International Journal of Pharmaceutics, 84 (1992) 107–116
Elsevier Science Publishers B.V.

IJP 02721

Preparation of submicron drug particles in lecithin-stabilized o/w emulsions: I. Model studies of the precipitation of cholesteryl acetate

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(Received 6 September 1991)
(Accepted 1 November 1991)

Key words: Submicron particle; Precipitation; Cholesteryl acetate; Emulsion; Suspension; Lecithin; Bile salt

Summary

Nanoparticles of a model drug, viz. cholesteryl acetate, were prepared. The cholesteryl acetate was dissolved in cyclohexane containing lecithin. The organic solution was emulsified in an aqueous solution containing a cosurfactant. A stable o/w emulsion resulted. The solvent was evaporated from the emulsion and cholesteryl acetate precipitated in the emulsion droplets. The size of the particles was almost unaffected by the concentration of cholesteryl acetate in cyclohexane. Furthermore, the increase in particle size as a result of an increased oil/water ratio was negligible. With a blend of phosphatidylcholine and sodium glycocholate as emulsifiers, particle sizes down to 25 nm were obtained. The ratio of phosphatidylcholine to sodium glycocholate appears to be critical. On increasing the ratio above 9:1, the suspension becomes more unstable as indicated by an increase in particle size during storage. The optimal conditions coincide with those giving an extensively swelling lamellar liquid crystalline phase containing phosphatidylcholine and sodium glycochololate.

Introduction

Poorly water-soluble drug substances may be administered using emulsions (Davis, 1982; Davis et al., 1985, 1987; Singh and Ravin, 1986; Levy and Benita, 1989; Collins-Gold et al. 1990; Prankerd and Stella, 1990), liposomes (Lopez-Berestein and Juliano, 1987; Weinstein, 1987; Weiner et al., 1989), or micellar systems (Florence, 1981; Westesen, 1988). All of these systems may be stabilized by surface-active lipids. The advantages of such systems are that they are biodegradable and may be composed of biocompatible emulsifiers and oils. Furthermore, the in vivo distribution of liposomes (Senior, 1987; Moghimi et al., 1991) and emulsion droplets (Mulley, 1974; Davis, 1982; Davis et al., 1985, 1987; Illum et al., 1989) may be influenced as a result of modification of their surfaces by using different emulsifiers and polymers. However, the use of emulsions, liposomes and micelles as delivery systems also involves several drawbacks. The amount of poorly water-soluble drug that can be incorporated into liposomes and micellar systems

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is usually limited and thus a considerable volume of drug solution is necessary due to the high emulsifier/drug ratio. Achieving a satisfactory shelf-life for liposomes is a difficult task and the stability during the process of dilution of micellar systems at the time of injection is a critical factor (Von Dardel et al., 1976). The use of emulsions as delivery systems is also limited, mainly due to the low solubility of high-melting-point, hydrophobic drugs in triglyceride oils. The possibility of introducing the drugs in the form of ground particles is usually restricted owing to the difficulty in preparing particles below 1 μm in size by normal grinding procedures. Moreover, grinding may not be feasible as a consequence of the degradation of compounds of low thermal stability that may be caused by locally generated thermal effects. Hence, there is a clear need for new drug delivery systems allowing for parenteral administration of high-melting-point hydrophobic substances.

In this paper, we describe how the previously reported method for preparing submicron particles by precipitation from an emulsion (Sjöström et al., 1992a) can be applied in order to produce particles of a model drug stabilized by biocompatible emulsifiers. Thus, particles with the drug in the core and a blend of biocompatible emulsifiers on the surface were prepared. Through the particulate nature we were able to increase the drug load in each delivery system and to reduce the emulsifier/drug ratio considerably. Particular attention was focussed on naturally occurring emulsifiers, i.e., phospholipids, due to their general acceptability as emulsifiers in biological systems.

In previous studies (Sjöström et al., 1992a,b) we showed that the final particle size is mainly determined by the initial droplet size in the emulsion. Hence, the conditions employed for emulsification govern the results obtained. One such important condition concerns the phase equilibrium of the emulsifier in the initial system (Friberg and Mandell, 1970; Friberg and Wilton, 1970; Bergenståhl and Claesson, 1990). The type of liquid crystalline phases formed by different phospholipids can be described as an average geometrical shape and depends on the balance between their interaction with water and the volume of the hydrophobic moiety of the molecule (Israelachvili et al., 1976; Bergenståhl and Claesson, 1990). Lysophospholipid molecules, being cone-shaped, with the polar end group as the base, form micelles (Arvidsson et al., 1985) while phosphatidylcholine and phosphatidylinositol, having a cylindrical shape, have a preference for lamellar phases, i.e., stacked lipid bilayers separated by thin layers of water (Small, 1967; Bergenståhl, 1990). On the other hand, the average shape of phosphatidic acid and phosphatidylethanolamine can be described as conic with the base at the end of the hydrophobic moiety and their molecules form reversed hexagonal phases, i.e., rod-like aggregates with an aqueous core surrounded by the hydrophobic tails (Cullis and De Kruijff, 1978; Bergenståhl, 1990; Lindblom et al., 1991). The upper limit of swelling for the lamellar phase provides an indication of both the range and the limits of the repulsive interactions in water generated by the emulsifier layers. For instance, the increased swelling of the sodium sulfosuccinate lamellar phase is strongly reduced on shielding from the electrostatic repulsion by replacement of the counterions with Ca^{2+} or Mg^{2+} (Khan et al., 1984).

