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Investigations on the physical state of lipid nanoparticles by synchrotron radiation X-ray diffraction

Kirsten Westesen [a], Britta Siekmann [a] and Michel H.J. Koch [b]

[a] *Institute of Pharmaceutical Technology, Technical University of Braunschweig, Mendelssohnstrasse 1, D-3300 Braunschweig (Germany) and* [b] *Hamburg Outstation, European Molecular Biology Laboratory, c/o DESY, Notkestrasse 85, D-2000 Hamburg 52 (Germany)*

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Summary

Submicron-sized parenteral drug carrier systems based on lipids solid at room temperature in the bulk phase were prepared by high pressure homogenization of an emulsion of molten lipids in an aqueous phase. Phospholipids, bile salts and block copolymers of polyoxyethylene and polyoxypropylene were used as emulsifiers. The mean diameters obtained under standardized homogenization conditions determined by photon correlation spectroscopy lie between 70 and 300 nm, and depend on the amount and type of emulsifier. The present study describes the results of an investigation on the physical state, the melting behaviour and the recrystallization of the lipids in these colloidal carrier systems using X-ray diffraction. Debye-Scherrer diffraction patterns were first obtained on a conventional X-ray source, but the long exposure times (8–24 h) precluded a systematic investigation. Therefore, simultaneous small and wide angle diffraction measurements were made using synchrotron radiation with exposure times around 3 min. All investigated carrier systems were in the β-crystalline modification at room temperature. At body temperature the carriers were either β-crystalline or amorphous liquids depending on the matrix constituent. Incorporation of coenzyme Q_{10} into the lipid particles did not significantly alter the recrystallization tendency of the nanoparticles. Time-resolved X-ray diffraction measurements during temperature scans demonstrated that the recrystallization of the lipid nanodispersions is different from that of bulk material. After heating above the bulk melting temperature and cooling down under controlled conditions the dispersed lipids recrystallize in the α-form whereas the bulk lipids recrystallize in the β'-modification and transform rapidly into the β-form.

Introduction

Several approaches have been investigated to develop submicron-sized delivery systems for parenteral administration of drugs that are insufficiently water soluble to be administered as simple aqueous solutions, as well as for use as drug targeting devices (Douglas et al., 1987). The conventional vehicles used as drug carriers are polymeric nanoparticles and microspheres, liposomes and lipid emulsions (Gupta, 1990). A number of disadvantages are, however, associated with the use of these systems for parenteral administration of hydrophobic drugs.

Polymeric nanoparticles and microspheres are

Correspondence to: K. Westesen, Institute of Pharmaceutical Technology, Technical University of Braunschweig, Mendelssohnstrasse 1, D-3300 Braunschweig, Germany.

prepared by emulsion polymerization and solvent evaporation techniques so that the risk of residual contaminations from the production process such as organic solvents, toxic monomers and toxic degradation products (Kante et al., 1982) cannot be eliminated.

In contrast, lipid-based carriers consist of physiological components only. Lipid systems, however, often display physicochemical instabilities. Liposomes, for instance, tend to fuse and are therefore relatively unstable on storage (Frøkjær et al., 1982). Due to lipid exchange with lipoproteins they are often also unstable in the vascular system (Allen, 1981). The reproducibility of liposomes in terms of vesicle size and properties is poor thus complicating large scale production.

Drug-free lipid emulsions of the oil-in-water type which are generally applied as calory source in parenteral nutrition have an acceptable long-term stability. These colloidal systems are, however, susceptible to the incorporation of drugs which can be attributed to drug crystallization within the oil droplets and perturbations of the stabilizing emulsifier layer by diffusing drug molecules or drug crystals.

An additional problem found with lipid systems is drug leakage, i.e., the loss of drug from the carrier on storage as well as in body fluids.

In order to reduce or circumvent the drawbacks of conventional drug delivery systems, a new type of submicron-sized drug carriers was developed (Siekmann and Westesen, 1992a). These systems contain physiologically tolerable components only and are based on lipids, predominantly glycerides, that are crystalline at room temperature as matrix constituents. The submicron-sized carrier systems are prepared by emulsification of the molten lipids in an aqueous phase.

The idea behind the use of lipids that are solid at room temperature as a carrier matrix is that the emulsified molten lipids resolidify on cooling creating solid particles. These rigid solid particles are expected to be stable against coalescence. The solid lipid matrix should reduce the mobility of incorporated drugs and thus prevent drug leakage from the carrier.

