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Preparation of submicron drug particles in lecithin-stabilized o/w emulsions. II. Characterization of cholesteryl acetate particles

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Summary

Submicron particles of a model drug, viz., cholesteryl acetate (CA), have been characterized. The particles were prepared by precipitation of CA in the dispersed phase of an o/w emulsion stabilized by two different emulsifier systems i.e., (a) a mixture of lecithin and sodium glycocholate and (b) a polyoxyethylene sorbitan fatty acid ester. The particle size has been determined by photon correlation spectroscopy (PCS) and transmission electron microscopy (TEM). The average particle size, by number, was determined by PCS to be 21–144 nm depending on the emulsifier system used in the particle preparation. The smallest mean particle size of 21 nm was achieved with a blend of lecithin and sodium glycocholate. According to TEM pictures, the particles have a smooth surface and are spherical, and the majority of the particles seem to be amorphous, exhibiting neither microporosity in the particles nor aggregation between the particles. The transition temperature of cholesteryl acetate in the particles prepared with the blend of lecithin and sodium glycocholate has been determined by differential scanning calorimetry (DSC). The DSC measurements indicate that the melting point at 105°C of the cholesteryl acetate particles prepared with lecithin/sodium glycocholate is lower than that of the pure macroscopic cholesteryl acetate crystals which is 116°C. The irreversible transition of cholesteryl acetate at about 80°C was not observed in the particle form of cholesteryl acetate prepared with the emulsifier blend of phosphatidylcholine and sodium glycocholate. The suspensions have also been investigated by small angle X-ray scattering (SAXS). The cyclohexane residue in the resulting suspensions was below 25 ppm according to analysis by gas chromatography.

Introduction

Many factors reduce the possibility of achieving a satisfactory delivery of lipophilic drug substances. Because of their solubility properties, it may be impossible to study some lipophilic substances in vivo, though they seem to be efficient in treating some diseases in studies on the cellular or subcellular level. Drug substances administered parenterally as aqueous solutions must be water soluble and stable in blood. The drug stability in blood can be critical for substances that are metabolized or enzymatically degraded. Furthermore, some drugs, such as anti-cancer drugs,

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show harmful effects on normal cells and therefore, a selective delivery of the drug to the malignant cells is preferred.

One way of solving these problems is to incorporate the drug substance in particles. It is possible by precipitation of the drug substance in the dispersed phase of a water continuous emulsion (Sjöström et al., 1992a,b), to achieve suspensions containing drug particles with a size that is acceptable for intravenous administration, i.e., a particle size far below 5 μm. Emulsions containing large droplets are known to cause adverse reactions (Fujita et al., 1971).

When the drug is injected intravenously in particle form, it is possible to obtain a selective or targeted drug delivery to specific organs by attachment for example of a monoclonal antibody having receptors on the target-cell antigenes (Illum et al., 1984; Gupta, 1990). It is also possible to achieve a specific uptake of the drug in the reticuloendothelial system (RES) after intravenous injection by incorporation of the substance in a particle, e.g., passive targeting. In addition, the particulate form of the drug may inhibit the affinity of the drug to serum components and thereby make it possible to achieve a reduction in the circulation time of the drug substance after intravenous injection, which can promote a RES-specific uptake of the drug.

In this study, cholesteryl acetate was chosen as a model substance for the drug. The drug substance was dissolved in a non-polar solvent. When lecithin was used as emulsifier, it was also added to the non-polar solvent. This phase was emulsified in an aqueous phase containing a water soluble emulsifier, and a stable water-continuous emulsion was formed. When the non-polar solvent was evaporated, the drug substance precipitated in the dispersed phase of the emulsion forming a suspension of submicron particles. In a previous study (Sjöström et al., 1992c), particles were prepared in lecithin-stabilized o/w emulsions in order to achieve a formulation allowed for parenteral administration. In our experiments, we have produced particles down to a mean diameter (by mass) of 25 nm using a blend of lecithin and sodium glycocholate (4 : 1, w/w). The emulsifiers, lecithin and sodium glycocholate, are also used in a commercial product for parenteral application (Valium® Novum). In this product the lecithin/sodium glycocholate forms mixed micelles which act as carriers for the lipophilic drug (Westesen, 1988).

Previous observations indicate that the particle size is the same as the emulsion droplet size (Sjöström et al., 1992b) when the CA concentration in the organic phase is close to saturation, despite a CA load in the organic solvent of only about 25–50% (w/w). Thus, there must exist a porosity in the particles and hence the particle structure is an important issue. Depending on the way of preparation, the particles may be spherical, may contain holes, or may be microporous. The solvent residue in the suspensions was analysed to see whether or not the particle porosity was due to the presence of organic solvent in the final particles.

Another important issue is the crystallinity/amorphism of the CA in particle form. This is important for the solubility of the particles which in turn will influence the bioavailability of the drug (Shefter, 1981). It may also be important if the purpose is to obtain a slow release of the drug. Hence, the particles were analysed by means of crystallinity and amorphism.