The swelling of neutral phospholipids is limited to a water layer of around 25–30 Å due to the short-range character of the repulsive interactions (Rand, 1981; Lis et al., 1982). The capacity for stabilization can be enhanced through the introduction of additives that increase the swelling into the emulsifier blend. Phase diagrams for phosphatidylinositol (Söderberg, 1991) and blends of phosphatidylinositol and phosphatidylcholine (Bergenståhl, 1991) show almost unlimited swelling. Extensive swelling of technical lecithin has also been shown to improve emulsifying ability (Rydhag and Wilton, 1981). Similarly, the introduction of bile salts into the lamellar phase formed by lecithin increases the capacity for swelling. The three-component phase diagram of sodium cholate, lecithin and water of a previous investigation (Small and Bourgès, 1966; Small et al., 1966) demonstrated a strongly swelling lamellar phase up to a lecithin/bile salt ratio of about 2:1. Below this ratio mixed micelles were formed.

When parts of the oil phase have crystallized,

a tendency toward destabilization of the emulsions is observed (Van Boekel and Walstra, 1981; Walstra, 1987). This becomes particularly pronounced at a high volume fraction of the dispersed phase. It is also known that large volume of the dispersed phase leads to a reduction in the efficiency of emulsification (Phipps, 1985). Hence, the volume fraction can be expected to be a sensitive parameter which limits the possibilities for using a given system.

The objective of the present study was to evaluate the most efficient blend of biocompatible emulsifiers for preparing the smallest (below 100 nm) possible drug particles by precipitation in an o/w emulsion (Sjöström et al., 1992a,b). In order to obtain preparations with the smallest possible particles, the potential for emulsification of a blend of lecithin and bile salt was thoroughly examined. In this article, the steroid, cholesteryl acetate (CA), was used as a model for a poorly water-soluble drug substance. CA and lecithin were dissolved in cyclohexane. The organic solution was emulsified in an aqueous solution containing a cosurfactant and a water continuous emulsion was formed. The cyclohexane was removed by evaporation. One particle precipitated in each emulsion droplet.

This article describes the detailed characterization of particles prepared using the emulsifier blend found to result in the optimum performance, as judged on the basis of particle size, namely, lecithin and sodium glycocholate.

Experimental

Materials

CA, water solubility < 0.1 mg/ml (Sigma Chemical Co., U.S.A.), cyclohexane pa (Merck AG, Germany), soya phosphatidylcholine (Epikuron 145 V (E145), Epikuron 180 (E180), Epikuron 200 (E200), Epikuron 200SH (E200SH)), de-oiled egg phospholipids (Ovothin 170 (O170)), (Lucas Meyer, Germany), sodium taurocholate, sodium glycocholate (both from Sigma Chemical Co., U.S.A.), polyoxyethylene sorbitan fatty acid ester (Tween 80 (T80); ICI, Belgium), sucrose fatty acid ester (Crodesta DKS F160 (F160); DKS

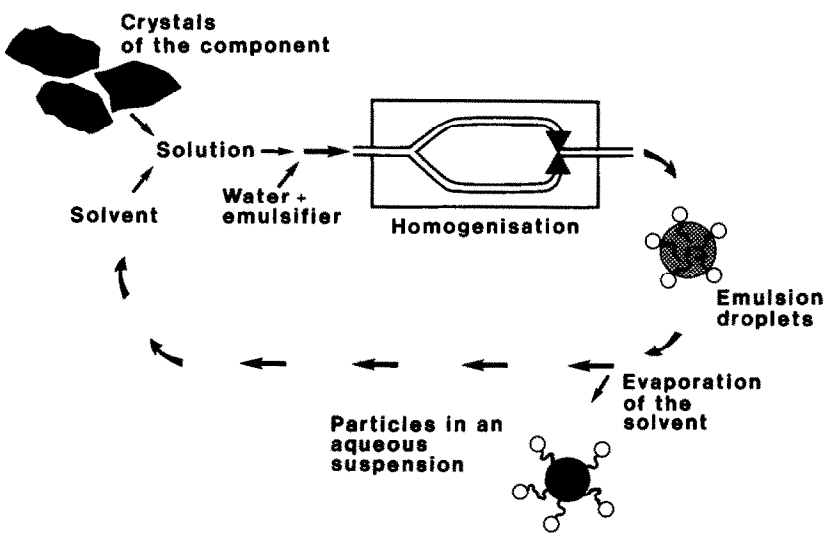

Fig. 1. Scheme illustrating the method of preparation of small particles. The drug is dissolved in an organic solvent containing lecithin and the solution is then emulsified, using an aqueous surfactant-containing solution, forming an o/w emulsion. On removal of the solvent by evaporation, the drug precipitates and the particles are stabilized by the emulsifier, adsorbed on the surface of the drug particles.

