Formation of lecithin-based microemulsions containing *n*-alkanol phosphocholines

M. Trotta *, F. Pattarino, G. Grosa

Dipartimento di Scienza e Tecnologia del Farmaco, via P. Giuria 9, 10125 Torino, Italy

Abstract

The formation of microemulsion phases was studied at 25°C for systems containing soybean phosphatidylcholine (lecithin), medium-chain-triacylglycerol (MCT) or isopropyl myristate (IPM), water, alcohol and *n*-alkanol phosphocholines (Cn), with chain length between 6 and 12 carbon atoms, as second hydrophilic surfactant. The lecithin/Cn mixing ratio was 1:1, while the surfactant/alcohol mixing ratios were 1:1 and 2:1 for the systems containing MCT, and 1:1 for the systems containing IPM. Alcohols used were ethanol, 1-propanol and 1-butanol. The maximum extension of the region of existence of the microemulsion systems containing MCT was found with butanol, while in the presence of IPM it was found with propanol. Except for the system containing IPM and butanol, the size of the microemulsion phase increased on increasing the length of the Cn chain, and moved towards the aqueous corner of the phase diagram. The use of a second hydrophilic amphiphile to adjust the spontaneous packing properties of the lecithin–alcohol system, or to increase the fluidity of the surfactant film, provided an increase in the region of existence of the microemulsion systems. © 1998 Published by Elsevier Science B.V. All rights reserved.

Keywords: Microemulsion; Lecithin; *n*-Alkanol phosphocholines; Phase diagram

1. Introduction

Microemulsions can be defined as a thermodynamically stable, isotropically clear dispersion of two immiscible liquids. The formation of microemulsions usually involves a combination of four components, namely oil, water, surfactant and cosurfactant. Microemulsions containing phosphatidylcholines (lecithin) as surfactant are of interest as potential drug delivery systems. Lecithin is a naturally-occurring, non-toxic and safe material but, when used as sole surfactant, is not capable of producing isotropic solutions of water and oil over a wide range of compositions. The majority of microemulsions require the presence of cosurfactant for their production. Numerous studies (Attwood et al., 1992; Aboofazeli et al.,

1993, 1994) report the use, as cosurfactant, of short chain alcohols, alkanoic acids, amines and glycols to increase the region of existence of lecithin-based microemulsions.

Recently a great deal of dosage-form development activity has focused on the formulation of lecithin-based microemulsions and some recent studies (Gallarate et al., 1993; Kovarik et al., 1994; von Corswant et al., 1998) have shown that microemulsions containing pharmaceutically acceptable oils, as vehicles for sparingly soluble substances, can be formulated by using lecithin and a short chain alcohol.

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 alkylphosphates (Trotta et al., 1995) or lysolecithin (Trotta et al., 1996). The extension of the microemulsion phase was found to be very dependent upon the nature of the cosurfactant; microemulsions covering most of the possible oil, water and surfactant compositions were obtained with short chain alcohols.

In this study, a series of *n*-alkanol phosphocholines, of chain-length between 6 and 12 carbon atoms, were synthesized, and the possibility of using them as a second amphiphilic molecule to increase the region of existence of lecithin-based microemulsions containing pharmaceutically acceptable oils and short chain alcohols was verified.

2. Materials and methods

2.1. Materials

Soybean phosphatidylcholine (Epikuron® 200) was obtained from Lucas Meyer (Hamburg, Germany) and used without further purification. Isopropyl myristate (IPM), ethanol, 1-propanol and 1-butanol were from Aldrich (Dorset, UK). The MCT Myritol 318®, a mixture of caprylic and capric triglycerides, was from Henkel (Dusseldorf, Germany).

n-Alkanol phosphocholines $ROPO_3(CH_2)_2N-(CH_3)_3$ with R = 6 (C6), R = 8 (C8), R = 10 (C10)

and R = 12 (C12) were synthesized and purified as described for *n*-alkanol phosphocholines (R = 12–18) by Nuhn et al. (1982).

