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# Investigation of the phase behaviour of systems containing lecithin and 2-acyl lysolecithin derivatives

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## Abstract

A series of modified phospholipids (m-PC) possessing different acyl chains in position 2, from butanoyl to hexadecanoyl, were prepared by partial synthesis from soybean lysolecithin. They were used with soybean lecithin to construct phase diagrams containing ethanol as cosolvent, water and medium chain triglycerides (MCT) or isopropyl myristate (IPM) as oils. The weight ratios lecithin:m-PC and surfactants:ethanol were kept constant at 1:1.The results indicate that the m-PCs have a strong effect on the microemulsion (L) and liquid crystalline (LC) domains in the water-rich/oil-poor part of the phase diagrams, although all diagrams correspond to a single lecithin:m-PC ratio. On decreasing the acyl chain length, and thus increasing the hydrophilicity of the surfactant, there was a corresponding increase in the L area, which moved towards the aqueous corner of the phase diagrams. The LC phase was detected only in the presence of the hexadecanoyl derivative for the systems containing MCT, and it was not detected only in the presence of the butanoyl derivative for the systems containing IPM. The use of a second hydrophilic surfactant to adjust the packing properties of the lecithin–alcohol systems, and/or to increase the fluidity of the surfactant film, increased the region of existence of the isotropic systems. This may be of importance in the formulation of drug delivery systems, especially those which are diluted by biological fluids upon administration. © 1999 Elsevier Science B.V. All rights reserved.

*Keywords:* Phospholipids; Isotropic systems; Microemulsions; Phase diagrams

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## 1. Introduction

The term ‘microemulsion’ has been widely used to describe complex systems consisting of oil, surfactant and water, which are a singly optically isotropic and thermodynamically stable liquid solution (Danielsson and Lindman, 1981). Microemulsions are currently the subject of many investigations because of their wide range of potential and actual utilization (Zana, 1994). Most of the investigations performed on microemulsions deal with phase diagrams studies. The phase diagrams of several water–surfactant–oil mixtures exhibit a complex variety of phases and also complex multiphase regions (Rosano and Clausse, 1987). In the singly isotropic region, the full determination of the phase boundaries requires addi-

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tional experimental measurements, because of the continuity among pure micellar solutions, swollen micelles and microemulsions. In most cases, it is not necessary to draw quantitatively the phase diagram. A schematic representation is often sufficient and serves as a guide to follow the evolution of phase equilibria. In practice, systems containing water, oil and surfactant and optically clear to the eye are identified as 'microemulsions'. One of the essential consequence of the simplification of the appearance of the diagrams is to make easier the understanding of the complicated evolution of phase equilibria occurring in multicomponent systems.

To increase the domain of existence of the microemulsion region, a fourth component, which acts as cosolvent for both water and oil, is usually added. There is an extensive literature on the formation of microemulsions using alcohols, glycols, alkanoic acids (which can help to solubilize large quantities of oil) and water (Rosano and Clausse, 1987). Generally, hydrophilic cosolvents are the best to produce a large optically clear isotropic region, but a problem with using water-soluble cosolvents is the destruction of the microemulsion phase on dilution of the cosolvent to below effective levels, for instance after administration into the body.

Microemulsions often have high solvent capacities for drugs (von Corswant et al., 1998), making them attractive formulations for pharmaceuticals, and they also offer several benefits for oral administration, including increased absorption, improved clinical potency and decreased toxicity (Bryan and Samuel, 1989; Ritschel, 1991). The non-dilutability of the microemulsion may cause obvious problems such as 'dose-dumping' of any incorporated drug (Pouton, 1997), leading to uneven absorption.

Recently, much dosage-form development activity has focused on the formulation of lecithin-based microemulsions, and some recent studies (Attwood et al., 1992; Gallarate et al., 1993; Kovarik et al., 1994; Aboofazeli et al., 1995) have shown that microemulsions containing pharmaceutically acceptable oils as vehicles for sparingly soluble substances can be formulated using lecithin and a cosolvent. Lecithin is a naturally occurring, non-toxic and safe material but, when used as sole surfactant, it is not capable of producing isotropic solutions of water and oil over a wide range of compositions.

In previous studies, we obtained a drastic increase of the region of existence of microemulsion systems containing lecithin, using a second hydrophilic amphiphilic molecule, such as lysolecithin (Trotta et al., 1996). The extension of the microemulsion phase was found to be closely dependent upon the nature of the cosolvent. Microemulsions covering most of the possible oil, water and surfactant compositions were obtained with short chain alcohols, and the transition from microemulsion to mixed micelles gave these systems dilutability.