The aim of the present investigation was to determine the physical state of the matrix in solid-lipid based carrier systems. Although the particles were produced from crystalline raw materials, the presence of emulsifiers (Garti et al., 1982), the preparative method and the high dispersity as well as the small particle size (Skoda and Van den Tempel, 1963) of the resulting systems may account for changes in the crystallization behaviour, the degree of crystallinity, and the crystal modifications of the matrix constituents compared to the bulk materials. This may lead to liquid, amorphous or only partially crystallized metastable systems.

Preliminary investigations on the physicochemical structure of the lipid nanoparticles were performed by transmission electron microscopy studies of freeze fractured specimen and by thermoanalytical methods (Siekmann and Westesen, 1992b). Since the results of the latter methods were in some cases difficult to interpret, X-ray diffraction was used as a more direct method allowing to distinguish between the various lipid polymorphs. These different polymorphic forms can be unambigiously characterized by their spacings (Hoerr and Paulicka, 1968).

The carrier systems investigated here have a relatively low concentration and contain approx. 10% lipid (w/w) dispersed as nanoparticles in aqueous media. Hence, only broad low intensity diffraction peaks characteristic of poorly ordered systems are obtained. Moreover, to compare the recrystallization of the lipid nanodispersions with that of the bulk material it is necessary to measure time-resolved X-ray diffraction during temperature scans. As illustrated below, synchrotron radiation X-ray diffraction provides an efficient way of performing such measurements.

Materials and Methods

Materials

The following materials were obtained from the indicated sources: Witepsol® W35 (Hüls AG); tripalmitin (Fluka); tripalmitin puriss. (Fluka); glycerol monostearate Ph. Eur. III (Goldschmidt); coenzyme Q_{10} (Kabi Invent AB); soya lecithin Lipoid S100 (Lipoid KG); sodium glycocholate (Sigma); Pluronic® PE 6800 (BASF); glycerol 85%

Ph. Eur. III (Chemie-Vertrieb CVH); thiomersal (Synopharm); bidistilled water.

Preparation of carrier systems

The glyceride was heated to approx. 10°C above its melting point. In the case of drug-loaded carriers, the drug was dissolved in the melt. The phospholipid was dispersed in the melt by probe sonication (MSE Soniprep 150) until the dispersion appeared optically clear. After addition of the heated aqueous phase a crude emulsion was produced by probe sonication for approx. 5 min. The crude emulsion was transferred to a thermostated high-pressure homogenizer (APV Gaulin Micron Lab 40) and emulsified at a homogenization pressure of 800 bar. Each dispersion was passed through the homogenizer for 10 cycles. The homogenized samples were allowed to stand at room temperature for cooling.

All preparations contained 2.25% glycerol to achieve blood isotony. For the present study the systems were not sterilized. Instead 0.01% thiomersal was added as a preservative.

Photon correlation spectroscopy

Dynamic light scattering 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 approx. 100000 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 calculated from the 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 subruns.

Wide angle X-ray diffraction (Debye-Scherrer)

Samples were filled into X-ray capillaries (Glas) and placed in a Debye-Scherrer camera (Debye and Scherrer, 1916) with a perimeter of 360 mm installed on a Philips PW 1730 X-ray generator equipped with a Philips PW 2253/11 copper anode X-ray tube and a Nickel K_β filter. The exposure time was varied between 8 and 24 h using Fuji 100 X-ray films.

Synchrotron radiation X-ray diffraction

Measurements were performed on the double focussing monochromator mirror camera X33 (Koch and Bordas, 1983) in HASYLAB on the storage ring DORIS of the Deutsches Elektronen Synchrotron (DESY) using quadrant or linear delay line readout detectors (Gabriel and Dauvergne, 1982) and the standard data acquisition and evaluation systems (Boulin et al., 1986, 1988). The observation range was $0.02 < s < 0.4$ nm^{-1} for the small angle range and $1.7 < s < 2.8$ nm^{-1} for the wide angle range, where $s = 2 \sin \vartheta/\lambda$, 2ϑ is the scattering angle and λ the wavelength (0.15 nm).

For simultaneous small and wide angle measurements two linear delay line detectors were connected in series.

The samples were filled in thermostatized cells. The patterns of the lipid dispersions and the corresponding dispersion media were measured in separate 1 min time frames to monitor radiation damage and beam stability. Data reduction, background subtraction and correction for detector response were done following standard procedures (see e.g. Koch, 1991) using the program SAPOKO (Svergun and Koch, unpublished).

For time-resolved measurements samples were molten and cooled down in steps of 5°C from the melting point to +5°C in a thermostated sample holder. At each step the diffraction pattern of the sample was recorded for 180 s.