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 Cn/lecithin –alcohol–oil mixtures with water at 25°C. MTC Myritol and IPM were used as oil phase.

The regions of existence of the microemulsion systems (L) were determined at the surfactant/alcohol weight ratio (K_m) of 1:1 and 2:1 for systems containing Myritol, and 1:1 for those containing IPM. The Cn/lecithin (surfactant mixture) weight ratio was 1:1.

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 (LC) were determined using cross polarizers.

No attempt was made to distinguish between oil-in-water, water-in-oil, or bicontinuous type microemulsions, or to characterize the different liquid crystalline phases. The final determinations of phase equilibria were made on selected samples inside and outside the preliminary phase limits, using long-term storage at 25° 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 alone is too lipophilic to form microemulsions that cover most of the possible oil, water

and surfactant compositions (Israelachvili et al., 1976). It has a fairly high critical packing parameter around 0.8 (Cornell et al., 1986) and this value is further increased if the oil phase penetrates into the alkyl chains of the lecithin molecule (Evans et al., 1986).

In order to produce microemulsions over a wide range of compositions, it is necessary to reduce this parameter by the use of cosurfactants, generally short chain alcohols (Shinoda et al., 1991). The alcohols act to reduce the packing parameter of the lecithin either by making the aqueous phase less hydrophilic, and/or by incorporating themselves into the interfacial film. In addition, a cosurfactant can also act to increase the fluidity of the surfactant film, thus allowing the interfacial film sufficient flexibility to take up the different curvatures required to form microemulsions. In general an elastic or flexible surfactant film promotes the formation of microemulsion, whereas a lamellar phase is formed with a more rigid film (Binks et al., 1989).

The pseudo-ternary diagrams of the systems containing water–lecithin–Myritol–alcohol at K_m 1:1 and 2:1, together with the pseudo-ternary systems obtained in the presence of Cn, with a lecithin/Cn mixing ratio of 1:1, are reported in Fig. 1 and Fig. 2, respectively.

In the absence of Cn, at a K_m of 1:1, an L phase and a restricted area of existence of LC phase was observed with all the alcohols. These phases slightly increased at a K_m of 2:1. Probably the alcohols used were not capable of reducing the packing parameter and the rigidity of the lecithin film sufficiently for any significant amount of microemulsion to form.

The lipophilicity of lecithin could be further reduced by using a second hydrophilic surfactant. The partial substitution of lecithin by a more hydrophilic amphiphile increases both the hydrophobic volume and the area per head group of the surfactant molecules at the interface, affecting the spontaneous curvature and thus promoting the formation of microemulsion systems.

Alkylphosphocholine derivatives are amphiphilic molecules, with a large hydrophilic head and a relatively small hydrocarbon volume. The presence of these molecules in the systems con-

taining Myritol tended to produce changes such that the microemulsion region became larger and shifted further towards the aqueous corner. The appearance and growth of the microemulsion phase observed in Figs. 1 and 2 can be explained by the fact that the partition at the o/w interface
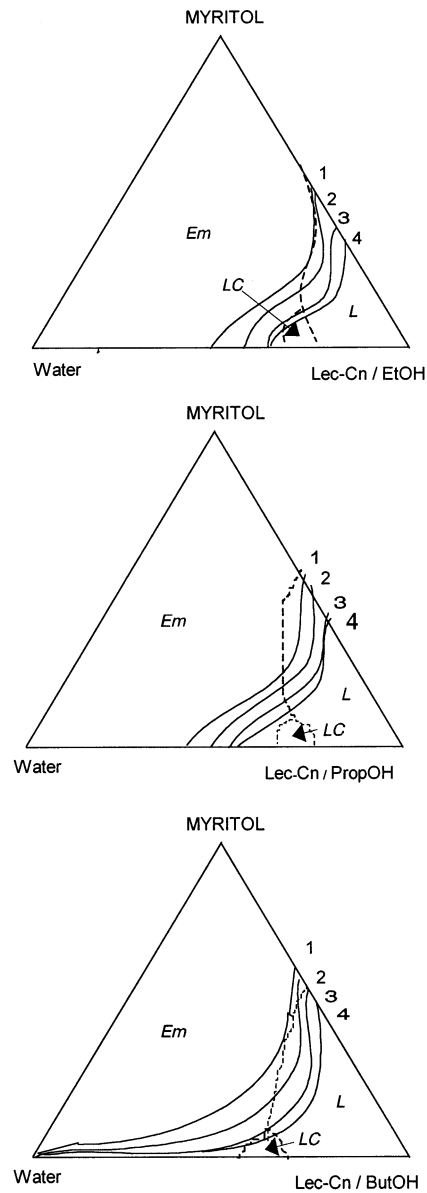