In this study, a series of modified phospholipids, obtained by acylation of the soybean lysolecithin in position 2 were synthetized and the possibility of using them to increase the region of existence of lecithin-based microemulsions containing pharmaceutically acceptable oils and ethanol as cosolvent was explored.

## 2. Materials and methods

### 2.1. Materials

Soybean lecithin (phosphatidylcholine > 95%, Epikuron® 200) and hydrogenated lecithin (Epikuron® H100) were obtained from Lucas Meyer (Hamburg, D) and used without further purification. Isopropyl myristate (IPM) and ethanol were from Aldrich Chemical. Co. (Dorset, UK). MCT Myritol 318®, a mixture of caprylic and capric triglycerides, was from Henkel (Dusseldorf, D). The modified phospholipids (m-PC) were prepared by partial synthesis from soybean lecithin (Epikuron® 200) as starting material. The fatty acid was split off in position 2 by enzyme phospholipase A2 (Lecitase, Novo Nordisk) and the resulting lysolecithin was converted to the 2-acyl derivative following the method described by Gupta et al. (1977). Butanoyl (m-PC4), hexanoyl (m-PC6), octanoyl (m-PC8), decanoyl (m-PC10) and hexadecanoyl (m-PC16) derivatives were synthetized. The iodine number was about 50 for the lysolecithin and decreased by from 25 to 12 on increasing the chain length of the 2 acyl lysolecithin derivative.

All other chemicals were reagent grade and were used as received. Water was freshly bidistilled.

### *2.2. Pseudo-ternary phase diagrams*

Phase diagrams were constructed by titrating a series of m-PC:lecithin/alcohol/oil mixtures with water at 25°C. MCT and IPM were used as oil phase. The regions of existence of the microemulsion systems (L) were determined at constant m-PC:lecithin (surfactant mixture) weight ratio and surfactant mixture:alcohol weight ratio ($K_m$) of 1:1. As reference standards, phase diagrams were constructed using lecithin alone or using a 1:1 lecithin:hydrogenated lecithin mixture.

Appropriate amounts of surfactant mixture, alcohol and oil were weighed into glass ampoules. Samples were shaken for sufficient time to attain equilibrium and then progressively enriched with water, added drop by drop, and the amounts of added water at which transition occurred were used to determine the phase domains. The phase domains were first determined by observation for transparency, and the liquid crystalline structures were determined using cross polarizers.

No attempt was made to distinguish between, micelles, swollen micelles, oil-in-water microemulsions, water-in-oil microemulsions, or bicontinuous type microemulsions (L phase), or to characterize the different liquid crystalline phases (LC phase). The final determinations of phase equilibria were made on selected samples inside and outside the preliminary phase limits, using long term storage at 25°C to ensure a state close to equilibrium.

By repeating this experimental procedure for other combinations of surfactant mixture to alcohol to oil weight ratio, the pseudo ternary phase diagrams were constructed.

## 3. Results and discussion

Lecithin is not capable of producing isotropic solutions of oil in water over a wide range of compositions because it is too lipophilic. The critical packing parameter, which is the ratio between hydrocarbon volume, optimum head group area and tail length (Israelachvili et al., 1976) is rather too high to form water-rich isotropic phase (o/w microemulsions) and when used as the only surfactant, lecithin tends to favour the formation of oil-rich isotropic phase (w/o microemulsions) and liquid crystal phase over a limited concentration range (Cornell et al., 1986).

To produce o/w microemulsions it is necessary to reduce this parameter by using a cosolvent, such as a short-chain alcohol. This alters the packing parameter either by making the aqueous phase less hydrophilic and/or by incorporating it into the interfacial film (Shinoda et al., 1991). The cosolvent can also act to increase the fluidity of the surfactant film, thus giving the interfacial film sufficient flexibility to take up the different curvatures required to form microemulsions (Binks et al., 1989).

In order to formulate lecithin-based microemulsions for drug delivery purposes, it is necessary to produce isotropic systems that form over a wide range of oil and water contents. This means that the domain of existence of these systems can incline toward the aqueous corner in the isothermal pseudo-ternary phase diagram, allowing considerable dilution with water or with biological fluids, as occurs after administration, without destroying the microemulsion phase in favour of a turbid phase, as frequently occurs.