Results

Preparation and particle size determination

In the present studies two series of lipid carrier systems were prepared, the first one comprising a number of empty carriers with various glycerides as matrix constituents and different emulsifiers. The compositions and the mean particle size determined by photon correlation spectroscopy (PCS) are presented in Table 1.

Systems containing the suppositoria mass Witepsol® W35 as the lipid component tended to

TABLE 1

Composition and mean particle size of drug-free carrier systems

System	Matrix component	Composition (% g/g)				Mean particle size ± SD (nm)
		Matrix	PL	SGC	F68	
A.1	W35	10	1.2	0.4	–	92 ± 4.0
A.2	W35	10	2.4	0.4	–	67 ± 2.7
A.3	W35	10	–	–	1.8	–
A.4	W35	10	–	–	3.6	–
A.5	TP	10	1.2	0.4	–	137 ± 10.1
A.6	TP	10	2.4	0.4	–	104 ± 9.7
A.7	TP	10	–	–	1.8	284 ± 41.5
A.8	TP	10	–	–	3.6	187 ± 14.3
A.9	TP/GMS [a]	10	1.2	0.4	–	135 ± 14.6
A.10	TP/GMS	10	2.4	0.4	–	102 ± 8.2
A.11	TP/GMS	10	–	–	1.8	304 ± 54.0
A.12	TP/GMS	10	–	–	3.6	–
A.13	TP puriss.	10	1.2	0.4	–	166 ± 14.3

PL, phospholipid (Lipoid S100); SGC, sodium glycocholate; F68, Pluronic® F68; W35, Witepsol® W35; TP, tripalmitate, 95% pure; GMS, glycerol monostearate; TP puriss., tripalmitate, 99% pure; SD, standard deviation.

[a] Tripalmitate and glycerol monostearate were mixed in a ratio of 19:1 (w/w).

form the finest dispersions with a mean equivalent sphere diameter around 67 nm (Table 1).

Systems prepared with Pluronic® F68 were unstable and tended to gel forming ointment-like structures. Gelation started either directly on cooling after preparation thus precluding a determination of particle size, or on storage at room temperature. Furthermore, it was observed that gelation may be induced by shear forces, e.g. pressing the dispersion through the needle of a syringe.

In a second series the drug coenzyme Q_{10} (6-decaprenyl-2,3-dimethoxy-5-methyl-1,4-benzoquinone) which is an endogenous quinone of the respiratory chain therapeutically used for heart diseases (Folkers et al., 1991) was incorporated into the lipid nanoparticles at different drug/lipid ratios. Coenzyme Q_{10} was chosen as a model substance because it is extremely hydrophobic and practically insoluble in water. The composition of the systems and their mean PCS particle size are listed in Table 2. Based on the experience with empty carrier systems, Pluronic® F68 was not used for the preparations.

TABLE 2

Composition and mean particle size of drug-loaded carrier systems

System	Matrix component	Composition (% g/g)				Mean particle size ± SD (nm)
		Matrix	PL	SGC	CoQ_{10}	
B.1	TP	10	1.2	0.4	0.2	103 ± 10.3
B.2	TP	10	1.2	0.4	0.5	112 ± 13.0
B.3	TP	10	1.2	0.4	1.0	100 ± 10.7
B.4	TP/GMS [a]	10	1.2	0.4	0.2	103 ± 8.7
B.5	TP/GMS	10	1.2	0.4	0.5	92 ± 7.8
B.6	TP/GMS	10	1.2	0.4	1.0	104 ± 4.2
B.7	W35	10	1.2	0.4	0.2	86 ± 7.2
B.8	W35	10	1.2	0.4	0.5	83 ± 3.2
B.9	W35	10	1.2	0.4	1.0	79 ± 3.5

PL, phospholipid (Lipoid S100); SGC, sodium glycocholate; CoQ10, coenzyme Q10; W35, Witepsol® W35; TP, tripalmitate, 95% pure; GMS, glycerol monostearate; SD, standard deviation.

[a] Tripalmitate and glycerol monostearate were mixed in a ratio of 19:1 (w/w).

Incorporation of coenzyme Q_{10} seems to result in a reduction of the measured mean particle sizes compared with the corresponding dispersions of the empty carriers (Table 2).

X-ray diffraction studies in a Debye-Scherrer camera

The different polymorphic forms of glycerides can be distinguished by their characteristic short spacing patterns (Table 3). Their long spacings depend on the carbon chain length.

