ELSEVIER

International Journal of Pharmaceutics 151 (1997) 35–45

international journal of pharmaceutics

Investigation of the gel formation of phospholipid-stabilized solid lipid nanoparticles

Kirsten Westesen [a,*], Britta Siekmann [b]

[a] *Institute for Pharmaceutical Technology, Friedrich Schiller University, Lessingstrasse 8, D-07743 Jena, Germany*
[b] *Department of Pharmaceutics, Astra Arcus AB, S-151 85 Södertälje, Sweden*

Received 5 August 1996; revised 16 January 1997; accepted 19 January 1997

Abstract

Despite the obvious similarities between colloidal lipid suspensions (solid lipid nanoparticles) and lipid o/w emulsions regarding the chemical composition and the preparation method, there are basic differences in the physicochemical behaviour of these systems. Phospholipid stabilized tripalmitate suspensions with a composition similar to commercial lipid emulsions for parenteral nutrition tend to form semi-solid ointment-like gels. Gel formation can be attributed to the recrystallization of melt-homogenized tripalmitate. As observed by transmission electron microscopy, recrystallization is associated with an increase in specific interfacial area due to the formation of anisometrical, platelet-like colloidal crystals with structured surfaces. Due to the limited mobility of phospholipid molecules in excess which form predominantly vesicles in the aqueous phase these emulsifiers are not able to immediately cover the newly created interfaces during platelet formation in an efficient way. Phospholipid molecules seem to be preferably associated with specific crystal interfaces during recrystallization causing variations in polarity and atomic/molecular order of different nanocrystal faces. Crystal interfaces with low concentrations of adsorbed emulsifier molecules represent preferred sites of particle aggregation over which gel formation can proceed. Gel formation can be prevented by the addition of co-emulsifying agents to the aqueous phase provided the concentration of co-surfactant is sufficiently high to constitute a reservoir of molecules immediately available for interfacial stabilization during recrystallization. Moreover, the co-emulsifier should preferably adsorb on crystal interfaces not or only incompletely covered by phospholipids. © 1997 Elsevier Science B.V.

Keywords: Solid lipid nanoparticles; Lipid emulsions; Parenteral drug carrier systems; Gel formation; Crystallization in dispersion

1. Introduction

In the course of the successful clinical introduction of submicron-sized oil-in-water (o/w) emul-

* Corresponding author.

PII S0378-5173(97)04890-4

sions for parenteral nutrition (Wretlind, 1981) and as colloidal drug carrier systems for poorly water soluble substances (Collins-Gold et al., 1990; Prankerd and Stella, 1990) two decades ago, first attempts were made to transfer the concept of these lipid emulsions to the preparation of colloidal lipid suspensions as an alternative parenteral drug delivery system (personal communications). According to the IUPAC definition a colloidal suspension consists of solid particles of colloidal dimensions dispersed in a liquid continuous medium (Everett, 1972). Colloidal lipid suspensions thus represent aqueous dispersions of lipids solid at room temperature. They can be prepared by emulsification of a molten lipid in an aqueous medium creating an oil-in-water emulsion in the heat which is supposed to transform into an aqueous colloidal suspension by recrystallization of the dispersed lipids. These systems have often been regarded as o/w emulsions with solid globules.

There are some advantages that can be theoretically deduced from the solid state of the dispersed lipid phase compared to the liquid (emulsions) or liquid crystalline state (liposomes, cubosomes) of lipidic carriers, such as improved physical stability, avoidance of drug leakage and sustained drug release. Due to these potential advantages colloidal lipid suspensions have found interest as an alternative colloidal drug carrier system. The lipid suspension concept could, however, not be realized until the beginning of the nineties. To our knowledge, there are no reports in the scientific literature on the preparation of colloidal lipid suspensions with an acceptable long-term stability dated from before 1992 when Siekmann and Westesen (1992) reported on 12 months stability data for solid lipid nanoparticles of optimized chemical compositions. Although not explicitly stated in the literature, it is known to the authors that numerous attempts to prepare colloidal triglyceride suspensions failed due to stability problems. In the late 1980s and the beginning of the 1990s, however, several patent applications on (colloidal) aqueous dispersions of lipids solid at room temperature prepared by a melt homogenization process were published (Speiser, 1990; Domb and Maniar, 1991; Müller and Lucks, 1993). Whereas the patent by Speiser (1990) and the patent application by Müller and Lucks (1993) do not contain any stability data, Domb and Maniar (1991) considered a 7 days stability of their aqueous lipid dispersions as 'exceptionally stable'. Kecht-Wyrsch (1987) who studied compositions described in Speiser's patent (Speiser, 1990) observed a storage stability of only 3 months for highly diluted suspensions with a lipid concentration ranging from 0.2 to 1 w%. Despite obvious similarities between colloidal lipid suspensions and o/w emulsions regarding the preparation method and the chemical composition, the instability of colloidal triglyceride suspensions containing the same type and concentration of emulsifiers as comparable o/w emulsions (Siekmann and Westesen, 1992) indicate that there are basic physicochemical differences between colloidal lipid emulsions and suspensions.