A possible way of obtaining lecithin-based microemulsions that are stable at high water content could be the partial replacement of lecithin by a more hydrophilic amphiphile. The modified phospholipids used in these experiments, especially those with short- to medium-chain, are more hydrophilic than lecithin, having the same large hydrophilic head and a relatively smaller hydrocarbon volume. They are not chemically homogeneous, but are mixtures obtained by acylation in position 2 from soybean lecithin previously treated with phospholipase A2; the influence of the acyl-chain structure in position 1 could therefore not be elucidated.

Figs. 1–4 report the pseudo ternary phase diagrams of systems containing oil/water/ethanol/

lecithin or a mixture of lecithin with modified phospholipids (m-PC) in a weight ratio of 1:1, together with the diagrams obtained using a 1:1 mixture of hydrogenated lecithin and lecithin.

All systems, regardless of the surfactant used, were capable of producing an isotropic phase. The extension of this phase and the presence and extension of the LC phase were found to be dependent both on the surfactant mixture and on the oil used. A comparison between the phase diagrams shows that the trend of the changes induced by the presence of m-PCs is that the

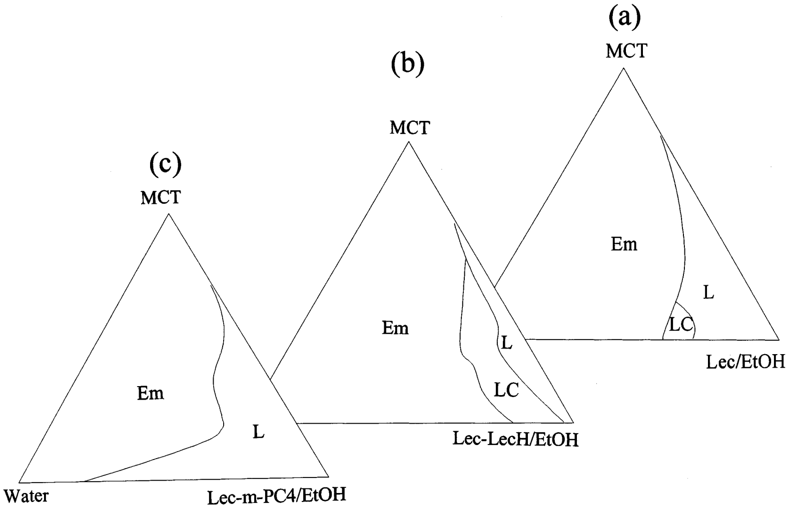

Fig. 1. Phase diagrams of systems containing (a) water–MCT–lecithin–ethanol, (b) water–MCT–lecithin/hydrogenated lecithin–ethanol, (c) water–MCT–lecithin/m-PC4–ethanol. $K_m = 1:1$. Weight ratio of lecithin/hydrogenated lecithin and lecithin/m-PC4 = 1:1.

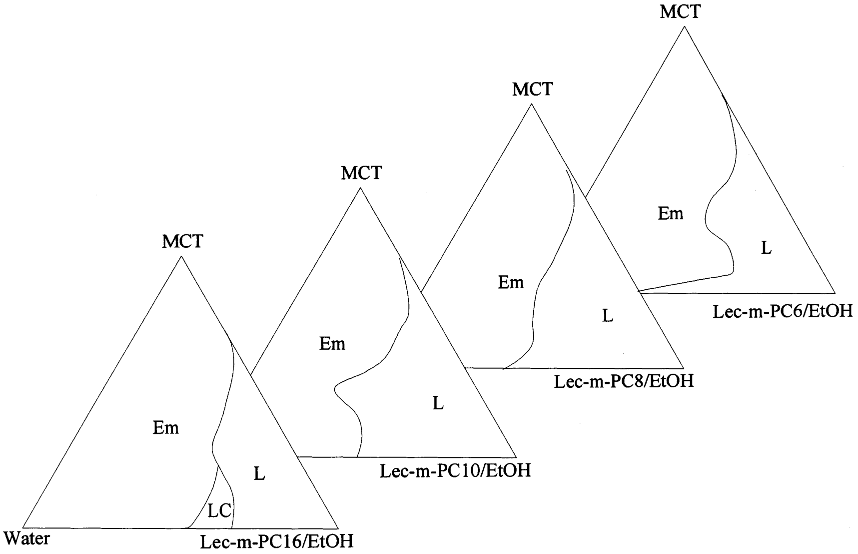

Fig. 2. Phase diagrams of systems containing water–MCT–lecithin/m-PC–ethanol at $K_m$ of 1:1 and lecithin/m-PC weight ratio of 1:1.