TABLE 3

Characteristic X-ray short spacings of glycerides [a]

Modification	Characteristic short spacings
α	a single strong line corresponding to approx. 0.415 nm
β'	usually two (but occasionally more) strong lines corresponding to approx. 0.42 and 0.38 nm
β	a strong (usually strongest) line corresponding to approx. 0.46 nm

[a] Chapman (1962).

For preliminary X-ray diffraction experiments in a Debye-Scherrer camera lipid systems of tripalmitate and Witepsol® W35 were prepared all containing 10% matrix constituent, 1.2% phospholipids and 0.4% sodium glycocholate. Dispersions of different mean particle sizes were produced by variation of the homogenization parameters. The very small crystal size required exposure times of 8 or 24 h. The particle size, spacings and relative intensities are given in Table 4.

The diffraction pattern of the tripalmitate dispersion with the smaller mean particle size displayed relatively weak reflections. Increased exposure times led to a considerable blackening of the film impeding the determination of the diffraction angles. For the larger tripalmitate particles an exposure time of 8 h was sufficient to obtain strong reflections. The Bragg spacings correspond to the β-modification of tripalmitate.

Dispersions of Witepsol® particles gave either no diffraction peaks (smaller mean particle size) or only very weak ones (larger mean particle size) with a pronounced line broadening so that no reliable spacings or intensity estimates could be obtained.

TABLE 4

Results of X-ray diffraction studies in a Debye-Scherrer camera

Matrix	Mean size (nm)	Exposure time (h)	Reflections d (nm)
10% TP	127	8	0.53 (vw) [a] 0.46 (w) 0.39 (vw) 0.37 (w)
10% TP	127	24	0.53 (vw) 0.46 (s) 0.39 (m) 0.37 (m)
10% TP	249	8	0.53 (w) 0.46 (vs) 0.39 (s) 0.37 (s)
10% W35	80	24	diffuse scattering
10% W35	150	8	0.46 (w) 0.42 (vw) 0.38 (w)

TP, tripalmitate, 95% pure; W35, Witepsol® W35.
[a] Values in parentheses refer to the scattering intensity: vw, very weak; w, weak; m, medium; s, strong; vs, very strong.

TABLE 5

Long spacings of β-modification of raw materials

Substance	Long spacings (nm)
Tripalmitate (99%)	4.04 (4.06 [a])
Tripalmitate (95%)	4.04; 4.52 (4.06 [a])
Glycerol monostearate	4.97 (5.00 [b])
Witepsol® W35	3.70; 4.20 (3.80; 4.25 [c])
Coenzyme Q_{10}	2.80; 5.67 (2.77; 5.52 [d]) [e]

Values in parentheses refer to the literature.
[a] Malkin (1954).
[b] Chapman (1962).
[c] Thoma et al. (1983a).
[d] Quinn and Katsikas (1985).
[e] For coenzyme Q_{10} the modification is not termed β-form.

Synchrotron radiation X-ray diffraction

Synchrotron radiation small angle X-ray (SAX) diffraction studies of the powdered raw materials yielded the characteristic long spacings of the stable β-modification of the glycerides (Table 5). The additional long spacing of 95% pure tripalmitate is not listed in the literature and may be attributed to a contamination of the raw material.

The reflections in SAX diffraction patterns of the drug-free lipid dispersions are broader than in those of the raw materials. The spacings found for the tripalmitate based systems suggest that the glyceride is in the β-form as illustrated in Fig. 1 for system A.13. The additional long spacing of 95% pure tripalmitate is not resolved in the diffraction patterns of the lipid dispersions containing this material. In comparison to system A.13, which is based on the highly pure tripalmitate, these dispersions reveal a broader and asymmetric peak. In the lipid dispersions based on the mixture of tripalmitate and glycerol monostearate (GMS) the reflection corresponding to GMS cannot be detected. The phospholipid-stabilized Witepsol® carriers do not display the double peak found for the raw material and, although less well resolved, for the gel-like systems stabilized by Pluronic® F68, so that a clear assignment of the crystal modification is not possible.

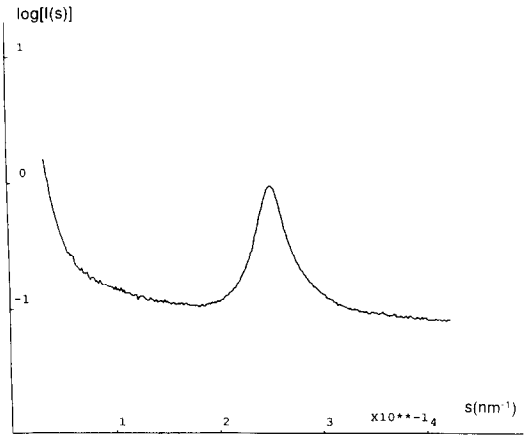

Fig. 1. Small angle X-ray diffraction pattern of system A.13. Exposure time: 3 min.