Fig. 1. Phase diagrams of systems containing water–Myritol–lecithin/Cn–alcohol at K_m of 1:1 and lecithin/Cn weight ratio of 1:1. (1) C12, (2) C10, (3) C8, (4) C6, lecithin (- - -).
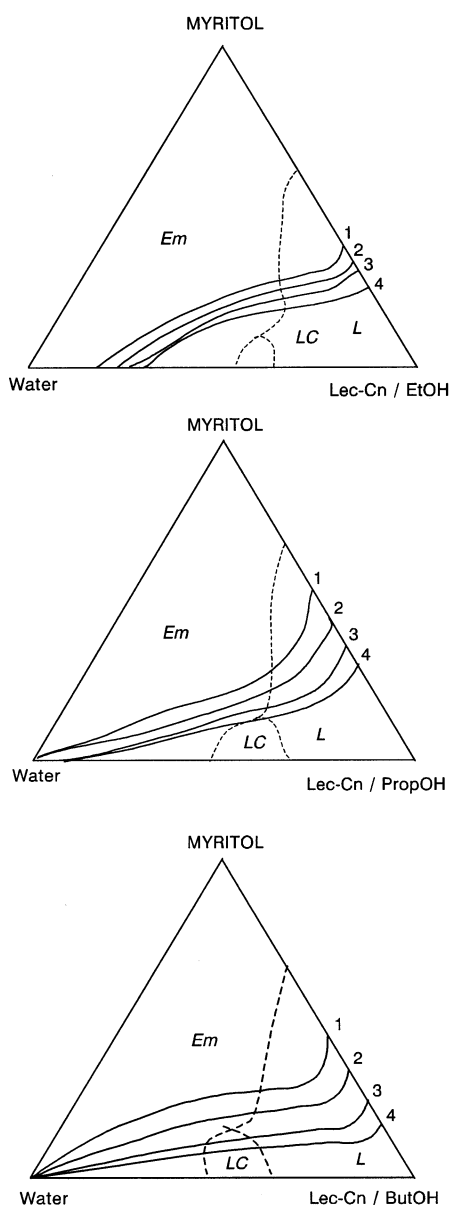
Fig. 2. Phase diagrams of systems containing water–Myritol–lecithin/Cn–alcohol at K_m of 2:1 and lecithin/Cn weight ratio of 1:1. (1) C12, (2) C10, (3) C8, (4) C6, lecithin (- - -).

The extent and position of the L phase was dependent largely upon the chain length of the derivative. Regardless of the K_m and the alcohol being investigated, the extension of the microemulsion region was found to decrease from C12 to C6. It is difficult to explain the differences observed in the extent of the L phase obtained with the different Cn. The differences in hydrocarbon volume should lead to a different location of these molecules: hexyl derivative, which is the most hydrophilic of the surfactants examined should be located in the polar head group region. By increasing the alkyl chain length a higher incorporation in the interfacial region should be favoured, allowing the extension of the L phase.