The emulsifier used in the particle preparation may also influence the particle structure. The precipitation of CA probably starts at the oil/water interface since the cyclohexane concentration is lowest and the CA concentration is highest at this part of the oil droplet. The emulsifier is accumulated at the oil/water interface and thus the orientation of the CA molecules and thereby the polymorphism of CA in the final particle or the particle morphology may be influenced by the molecular shape of the emulsifier. Two different types of emulsifier systems, a blend of soybean phosphatidylcholine and sodium glycocholate (4 : 1, w/w) and a polyoxyethylene sorbitan fatty acid ester were used in the particle preparation, the latter emulsifier being included as a reference. The presence of liposomes is an issue of special interest in the suspensions prepared with lecithin since liposome structures in intravenous emulsions sometimes cause adverse side effects (Hajri et al., 1990).

The aim of this study was to characterize the particles prepared in a lecithin-stabilized o/w emulsion (Sjöström et al., 1992c). The particles were characterized in terms of crystallinity/amorphism, morphology and size, and the solvent residue in the final suspensions was analysed. Particles prepared using a polyoxyethylene sorbitan fatty acid ester as emulsifier were included for comparison.

Experimental

Materials

Cholesteryl acetate (99%, Sigma Chemical Co., U.S.A.), cyclohexane pro analysi (Merck AG, Germany), deuterium oxide (99.9 atom% D, Sigma Chemical Co., U.S.A.), polyoxyethylene sorbitan fatty acid ester (Tween 80, ICI, Belgium), soybean phosphatidylcholine (Epikuron 200, Lucas Meyer, Germany) and the sodium salt of glycocholic acid, (Sigma Chemical Co., U.S.A.) were all used without further purification.

The water used was purified in a closed system as follows: 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 which was a 100 nm Zetapore filter.

Methods

Small steroid particles were prepared by precipitation in an o/w emulsion. The method, presented in Fig. 1, has previously been described in detail (Sjöström et al., 1992a,b). The cholesteryl acetate (CA) was dissolved in cyclohexane. The organic solution was emulsified with an aqueous solution to form a water-continuous emulsion. The emulsifier concentration was 5% (w/w) calculated with respect to the non-polar solvent. The emulsifiers used were ethoxylated sorbitan ester and a blend of soybean phosphatidylcholine (PC) and sodium glycocholate (4:1, w/w). The ethoxylated sorbitan ester and the sodium glycocholate were added to the water phase and the PC was added to the non-polar solvent. The emulsifier-containing phases were stirred until they appeared macroscopically homogeneous. The con-

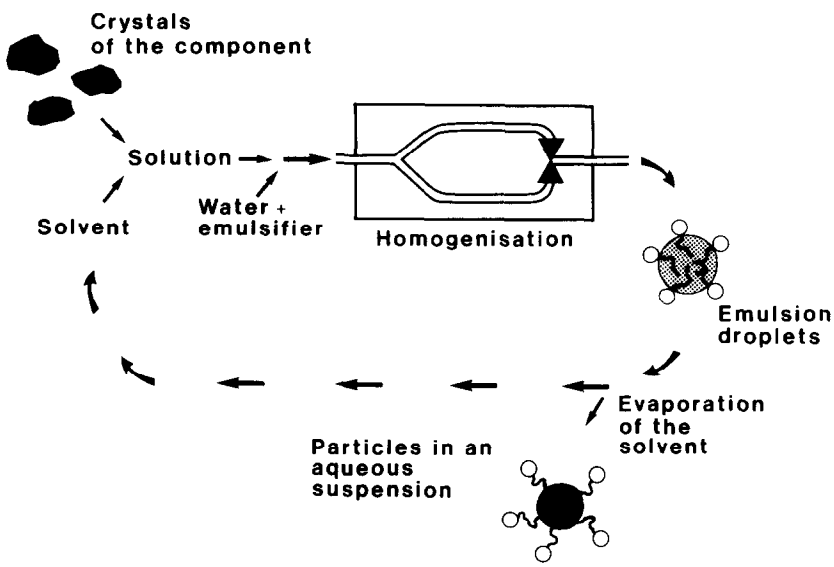


Fig. 1. An illustration of the method of preparation of the CA particles by precipitation in the dispersed phase of a water-continuous emulsion. The drug is dissolved in an organic solvent. After addition of emulsifier, the solution is emulsified with a water solution, thus forming an o/w emulsion. On removal of the organic solvent by evaporation, the drug precipitates and the emulsifier adsorbs on the particle surface.

centration of CA in cyclohexane was 5 and 25% (w/w). The oil/water ratio in the emulsions was 10:90 (w/w).

The mixture to be emulsified was placed in a colloid mill for two minutes at room temperature (Ultra-Turrax T18/10, Shaft TP10N, Janke & Kunkel GmbH & Co, Staufen, Germany) followed by high pressure homogenization under a 1000 bar pressure drop 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 at a pressure of about 100 mbar.

The particle size was measured by photon correlation spectroscopy at a fixed angle of 90°. The distribution of mass was used for data presentation, (Malvern Autosizer IIc, Malvern Instruments, Malvern, U.K.). Further studies were performed at four different angles: 50°, 70°, 90° and 110°. A commercial 'multiangle software' was used for multiangle analysis. The mean diameters are presented as mass and number distributions (Malvern ZetaSizer IIIc, Malvern Instruments, Malvern, U.K.).