The SAX diffraction patterns of the coenzyme Q_{10} loaded carrier systems are similar to those of the drug-free lipid dispersions. The coenzyme Q_{10} containing dispersions exhibit a peak broadening comparable to that of the drug-free carrier systems. The additional long spacing of 95% pure tripalmitate is not resolved in the drug-loaded dispersions of this glyceride. Long spacings corresponding to coenzyme Q_{10} could not be detected. Increasing amounts of coenzyme Q_{10} incorporated into the lipid particles have no effect on the diffraction patterns. As with the drug-free systems, the patterns of the drug-loaded carriers based on the tripalmitate/GMS mixture have no peak corresponding to the monoglyceride.

Although the results of the SAX diffraction studies suggest that all investigated lipid carriers are crystalline, they do not permit a clear distinction between the crystal modifications in all cases. Therefore, further studies on the polymorphism of the glyceride dispersions were performed by wide angle X-ray (WAX) diffraction. The diffraction maxima of the raw materials are listed in Table 6.

The reflection at 0.415 nm detected for GMS is not described for the β-modification in the literature. Possibly, GMS is present as a mixture of the β'- and β-forms which cannot be distinguished by their long spacings. No spacings corresponding to the α- or sub-α-form were detected. Therefore, the additional spacing may be attributed to a contamination. The reflections of tripalmitate are sharper than those of the complex glyceride mixture Witepsol® W35 as well as those of GMS.

TABLE 6

Wide angle X-ray diffraction patterns of raw materials

Substance	Short spacings [a] (nm)
Tripalmitate (99%)	0.46; 0.37; 0.39; 0.53 (β) [b]
Tripalmitate (95%)	0.46; 0.39; 0.37; 0.53 (β)
Glycerol monostearate	0.46; 0.39; 0.415 (β)
Witepsol® W35	0.38; 0.42; 0.46 (β)
Coenzyme Q_{10}	0.49; 0.395

[a] The reflections are named in order of decreasing intensity.
[b] Crystal modification.

The WAX diffraction patterns of the drug-free lipid dispersions all reveal a short spacing at approx. 0.46 nm characteristic of the β-modification of glycerides. Fig. 2 represents the diffraction pattern of system A.5. As with the long spacings, short spacings corresponding to GMS could not be detected in the carrier systems based on the mixture of tripalmitate and the monoglyceride. Differences in the intensities of certain carriers can be observed. The intensity seems to be reduced by the addition of GMS.

The WAX diffraction patterns of the coenzyme Q_{10} loaded carriers are similar to those of comparable drug-free glyceride dispersions as illustrated in Fig. 3. Reflections corresponding to

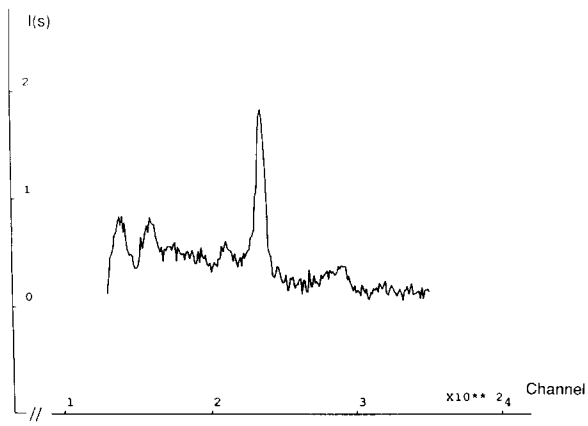

Fig. 2. Wide angle X-ray diffraction pattern of system A.5. Exposure time: 3 min.

crystalline coenzyme Q_{10} could not be detected. The scattering intensities of drug-loaded Witepsol® carriers are very low.

The WAX diffraction patterns of selected carrier systems were also recorded at body temperature. The carriers based on a Witepsol® W35 matrix did not yield any reflections whereas tripalmitate containing carriers displayed the same reflections as at 20°C. The different behaviour of Witepsol® W35 and tripalmitate can also be observed for the raw materials.

Time-resolved X-ray diffraction during temperature scans

Investigations on the recrystallization behaviour of the lipid dispersions after heating above the melting temperature were performed by time-resolved X-ray diffraction studies during temperature scans. A drug-free (system A.1) and a coenzyme Q_{10} loaded Witepsol® carrier (system B.8) were investigated and compared to the recrystallization of the raw material Witepsol® W35.

