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

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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 showed only minor variations with the cholesteryl acetate concentration 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 between phosphatidylcholine and sodium glycocholate appears to be critical. On increasing the ratio above 9:1, the suspension becomes more unstable as demonstrated by an increase in particle size during storage The optimal conditions coincide with those given an extensively swelling lamellar liquid crystalline phase containing phosphatidylcholine and sodium glycocholate.

Introduction

Poorly water soluble drug substances may be administered using emulsions (Davis, 1982, 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 with these systems are that they are biodegradable and may be composed of biocompatible emulsifiers and oils. Furthermore, the in vivo distribution of the liposomes (Senior, 1987; Moghimi et al., 1991) and the emulsion droplets (Mulley, 1974; Davis, 1982, 1987; Illum et al., 1989) may be influenced by modification of their surfaces by different emulsifiers and polymers. However, there are also several disadvantages of emulsions, liposomes and micelles as delivery systems. The amount of poorly water soluble drug that it is possible to incorpo-

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rate in liposomes and micellar systems is usually limited, which makes the amounts of the solutions required large, due to the high emulsifier/drug ratio. A satisfactory shelf stability for liposomes is hard to achieve and the stability during the dilution of micellar systems at the time of injection is critical (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 as ground particles is usually hindered by the difficulties in obtaining particles of a size below 1 μm through normal grinding procedures. Furthermore, grinding processes may be impossible to use owing to degradation of compounds of low thermal stability due to the creation of local heat effects. Thus, there is a clear need for new drug delivery systems allowing for parenteral administration of high melting hydrophobic substances.

In this paper, we describe how the method of preparing submicron particles by precipitation from an emulsion reported in a preceding paper (Sjöström et al., 1992a) can be applied to prepare 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 character we are able to increase the load of drug in each entity and to reduce the emulsifier/drug ratio considerably. Particular attention was focused on the naturally occurring emulsifiers, 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 of the emulsification govern the results obtained. One such important condition is 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). Lysophospholipids, having the molecular shape of a cone 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 they 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 swelling limit of the lamellar phase is an indication of the range and of the limits of the repulsive interactions in water generated by the emulsifier layers. For instance, the expanded swelling of the sodium sulfosuccinate lamellar phase is strongly reduced if the electrostatic repulsion is shielded by replacement of the counterions by 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 stabilizing power can be enhanced if additives that expand the swelling are introduced into the emulsifier blend. Phase diagrams of phosphatidylinositol (Söderberg, 1991) and blends of phosphatidylinositol and phosphatidylcholine (Bergenståhl, 1990) show almost unlimited swelling. Extensive swelling of technical lecithin has also been shown to improve the emulsifying ability (Rydhag and Wilton, 1981). Similarly, the introduction of bile salts into the lamellar phase formed by lecithin increases the swelling properties. The three-component phase diagram of sodium cholate, lecithin and water (Small and Bourgès, 1966; Small et al., 1966) shows a strongly swelling lamellar phase up to a lecithin/bile salt ratio of about 2:1. Below this ratio mixed micelles are formed.

When part of the oil phase is crystallized it tends to destabilize emulsions (Van Boekel and Walstra, 1981; Walstra, 1987). This is particularly

pronounced at a high volume fraction of the dispersed phase. It is also known that a high volume of dispersed phase will reduce the efficiency of emulsification (Phipps, 1985). Hence, the volume fraction can be expected to be a sensitive parameter limiting the possibilities for using a given system.

The objective of this study is to evaluate the most efficient blend of biocompatible emulsifiers for preparing the smallest (below 100 nm) drug particles possible by precipitation in an o/w emulsion (Sjöström et al., 1992a,b). In order to prepare the smallest particles possible, the emulsifying properties of a blend of lecithin and bile salt were 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 so that a water continuous emulsion was formed. The cyclohexane was removed by evaporation upon which one particle precipitated in each emulsion droplet.

This study will continue with a thorough characterization of the particles prepared with the best performance by means of particle size, emulsifier blend, e.g., lecithin and sodium glycocholate.

Experimental

Materials

Cholesteryl acetate, 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)), deoiled egg phospholipids (Ovothin 170 (O170)) (Lucas Meyer, Germany), sodium taurocholate and 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 F 160 (F160)) (DKS Int, Inc., Japan) and polyvinyl pyrrolidone K-25 (PVP25) (Fluka AG, Switzerland) were all used without purification.