For the TEM pictures, the suspensions were stored at room temperature for four days prior to the preparation of the specimens and then were frozen by a slush method in melting propane. The suspensions were sandwiched between flat golden holders, after which the samples were freeze fractured at 173 K with a BAF 400, Balzers AG, Lichtenstein. The fractured frozen samples were shadowed at 45° with platinum/carbon (layer thickness: 2 nm) and at 90° with pure carbon for replica production. Replicas were cleaned with chloroform-ethanol (1:1, v/v), picked up on uncoated microscope grids, and examined by transmission electron microscopy (EM 300, Philips, Kassel, Germany). A three-dimensional analysis of the fractures was performed according to Rose (1980), to obtain information on the particle diameters which are presented as a number distribution.

Small angle X-ray scattering measurements were performed in a Kratky compact small angle system KCLC connected to a Philips PW 2253/11 X-ray tube with copper anode. A nickel foil served as a K_β filter. A tube voltage of 40–50 kV and an anode current of 35–40 mA were used. An OED 50 from Braun GmBH, München, Germany, was used as a position sensitive detector with argon/methane (9:1) as filling gas. The linked multichannel analyser was a Canberra MCA 8100. Liquid samples were measured in a capillary holder K-PR with Peltier temperature control device from Anton Paar, Graz, Austria. Exposure times of 3000 or 5000 seconds were chosen for SAXS measurements. Scattering curves were analysed with the computer program collection of Glatter (1983).

The particle density was observed by comparing the sedimentation after ultracentrifugation for 1.5 h at 45000 rpm with a Beckman L8-M, rotor SW 50.1 (Beckman Instr. Inc., U.S.A.). In these studies, the density of the aqueous phase was varied by addition of deuterium oxide to the suspension.

The DSC analysis of the suspensions was performed between 40 and 130°C, at a heating rate of 2°C/min (DSC 120, Seiko Instruments Inc., Tokyo, Japan). The samples were stored for 2 months prior to the measurements, which were performed by The Application Laboratory, Kameido at the facilities of Seiko Instruments Inc., Japan.

The cyclohexane residues in the suspensions were analysed by gas chromatography. The carrier gas was helium, the stationary phase was 10% Apiezon L and the support was Chromosorb W HP (Middelburg, The Netherlands). The temperature at the site of injection was 100°C, the column temperature was 70°C and the temperature of the flame ionization detector was 140°C (Carlo Erba 4200, Milano, Italy).

Results and Discussion

Particles with two concentrations, 5 and 25% (w/w), of CA in cyclohexane were prepared in order to see whether the porosity and/or crystallinity of the particles depends on the CA concentration in the dispersed phase of the emulsion. The influence of the emulsifier on the particle structure was also investigated by using two

TABLE 1

Reproducibility of the preparation process monitored by the mean particle size, determined by PCS at a fixed angle of 90° (95% confidence interval)

Surfactant	Diameter (nm) (distribution by mass)	
	5% (w/w) CA in cyclohexane	25% (w/w) CA in cyclohexane
Lecithin/ sodium glycocholate	40 ± 5 ($n = 14$)	67 ± 6 ($n = 16$)
Ethoxylated sorbitan ester	116 ± 15 ($n = 6$)	156 ± 16 ($n = 7$)

different emulsifier systems, PC/sodium glycocholate and polyoxyethylene sorbitan fatty acid ester, in the particle preparation.

The process for the preparation of particles is fairly reproducible in terms of the achieved particle size. Table 1 shows the particle sizes obtained in six to sixteen independent preparations of particles. The mean diameter of the particles prepared with an emulsifier blend of lecithin and sodium glycocholate with 5 and 25% (w/w) CA in cyclohexane was 40 and 67 nm, respectively, by mass according to size determinations by PCS at a fixed angle of 90°. With polyoxyethylene sorbitan fatty acid ester as emulsifier, a larger particle diameter was obtained, viz., 116 nm when the CA concentration in cyclohexane was 5% (w/w) and 156 nm when the CA concentration was 25% (w/w) in cyclohexane. According to the data, the mean particle size deviated by 13% at most in different preparations (Table 1). Some of the particle size deviations may have been caused by fluctuations in the homogenization pressure due to fluctuations in the air supply to the homogenizer pump. This fluctuation in air supply results in a variation in droplet size of less than 10%.

TEM studies indicate that the majority of the CA particles of all systems studied are spherical and have a smooth surface. These observations are in agreement with earlier findings from scanning electron microscopy (Sjöström et al., 1992a). The majority of the particles in all the systems studied seem to be amorphous, but the larger particles, e.g., above 100 nm, prepared with the blend of PC and sodium glycocholate were crystalline. Some non-spherical particles with a layered structure were also found in a few of the TEM pictures of the particles (A–D), as is illustrated in Fig. 2, which presents particles prepared with 25% (w/w) CA in cyclohexane and the emulsifier blend of PC/sodium glycocholate (B). The occurrence of these layered crystals can be the result of a modification of the particles dur-