The dispersions were heated above the melting point of the lipid, kept at 45°C for approx. 15 min, and then cooled down to +5°C in steps of 5°C. At each step the WAX diffraction pattern of the sample was recorded for 180 s after an equilibration time of 3 min.

The drug-free and the coenzyme Q_{10} loaded

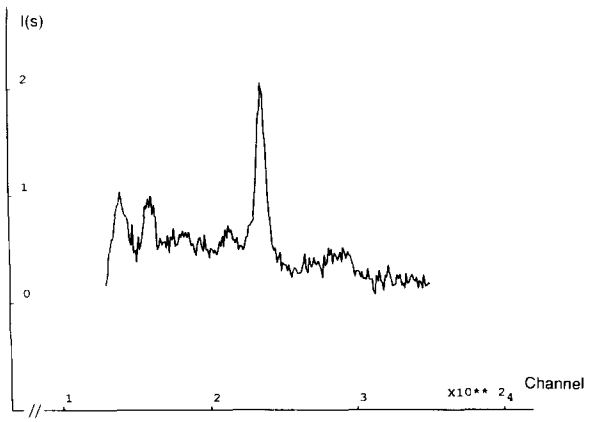

Fig. 3. Wide angle X-ray diffraction pattern of coenzyme Q_{10} loaded carrier system B.3. Exposure time: 3 min.

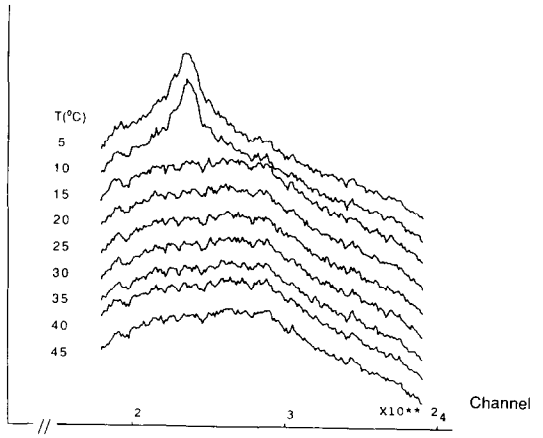

Fig. 4. Wide angle X-ray diffraction patterns of system B.8 from time resolved measurements during a temperature scan from 45 to 5°C in steps of 5°C. The peak corresponds to approx. 0.415 nm.

carriers behave similarly with regard to their recrystallization temperature and crystal modification. No reflections were observed in the diffraction pattern between 45 and 15°C. At 10°C a strong reflection at approx. 0.415 nm characteristic of the α-modification of complex glyceride mixtures (Thoma et al., 1983a) could be detected (Fig. 4).

The raw material Witepsol® W35 was exposed to a similar temperature program. During the temperature scan, however, the small angle and wide angle diffractions were recorded simultaneously. The recrystallization behaviour of Witepsol® W35 is different from that of the lipid dispersions. A long spacing at approx. 3.7 nm corresponding to the β'-modification could already be observed at 35°C. At 30°C the long spacing was more pronounced and accompanied by two weak reflections in the WAX range. An additional reflection in the small angle range corresponding to approx. 4.2 nm appeared at 25°C indicating a transformation into the so-called β_2- or intermediate β-form described for complex glyceride mixtures (Larsson, 1966). This reflection became stronger at 20°C while the WAX diffraction pattern was also changing to the characteristic spacings of the β-modification (Fig. 5). However, the intensities of the short spacings were

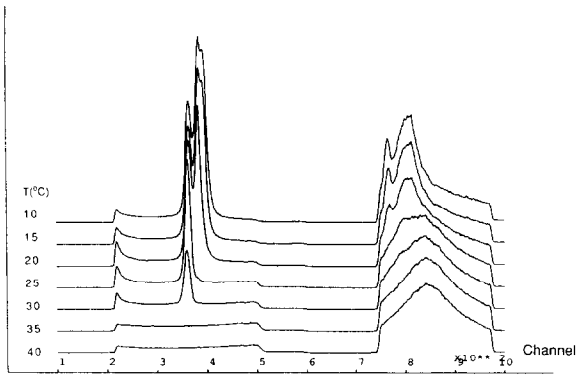

Fig. 5. Simultaneously recorded small and wide angle X-ray diffraction patterns of Witepsol® W35 from time resolved measurements during a temperature scan from 45 to 5°C. Due to the geometry of the measurements the intensities in the small and wide angle ranges are not on the same scale.

relatively low hinting at an incomplete or delayed recrystallization of the lipid.