Our previous studies revealed that the gel formation can be circumvented by a proper choice of emulsifier blends, their concentrations and process parameters. Satisfactory long-term stability can be obtained by using the ionic surfactant sodium glycocholate (Siekmann and Westesen, 1992; Westesen et al., 1993) or the non-ionic polymer tyloxapol (Siekmann and Westesen, 1994a,b) as co-surfactants in combination with lecithin.

The aims of the present study were to compare the physicochemical properties of colloidal o/w emulsions and solid tripalmitate nanoparticles and to develop a model describing the instability phenomena observed for these colloidal lipid suspensions with similar compositions as o/w emulsions.

2. Materials and methods

2.1. Materials

Tripalmitate Dynasan 116 (Hüls AG); soya lecithins Lipoid S75 and S100, egg lecithin Lipoid E 80 (Lipoid KG); sodium glycocholate (Sigma); Pluronic® F127 (BASF); Tetronic® 908 (BASF); tyloxapol (Eastman Kodak); glycerol 85% Ph. Eur. III (Chemie-Vertrieb CVH); thiomersal (Synopharm); bidistilled water.

2.2. Preparation of carrier systems

Tripalmitate was heated to approximately 80°C. The phospholipid was dispersed in the melt by probe sonication (MSE Soniprep 150) until the dispersion appeared optically clear. Optionally, the co-emulsifying agent was dissolved in the aqueous phase. After addition of the heated aqueous phase to the melt a crude emulsion was produced by probe sonication for approximately 3 min. The crude emulsion was transferred to a thermostatized high pressure homogenizer (APV Gaulin Micron Lab 40). The number of homogenization cycles and the homogenization pressure are specified in the text. The homogenized samples were allowed to stand at room temperature for cooling.

All preparations contained 2.25% glycerol for isotonicity and 0.01% thiomersal as a preservative.

2.3. Photon correlation spectroscopy (PCS)

Particle size measurements were performed on a Zetasizer 3 (Malvern Instruments) at 90°. Samples were diluted with dust-free water to a scattering intensity of approximately 100 000 cps as recommended by the manufacturer. The autocorrelation function was transformed into the size distribution using cumulant analysis and exponential sampling method (McNeil-Watson and Parker, 1991). Mie correction was not applied. The mean particle size was approximated from the linearized number distribution as the diameter of the equivalent hydrodynamic sphere. The values are the mean of five measurements of 120 s each divided into 10 sub-runs.

2.4. Transmission electron microscopy (TEM) of freeze-fractured replica

Samples were freeze-fractured at 173 K in a BAF 400 (Balzers AG, CH-Liechtenstein). Fast freezing was accomplished by slush into melting propane or by jet freezing in propane (Balzers JFD 030). Shadowing of the samples was performed with platinum/carbon (layer thickness: 2 nm) at 45° and with pure carbon at 90° for replica preparation. Replica were cleaned with a 1:1 (v/v) chloroform/ethanol mixture. Replica on uncoated grids were viewed with an electron microscope EM 300 (Philips, D-Kassel).

2.5. Cryo-transmission electron microscopy of frozen-hydrated specimen

A few μl of the dispersion were placed on a hydrophilic copper grid. The sample was cryofixed on the copper grid by shooting into liquid ethane. The frozen-hydrated specimen was transferred to the cryo-chamber of an energy-filtered transmission electron microscope EM 902A (Zeiss, D-Oberkochen) and was viewed using the low dose technique.