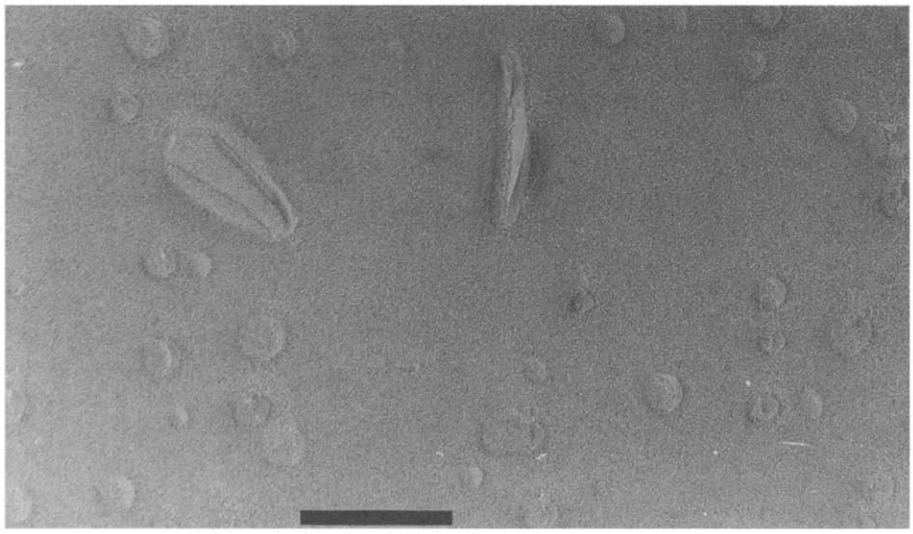


Fig. 2. TEM picture of CA particles prepared by precipitation in an emulsion system. The CA concentration was 25% (w/w) in cyclohexane and the surfactants were phosphatidylcholine and sodium glycocholate. Bar: 100 nm.

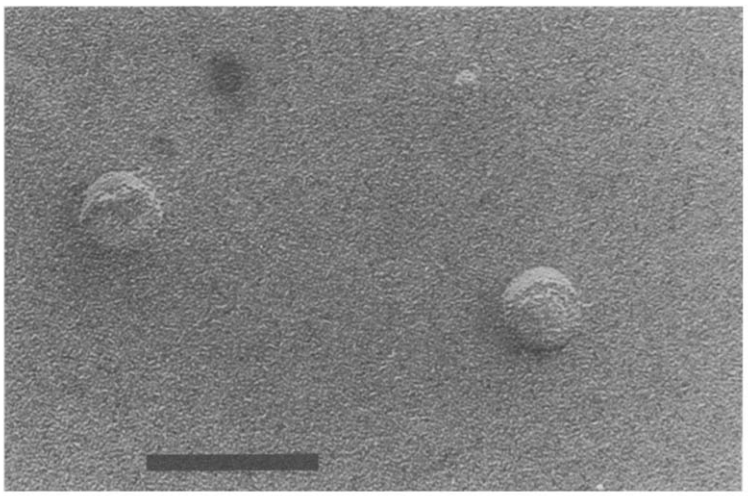


Fig. 3. TEM picture of CA particles prepared by precipitation in an o/w emulsion. The CA concentration was 5% (w/w) in cyclohexane. The surfactants were phosphatidylcholine and sodium glycocholate. Bar: 100 nm.

ing the preparation of the specimens prior to photography. The existence of micropores which are too small to be observed by TEM cannot be excluded. No aggregation between the particles was observed in any of the systems studied.

Phosphatidylcholine and sodium glycocholate; 5% (w/w) CA in cyclohexane

Regardless of size, the particles seem to be spherical according to the TEM pictures. The TEM pictures give the impression that the particles with a diameter below 100 nm are amorphous, e.g., their appearance 4 days after preparation is smooth (Fig. 3), whereas the larger particles appear to be spherical and crystalline (Fig. 4). The coarse appearance of crystalline particles is clearly shown in Fig. 4. The particle structure thus seems to be influenced by the particle diameter. The smaller particles ($d < 100$ nm) may be formed more rapidly so that the time available

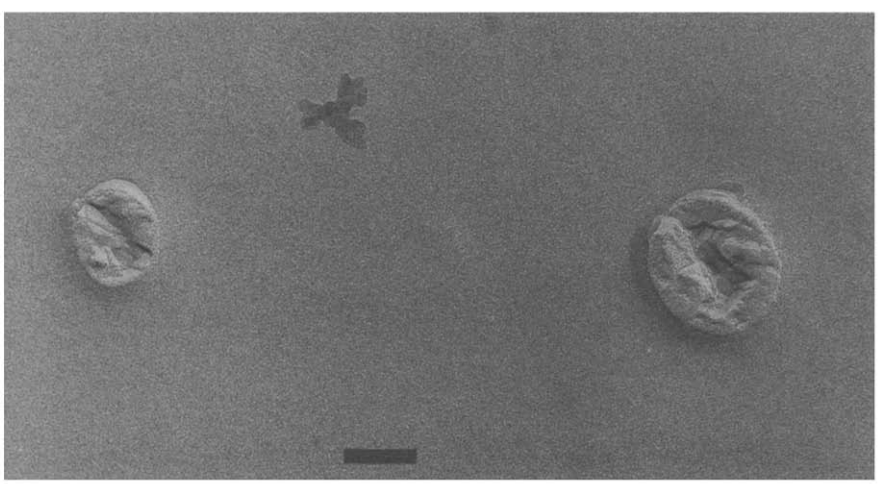


Fig. 4. TEM picture of CA particles prepared by precipitation in an o/w emulsion. The CA concentration was 5% (w/w) in cyclohexane and the surfactants were phosphatidylcholine and sodium glycocholate. Bar shows 100 nm.