Discussion

Preparation and particle size determination

It has been demonstrated before that nanoparticles based on glycerides that are crystalline at room temperature in the bulk phase can be produced by emulsification of the molten lipid in an aqueous phase employing phospholipids as emulsifiers (Siekmann and Westesen, 1992b). Emulsification by high pressure homogenization yielded a smaller mean particle diameter and more narrow size distribution than sonication. Addition of sodium glycocholate led to a reduction in particle size and yielded a stable product compared with the use of phospholipids alone that tended to gel.

Since preliminary studies by transmission electron microscopy of freeze-fractured samples revealed that carriers prepared from tripalmitate recrystallize as anisometric particles (Siekmann and Westesen, 1992b), the mean particle size calculated from the PCS number distribution corresponds to the diameter of the equivalent hydrodynamic sphere. Moreover, Mie theory could not be applied to the PCS data because crystalline materials are anisotropic so that the refractive index is changing with the orientation of the molecular planes in the beam. Computer programs for the conversion of light scattering data into size distributions which consider the anisometry of particles and alterations of the refractive index with the orientation are, to our knowledge, not commercially available.

The results from PCS measurements demonstrate that the lipid dispersions consist predominantly of particles in the lower nanometer size range. The particle size depends on the matrix constituent as well as on the type and amount of emulsifying agents. The use of the glyceride mixture Witepsol® W35 as matrix constituent yielded the smallest particles. Since Witepsol® W35 is composed of approx. 5% monoglycerides, 29% diglycerides and 65% triglycerides of different fatty acids, the reduced particle size compared to tripalmitate containing systems may be attributed to the presence of surface-active mono- and diglycerides which probably facilitate emulsification. Addition of 5% GMS to tripalmitate could, however, not significantly reduce the particle size determined by PCS.

Increasing the amount of emulsifier generally decreased the mean particle size. The combination of phospholipids and sodium glycocholate yielded smaller particle sizes than the nonionic block copolymer Pluronic® F68. Moreover, systems prepared with Pluronic® F68 were unstable and tended to gel and to form ointment-like structures.

As found with the drug-free lipid dispersions, Witepsol® W35 yields drug-loaded carriers of a smaller particle size than tripalmitate or the mixture of tripalmitate and monostearate. Unexpectedly, the incorporation of the drug decreases the mean particle size detected by PCS. The decrease amounts to approx. 10 to 20% compared to the corresponding drug-free carrier systems. The reason for this effect of coenzyme Q_{10} is under investigation.

Investigations by X-ray diffraction

There is a very pronounced dependence of the wide angle diffraction pattern on the particle size. Besides the actual grain size it seems that the increase in surface/volume ratio of the particles considerably increases their mosaicity. As a

result, the number of observable spacings is reduced.

Powder diffraction indicates that tripalmitate carriers are in the β-modification. The exact determination of the Bragg spacings of Witepsol® dispersions was, however, not possible since either no or only very weak diffraction maxima were obtained.

For all systems the exposure times were too long to allow time-resolved investigations on a conventional X-ray source.

The comprehensive studies on the physical state of the lipid carrier systems by synchrotron radiation small angle and wide angle X-ray diffraction revealed that all investigated lipid carriers are crystalline at 20°C.

The SAX diffraction studies alone were not sufficient to identify the crystal modification of the phospholipid-stabilized carriers based on Witepsol® W35 since the broad peaks in the diffraction pattern did not permit a clear distinction between β'- and β-form. For the semi-solid Witepsol® carriers stabilized by Pluronic® the double peak characteristic of the intermediate β-modification was, however, found. The stable β-modification could also be detected in the tripalmitate carriers.

The crystal modifications of all carrier systems could also be unambiguously identified by WAX. The short spacings indicate that the finely dispersed glycerides are present in the stable β-form in all systems.

Carriers based on the complex glyceride mixture Witepsol® W35 displayed particularly weak and broad maxima at wide angles. Obviously, the crystal lattice of these lipid dispersions is highly perturbed since the complex composition of Witepsol® W35 precludes the formation of a true lattice. Further, the accumulation of lattice imperfections may be attributed to the small crystallite size in the dispersions. The effect of the distorted lattice of Witepsol® W35 is also visible in the WAX diffraction patterns of the raw materials. The diffraction peaks of the highly pure tripalmitate are much sharper than those of Witepsol® W35.

Incorporation of GMS into tripalmitate carriers reduced the scattering intensity of the 19:1 mixture compared with the pure tripalmitate dispersions. The absence of spacings corresponding to GMS in the liquid dispersions indicates that GMS is incorporated in the tripalmitate lattice, as also suggested by the reduced scattering intensity, or that the surface-active GMS is located in the surface of the lipid nanoparticles.