Int., Inc., Japan) and polyvinyl pyrrolidone K-25 (PVP25; Fluka AG, Switzerland) were all used without purification.

The water used was purified, without exposure to laboratory air, by carrying out the following procedures: decalcination and prefiltration, followed by reverse osmosis, treatment with two mixed bed ion exchangers, activated charcoal, Organex® and finally a second filtration. The purification units were all Millipore products except for the final filtration stage through a 100 nm Zetapore filter.

Methods

The method for the preparation of small drug particles, represented in Fig. 1, has been described more thoroughly in a previous paper (Sjöström et al., 1992a). Various amounts of CA were dissolved in a solution of lecithin and cyclohexane. The lecithins used were different soybean and egg lecithins (Table 1). The organic solution was emulsified with an aqueous solution containing a cosurfactant to create a water continuous emulsion. The cosurfactants employed were bile salts, a polyethylene oxide sorbitan ester, a sucrose fatty acid ester and a polymer. The CA concentrations in oil were 5 and 25% (w/w). The concentration of the surfactant was 5% (w/w), calculated with respect to the oil except for lecithin/polyvinylpyrrolidone where the concentration was 14% (w/w). The oil/water ratio was varied from 10:90 to 40:60 and the lecithin/cosurfactant ratio was 4:1 except in the case of lecithin/polyvinylpyrrolidone where it was 4:10.

Emulsification was accomplished by treatment in a colloid mill for 2 min at room temperature (Ultra-Turrax T18/10, Shaft TP10N, Janke & Kunkel GmbH & Co, Staufen, Germany) followed by high-pressure homogenization at a pressure difference of 1000 bar for about 5 min with the systems cooled in an iced water bath (Microfluidizer TM 110, Microfluidizer Co., Newton, MA, U.S.A.). The organic solvent in the emulsions was removed by evaporation at room temperature in a rotavapor flask using a pressure of about 100 mbar. The solvent concentration in the final suspension was not determined (in production processes, it will depend on the process parameters) but it was very low. The CA particle size determined was the same irrespective of the evaporation time, e.g., 8 and 48 h.

The particle size was determined from quasi-elastic light scattering. The instrument (Malvern Autosizer IIc, Malvern Instruments, Malvern, U.K.) measures the scattering at a fixed angle of 90°. The hydrodynamic radius of the particles was calculated assuming a spherical geometry. TEM pictures have verified the validity of this assumption for the investigated system. The mean values of the particle diameters are given as a distribution of mass.

Results and Discussion

Emulsifier composition

A number of different bioacceptable lecithin/coemulsifier combinations were screened in order to identify possible alternatives for well-perfor-

TABLE 1

Lecithins used in the study

	Composition (% (w/w)) [a]				
	Ovothin 170 (egg)	Epikuron 145 V (soybean)	Epikuron 180 (soybean)	Epikuron 200 (soybean)	Epikuron 200SH (soybean)
Phosphatidylcholine	≈ 70	44–47	80–85	≈ 95	≈ 98
Lysophosphatidylcholine		< 4		< 3	≈ 0.5
Phosphatidylethanolamine	≈ 18	12–15	4–6		
Phosphatidylinositol		0–3	0		
Phosphatidic acid		0–3			

[a] As given by the supplier.

Trade names are listed with the source of the lecithin being indicated in parentheses.

ming combinations. The results are displayed in Fig. 2. The CA concentration in oil was 25% (w/w). It was not possible to prepare stable emulsions using pure phosphatidylcholine (E200 or E200SH) without a cosurfactant. This might be due to the absence of phosphatidylethanolamine in these preparates, which reduces the degree of formation of liposomes during the emulsification process. All other mixtures and pure emulsifiers listed in Table 1 resulted in the formation of stable emulsions. The majority of the particles prepared had a diameter between 50 and 200 nm (Fig. 2). Some of the measurements on emulsifier combinations plotted in Fig. 2 were repeated two or three times with acceptable reproducibility. The particles prepared with bile salt as cosurfactant were significantly smaller (below 100 nm) than those formed using the other cosurfactants (100–200 nm). The smallest particles (diameter 20 nm) were obtained with a system containing a blend of soybean phosphatidylcholine (E200) and sodium glycocholate.

In general, the results indicate that the bile salts, sodium glycocholate and sodium taurocholate, are efficient cosurfactants for use in the preparation of CA particles of small sizes.