TABLE 2

The mean particle diameter of one batch according to measurements by photon correlation spectroscopy performed under four angles and TEM pictures

	Mean particle diameter (nm) (distribution by mass and by number)			
	5% (w/w) CA in cyclohexane		25% (w/w) CA in cyclohexane	
	PCS	TEM	PCS	TEM
Lecithin/ (by mass)	33		48	
sodium (by number)	21	49 ($n = 613$)	41	45 ($n = 2336$)
glycocholate				
Ethoxylated (by mass)	59		152	
sorbitan (by number)	40	76 ($n = 125$)	144	88 ($n = 283$)
ester				

TABLE 3

Transition temperatures (°C) of the particles according to DSC measurements

Pure cholesteryl acetate	79 and 116	
	5% (w/w) CA in cyclohexane	25% (w/w) CA in cyclohexane
Lecithin/sodium glycocholate	104	105

for the CA molecules to achieve a crystalline order is shorter than in the larger particles. Moreover, the ratio between the surface area and the amount of CA in each particle increases as the particle size is reduced and hence the CA molecules are influenced by the emulsifier to a greater extent in the smaller particles than in the larger ones. It is not possible to determine beyond doubt from the TEM micrographs whether the particles are dense or show microporosity (Figs 3 and 4). The mean particle size by number achieved by a three-dimensional analysis of the TEM pictures was 49 nm ($n = 613$) (Table 2), and the mean particle diameter by number was 21 nm (Table 2) according to multiangle PCS analysis. The main cause of the discrepancy between the mean diameters achieved by TEM and PCS is probably the broad size distribution that is weighted differently in the two methods.

The DSC measurements show that the melting point of the CA particles at 104°C is 12°C lower than that of pure macroscopic CA crystals (Table 3). This is probably due to the disturbed crystallization of CA due to the high curvature of the particle and the existence of surfactant at the outer layer of the particles and also due to the

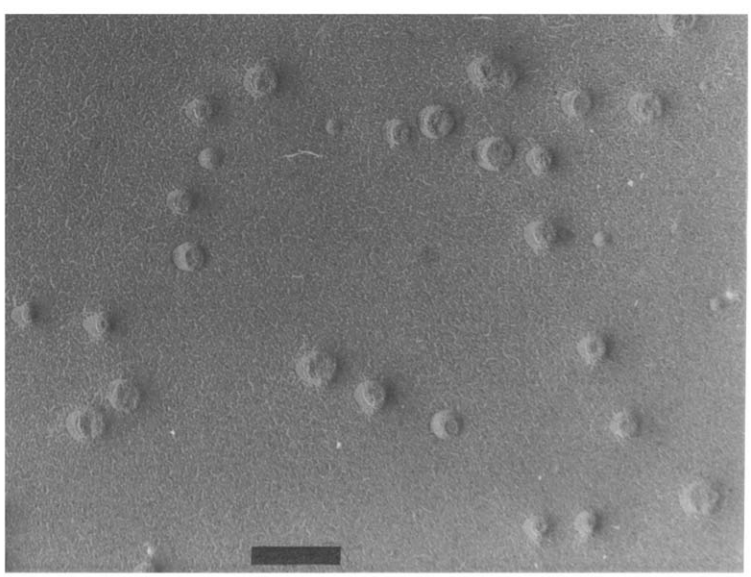


Fig. 5. TEM picture of CA particles prepared by precipitation in an emulsion system. The CA concentration was 25% (w/w) in cyclohexane. The surfactants were phosphatidylcholine and sodium glycocholate. Bar shows 100 nm.

probable amorphous structure of CA in the particle. The presence of traces of cyclohexane in the particles may also contribute to the lowering of the melting point. The irreversible transition temperature of macroscopic CA crystals at 68–87°C (Kunihisa and Gotoh, 1977) has not been observed in the thermograms of the particles. This may be an indication of an amorphous structure of CA in the particle or of a different polymorphism due to CA precipitated in the dispersed phase of the emulsion.

The amount of cholesteryl acetate solubilized by phosphatidyl choline dispersed in water is 16.7% (w/w) (Kellaway and Saunders, 1967) and is this therefore not a source of error in our studies since the amount of cholesteryl acetate is here greater than 55% (w/w) calculated on phosphatidylcholine.

Phosphatidylcholine and sodium glycocholate; 25% (w/w) CA in cyclohexane

According to the TEM pictures, most of the particles seem to be spherical and amorphous (Fig. 5), however, it is not possible to determine unambiguously from the TEM micrographs whether the particles are dense or show microporosity (Figs 2 and 5). The particle structure is similar in this system to that in the previous sample (A) and thus not dependent on the CA concentration in cyclohexane in the initial emulsion. It should be noted that the emulsifier concentration calculated with respect to the cyclohexane concentration is the same in systems A and B, so that the packing of the emulsifier on the emulsion droplets in the two systems (A and B) is likely to be similar. A three-dimensional analysis of the TEM pictures resulted in a mean particle size of 45 nm ($n = 2336$) by number (Table 2). A mean particle size of 41 nm by number was achieved from a multiangle analysis by PCS (Table 2). The similarity in the particle sizes determined by the two methods is an indication that the particle size distribution is fairly narrow in this system.