The L phase was also dependent upon the K_m and alcohol. All systems containing ethanol were not capable of producing a microemulsion phase at high water content. On the contrary the water corner of the diagrams was achieved with all the Cn in the presence of butanol, and with C10 and C12 at a K_m of 2:1 in the presence of propanol. On decreasing the chain length of the alcohol an increase of the alkyl chain of the surfactant, and then of the hydrophobicity, is required to obtain microemulsion at high water content.

In the systems containing the higher levels of surfactants the differences between the observed phase behaviour with the three alcohols are less than at the lower K_m, when the alcohol seemed to dominate the phase behaviour. The differences observed among the alcohols were related to their different partition coefficients among the aqueous phase, the oil phase and the interface (Trotta et al., 1989). The ideal cosurfactant would exert an effect only on the interfacial layer. In this way, it would allow the production of microemulsions at high water–low alcohol content and then the infinite dilution of the microemulsions without destruction due to dilution of the cosurfactant below effective levels.

Butanol is expected to be partitioned mainly in the interfacial layer and the area of existence of microemulsion systems increased significantly because of the better packing of the molecules at the interface. No attempt was made to recognise microemulsion structures at different parts of the microemulsion region but, for systems that

of these molecules will destabilise the LC phase forming a mixed monolayer with lecithin between the water and oil domains, and the flexibility of this mixed film is greater than that of lecithin, because the different structures of the two molecules prevent close packing at the interface.

showed a continuity between the water-poor and the water-rich regions, it may be predicted that the microemulsion structure varies greatly but progressively from water-in-oil to oil-in-water, as the composition varies.

The most hydrophilic alcohols are expected to be mainly in the aqueous phase and partially in the polar part of the surfactant layer. The increase in the area of existence of the microemulsion domain was attributed primarily to a decrease in the polarity of the medium, that led to a decrease in the spontaneous mean curvature of the surfactant film separating the water and oil domains.

When a water-soluble cosurfactant is an important component of the formulation, dilution with water will usually produce a substantial change in phase behaviour. In the presence of ethanol, because the microemulsion region is not connected to the water corner, any dilution of the microemulsion with water will induce emulsion formation. Upon dilution the ethanol concentration in the aqueous phase will decrease, increasing the polarity of the polar medium and then the mean curvature of the surfactant film.

The behaviour of systems containing propanol was intermediate between that of butanol and that of ethanol: the clear regions obtained with propanol were smaller than that achieved with butanol. This result suggests that, due to its high aqueous solubility, propanol resides almost exclusively in the aqueous phase, exerting a lower effect on the packing parameter than butanol does.

Fig. 3 reports the pseudo phase diagrams of the systems at K_m 1:1 obtained by substituting the MCT Myritol with IPM. In the absence of Cn, IPM gave rise to systems that produce isotropic regions especially along the surfactant–oil axis: this region increased by increasing the chain length of the alcohol. Irrespective of the alcohol used, LC phases were not observed.

In the presence of Cn, except for the systems containing butanol, a remarkable increase in the extent of the L phase was obtained. By increasing the chain length of the phosphocholine derivative an increase of the L phase was obtained and the water corner of the diagrams was achieved with all the Cn in the presence of propanol and with C12 in the presence of ethanol. As noted using

Myritol, the second surfactant should have a relative hydrophobicity to gave L phases at high water content.