2.6. Zetapotential measurements

Samples were dispersed in phosphate buffer pH 6.88 diluted 1:25 with bidistilled water. The electrophoretic mobility was determined by laser Doppler anemometry in the microelectrophoresis cell AZ4 of a Zetasizer 3 (Malvern Instruments). The instrument was operated in cross beam mode at a modulation frequency of 1000 Hz and an applied voltage of 150 V. Zetapotentials were approximated using the Smoluchowski equation.

3. Results and discussion

3.1. Gel formation of melt-homogenized tripalmitate dispersions

Aqueous dispersions of tripalmitate have been prepared by melt homogenization using different commercially available lecithin mixtures (Table 1). Hot tripalmitate emulsions containing exclusively the phosphatidylcholine rich soya lecithin product Lipoid S100 (S100) as an emulsifier became semi-solid already on cooling whereas dispersions stabilized by the egg lecithin mixture Lipoid E80 formed gels within only several hours after preparation (Table 2). The Lipoid S75 (S75) stabilized tripalmitate dispersions exhibited fast and considerable particle growth on cooling of the colloidally dispersed tripalmitate (Table 2). These

Table 1
Composition and source of commercially available lecithins according to manufacturer's specification

Components[a]	Lipoid S100	Lipoid S75[b]	Lipoid E80
Phosphatidylcholine	min. 94.0	66.0–70.0	80.0–85.0
Phosphatidylethanolamine	n. sp.	7.0–10.0	7.0–9.5
N-Acyl-phosphatidylethanolamine	max. 1.0	n. sp.	n. sp.
Phosphatidylinositol	max. 0.1	max. 0.5	n. sp.
Lysophospholipids	max. 3.0	max. 3.5	max. 3.5
Triglycerides	max. 2.0	max. 3.0	max. 3.0
Free fatty acids	max. 0.5	max. 0.5	max. 0.05
Sphingomyelin	n. sp.	n. sp.	2.0–3.0
Cholesterol	n. sp.	n. sp.	max. 1.5
DL-α-Tocopherol	0.15–0.25	0.1–0.2	0.05–0.1
Source	Soya	Soya	Egg

n. sp. = not specified
[a] Concentration are given in % (by weight).
[b] According to information from the manufacturer Lipoid S75 contains 10–15% (by weight) glycolipids which are not specified.

dispersions always contained significant concentrations of microscopically and macroscopically observable tripalmitate aggregates directly after cooling, which would exclude their intravenous administration and point to a poor long-term stability. A high tendency to spontaneous gel formation was observed under shear stress such as passage through the tight needle of a syringe.

Table 2
Comparison of differently composed phospholipid dispersions[a]

Composition[b]	Size[c]	Macroscopic observations
10% TP, 2.4% E80	n. d.	Gel formation after 2 h
10% TP, 2.4% S75	146 nm	Particle growth; gel formation under shear
10% TP, 2.4% S100	n. d.	Gel formation during cooling
10% TP, 2.4% S100, 0.6% SGC	136 nm	Fluid dispersion, stable on storage
10% TP, 2.0% S100, 2.0% Tyl	135 nm	Fluid dispersion, stable on storage

TP, tripalmitate; E80, Lipoid E80; S75, Lipoid S75; S100, Lipoid S100; SGC, sodium glycocholate; Tyl, tyloxapol.
n. d., not determined.
[a] The dispersions were homogenized 5 times at 800 bar.
[b] In % (by weight), difference to 100% is bidistilled water containing 2.25% glycerol and 0.01% thiomersal.
[c] Mean particle size determined by PCS.

Transformation of the crude S75 stabilized tripalmitate suspensions into semi-solid products is obviously retarded but not prevented. The less pronounced gelation tendency of the S75 stabilized systems compared to those stabilized by S100 or E80 may be explained by an improved but still not sufficient steric or electrostatic stabilization caused by the minor components of the cruder lecithin mixtures, such as glycolipids. According to the manufacturer of S75, the lecithin may contain up to 15 w% glycolipids. In contrast to tripalmitate suspensions, soy bean oil-in-water emulsions stabilized by E80, S75 or S100 did neither exhibit comparably fast particle growth nor gel formation independently of the phospholipid mixture used as emulsifier.