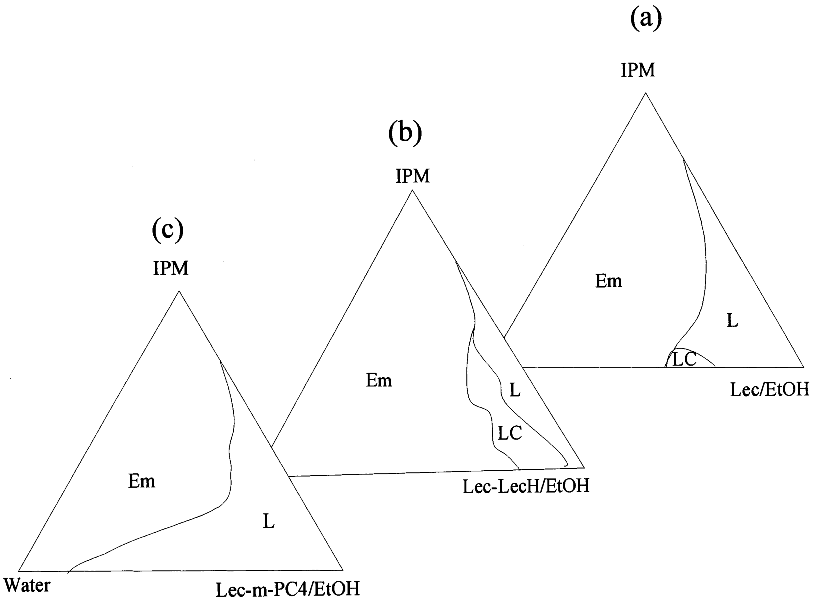

Fig. 3. Phase diagrams of systems containing (a) water–IPM–lecithin–ethanol, (b) water–IPM–lecithin/hydrogenated lecithin–ethanol, (c) water–IPM–lecithin/m-PC4–ethanol. $K_m = 1:1$. Weight ratio of lecithin/hydrogenated lecithin and lecithin/m-PC4 = 1:1.

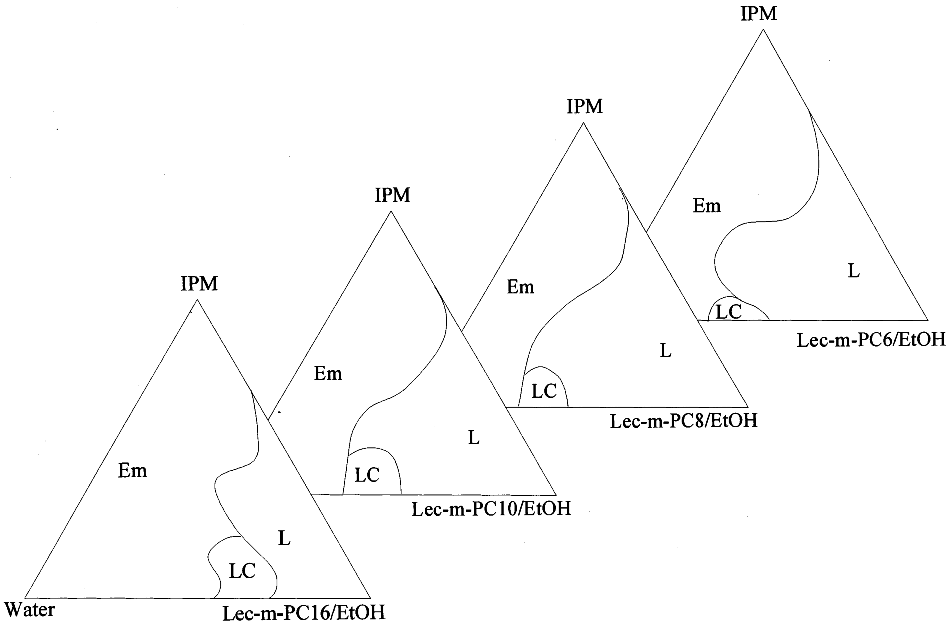

Fig. 4. Phase diagrams of systems containing water–IPM–lecithin/m-PC–ethanol at $K_m$ of 1:1 and lecithin/m-PC weight ratio of 1:1.

isotropic realm shifts to the aqueous corner of the diagrams as the acyl chain of the m-PC decreases, that is, as the hydrophilicity of the m-PC increases.