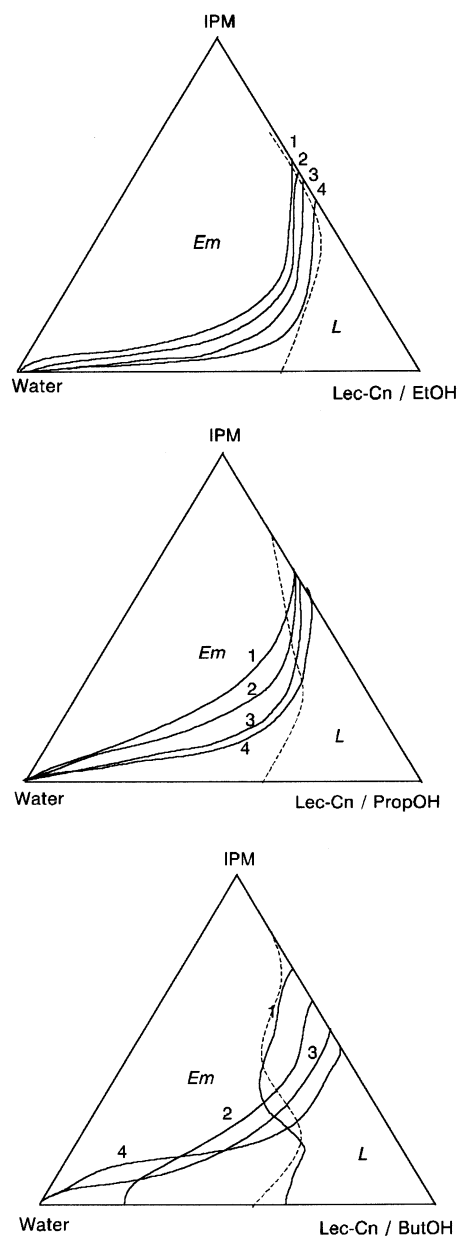In a previous study (Trotta et al., 1996), we have investigated the effect of lysolecithin on the

Fig. 3. Phase diagrams of systems containing water–IPM–lecithin/Cn–alcohol at K_m of 1:1 and lecithin/Cn weight ratio of 1:1. (1) C12, (2) C10, (3) C8, (4) C6, lecithin (- - -).

formation of lecithin microemulsions containing IPM, and L phases at high water content were obtained in the presence of ethanol or propanol. The gradual transition from the oil-droplet-in water continuum to mixed micelles reached the water corner of the diagram. Also with Cn the presence of L phase in the water corner region should be due to the formation of mixed micelles between Cn and lecithin.

In the presence of Cn the regions of microemulsion existence for systems containing ethanol or propanol are larger than those with Myritol at a K_m of 1:1, and quite similar to those obtained with Myritol at a K_m of 2:1: on replacing short chain triglycerides with the alkyl ester, less surfactant was required to dissolve the same amount of oil. The formation of a microemulsion phase is very sensitive to the oil being incorporated and generally triglycerides produce a narrow L phase with an LC area (Alander and Werenheim, 1989). Aboofazeli et al. (1995) compared triglyceride behaviour to that of alkanoic acids and their ethyl esters, and they too found that triglycerides, in particular large molecules such as those found in vegetable oil, are more difficult to dissolve than acids or alkyl esters as a consequence of their size and polarity. It is expected that, depending upon the chain length and volume of the oil molecule, its penetration in the hydrocarbon tails of the surfactant will vary, altering the elasticity of the surfactant film or the effective hydrocarbon chain volume of the surfactant molecule and thus the effective geometric packing parameter.

In marked contrast to the trend seen with ethanol and propanol, in the presence of butanol, the region of existence of microemulsion systems decreased on increasing the chain length of the alkanol derivative, and microemulsions that cover most of the possible oil, water and surfactant compositions were obtained with C6 and C8. C10 and C12 might be too lipophilic to produce water-rich microemulsions in the presence of butanol. The extension and position of the L phase obtained with C12-butanol is similar to that obtained with lecithin-butanol; the one obtained with C6-butanol is similar to that with C12-propanol.

The detailed interpretation of phase behaviour of systems containing lecithin and a second surfactant is complicated and depends upon the delicate balance between the nature of the surfactants, oil and cosurfactant.

In conclusion, the use of *n*-alkanol derivatives, to adjust the spontaneous packing properties of lecithin-alcohol system or to increase the fluidity of the surfactant film, generally provided an increase of the region of existence of the microemulsion systems, and enabled stable clear regions at high water content.

References

Aboofazeli, R., Lawrence, M.J., 1993. Investigations into the formation and characterisation of phospholipid microemulsions: I. Pseudo-ternary phase diagrams of systems containing water–lecithin–alcohol–isopropyl myristate. Int. J. Pharm. 93, 161–175.