Gel formation was generally not observed with phospholipid stabilized tripalmitate dispersions stored at temperatures above the melting temperature of the triglyceride indicating that gel formation does not occur as long as the systems exist in the emulsion state. Tripalmitate suspensions stabilized by mixtures of the insufficient stabilizer S100 with the ionic surfactant glycocholate or the nonionic surfactant tyloxapol did, however, not contain observable amounts of microscopically and macroscopically detectable tripalmitate aggregates and retained their colloidal state (Table 2) as has been observed earlier (Siekmann and Westesen, 1992, 1994a,b,c). Moreover, gel formation of S100

Table 3
Zetapotential of tripalmitate nanoparticles after adsorption of block copolymers[a]

Composition (% w/w)			Zetapotential (mV)
TP	PL	Polymer	
5%	2%	–	−29.6
5%	2%	3% F127	−1.9
5%	2%	3% T908	−2.9

TP, tripalmitate; PL, phospholipids; F127, Pluronic F127; T908, Tetronic 908.
[a] The dispersions were homogenized 5 times at 1200 bar, followed by overnight incubation with block copolymer. The dispersion with phospholipids only was stored at 80°C prior to zetapotential measurement in order to prevent gel formation.

stabilized tripalmitate dispersions could be prevented by subsequent adsorption of polyoxyethylene-polyoxypropylene block copolymers of the poloxamer and poloxamine type. Polymer adsorption was verified by zetapotential measurements (Table 3).

3.2. Electron microscopy of tripalmitate suspensions

Electron microscopic pictures of the replica of freeze fractured specimen display platelet-like particles independent of the emulsifier composition (Fig. 1). Due to the highly anisometrical shape of the tripalmitate nanoparticles the transformation of the spherical emulsion droplets of the tripalmitate melt into solid tripalmitate platelets is accompanied by a significant increase in the particle surface area. Moreover, the molecular organization of the tripalmitate molecules can be derived from electron microscopic pictures. The tripalmitate molecules form a lamellar structure with an orientation of the layers rectangular to the platelet normal. The thickness of single molecular layers is about 4 nm. This observation is in accordance with X-ray data which demonstrate that the resolidified tripalmitate particles are in the β-crystalline state (Westesen et al., 1993). Often the platelet-like suspension particles exhibit a thickness of less than 50 nm, that means only several molecular layers. In addition, spherical particles can be observed which were identified as small unilamellar vesicles by their low fracture tendency.

The occurrence of small unilamellar vesicles representing the excess of phospholipids in the system was also verified by cryo-electron microscopy (Fig. 2). The phospholipid bilayers of small unilamellar vesicles exhibit a high electron density resulting in a high contrast against the aqueous phase. The ring shaped structures in the cryo-preparations represent the concentric shell structures of phospholipid vesicles. Moreover, the photographs taken from cryo-preparations support the interpretation of the pictures taken from replica of freeze fractured specimen with respect to the shape of the suspension particles. Anisometrical shapes with low contrast dominate pointing to their low thickness and thereby to their platelet-like nature (Fig. 2). In addition, needle-like structures were found (Fig. 2) and their darkness is in accordance with the expected high contrast of a low contrast platelet viewed perpendicular to the platelet normal.

3.3. Electron microscopy of tripalmitate gels

Electron microscopy studies of freeze fractured specimen gave insight into the structure and characteristics of the phospholipid stabilized tripalmitate systems which became semi-solid after melt-homogenization. The semi-solid systems immobilize the complete aqueous phase of the compositions which amounts to more than 80 w%. The immobilization of such a high portion of aqueous media points to the existence of a gel, that means the occurrence of a superfine three-dimensional network interpenetrated by the aqueous phase.

The semi-solid tripalmitate samples represent 2-phase systems according to the phase definition by Gibbs since the thickness of the cross fractured network corresponds to the thickness of at least several triglyceride layers (Fig. 3). The thickness of the cross fractured network is in the order of the thickness of suspension particles. No significant numbers of small vesicles were found in the gels pointing to the contribution of the phospholipids to the network structure. Fig. 4 shows a replica of a freeze fractured gel-specimen where