The effect on particle size of the composition of the lecithin used as emulsifier in combination with sodium glycocholate as cosurfactant was examined. Various lecithins from egg and soybean of different degrees of purification were used. The emulsifier concentration was 5% (w/w) as calculated with respect to the oil. The o/w ratio in the emulsion was 10:90, the lecithin/sodium glycocholate ratio being 4:1. The concentration of CA in the dispersed phase of the emulsion was 25% (w/w).

The size of the resulting particles was around 100 nm. In most cases, the size appeared to be independent of the composition of the lecithin, except for pure phosphatidylcholine (E200), which gave rise to particle sizes around 25 nm (Fig. 3).

In order to achieve the smallest particle size possible, the ratio of phospholipid/sodium glyco-

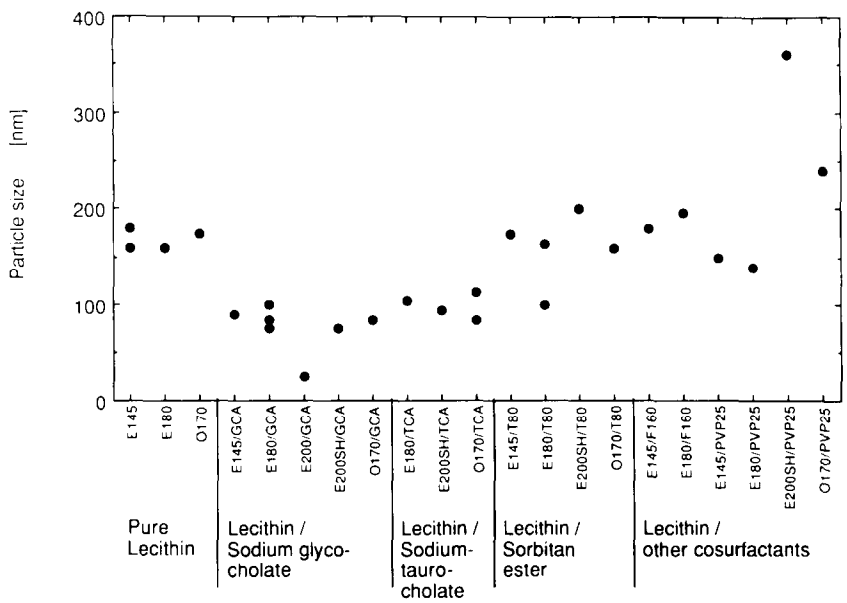

Fig. 2. Particle size 1 day after precipitation as a function of surfactant. Surfactant concentration in the emulsion was 5% (w/w) as measured with respect to the oil except for lecithin/polyvinylpyrrolidone (PVP), in which the value was 14% (w/w). Lecithin/surfactant ratio: 4:1 (except for lecithin/PVP, being 4:10). Concentration of CA was 25% (w/w) in the oil. o/w ratio of emulsion: 10:90.

cholate was varied. The concentration of CA was 5 or 25% (w/w) in the oil. Particles down to a size of 20 nm were obtained for an E200/GCA ratio of 4:1 at a CA concentration in the oil of 5% (w/w) (Fig. 4). The particle size appeared to decrease to a slightly greater extent for E200/GCA ratios of 3:2 and 4:1 as compared to those for 9:1 and 19:1, respectively. The clear difference between the results for the phosphatidylcholine/sodium glycocholate blend as compared to the other mixtures suggests that the mechanism of emulsification differs. The phase diagram shows that mixed micelles are formed for lecithin/sodium cholate ratios up to 2:1. The increased solubility of the emulsifier in the aqueous phase might contribute to the process of emulsification through the Gibbs-Marangoni effect (Walstra, 1983), thereby favoring an extremely small droplet size. We have also observed a strong tendency for bimodal distributions to be adopted when operating close to the optimal conditions. Phosphatidylethanolamine remaining in several phospholipid preparates (E145, E180, O170) would be expected to reduce the extent of micelle formation due to its hydrophobicity. More saturated phospholipids, for example, egg phosphatides, might be less effective due to their lower fluidity and thereby slower kinetic process during emulsification.

Storage stability

The stability during storage with respect to particle size was monitored by analysis using quasi-elastic light scattering. The change in size of the particles during a storage period of 2.5 weeks at room temperature (Fig. 3) appears to be independent of lecithin composition.

