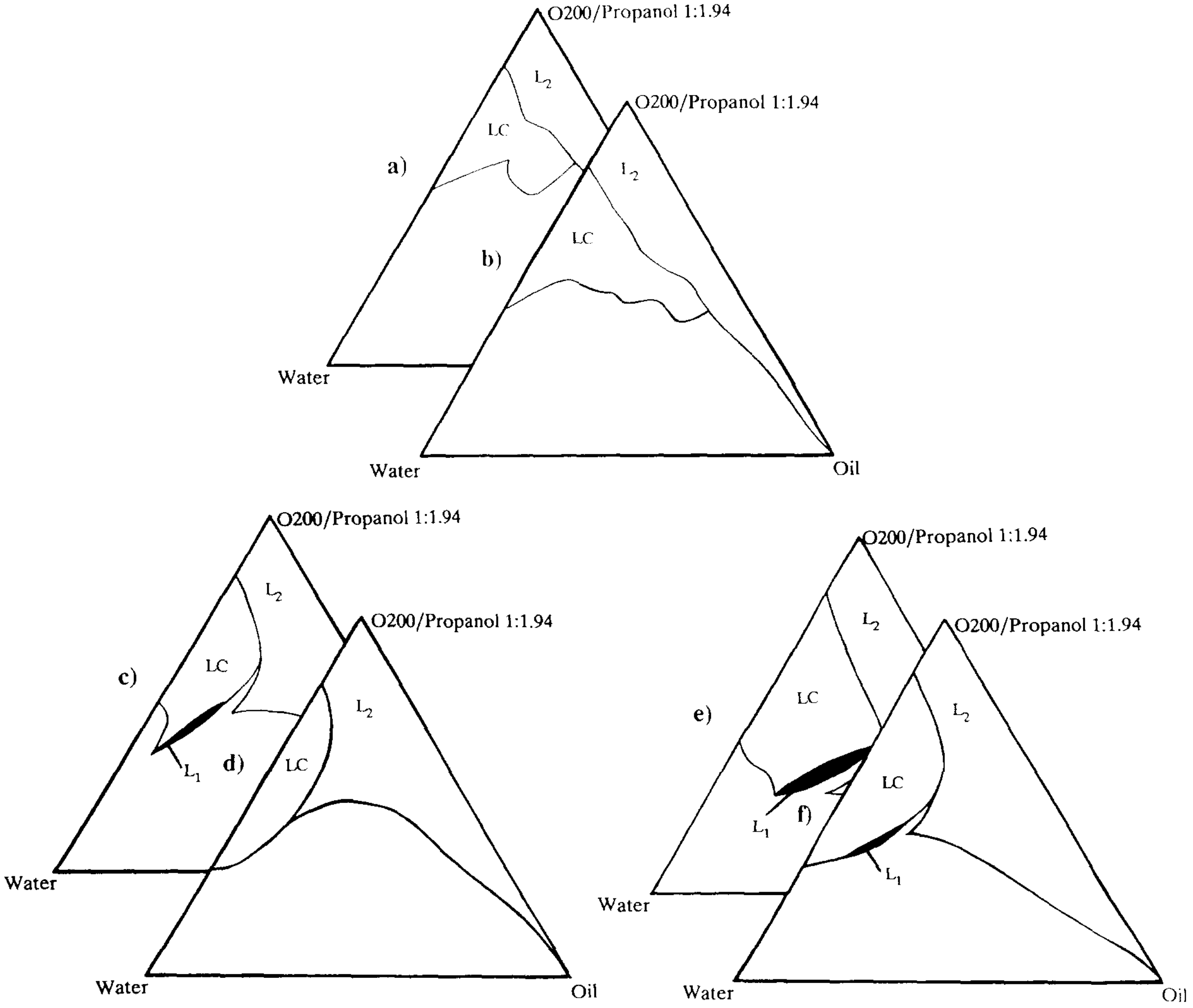

Fig. 4. Phase diagrams at room temperature (21°C) of the quaternary systems containing oil/water/O200/propanol and a K_m of 1 : 1.94; the triglycerides, soybean oil (a) and Miglyol 812 (b), the alkanoic acids, oleic acid (c) and octanoic acid (d), and the ethyl esters of alkanoic acids, ethyl oleate (e) and ethyl octanoate (f).