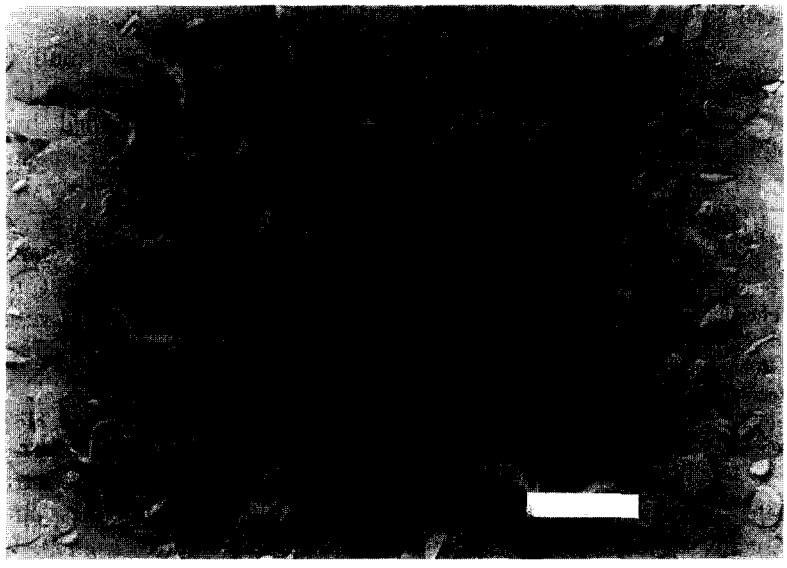

Fig. 1. Transmission electron micrograph of a 10% tripalmitate dispersion stabilized by 1.2% S100 and 0.4% sodium glycocholate (10 times homogenized at 800 bar). The bar indicates 300 nm.

the fracture of the sample runs parallel to a plane of the network. The network lamellas are arranged parallel to the areas of immobilized aqueous media characterized by their amorphous appearance. The network seems to consist of sintered particles which have been sintered predominantly via the platelet heights. The surfaces of the network must have extremely low interfacial tensions in contact with the immobilized aqueous media because of its extremely high specific surface area in combination with a high macroscopic long-term stability of the tripalmitate gels. They do not show any significant syneresis on storage for several months. It can be deduced, therefore, that the gels do not contract significantly on storage. Gel contraction would correspond to additional crystallization or the formation of additional contact points on ageing. Obviously the gel structure does not change significantly on storage.

3.4. Structure information derived from electron microscopy

The differences between colloidal lipid emulsions and suspensions of solid tripalmitate nanoparticles can be explained by the presented results considering the following aspects. Recrystallization of the colloidally dispersed tripalmitate in the β-modification is accompanied by a tremendous increase in the surface area of the particles due to the formation of platelet-like colloidal crystals with surfaces being structured by kinks, edges and steps as could be seen on TEM micrographs of freeze fractured specimen. Model calculations demonstrate that the increase in specific surface area can easily exceed 100% (Fig. 5) since the change of the particle shape is not only due to changes in the overall geometrical shape of the particles (model particle II) but is multiplied by the formation of surface structures such as the two steps of the model particle III. The emulsion droplets are stabilized by a monomolecular layer of phospholipid molecules, which can be deduced from the chosen production parameters, and the excess of phospholipid molecules can be expected to form vesicles (Westesen and Wehler, 1992). The change in particle shape during recrystallization results in a sudden demand for additional emulsifier molecules in the particle surfaces. Formation of a network via the platelet heights reduces the number of surfaces from six for the isolated platelets to only two in the network.

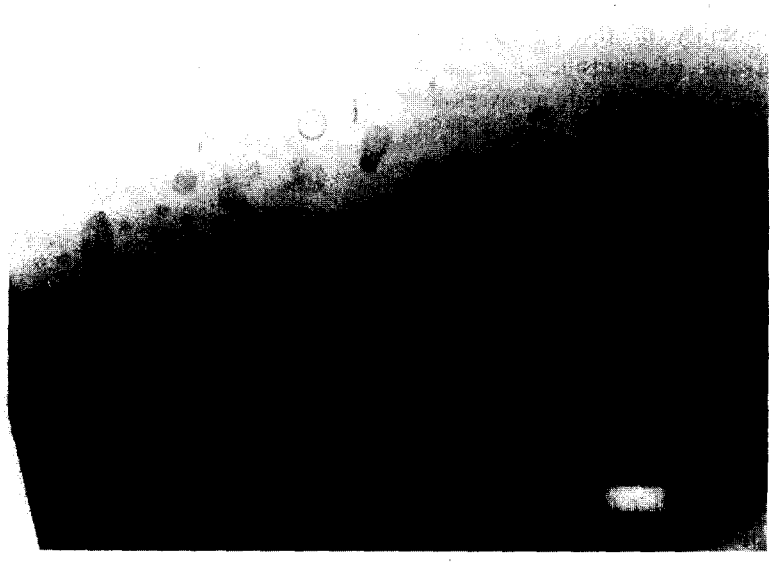

Fig. 2. Cryo-electron microscopic picture of a frozen-hydrated 10% tripalmitate dispersion stabilized by 2.4% S100 and 0.4% sodium glycocholate (10 times homogenized at 800 bar). The bar indicates 110 nm.