The water used was purified, without being exposed to laboratory air, as follows: decalcination and prefiltration, followed by reverse osmo-

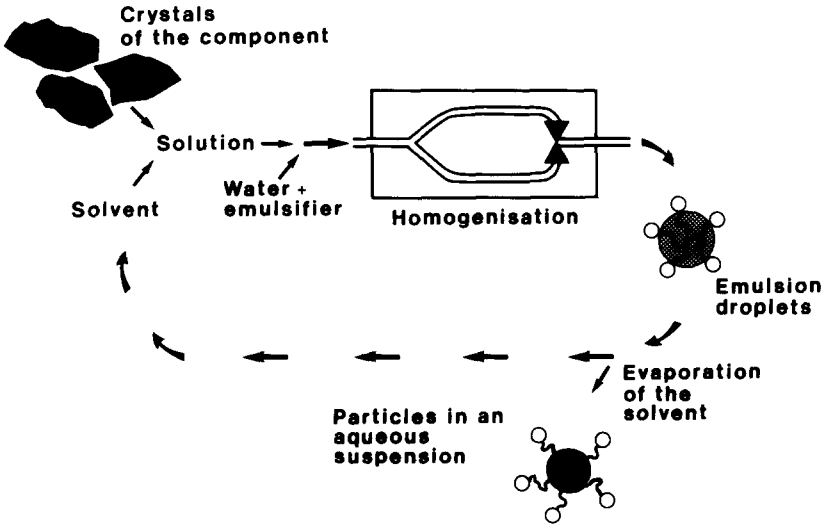

Fig. 1. A schematic illustration of the method of manufacturing small particles. The drug is dissolved in an organic solvent containing lecithin and the solution is emulsified, with an aqueous solution containing a cosurfactant, forming an o/w emulsion. The organic solvent is evaporated, the drug precipitates and the particles are stabilized by the emulsifier, adsorbed on the surface of the drug particles

TABLE 1

The 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 given with the source of the lecithin being indicated in parentheses

sis, 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, presented in Fig. 1, has been described more thoroughly previously (Sjöström et al., 1992a). Various amounts of cholesteryl acetate

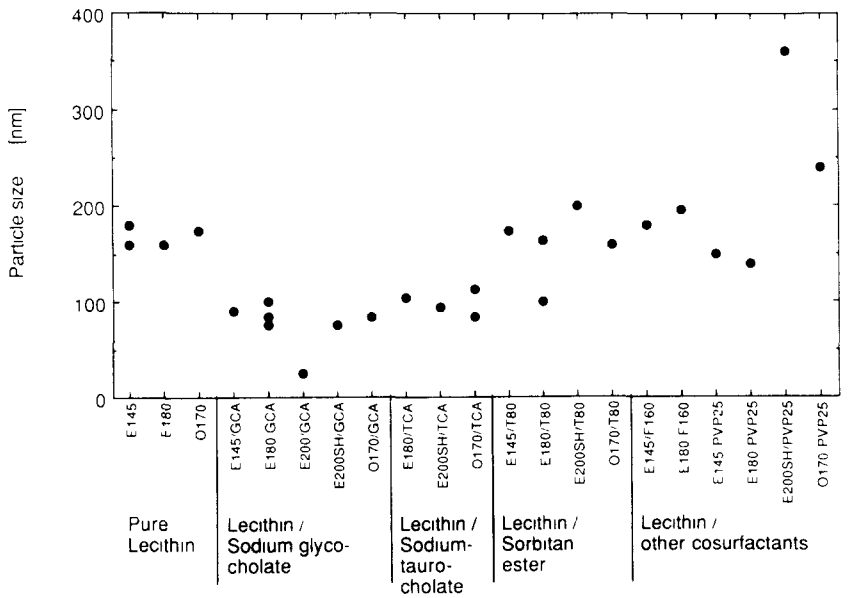

Fig. 2 Particle size at the first day after the precipitation as a function of surfactant The surfactant concentration in the emulsion was 5% (w/w) measured with respect to the oil except for lecithin/polyvinylpyrrolidone where it was 14% (w/w) The lecithin/cosurfactant ratio was 4 1 except in the case of lecithin/PVP where it was 4:10 The concentration of CA was 25% (w/w) in oil The o/w ratio of the emulsion was 10·90.