Although all the diagrams correspond to only one Km ratio, these results indicate that the m-PCs have a big effect on the isotropic phase in the water-rich part of the phase diagrams. Even if no attempt was made to recognise the structure in different parts of the isotropic realm, it may be predicted that short acyl derivatives have a big effect, increasing the realm of existence of o/w microemulsions.

In the presence of MCT, an optically clear region was noted along the surfactant–oil axis. This region moved toward the water axis in the oil-poor part of the diagram for the systems containing short m-PC derivatives (m-PC4 and m-PC6) and toward the middle of the phase diagram for the systems containing medium m-PC (m-PC8 and m-PC10). A LC phase was only observed in the system containing lecithin alone and in the systems containing lecithin and m-PC16 or lecithin and hydrogenated lecithin. It is well known that large oil molecules, like the triglycerides, cannot easily penetrate the hydrophobic chains of the surfactant (Alander and Werenheim, 1989) and as a consequence they tend to promote the formation of LC phases. The difference in the phase diagrams could be related to the different packing parameters of the surfactant at the interface. The presence of an unsaturated chain in the lecithin, and to a lesser extent in the m-PC16, leads to a more appropriate packing parameter compared to the system containing hydrogenated lecithin, thus promoting the formation of the isotropic phase and reducing the extent of the LC phase. In the presence of short- to medium-chain m-PCs no LC phase and different isotropic domains were found. This could be ascribed to the incorporation of m-PC molecules in the interfacial layer, reducing the packing parameter of lecithin due to a decrease in the hydrocarbon volume of these derivatives: the packing parameter could be shifted as appropriate to form o/w microemulsions. Short-chain m-PCs should be more effective to reduce the packing parameter than medium-chain PCs: the extension of the isotropic area toward the aqueous corner was found to increase as the chain length of modified phospholipid decreased.

In the presence of medium-chain m-PCs, an isotropic area that protrudes into the central part of the phase diagrams is found. As the zone corresponding to equal amounts of oil and water is approached, the structure becomes complicated and the experimental results become difficult to analyze. Several theoretical developments have emerged (Larch et al., 1987) and it is generally accepted that in these optically isotropic, light-scattering microemulsion phases, referred to as bicontinuous, the distinct interface is lacking and oil and water merge into each other to form a single domain

For all the systems, because the isotropic region is not connected to the water corner, any dilution with water will induce emulsion formation. This is because ethanol, as a result of its high water solubility, exerts its influence predominanty by reducing the hydrophilic nature of the aqueous phase. It has been shown that cosolvents acting predominantly in the aqueous phase are generally more effective at producing microemulsions over a wide range of compositions than those mainly acting in the interfacial film region (Aboofazeli et al., 1994); but a problem with using water-soluble cosolvents is the destruction of the microemulsion by dilution of the cosolvent below effective levels.

In the presence of IPM, all m-PCs were capable of producing an isotropic phase and the extent and position of this region depended upon the length of the modified phospholipid, as it did in the presence of MCT. In contrast to systems containing MCT, the LC phase was observed for all the m-PCs, the only exception being the system containing the most hydrophilic member, m-PC4. This region, observed along the water-surfactant axis in the oil-poor part of the diagrams, increased in size and progressively shifted toward the alcohol-plus-surfactant side of the diagrams as the chain length of the m-PC increased. Extension was maximum with the hydrogenated lecithin.

The isotropic phase extensions of the systems containing MCT and IPM were quite similar. The small differences might be ascribed to the different influence of the oil on the packing parameter of the surfactant at the interface. It is well known that the phase behaviour is strongly influenced by the size of the oil used, with microemulsion formation tending to be favoured with small oils (Aboofazeli et al., 1995). IPM is small in size compared to MCT, thus an increase of the isotropic area in the water-rich region would be expected; this is because, depending upon the chain length and volume of the oil molecule, its penetration in the hydrocarbon tails of the surfactant will vary, altering the effective hydrocarbon chain volume of the surfactant molecule and thus the effective geometric packing parameter.

As with MCT, in the presence of IPM isotropic systems stable to water dilution could not be obtained, even if there was a drastic increase in the area of existence compared to those containing lecithin alone. The maximum extension of the isotropic area toward the water corner was found with m-PC4, while m-PC6 gave the maximum extension in the central part of the diagram.

In conclusion, the use of a second hydrophilic surfactant to adjust the packing properties of the lecithin–alcohol system, and/or to increase the fluidity of the surfactant film, provided an increase in the domain of existence of the isotropic systems and may be of importance in the formulation of drug delivery systems, especially those which are diluted by biological fluids upon administration.

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