Comparison of the characteristics of a triglyceride single crystal (Skoda et al., 1967) and the TEM micrographs of freeze fractured specimen of the colloidal tripalmitate crystals indicates that the platelet planes probably correspond to the (001)-face, the plane of the methyl end groups, and represent the most hydrophobic surfaces of a colloidal tripalmitate crystal provided these surfaces are not covered by amphiphilic molecules. The gels contain, however, size-maximized platelet planes pointing to neglectable interfacial tensions of these planes. From its preferential formation in an aqueous medium it can be deduced that the (001)-face of the nanocrystals is hydrophilic. This is confirmed by decoration of the fractures with water which condenses preferably on the huge (001)-face (Fig. 6). The detected hydrophilicity of the (001)-face points to the association of surfactant molecules with these crystal planes. Obviously, phospholipids are preferably adsorbed or incorporated in these crystal planes resulting in a sufficient stabilization. The phospholipids originate on the one hand from the initial tripalmitate emulsion droplets in the heat, and on the other from the small vesicles representing the excess of emulsifier in the initial emulsion since vesicles could not be detected in the cavities of the three dimensional networks filled with the immobilized aqueous media. Considering the properties of triglyceride single crystals and the diversity in polarity and atomic as well as molecular order of different crystal faces, preferred sites of aggregation can be derived. These are the planes oriented perpendicular to the platelet plane as supported by TEM observations (Figs. 3, 4 and 6).

3.5. Theoretical considerations

The phospholipids used in the preparations under investigation are neither soluble in the continuous phase, nor do they form highly dynamic micelles. Those phospholipid molecules representing the excess emulsifier during the homogenization process form small, predominantly unilamellar vesicles under the chosen production conditions (Fig. 2). Phospholipid molecules bound to vesicles exhibit, however, only a limited mobility. Therefore, they are not able to immediately cover the newly created interfaces during recrystallization. It has to be considered that during the recrystallization process there is no external energy input promoting disruption of the

bilayers and reducing the diffusional pathways. Due to the low mobility of the phospholipid molecules a sudden lack of emulsifier is created locally on the particle surfaces despite an excess of emulsifier in the overall system.

The admixture of an ionic co-emulsifier, e.g. glycocholate (Siekmann and Westesen, 1992), as well as the admixture of a non-ionic polymer, e.g. tyloxapol (Siekmann and Westesen, 1994a,b), resulted in a stabilization of the colloidally dispersed state of the recrystallizing tripalmitate. The common property of both amphiphiles is their ability to form micelles and to exhibit a significant solubility in aqueous media. Dissolved surfactant and polymer molecules are able to diffuse to the particle surfaces in a much shorter time than vesicles diffuse. Moreover, micelles, in contrast to phospholipid vesicles, represent highly dynamic colloidal structures and may serve as reservoirs for mobile surface active molecules required for the immediate coverage of unprotected particle surfaces created during the recrystallization process.

Fig. 3. Transmission electron micrograph of a 10% tripalmitate gel stabilized by 1.2% S100 only (10 times homogenized at 800 bar), cross-layer section. The bar indicates 250 nm.

Fig. 4. Transmission electron micrograph of a 10% tripalmitate gel stabilized by 1.2% S100 only (10 times homogenized at 800 bar), plane-parallel layer. The bar indicates 250 nm.

3.6. Model of the gel formation of tripalmitate suspensions

Based on the experimental results and theoretical considerations on crystal structure and molecular mobility a model as depicted in Fig. 7 has

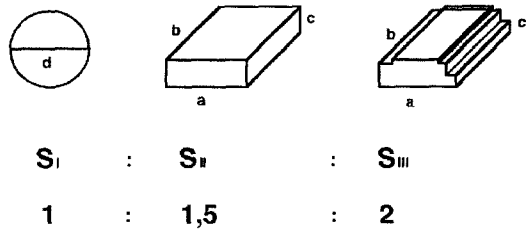

Fig. 5. Model calculations illustrating the increase in particle surface area (S) upon platelet formation. a, 120 nm; b, 180 nm; c, 42 nm; d, 120 nm.