The melting point of the particles, 105°C, is about the same as that of the previous sample (A)

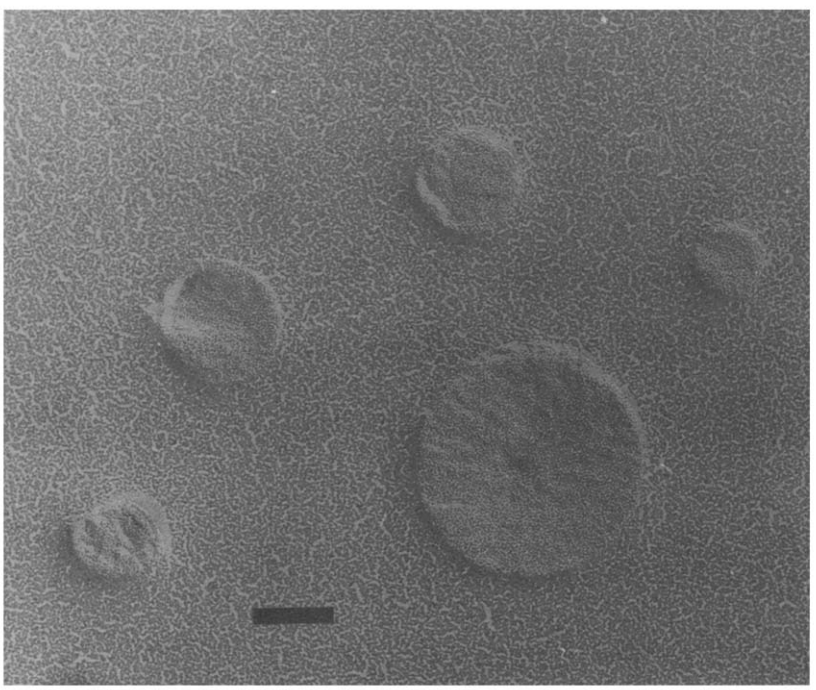


Fig. 6. TEM picture of CA particles prepared by precipitation in an emulsion system. The CA concentration was 5% (w/w) in cyclohexane and the surfactant was an ethoxylated sorbitan ester. Bar: 100 nm.

and no transition temperature at 80°C was observed (Table 3). Hence, the thermogram of the CA particles prepared with PC and sodiumglycocholate is not dependent on the CA concentration in cyclohexane in the initial emulsion.

The particle density, as observed by ultracentrifugation experiments in different D_2O/H_2O mixtures, is about 1.03 g cm^{-3}, which is slightly lower than that of the pure CA crystals which is 1.05–1.06 g cm^{-3}. These data indicate that the polymorphism of CA in the nanoparticles is different from that of CA in macroscopic crystals or that the particles are amorphous. The surfaces of the CA particles are covered by lecithin (density = 1.01 g cm^{-3}) which slightly reduces the particle density but not to the extent observed (CA/lecithin ratio, 6.25 : 1, w/w).

Ethoxylated sorbitan ester; 5% (w/w) CA in cyclohexane

Irrespective of size, the particles appear to be spherical and amorphous (Fig. 6). The particle structure seems to be independent of the emulsifier selected, since the structure of the particles in this sample is the same as that of particles prepared from the PC/sodium glycocholate emulsion (A and B). A few particles in the sample deviate from spherical shape but such particles appear to consist of two fused particles (Fig. 7). The occurrence of microporosity in the particles cannot be excluded on the basis of the TEM micrographs (Figs 6 and 7). A three-dimensional analysis of the TEM pictures resulted in a mean particle size of 76 nm (n = 125) by number (Table 2). The use of an ethoxylated sorbitan ester in the particle preparation therefore results in a larger mean particle size than that achieved in the preparation with a blend of lecithin and a sodium salt of glycocholic acid (Table 2). According to multiangle PCS analysis, the particle size is 40 nm by number. The main reason for the discrepancy between the mean diameters of the particles achieved by TEM and by PCS is that the broad size distribution is weighted differently by the two methods. Further, the small number of particles taken into account in the TEM analysis decreases the precision in the particle diameter determination.

The amount of CA dissolved by the surfactant is likely to be negligible (Attwood and Florence, 1983) and is therefore not a source of error in our studies.

Fig. 7. TEM picture of CA particles prepared by precipitation in an o/w emulsion. The CA concentration was 5% (w/w) in cyclohexane and the surfactant was an ethoxylated sorbitan ester. Bar: 100 nm.

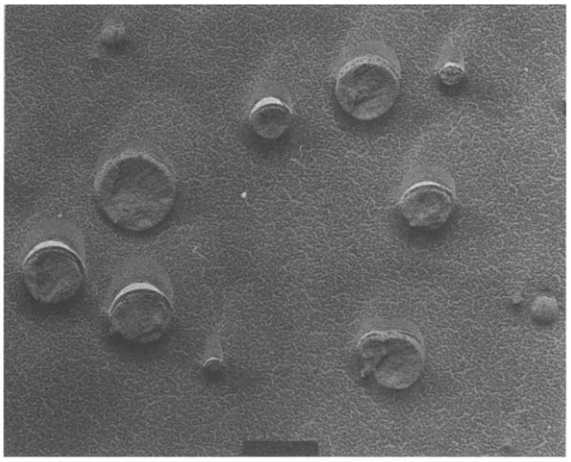


Fig. 8. TEM picture of CA particles prepared by precipitation in an emulsion system. The CA concentration was 25% (w/w) in cyclohexane and the surfactant was an ethoxylated sorbitan ester. Bar: 100 nm.