The diffraction peaks of the semi-solid systems, i.e. those stabilized by Pluronics® which tended to gel, are sharper than those of the corresponding liquid dispersions. In the semi-solid systems the glyceride probably forms a network-like crystalline gel structure so that the crystal dimensions are extended compared to the nanoparticulate lipid crystals of the liquid dispersions.

The incorporation of coenzyme Q_{10} did not substantially alter the recrystallization tendency of the lipid carriers. Differences in scattering profiles appear to be insignificant. The absence of spacings corresponding to crystalline coenzyme Q_{10} cannot definitely be explained. Tentatively, there could be an association of coenzyme Q_{10} with the emulsifier layer at the surface of the lipid particles. It has been demonstrated before by ^{2}H-NMR spectroscopy studies (Westesen and Wehler, 1991) that in a submicron-sized model o/w emulsion coenzyme Q_{10} is predominantly located in phospholipid layers.

The results of the WAX diffraction studies at body temperature display differences in the behaviour of tripalmitate and Witepsol® dispersions. Whereas tripalmitate carriers are still crystalline at 38°C, Witepsol® carriers do not give any diffraction peaks. This suggests that this glyceride mixture is molten. The different physical state of the carriers at body temperature may influence their biopharmaceutical properties. The liquid Witepsol® carriers give rise to fast drug release, whereas tripalmitate carriers may be suitable as sustained release delivery systems. Being crystalline at room temperature, both carrier systems may provide an enhanced in vitro stability and a decreased risk of drug leakage on storage.

Time-resolved X-ray diffraction studies during temperature scans indicate that the recrystallization of Witepsol® W35 in the dispersion is different from that of bulk material. After heating

above their bulk melting temperature the dispersed glycerides recrystallize in the α-modification. In contrast, the bulk lipid recrystallizes in the β'-form which is rapidly transformed into the intermediate β-modification. As indicated by the low scattering intensities in the wide angle range, this transformation appears to be incomplete. It has been demonstrated by thermoanalytical investigations that the transition from the β'- to the β-form is temperature-dependent and proceeds over several months (Thoma and Serno, 1983b).

The recrystallization of the bulk material starts at a higher temperature than the recrystallization of the dispersed lipids. This behaviour suggests that in the lipid dispersions Witepsol® W35 represents an undercooled melt and may explain the less ordered structure of the crystal lattice indicated by the low scattering intensities of the Witepsol® carriers.

Since dispersed Witepsol® W35 particles recrystallize in the α-modification after heating, there must be a transformation into the intermediate β-form which was detected by WAX diffraction studies of the carrier systems stored for several weeks. This transformation was not observed in the time-resolved measurements and might be retarded due to the presence of emulsifiers or the dispersity of the systems since crystallization in dispersed systems proceeds more slowly than in bulk material (Van den Tempel, 1978).

The simultaneous detection of the small angle and wide angle diffraction range as performed for Witepsol® W35 permits the simultaneous observation of the formation of the long and short range order.

Conclusions

The colloidal dispersions of different glycerides display both long and short range order originating from the packing of the lipid molecules in the solid particles. No interparticle interactions were observed.

The considerable brightness of synchrotron radiation allows one to identify the crystal modification even in dilute suspensions of submicron particles. The ability to perform simultaneous time-resolved small and wide angle diffraction measurements should increase the efficiency with which new carrier systems and the influence of physicochemical parameters on their behaviour can be characterized.

The results from synchrotron radiation X-ray diffraction studies demonstrate that the lipid carriers are crystalline at 20°C. The stored systems are present in the stable β-modification. The crystal structure of the lipid nanoparticles is less ordered than bulk materials. The incorporation of coenzyme Q_{10} did not substantially alter the recrystallization tendency of the lipid carriers.

Time-resolved X-ray diffraction measurements during temperature scans revealed that the dispersed glycerides recrystallized in the α-form whereas the bulk lipids recrystallized in the β'-modification and transformed rapidly into the β-form after heating above the melting point and slow cooling.

Depending on the matrix constituent the lipid carriers display differences of their physical state at body temperature which may influence their biopharmaceutical behaviour. Whereas Witepsol® carriers are liquid at 38°C, tripalmitate carriers are crystalline and may therefore give rise to a sustained drug release.

The solid lipid nanoparticles present an interesting approach to the parenteral administration of poorly water soluble drugs. Due to their solid physical state they might overcome a number of disadvantages of conventional parenteral drug carriers.

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