The influence of the phosphatidylcholine/sodium glycocholate ratio on storage stability was evaluated according to the changes in particle size. The results are shown in Figs 5 and 6. The particle size was found to remain constant at E200/GCA ratios of 3:2, 4:1 and 9:1. On reducing the proportion of sodium glycocholate to result in an E200/GCA ratio of 19:1, the stability was found to be lowered. This may be due to crystal growth and/or to agglomeration (Fig. 6). Thus, by reducing the concentration of sodium

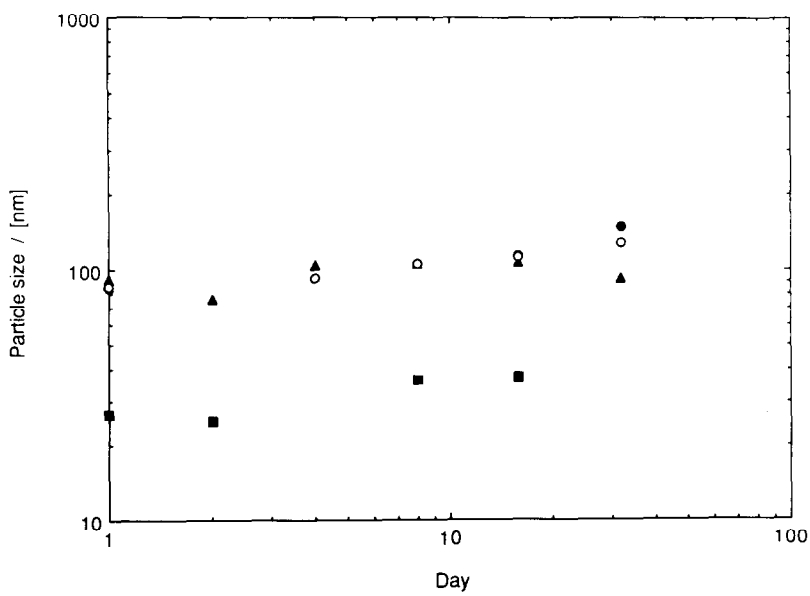

Fig. 3. Particle size as a function of time (in days). Surfactant concentration in the emulsion was 5% (w/w) as measured with respect to the oil phase and that of CA was 25% (w/w) in the oil. o/w ratio of emulsion: 10:90 and lecithin/GCA ratio: 4:1. The surfactants used were: (●) O170/GCA, (▲) E145/GCA, (○) E180/GCA and (■) E200/GCA.

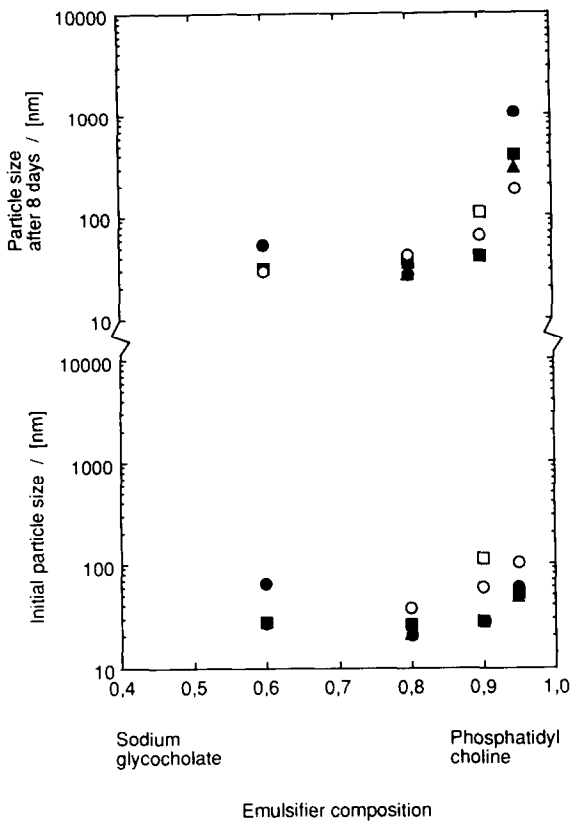

Fig. 4. Particle size at 1 and 8 days after preparation as a function of E200/GCA ratio. Surfactant concentration in the emulsion was 5% (w/w) as measured with respect to the oil phase. Concentrations of CA are expressed as percent (w/w) in the oil. o/w ratio 10:90, 5 (●) and 25 (■) % (w/w) CA; o/w ratio 20:80, 5 (▲) and 25 (○) % (w/w) CA; o/w ratio 40:60, (□) 25% (w/w) CA.

glycocholate a more unstable system is created. This could result from the presence of liposomes and weaker electrostatic repulsion.

Particles with a lecithin/sodium glycocholate ratio of 19:1 and above are clearly unstable.

Two destabilization processes are possible: Ostwald ripening and flocculation (Bergenståhl and Claesson, 1990). Both processes are strongly favored by a small particle size. However, one would expect Ostwald ripening to be the preferred route for emulsifiers that enhance diffusional transport by forming hydrophilic micelles. On the other hand, flocculation would be expected to be enhanced by hydrophobic emulsi-