ium water uptake of about 20 wt%, together with a fairly extensive LC area. Although the SBO containing system produced a very similar L_2 region to that observed with MIG it exhibited a very much smaller LC region. Interestingly there was considerable similarity between the phase diagrams produced by both triglyceride oils at a K_m of 1 : 1.94 (see Fig. 2 and 3). The main difference is the extent of the LC region in both cases; in the systems containing propanol the LC regions extend to higher oil concentrations, while in the butanol containing systems the LC areas tended towards higher water concentrations.

3.5. Relative polarity of the oils

As oil polarity increases (or hydrophobicity decreases), a $\underline{2}$, $-\!\!\!\!2$ (or 3), $\bar{2}$ phase progression results; similarly as j of C_4E_j decreases the same phase progression is seen (Kahlweit et al., 1983). The rank order of oil polarity was established by constructing a grid of phase behaviour as a function of cosurfactant and oil type with all the $\bar{2}$, $\underline{2}$ or $-\!\!\!\!2$ (3) transitions properly ordered. The most polar oils are placed at the top of the grid. It is clear from the grid that the triglycerides oils were the least polar of the oils investigated, while the alkanoic acids were the most polar. In spite of the fact that OCA and OLA exhibited the same progression of phase behaviour, it is reasonable to assume that, due to the fact that it has the shortest hydrocarbon chain, OCA was the most polar oil. A more accurate ranking of polarity would have been possible if the cosurfactant series could have been increased to j values greater than 3. Unfortunately, this was not possible as these compounds are not readily commercially available.

	COSURFACTANT			
OIL	C_4OH	C_4E_1	C_4E_2	C_4E_3
OCA	$\bar{2}$	$\bar{2}$	$\bar{2}$	$\bar{2}$
OLA	$\bar{2}$	$\bar{2}$	$\bar{2}$	$\bar{2}$
EOA	$\bar{2}$	$\bar{2}$	$\bar{2}$	$\underline{2}$
EOL	$\bar{2}$	$\bar{2}$	$\underline{2}$	$\underline{2}$
MIG	$\bar{2}$	$\underline{2}$	$\underline{2}$	$\underline{2}$
SBO	3	$\underline{2}$	$\underline{2}$	$\underline{2}$

Fig. 5. Ranking of oil polarity from the partitioning of 25 wt% cosurfactant in equal masses of oil and water (T, room temperature; 21°C). Octanoic acid (OCA) is the most polar oil examined.

3.6. Mutual solubility

In all instances the solubility of the oil in water and vice versa was less than a few percent. This result meant that, inspite of the oils being relatively polar, the observed phase behaviour was not complicated by one or both of the phase exhibiting a high solubility in the other.

4. Discussion

In order to exploit lecithin microemulsions for drug delivery purposes, it is beneficial to produce balanced microemulsions, ie microemulsions that form over a wide range of oil and water concentrations. The production of balanced microemulsions has several important pharmaceutical advantages. For example, balanced microemulsions would not be expected to break up after the large dilution that frequently occurs following administration. In addition it should be possible to store such microemulsions as a concentrated form that can be diluted just prior to administration. Previous studies have shown that the nature of the cosurfactant used is critical in determining whether a balanced microemulsion will form when using lecithin as the primary surfactant (Aboofazeli et al., 1994). However, it is also well known that the nature of the oil can influence the production of microemulsions, with microemulsion formation tending to be favoured when small oils are present. Unfortunately pharmaceutically acceptable oils tend to be large and semi-polar in nature. This, together with the fact that the nature (and concentration) of the oil is important in determining the drug loading capacity of any microemulsion (Malcolmson, 1992) makes it essential to examine the effect of the oil on microemulsion formation. In the present study therefore the influence of the size and polarity of the oil on the production of lecithin-based microemulsions was studied.