Ethoxylated sorbitan ester; 25% (w/w) CA in cyclohexane

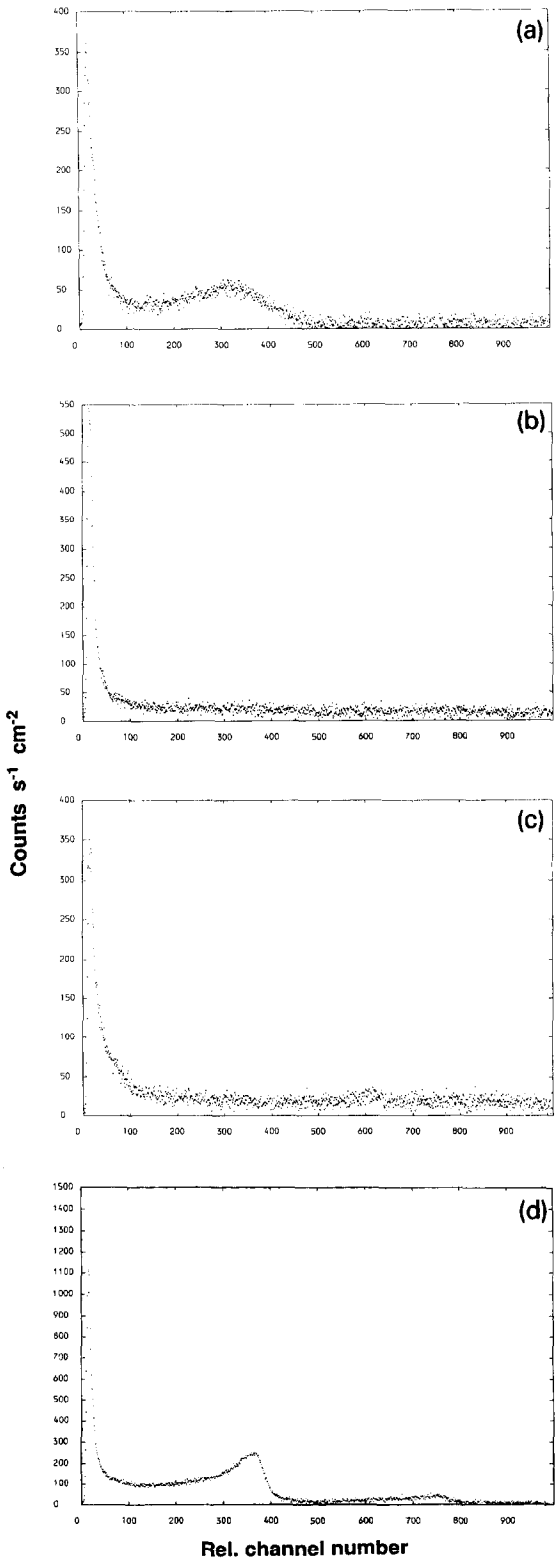
The particles seem, regardless of size, to be spherical and amorphous (Fig. 8). Thus, the structure is not influenced by the CA content in the organic solvent of the initial emulsion, since the particle structure is similar to that in the previous system (C). It is not possible to determine conclusively from the TEM micrographs whether the particles are dense or microporous (Fig. 8). The three-dimensional analysis of the TEM pictures resulted in a mean particle size of 88 nm ($n = 283$) by number distribution. According to multiangle PCS analysis the mean particle size in this sample is 144 nm by number (Table 2). The discrepancy between the mean diameters obtained by TEM and PCS is probably a result of the broad size distribution of the particles that is weighted differently in the two methods. The small amount of particles taken into account in the analysis of the TEM pictures is also a weakness.

When comparing of the size measurements made by three-dimensional analysis of TEM pictures and by the multiangle PCS, one has to consider the weaknesses of the two methods. In the TEM picture analysis, one weakness of the measurement is the lack of precision in the manual particle diameter determination. Shadows occurring around the particles may introduce an error in the diameter determination of about 2–4 nm. Moreover, the possibility that the particles are modified during the preparation of the specimens prior to the photography also cannot be excluded. It should also be emphasized that the particle size determinations performed by three-dimensional analysis of the TEM pictures are only rough estimations due to the limited number of particles taken into account. In the TEM picture analysis, the size of each particle is taken into account, whereas in the PCS measurements, the diameter of the mean population of particles is measured. Since the scattering of the particles is proportional to the sixth power of the particle radius this phenomenon leads to difficulties in determining the particle size of broad size distributions by PCS. The data are influenced more by the larger particles in the population and thus the mean diameter of the particle population appears to be larger than it really is. Moreover, in the PCS measurements, it is the hydrodynamic radius which incorporates the adsorbed, highly hydrated, surfactant layer that is measured.