(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 used 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 1000 bar pressure difference 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 emulsion was removed at room temperature by evaporation in a rotavapor flask at a pressure of about 100 mbar. The solvent residue in the final suspension was below 150 ppm, as determined by gas chromatography. The resultant CA particle size was the same irrespective of the evaporation time, e.g., 8 and 48 h.

The particle size was determined by 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 shown 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 to identify possible alternatives for well-performing com-

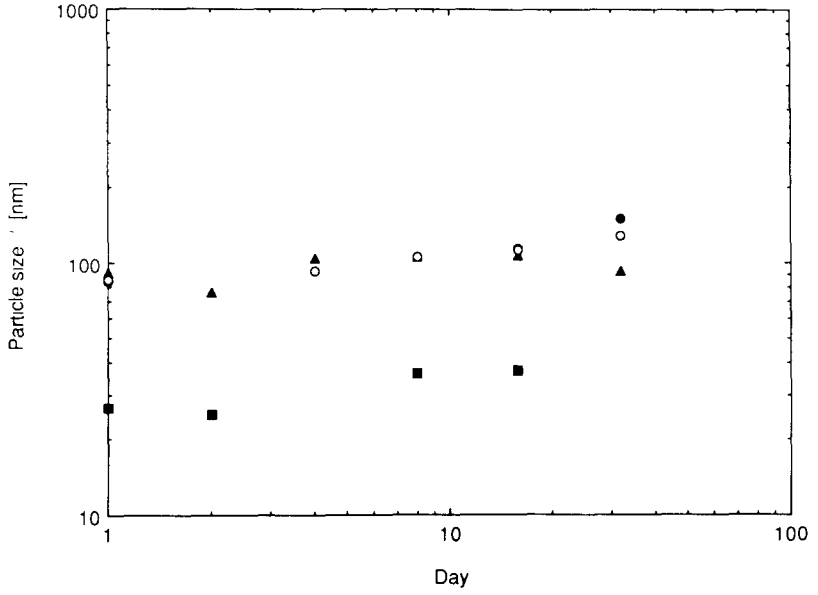

Fig. 3 Particle size as a function of time (in days) The surfactant concentration in the emulsion was 5% (w/w) calculated with respect to the oil phase and the CA concentration was 25% (w/w) in the oil The o/w ratio of the emulsion was 10·90 and the lecithin/sodium glycocholate ratio was 4 1. The surfactants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

binations. 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 the result of the absence of phosphatidylethanolamine in these preparations, which reduces the production of liposomes during the emulsification process. All other mixtures and pure emulsifiers listed in Table 1 were successfully used to prepare stable emulsions. Most of the particles prepared had a diameter between 50 and 200 nm (Fig. 2). Some emulsifier combinations in Fig. 2 were re-examined two or three times and showed acceptable reproducibility. The particles prepared with bile salt as cosurfactant were significantly smaller, below 100 nm, than those prepared with the other cosurfactants which were between 100 and 200 nm. The smallest particles, with a diameter of 20 nm, were prepared with a system containing a blend of soybean phosphatidylcholine (E200) and the sodium glycocholate. In general, the results indicate that the bile salts, sodium glycocholate and sodium taurocholate are efficient cosurfactants in the preparation of CA particles with small sizes.

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

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

In order to achieve the smallest particle size possible, the ratio of phospholipid/sodium glycocholate was varied. The CA concentration was 5 or 25% (w/w) in the oil. Particles down to a size of 20 nm were obtained when the E200/sodium glycocholate ratio was 4:1 and the CA concentration in oil was 5% (w/w) (Fig. 4). The particle size seemed to be slightly smaller when the ratio of E200/sodium glycocholate was 3:2 and 4:1 as compared to 9:1 and 19:1, respectively. The clear difference of the phosphatidylcholine/sodium glycocholate mixture compared to the others suggests that the emulsification mechanism is different. The phase diagram shows that mixed micelles are formed at lecithin/sodium cholate ratios up to 2:1. The increased emulsifier solubility in the aqueous phase might contribute to emulsification through the Gibbs-Marangoni effect (Walstra, 1983), thereby favoring an ex-