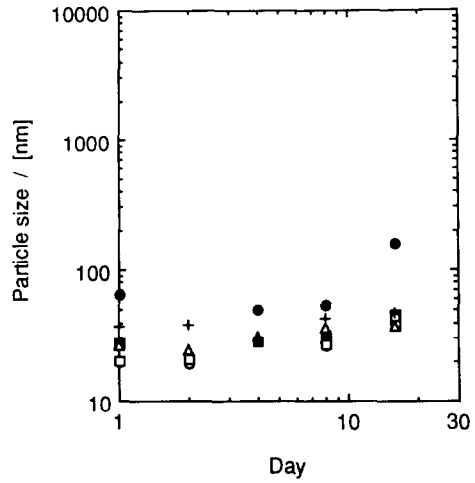

Fig. 5. Particle size as a function of time (in days). Surfactant concentration was 5% (w/w) as measured with respect to the oil phase. Concentrations of CA are expressed as percent (w/w) in the oil. (E200/GCA 3:2) o/w ratio 10:90, 5 (●) and 25 (■) % (w/w) CA; o/w ratio 20:80, (▲) 25% (w/w) CA. (E200/GCA 4:1) o/w ratio 10:90, 5 (○) and 25 (△) % (w/w) CA; o/w ratio 20:80, 5 (□) and 25 (+) % (w/w) CA.

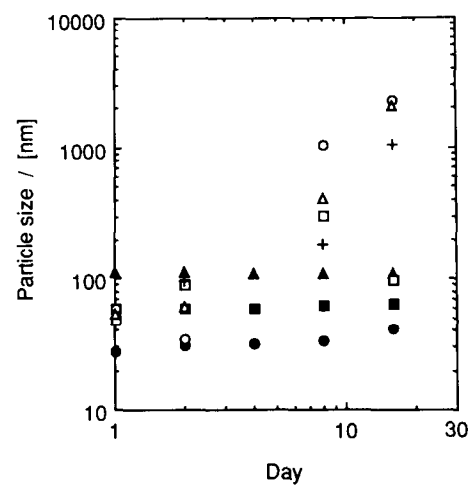

Fig. 6. Particle size as a function of time (in days). Surfactant concentration was 5% (w/w) as measured with respect to the oil phase. Concentrations of CA are expressed as percent (w/w) in the oil. (E200/GCA 9:1) o/w ratio 10:90, (●) 25% (w/w) CA; o/w ratio 20:80, (■) 25% (w/w) CA; o/w ratio 40:60, (▲) 25% (w/w) CA. (E200/GCA 19:1) o/w ratio 10:90, 5 (○) and 25 (△) % (w/w) CA; o/w ratio 20:80, 5 (□) and 25 (+) % (w/w) CA.

fiers. During the course of the experiments, we observed that the less hydrophobic blend reduced the stability. Hence, we drew the conclusion that the most probable cause of the instability observed at high ratios of lecithin to sodium glycocholate (Fig. 6) is the process of flocculation.

o/w ratio of the emulsion

The possibility of preparing more concentrated suspensions of CA particles was assessed by varying the o/w ratio of the emulsion from 10:90 to 40:60. The difference in particle size as a result of preparation using a system having an o/w ratio of 10:90 as compared with that produced at a ratio of 20:80 is negligible (Fig. 4). At higher o/w ratios, emulsification of the system was found to become an increasingly more difficult task to perform. At an o/w ratio of 40:60, the sole combination of emulsifiers that enabled the emulsification of the system was that in which the E200/GCA ratio was 9:1. The particles formed using an emulsion having an o/w ratio of 40:60 and CA concentration of 25% (w/w) in cyclohexane were determined as being 110 nm in diameter (Fig. 4). Clearly, the task of performing emulsification entails much greater difficulty at high volume fractions as compared with the use of low ratios. In the case of E200/GCA, since the size of particles is very small and the system is strongly charged, the strength of interaction between droplets of the emulsion is of considerable magnitude and of course becomes even more pronounced on increasing the o/w ratio. However, such drawbacks appear to arise during the stage in which homogenization is being carried out. No additional instability was observed during the step of solvent evaporation.

CA concentration in the solvent

The final step in the characterization of particles concerned evaluation of the effect of varying the CA concentration in the cyclohexane phase on the resulting particle size (Fig. 4). CA concentrations of 5 and 25% (w/w) in the oil were selected for study. At 25% (w/w) in cyclohexane, the concentration of CA approaches saturation. The size of particles prepared using an emulsion with a CA level of 5% (w/w) in the oil is comparable with that for the concentrated oil phase. The surfactant concentration was identical in both cases. The amount of CA solubilized by lecithin is 0.20 g/g (Kellaway and Saunders, 1967). During our preceding investigations, we observed increases in the porosity of the particles on lowering the CA concentration in oil (Sjöström et al., 1992a,b). The results suggest that the limits on particle size are governed by the droplet size rather than the amount of material available. A possible mechanism for particle formation may involve the following stages: (i) the removal of the oil by evaporation; (ii) a rise in concentration of CA in the oil phase; and (iii) the development of a concentration gradient of CA in the emulsion droplet, with the greatest level of CA occurring near the oil/water interface. Due to the existence of local saturation and as a result of the presence of surfactant at the oil/water interface, the precipitation of CA probably begins close to the surface of the emulsion droplet, subsequently leading to the formation of a porous aggregate of CA. Hence, the particle size depends on the size of the emulsion droplet during the period in which saturation is being reached at a location close to the interface of the droplet.