Small angle X-ray scattering

Small angle X-ray scattering studies were performed on the samples because SAXS can be used to obtain information on the particle shape, the particle size and also the internal structure of small colloidal particles such as serum lipoproteins (Laggner, 1983). Small angle X-ray scattering curves of all systems (A–D) were recorded as illustrated in Fig. 9. Systems A–C exhibit smooth small angle X-ray scattering curves over the whole range of observation suggesting that the particles have no ordered internal structure. In addition, in sample A, where the particles were prepared

Fig. 9. Small angle X-ray scattering curves obtained from particles prepared by precipitation in an o/w emulsion. (a) 5% (w/w) CA in cyclohexane; phosphatidylcholine and sodium glycocholate, (b) 25% (w/w) CA in cyclohexane; phosphatidylcholine and sodium glycocholate, (c) 5% (w/w) CA in cyclohexane; ethoxylated sorbitan ester and (d) 25% (w/w) CA in cyclohexane; ethoxylated sorbitan ester.



from an emulsion system in which the CA concentration in cyclohexane was 5% (w/w) and the emulsifiers were PC and the sodium salt of glycocholic acid, the results indicate the presence of phospholipid bilayers with a thickness of the single bilayer of 45 Å (Fig. 10). This indicates the existence of liposomes in sample A. According to the SAXS measurements, sample B does not contain liposomes. The curve of system D deviates in that the scattering curve of this system exhibits diffraction peaks of the first and the second order (35 and 17 Å). The particle sizes in all the studied systems were too large and the distributions too broad to allow conclusions to be drawn from the SAXS data regarding the sizes and shapes of the particles.

Solvent residue

The cyclohexane residues in the suspensions seem to depend on the emulsifier used. In the system where an ethoxylated sorbitan ester was used as emulsifier, the cyclohexane content was lower, 12 ppm (calculated on the total suspension), than when a blend of phosphatidylcholine and sodium glycocholate was used in the particle preparation where the concentration of cyclohexane is 25 ppm (Table 4). This indicates that the solvent was solubilized to a greater extent in the more hydrophobic emulsifier, lecithin, than in the ethoxylated sorbitan ester. It seems that the amount of solvent in the final suspension is independent of the CA content (Table 4) which suggests that the organic solvent is distributed mainly in the emulsifier layer surrounding the particles. It should be pointed out that the experiments were not optimized to reduce the organic solvent residue.

Conclusions

In this article, it has been shown that the majority of the cholesteryl acetate particles prepared by precipitation in the dispersed phase of an o/w emulsion are amorphous and spherical and that they have a smooth surface independent of the type of emulsifier and of the CA concentration in the organic solvent of the initial emul-

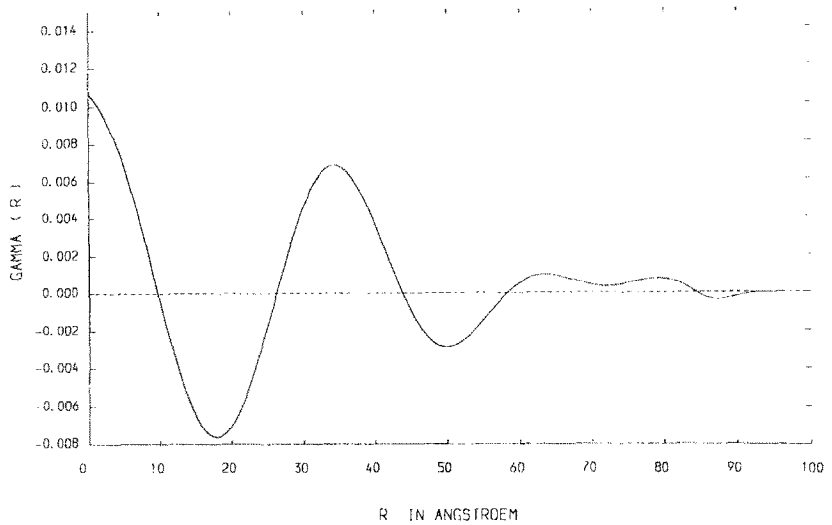


Fig. 10. The distance distribution function, $g(r)$, was derived from the scattering curve of system A (Fig. 9a) with the indirect Fourier transformation method applying the flat sheet approximation (Glatter, 1983).

sion. Moreover, according to the TEM data, no particle aggregation occurs. The method for preparation of particles is fairly reproducible in terms of the particle size achieved. The smallest particle size, 21 nm, by number was obtained using a blend of lecithin and sodium glycocholate (4:1, w/w) in the particle preparation. The melting point of cholesteryl acetate in the particulate form prepared with phosphatidylcholine and sodium glycocholate is about ten degrees Celsius lower than that of pure macroscopic cholesteryl acetate crystals. The cyclohexane residue in the suspensions is 11–25 ppm depending on the emulsifer used in the particle preparation but independent of the concentration of cholesteryl acetate.

TABLE 4

Cyclohexane residue in the suspensions (ppm)

Surfactant	5% (w/w) CA in cyclohexane	25% (w/w) CA in cyclohexane
Lecithin/sodium glycocholate	25	25
Ethoxylated sorbitan ester	11	12

It has been reported that it is possible to prepare spherical particles down to a diameter of 21 nm by number of a lipophilic compound by precipitation in an o/w emulsion. Further, this method permits a high load of drug in each entity and it also allows the particle size and surface to be varied by the use of different emulsifiers.

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