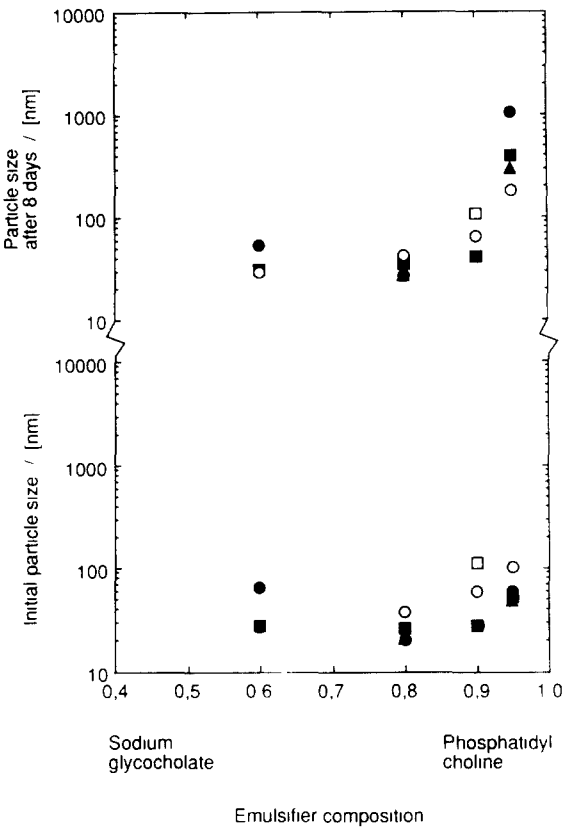

Fig. 4. Particle size at the first day after preparation and the particle size after 8 days as a function of the ratio between E200 and sodium glycocholate. The surfactant concentration was 5% (w/w) calculated with respect to the oil phase Concentrations of CA are given as (w/w) % in the oil. o/w ratio 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

tremely small droplet size. We have also observed that there is a strong tendency to adopt bimodal distributions when operating close to optimal conditions. Phosphatidylethanolamine, which remains in several phospholipid preparations (E145, E180, O170), can be expected to reduce the formation of micelles due to its hydrophobicity. More saturated phospholipids, such as egg phosphatides, might be less effective due to a lower fluidity and thereby slower kinetic process during emulsification.

Storage stability

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

The influence of the phosphatidylcholine/sodium glycocholate ratio on the storage stability was examined by means of following the changes in size of the particles. The results are shown in Figs 5 and 6. The particle size appears to be stable when the E200/sodium glycocholate ratio is 3:2, 4:1 and 9:1. When the sodium glycocholate concentration was reduced to a E200/sodium glycocholate ratio of 19:1, the stability was found to be reduced. This is due to crystal growth and/or to agglomeration (Fig. 6). Thus, in reducing the concentration of sodium glycocholate, a more unstable system is created, probably as a result of the presence of liposomes and of the reduced electrostatic repulsion.

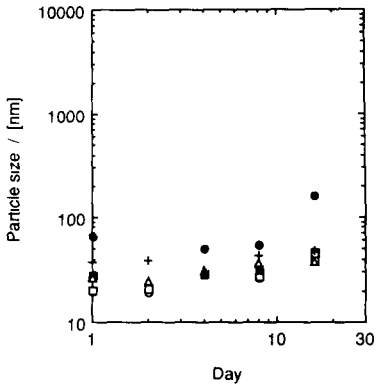

Fig. 5. Particle size as a function of time (in days). The surfactant concentration was 5% (w/w) calculated with respect to the oil phase. CA concentrations are given as % (w/w) in the oil (E200/sodium glycocholate 3·2) o/w ratio 10:90, 5 (●) and 25 (■) % (w/w) CA; o/w ratio 20:80, (▲) 25% (w/w) CA. (E200/sodium glycocholate 4:1) o/w ratio 10 90, 5 (○) and 25 (w/w) % CA; o/w ratio 20:80, 5 (□) and 25 (+) % (w/w) CA.

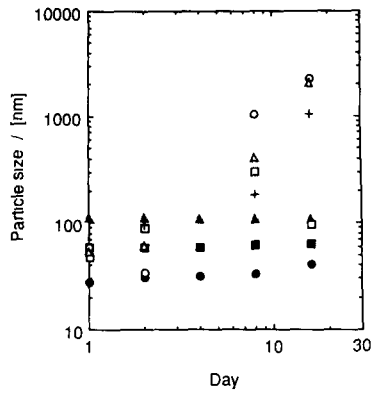

Fig 6 Particle size as a function of time (in days). The surfactant concentration was 5% (w/w) in the oil phase. CA concentrations are given as % (w/w) in the oil. (E200/sodium glycocholate 9:1) o/w ratio 10·90, (●) 25% (w/w) CA, o/w ratio 20·80, (■) 25% (w/w) CA; o/w ratio 40.60, (▲) 25% (w/w) CA. (E200/sodium glycocholate 19:1) o/w ratio 10:90, 5 (○) and 25 (△) % (w/w) CA; o/w ratio 20 80, 5 (□) and 25 (+) % (w/w) CA.