It must be remembered that lecithin is slightly too lipophilic to form balanced microemulsions when used as the only surfactant, tending rather to favour the formation of reverse microemulsions over a limited concentration range (Schurtenberger et al., 1993). This is because lecithin has a fairly high critical packing parameter (CPP) of around 0.8 (Cornell et al., 1986). If lecithin containing systems are to form microemulsions over a reasonably wide range of oil and water concentrations, the effective CPP of lecithin needs to be reduced. One way this can be achieved is by the addition of a short chain cosurfactant. A short chain cosurfactant can act to reduce the effective CPP in two ways, firstly by its incorporation into the interfacial film and/or secondly by dissolving in the aqueous phase thereby making it less hydrophilic. In addition, in the case of a long hydrophobic chain surfactant like lecithin, a short chain cosurfactant can also act to increase the fluidity of the interfacial surfactant layer, thereby reducing the tendency of lecithin to form highly rigid films (Binks et al., 1989) and allowing the interfacial film sufficient flexibility to take up the different curvatures required to form balanced microemulsions (De Gennes and Taupin, 1982).

With respect to the phase behaviour observed in the present study some general comments can be made. These are: that none of the oils investigated formed balanced lecithin-based microemulsions, possibly because of the particular cosurfactants used in this study (Aboofazeli et al., 1994). All systems, regardless of the K_m being investigated, were however capable of producing L_2 regions. With few exceptions, for example when using butanol at a K_m of 1:1, most systems also exhibited an LC region. In contrast relatively few systems were capable producing an L_1 region.

The extent and position of the various regions observed was dependent largely upon either the alcohol and the K_m, or the nature of the oil being incorporated. For example when using butanol at a K_m of 1:1, the predominate factor influencing the phase diagram appears to be the alcohol as there are only very slight differences observed between the phase diagrams produced by the different oils. In contrast, marked differences in phase behaviour were observed between the systems with the various oils when using propanol at the same K_m. This observation can be explained by the nature of the alcohol cosurfactant present. It has been shown in a previous study (Aboofazeli et al., 1994) that cosurfactants acting predominately in the aqueous phase are generally more effective at producing microemulsions over a wider range of compositions than those mainly acting in the interfacial film region. Propanol, as a result of its high water solubility, predominately exerts its influence by reducing the hydrophilic nature of the aqueous phase, thereby favouring the formation of an extensive clear area (although designated L_2 for the purposes of the present study, it should be remembered that more than one type of phase structure maybe present in this region). The only exception to this observation was when SBO was used as the oil. Here the very large size of the SBO molecule, the small amount of cosurfactant in the interfacial region and the subsequent low flexibility of the interfacial film this affords means that the SBO is not as readily able to penetrate the hydrophobic chains of the lecithin molecules, a necessary pre-requisite for the formation of a microemulsion. The low amount of cosurfactant present in the interfacial film also accounts for the differences observed between the phase diagrams obtained with the various systems at high water content. In contrast to propanol, butanol resides mainly in the interfacial film region, influencing the effective CPP of lecithin in this manner. In the butanol containing systems, the higher levels of alcohol present in the interfacial film are less effective at reducing the CPP, thereby reducing the ability of these systems to form microemulsions over such a large range of compositions compared to those containing propanol.