Fig. 6. Transmission electron micrograph of a 10% tripalmitate gel stabilized by 1.2% S100 only (10 times homogenized at 800 bar). The fractures were decorated with water. The bar indicates 230 nm.

been developed to describe the instability phenomena of colloidal tripalmitate suspensions. The recrystallization of the colloidally emulsified tripalmitate results in an increase in particle surface area due to the change in the overall particle shape and in a sudden local lack of emulsifier on the particle surfaces. The time t_1 corresponds to the time which the recrystallization process takes. During the emulsification process the excess of phospholipids has formed small vesicles which are randomly distributed in the continuous phase. The time t_2 corresponds to the time which the phospholipid vesicles would need to diffuse to the newly created insufficiently stabilized particle surfaces. The time t_2 is, however, significantly longer than the time t_1 resulting in an instability of the dispersed state and gelation via the lateral faces of the crystals.

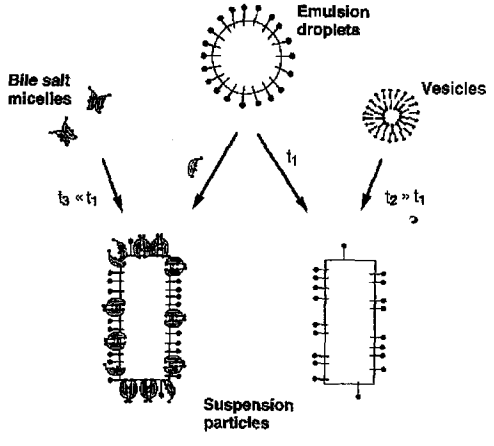

Fig. 7. Model describing the instability phenomena of colloidal tripalmitate suspensions.

In contrast, the dispersed state can be stabilized by an admixture of a soluble, micelle-forming component, since the soluble, micelle-forming component is able to cover the newly created surfaces in a time t_3 which is significantly shorter than the time t_2 for the phospholipid molecules and which is short enough that no gelation of the dispersed particles can occur. An additional effect might be that co-surfactants such as glycocholate destabilize the phospholipid vesicles and thus facilitate the disruption of the bilayers thereby reducing the time t_2. Furthermore, it has to be considered that phospholipids and co-surfactants adsorb preferably to different nanocrystal faces.

From the TEM micrographs it could be derived that phospholipids are preferentially adsorbed to the (001)-face so that the lateral faces are insufficiently stabilized, and aggregation can occur via these faces. Moreover, it seems likely that phospholipids, in contrast to the hydrophilic co-surfactants, are at least partially incorporated into the tripalmitate crystal lattice due to similarities in molecular geometry. Particle aggregation and sintering can thus proceed over adsorbed phospholipid molecules due to the similar molecular geometries, but is interrupted where co-surfactants are adsorbed to the nanocrystal surface.

4. Conclusions

Whereas S100-stabilized soy bean oil-in-water emulsions generally display a reasonable stability, the corresponding melt-homogenized tripalmitate suspensions exhibit fast gelation already on cooling of the hot tripalmitate-in-water emulsions indicating that such colloidal tripalmitate suspensions should not simply be regarded as colloidal lipid emulsions with solidified droplets. Complex additional stability aspects which originate from the crystalline nature, the crystallization kinetics and the polymorphism of the dispersed lipid have to be taken into consideration. Due to the underlying kinetics of the suspensions (surface formation, emulsifier mobility) steric and electrostatic stabilization do not give the same stabilizing effects generally obtained with emulsion systems. The stability of solid lipid based dispersions is closely related to the phase transitions of the dispersed phase and is affected, e.g. by the recrystallization tendency of the dispersed lipid (Bunjes et al., 1996). The observed long-term stability of melt-homogenized, phospholipid stabilized trilaurate dispersions recently described by Schwarz et al. (1994) can probably be attributed to the absence of recrystallization. As has been demonstrated by Westesen and Bunjes (1995) trilaurate dispersions remain in the state of a supercooled melt, even when stored at refrigerator temperature, and therefore represent emulsions according to the IUPAC definition (Everett, 1972).