Conclusions

It has been demonstrated in this article that a combination of sodium glycocholate and phosphatidylcholine (PC) constitutes a very efficient emulsifier blend for use in the production of extremely small drug particles. The ratio of PC to sodium glycocholate appears to be critical. With increasing PC/GCA ratios above 9:1, the suspension becomes more unstable due to crystal growth and/or agglomeration, which may be the consequence of the presence of liposomes and weaker electrostatic repulsion. Furthermore, the PC/GCA ratio must be maintained above 2:1 in order to exclude the possible occurrence of mixed micelles (Small and Bourgès, 1966; Small et al., 1966), thereby preventing an increase in solubility of the drug which would lead to enhancement of the ripening process of the system. The optimum conditions are consistent with those employed for

the preparation of an extensively swelling lamellar liquid-crystalline phase containing PC and GCA.

The method described above allows one to manufacture spherical particles of size as small as 25 nm in diameter by using a mixture of bile salts and lecithin. A high load of drug in each component is achievable together with the benefit from requiring a relatively low emulsifier/drug ratio as compared to liposomal or micellar preparations. In addition, modification of the surface properties and particle sizes by using a wide range of biocompatible emulsifier is feasible.

Acknowledgements

The authors gratefully acknowledge Professor Per Stenius for valuable comments on the manuscript and Pernilla Eriksson for performing parts of the laboratory work. This project was financed by a grant from the Swedish Board for Technical Development.

References

Arvidsson, G., Brentel, I., Khan, A., Lindblom, G. and Fontell, K., Phase equilibria in four lysophosphatidylcholine/water systems. Exceptional behaviour of 1-palmitoyl-glycerophosphocholine. *Eur. J. Biochem.*, 152 (1985) 753–759.

Bergenståhl, B., Phase diagrams of mixed soybean phospholipids. In Dickinson, E. (Ed.), *Food Polymers, Gels and Colloids*, Royal Society of Chemistry, Cambridge, 1991, pp. 123–131.

Bergenståhl, B.A. and Claesson, P.M., Surface forces in emulsions. In Larsson, K. and Friberg, S.E. (Eds), *Food Emulsions*, 2nd Edn, Dekker, New York, 1990, pp. 41–96.

Collins-Gold, L.C., Lyons, R.T. and Bartholow, L.C., Parenteral emulsions for drug delivery. *Adv. Drug Delivery Rev.*, 5 (1990) 189–208.

Cullis, P.R. and De Kruijff, B., The polymorphic phase behaviour of phosphatidylethanolamines of natural and synthetic origin. *Biochim. Biophys. Acta*, 513 (1978) 31–42.

Davis, S.S., Emulsion systems for the delivery of drugs by the parenteral route. In Bundgard, H., Bagger Hansen, A. and Kofod, H. (Eds), *Optimization of Drug Delivery*, Munksgaard, Copenhagen, 1982, pp. 333–347.

Davis, S.S., Hadgraft, J. and Palin, K.J., Medical and pharmaceutical applications of emulsions. In Becher, P. (Ed.), *Encyclopedia of Emulsion Technology*, Vol. 2, Dekker, New York, 1985, pp. 159–238.

Davis, S.S., Washington, C., West, P., Illum, L., Liversidge, G., Sternson, L. and Kirsh, R., Lipid emulsions as drug delivery systems. *Ann. N.Y. Acad. Sci.*, 507 (1987) 75–88.

Florence, A.T., Drug solubilization in surfactant systems. In Yalkowsky, S.H. (Ed.), *Techniques of Solubilization of Drugs*, Dekker, New York, 1981, pp. 15–89.

Friberg, S. and Mandell, L., Influence of phase equilibra on properties of emulsions. *J. Pharm. Sci.*, 59 (1970) 1001–1004.

Friberg, S. and Wilton, I., Liquid crystals – the formula for emulsions. *Am. Perf. Cosm.*, 85 (1970) 27–30.

Illum, L., West, P., Washington, C. and Davis, S.S., The effect of stabilising agents on the organ distribution of lipid emulsions. *Int. J. Pharm.*, 54 (1989) 41–49.

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

Kellaway, I.W. and Saunders, L., The solubilization of some steroids by phosphatidylcholine and lysophosphatidylcholine. *Biochim. Biophys Acta.*, 144 (1967) 145–148.

Khan, A., Fontell, K. and Lindman, B., Liquid crystallinity in systems of magnesium and calcium surfactants. *J. Colloid Interface Sci.*, 101 (1984) 193–200.

Levy, M.Y. and Benita, S., Design and characterization of a submicronized o/w emulsion of diazepam for parenteral use. *Int. J. Pharm.*, 54 (1989) 103–112.