Aboofazeli, R., Lawrence, C.B., Wicks, S.R., Lawrence, M.J., 1994. Investigation into the formation and characterization of phospholipid microemulsions. III. Pseudo-ternary phase diagrams of systems containing water–lecithin–isopropyl myristate and either an alkanoic acid, amine, alkanediol, polyethylene glycol alkyl ether or alcohol as cosurfactant. Int. J. Pharm. 111, 63–72.

Aboofazeli, R., Patel, N., Thomas, M., Lawrence, M.J., 1995. Investigations into the formation and characterisation of phospholipid microemulsions: IV. Pseudo-ternary phase diagrams of systems containing water–lecithin–alcohol and oil; the influence of oil. Int. J. Pharm. 125, 107–116.

Alander, J., Werenheim, T., 1989. Model microemulsions containing vegetable oils. Part 1: Non ionic surfactant systems. J. Am. Oil Chem. Soc. 66, 1656–1660.

Attwood, D., Mallon, C., Taylor, C.J., 1992. Phase studies on oil-in-water microemulsions. Int. J. Pharm. 84, R5–R8.

Binks, B.P., Meunier, J., Langevin, D., 1989. Characteristic sizes, film rigidity and interfacial tension in microemulsion systems. Prog. Colloid Polym. Sci. 79, 208–213.

Cornell, B.A., Middlehurst, J., Separovic, F., 1986. Small unilamellar phospholipid vesicles and the theory of membrane formation. Faraday Disc. Chem. Soc. 81, 163–167.

von Corswant, C., Thoren, P., Engstrom, S., 1998. Triglyceride-based microemulsion for intravenous administration of sparingly soluble substances. J. Pharm. Sci. 87, 200–208.

Evans, D.F., Mitchell, D.J., Ninham, B.W., 1986. Oil, water and surfactant: Properties and conjectured structure of simple microemulsions. J. Phys. Chem. 90, 2817–2835.

Gallarate, M., Gasco, M.R., Trotta, M., Chetoni, P., Saettone, M.F., 1993. Preparation and evaluation in vitro of solution and o/w microemulsions containing levobunolol as ion-pair. Int. J. Pharm. 100, 219–225.

Israelachvili, J.N., Mitchell, D.J., Ninham, B.W., 1976. Theory of self assembly of hydrocarbon amphiphiles into micelles and bilayers. J. Chem. Faraday Trans. II. 72, 1525–1568.

Kovarik, J.M., Mueller, E.A., Van Bree, J.B., Tetzloff, W., Kutz, K., 1994. Reduced inter- and intra-individual variability in cyclosporine pharmacokinetics from microemulsion formulation. J. Pharm. Sci. 83, 444–446.

Nuhn, P., Kertscher, P., Dobner, B., Braune, K.D., Kluge, S., 1982. Synthese und biologische aktivität einiger lysierend und fusogen wirkender phosphocholinderivate. Pharmazie 37, 706–708.

Shinoda, K., Araki, M., Sadaghiani, A., Khan, A., Lindman, B., 1991. Lecithin-based microemulsions: phase behaviour and microstructure. J. Phys. Chem. 95, 989–993.

Trotta, M., Gasco, M.R., Pattarino, F., 1989. Effect of alcohol cosurfactants on the diffusion coefficients of microemulsions by light scattering. J. Dispers. Sci. Technol. 10, 15–32.

Trotta, M., Ugazio, E., Gasco, M.R., 1995. Pseudo-ternary phase diagrams of lecithin-based microemulsions: influence of monoalkylphosphates. J. Pharm. Pharmacol. 47, 451–454.

Trotta, M., Cavalli, R., Ugazio, E., Gasco, M.R., 1996. Phase behaviour of microemulsion systems containing lecithin and lysolecithin as surfactants. Int. J. Pharm. 143, 67–73.