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 small particle size. However, Ostwald ripening is favored by emulsifiers that enhance diffusional transport by forming hydrophilic micelles. On the other hand, flocculation is enhanced by hydrophobic emulsifiers. In the experiments, we observe that the more hydrophobic blend reduced the stability and therefore we conclude that flocculation is the most probable cause of the instabilities observed at high lecithin/sodium glycocholate ratios (Fig. 6).

Oil / water ratio of the emulsion

The possibility of preparing more concentrated suspensions of CA particles was studied by varying the o/w ratio of the emulsion from 10:90 to

40:60. The difference between the particle size resulting from a system with an o/w ratio of 10:90 and that of an o/w ratio of 20:80 is negligible (Fig. 4). At higher o/w ratios, the system is more difficult to emulsify. The only emulsifier combination allowing emulsification with an o/w ratio of 40:60 was the system in which the E200/sodium glycocholate ratio was 9:1. Particles prepared from an emulsion with an o/w ratio of 40:60 and a CA concentration of 25% (w/w) in cyclohexane had a diameter of 110 nm (Fig. 4). Clearly, emulsification at high volume fractions is much more difficult than at low volume fractions. As the particle size is very small and the system is highly charged in the case of E200/sodium glycocholate, the interaction between the emulsion droplets is very strong and this of course becomes more pronounced as the o/w ratio is increased. However, the difficulties seem to occur during the process of homogenization. No additional instability was observed during the evaporation procedure.

CA concentration in the solvent

The influence of the concentration of CA in cyclohexane on the particle size was also examined (Fig. 4). The CA concentration in oil was 5 and 25% (w/w). At 25% (w/w) CA in cyclohexane the concentration is close to saturation. The size of the particles prepared from an emulsion containing 5% (w/w) CA in the oil is roughly comparable with that from the concentrated oil phase. The surfactant concentration was the same in both cases. The amount of CA solubilized by lecithin is 0.20 g/g lecithin (Kellaway and Saunders, 1967). In previous studies, we found that the particles become more porous when the concentration of CA in oil is lowered (Sjöström et al., 1992a,b). The results suggest that the limits of particle size are determined by the size of the droplets rather than by the amount of available material. A possible mechanism for the formation of particles is that as the oil is removed by evaporation, the CA concentration in the oil is increased and a concentration gradient of CA develops in the emulsion droplet with the highest concentration of CA close to the oil/water interface. Because of local saturation and the presence of surfactant at the oil/water interface, the precipitation of CA will probably begin close to the surface of the emulsion droplet and lead to the formation of a porous aggregate of solid CA. Hence, the particle size depends on the size of the emulsion droplet at the time of saturation close to the interface of the droplet.

Conclusions

In this article, we have shown that a combination of sodium glycocholate and phosphatidylcholine is a very efficient emulsifier blend in the production of extremely small drug particles. The ratio between phosphatidylcholine and sodium glycocholate appears to be critical. In increasing the PC/sodium glycocholate ratio above 9:1, the suspension becomes more unstable due to crystal growth and/or agglomeration, which might be the result of the presence of liposomes and reduced electrostatic repulsion. Furthermore, the PC/sodium glycocholate ratio must be above 2:1 in order to avoid the presence of mixed micelles (Small and Bourgès, 1966; Small et al., 1966), thereby preventing increased solubility of the drug which would enhance the ripening process of the system. The optimal conditions coincide with those giving an extensively swelling lamellar liquid crystalline phase containing PC and sodium glycocholate.

By the method described in this article, it is possible to manufacture spherical particles down to a diameter of 25 nm by the use of a mixture of bile salts and lecithin. This method allows one to produce a high load of drug in each entity and the emulsifier to drug ratio is relatively low compared to liposomal or micellar preparations. Furthermore, the method allows the surface characteristics and the particle sizes to be altered by the use of a wide range of biocompatible emulsifiers.

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