Lindblom, G., Rilfors, L., Hauksson, J.B., Brentel, I., Sjölund, M. and Bergenståhl, B., Effect of head-group structure and counterion condensation on phase equilibra in anionic phospholipid-water systems studied by ^{2}H, ^{23}Na, and ^{31}P NMR, and X-ray diffraction. *Biochemistry*, 30 (1991) 10938–10948.

Lis, L.J., McAlister, M., Fuller, N., Rand, R.P. and Parsegian, V.A., Interactions between neutral phospholipid bilayer membranes. *Biophys. J.*, 37 (1982) 657–666.

Lopez-Berestein, G. and Juliano, R.L., Application of liposomes to the delivery of antifungal agents. In Ostro, M.J. (Ed.), *Liposomes. From Biophysics to Therapeutics*, Dekker, New York, 1987, pp. 253–276.

Moghimi, S.M., Porter, C.J.H., Illum, L. and Davis, S.S., The effect of poloxamer-407 on liposome stability and targeting to bone marrow: comparison with polystyrene microspheres. *Int. J. Pharm.*, 68 (1991) 121–126.

Mulley, B.A., Medicinal emulsions. In Lissant, K.J. (Ed.), *Emulsions, Emulsion Technology*, Vol. 1, Dekker, New York, 1974, pp. 291–349.

Phipps, L.W., Aspects of dispersed phase disruption. In *The High Pressure Dairy Homogenizer, Technical Bulletin No. 6*, The National Institute for Research in Dairying, Reading, U.K., 1985, pp. 46–78.

Prankerd, R.J. and Stella, V.J., The use of oil-in-water emulsions as a vehicle for parenteral drug administration. *J. Parent. Sci. Technol.*, 44 (1990) 139–149.

Rand, R.P., Interacting phospholipid bilayers: Measured forces and induced structural changes. *Annu. Rev. Biophys. Bioeng.*, 10 (1981) 277–314.

Rydhag, L. and Wilton, I., The function of phospholipids of soybean lecithin in emulsions. *J. Am. Oil Chem. Soc.,* 58 (1981) 830–837.

Senior, J.H., Fate and behavior of liposomes in vivo: a review of controlling factors. *CRC Crit. Rev. Ther. Drug Carrier Syst.,* 3 (1987) 123–193.

Singh, M. and Ravin, L.J., Parenteral emulsions as drug carrier systems. *J. Parenter. Sci. Technol.,* 40 (1986) 34–41.

Sjöström, B., Bergenståhl, B. and Kronberg, B., A method for the preparation of submicron particles of sparingly water soluble drugs by precipitation in o/w-emulsions: II. The influence of the emulsifier, the solvent and the drug substance. *J. Pharm. Sci.,* (1992b), submitted.

Sjöström, B., Kronberg, B. and Carlfors, J., A method for the preparation of submicron particles of sparingly water soluble drugs by precipitation in o/w-emulsions: I. *J. Pharm. Sci.,* (1992a) submitted.

Small, D.M., Phase equilibria and structure of dry and hydrated egg lecithin. *J. Lipid Res.,* 8 (1967) 551–561.

Small, D.M. and Bourgès, M., Lyotropic paracrystalline phases obtained with ternary and quaternary systems of amphiphilic substances in water: studies on aqueous systems of lecithin, bile salt and cholesterol. *Mol. Cryst.,* 1 (1966) 541–561.

Small, D.M., Bourgès, M. and Dervichian, D.G., Ternary and quaternary aqueous systems containing bile salt, lecithin, and cholesterol. *Nature,* 211 (1966) 816–818.

Söderberg, I., The binary system soybean phosphatidylinositol-water. *Chem. Phys. Lipids,* (1991) submitted.

Van Boekel, M.A.J.S. and Walstra, P., Stability of oil-in-water emulsions with crystals in the disperse phase. *Coll. Surf.,* 3 (1981) 109–118.

Von Dardel, O., Mebius, C. and Mossberg, T., Diazepam in emulsion form for intravenous usage. *Acta Anaesth. Scand.,* 20 (1976) 221–224.

Walstra, P., Formation of emulsions. In Becher, P. (Ed.), *Encyclopedia of Emulsion Technology,* Vol. 1, Dekker, New York, 1983, pp. 57–127.

Walstra, P., Overview of emulsion and foam stability. In Dickinson, E. (Ed.), *Food Emulsions and Foams,* Royal Society of Chemistry, London, 1987, pp. 242–257.

Weiner, N., Martin, F. and Riaz, M., Liposomes as a drug delivery system. *Drug. Dev. Ind. Pharm.,* 15 (1989) 1523–1554.

Weinstein, J.N., Liposomes in the diagnosis and treatment of cancer. In Ostro, M.J. (Ed.), *Liposomes. From Biophysics to Therapeutics,* Dekker, New York, 1987, pp. 277–338.

Westesen, K., Beitrag zur Strukturaufklärung eines arzneistoffhaltigen hochkonzentrierten Tensidsystems und ausgewählter Verdünnungen. Dissertation, Technical University Carolo-Wilhelmina, Braunschweig, Germany, 1988.