In the systems containing the higher levels of lecithin the differences between the observed phase behaviour when using the two alcohols is much less than at the lower K_m when the alcohol present seemed to dominate phase behaviour. Interestingly, at this level of lecithin, the phase behaviour was heavily influenced by the oil used, in that the oils could be divided into two groups, namely the alkanoic acids and their ethyl esters and the triglyceride oils. Inspite of the triglycerides being the most nonpolar of the oils, the differences between the two classes of oil are probably a consequence of their size rather than their polarity, especially as the differences in the phase behaviour seen between the alkanoic acids and their corresponding esters were quite small. It can be seen that the systems containing the alkanoic acids and their ethyl esters produced a L_1, L_2 and an LC region, the only exception being the system containing OCA and propanol, in this instance no L_1 area was exhibited. In contrast the triglycerides generally produced a fairly narrow L_2 region together with a fairly extensive LC area. This observation was not unexpected since it is well known that large oils like the triglycerides used in the present study are too large to easily penetrate the hydrophobic chains of the surfactant (Alander and Warnheim, 1989) and as a consequence tend to promote the formation of LC lamellar phases as opposed to a L_2 thereby reducing the extent of this latter region.

Furthermore it was not suprising that the systems containing the other oils, at the lower K_m, allowed the formation of a L_1 area, as these oils were relatively small compared to the length of the lecithin hydrophobe and it is well recognised that the formation of an L_1 is very sensitive to the size of the oil being incorporated (Malcolmson and Lawrence, 1995): it is generally considered that oils do not readily form o/w microemulsions unless the hydrophobic chain(s) of the surfactant stabilizing the system are longer than those of the oil being incorporated.

Although the alkanoic acids and their esters exhibited similar phase diagrams, the position of the L_1 and LC regions appeared to be slightly influenced by the oil being studied, with the smaller oils, OCA and EOC tending to favour the formation of L_1 and LC regions at lower oil concentrations than their longer chain counterparts, implying possibly that size rather than polarity was more important in determining phase behaviour.

Overall, although differences in phase behaviour were seen when using different oils, these differences were generally small, except when using the large triglycerides as oils. Indeed the influence on the phase behaviour of the various oils is comparable with that seen when comparing grades of lecithin (Aboofazeli and Lawrence, 1994). Interestingly the phase diagrams obtained using ethyl oleate as oil are virtually identical to those previously seen when using the closely related oil, isopropyl myristate (Aboofazeli and Lawrence, 1993). It appears that the size, rather than the polarity of the oils used in this study is the major factor influencing phase behaviour, with the larger microemulsion regions seen when the smaller oils were used. Indeed, it is well known that up to a limit reducing the chain length of the incorporated oil tends to favour microemulsion formation. This observation suggests that wherever possible relatively small oils are preferred from a formulation point of view. However, care needs to be taken when using small oils as these may significantly penetrate the surfactant molecules of the interfacial region (Malcolmson, 1992) thereby acting more as a cosurfactant thereby possibly altering an important locus of drug solubilization, namely the polar head group region (Attwood and Florence, 1983). In this context it is important to note that while OCA has not been previously used as a cosurfactant in lecithin-based microemulsions, the slightly shorter chain hexanoic acid has (see, for example, Gasco et al. (1990) and Aboofazeli et al. (1994)). However, as hexanoic acid was not very efficient as a cosurfactant, it would seem very unlikely that the larger, more hydrophobic OCA would act as a cosurfactant.

It can be concluded from this and our previous studies that the major factor influencing the phase behaviour of lecithin-based microemulsions is the nature and mixing ratio of the cosurfactant used. The results suggest that generally hydrophilic cosurfactants are best from the point of producing balanced microemulsions. Indeed, recent work by Saint Ruth (1994) has shown that it is possible to produce a large clear isotropic region extending over a large range of oil and water compositions, that is a balanced microemulsion, when using very high amounts of ethanol (in the range 60–80%w/w of the aqueous phase) as cosurfactant in a lecithin-based system. Here the ethanol cosurfactant is undoubtably acting wholely in the aqueous phase. A potential problem with using water-soluble cosurfactants is the danger of their being diluted below an effective level after administration of the microemulsion into the body. This none dilutability of the microemulsion may cause obvious problems such as the dose dumping of any incorporated drug. It may be therefore that the range of concentrations over which a microemulsion is formed has to be compromised in order to produce a microemulsion that is infinitely dilutable under its expected conditions of use.

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