Gel formation of colloidal tripalmitate suspensions can be retarded to a certain extent by the use of phospholipid mixtures containing significant amounts of glycolipids such as Lipoid S75, but the resulting lipid suspensions exhibit a considerable particle growth on storage. Sufficiently stable colloidal suspensions can, however, be reproducibly prepared by a proper choice of emulsifier blends containing lecithin and a micelle-forming co-surfactant with high molecular mobility in the aqueous phase.

Acknowledgements

Most of this work was performed at the Institute of Pharmaceutical Technology, Technical University of Braunschweig, Germany. The authors would like to thank Dr. Sierralta, Max Plank Institute of Experimental Endocrinology, and Mr. Zellmann, Carl Zeiss AG, Oberkochen, for their support in the cryo-transmission electron microscopy studies, the Institute of Botanics, Technical University of Braunschweig, Germany, for the use of the transmission electron microscope and Pharmacia Oncology/Immunology, Helsingborg, Sweden, for financial support.

References

Bunjes, H., Westesen, K., Koch, M.H.J., Recrystallization tendency and polymorphic transitions in triglyceride nanoparticles. *Int. J. Pharm.*, 129 (1996) 159–173.

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

Domb, A. and Maniar, M., Lipospheres for controlled delivery of substances. *International Patent Application* WO 91/07171, 1991.

Everett, D.H., Report of the Commission on colloid and surface chemistry of the physical chemistry division. *Pure Appl. Chem.*, 31 (1972) 577–638.

Kecht-Wyrsch, P., Hochdisperse Glycerid-Mikropartikel als perorales Arzneiträgersystem, Ph.D. Thesis, Eidgenössische Technische Hochschule Zürich, 1987.

McNeil-Watson, F. and Parker, A., Comparison of methods for high resolution sub-micron sizing by quasi-elastic light scattering (PCS). In Williams, R.A. and de Jaeger, N.C. (Eds.), *Advances in Measurement and Control of Colloidal Processes*, Butterworth-Heinemann, Oxford, 1991, pp. 421–434.

Müller, R.H. and Lucks, J.S., Arzneistoffträger aus festen Lipidteilchen—Feste Lipid Nanosphären (SLN). *German Patent Application* DE 4131562, published 1993.

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

Schwarz, C., Mehnert, W., Lucks, J.S. and Müller, R.H., Solid lipid nanoparticles (SLN) for controlled drug delivery. I. Production, characterization and sterilization. *J. Control. Release*, 30 (1994) 83–96.

Siekmann, B. and Westesen, K., Submicron-sized parenteral carrier systems based on solid lipids. *Pharm. Pharmacol. Lett.* 1 (1992) 123–126.

Siekmann, B. and Westesen, K., Melt-homogenized solid lipid nanoparticles stabilized by the nonionic surfactant tyloxapol. I. Preparation and particle size determination. *Pharm. Pharmacol. Lett.*, 3 (1994a) 194–197.

Siekmann, B. and Westesen, K., Melt-homogenized solid lipid nanoparticles stabilized by the nonionic surfactant tyloxapol. II. Physicochemical characterization and lyophilisation. *Pharm. Pharmacol. Lett.*, 3 (1994b) 225–228.

Siekmann, B. and Westesen, K., Thermoanalysis of the recrystallization process of melt-homogenized glyceride nanoparticles. *Colloids Surf. B: Biointerfaces*, 3 (1994c) 159–175.

Skoda, W., Hoekstra, L.L., van Soest, T.C., Bennema, P., van den Tempel, M., Structure and morphology of β-crystals of glyceryl tristearate. *Kolloid Zeitschrift Zeitschrift Polym.*, 219 (1967) 149–156.

Speiser, P., Lipidnanopellets als Trägersystem für Arzneimittel zur peroralen Anwendung. *European Patent* EP 0167825, 1990.

Westesen, K. and Wehler, T., Physicochemical characterization of a model intravenous oil-in-water emulsion. *J. Pharm. Sci.*, 81 (1992) 777–786.

Westesen, K. and Bunjes, H., Do nanoparticles prepared from lipids solid at room temperature always possess a solid lipid matrix? *Int. J. Pharm.*, 115 (1995) 129–131.

Westesen, K., Siekmann, B. and Koch, M.H.J., Investigations on the physical state of lipid nanoparticles by synchrotron radiation X-ray diffraction. *Int. J. Pharm.*, 93 (1993) 189–199.

Wretlind, A., Development of fat emulsions. *J. Parenter. Enter. Nutr.*, 5 (1981) 230–235.