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Preparation and structural investigations of colloidal dispersions prepared from cubic monoglyceride–water phases

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Abstract

Dispersions of bicontinuous cubic monoglyceride–water phases, so-called ‘cubosomes’, have been proposed as parenteral sustained release delivery systems. For the present study, dispersions of monoolein-rich monoglycerides (MO), with or without purified soya phospholipids (PL), were prepared by equilibration of a MO/(PL)/water cubic phase, subsequent fragmentation with a poloxamer 407 (P407) solution, sonication and homogenization. This yielded systems of very different macroscopic appearance: Almost transparent dispersions, slightly turbid systems, opaque dispersions or milky emulsions. The mean z-average particle diameters ranged from 80 nm to well above 350 nm. Considerable particle growth could be detected in most systems during storage at room temperature. Storage at 5 °C resulted in the formation of ointment-like gels, which may be attributed to the crystallization of MO. Freeze-fracture transmission electron micrographs of MO dispersions revealed predominantly spherical particles with a low fracturing tendency. Synchrotron radiation X-ray diffraction indicated that high energy input during disintegration of the cubic phase leads to very complex systems in which particles with a cubic structure and MO/(PL) vesicles may coexist. The characteristic reflections of cubic systems were absent in the diffraction patterns of almost transparent or slightly turbid dispersions. The results indicate a strong dependence of ultrastructure of the dispersions on the preparation parameters. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Cubosome®; Cubic phase; Colloidal dispersion; Monoolein; Drug delivery; Homogenization; Structure investigation; Synchrotron radiation X-ray diffraction

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1. Introduction

Unsaturated long chain monoglycerides such as monoolein (MO) are able to form lyotropic liquid crystalline cubic phases in water. For monoolein–water-mixtures at room temperature there are two

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bicontinuous cubic phases in the concentration range between approximately 20 and 45% (w/w) water content corresponding to the space groups *Ia*3*d* (Q^{230}) and *Pn*3*m* (Q^{224}) (Qiu and Caffrey, 2000). Since the structures formed by the monoolein bilayers in these cubic mesophases correspond to specific infinite minimal periodic surfaces, IMPS, they are also often referred to as G-type (gyroid surface) and D-type (diamond surface; Hyde et al., 1984). For more complex systems, e.g. in the monoolein–poloxamer 407–water system, another mesomorphic cubic phase, *Im*3*m* (Q^{229}) or P-type (primitive surface) has been described (Landh, 1994). Phospholipids (PL) can be incorporated into cubic mesophases (Landh, 1991).

The lipid bilayer units in simple MO/water systems form a three-dimensional network which separates two identical water-channel systems that have a water pore diameter of about 5 nm in the fully hydrated cubic phase (Landh, 1991).

Due to its lipid and water domains the cubic phase may in principle solubilize both water- and lipid-soluble substances, and molecules with amphiphilic character may partition at the lipid/water-interface. Biodegradability, the ability to incorporate and slowly release a variety of drugs with different physicochemical properties and the possibility to enhance the chemical, physical and/or enzymatic stability of incorporated drugs and proteins have made the cubic phase an interesting candidate for use in drug delivery (Engström, 1990a; Ericsson et al., 1991; Ganem-Quintanar et al., 2000; Shah et al., 2001; Wyatt and Dorschel, 1992). With respect to parenteral administration the use of cubic bulk phases is, however, limited by their high viscosity which makes them difficult to inject, and their general incompatibility with the intravenous route. Since bicontinuous cubic monoglyceride–water phases are stable in excess water and can be dispersed in appropriate surfactant and protein solutions (Lindström et al., 1981; Larsson, 1989; Ljusberg-Wahren et al., 1996), submicron-sized colloidal dispersions of these cubic structures, so-called 'cubosomes', have been proposed as parenteral sustained release delivery systems, especially for peptide and protein drugs (Landh, 1991; Engström, 1990b; Engström et al., 1996). It is assumed that the nanostructured lipid/water networks are preserved in the nanoparticles, and so are the properties of the cubic phase suggesting that the nanoparticles offer advantages similar to those of cubic bulk phases. Compared with liposomes, the high bilayer area to particle volume ratio of the cubic nanoparticles may increase the relative payload of lipophilic and amphiphilic drugs.

Several techniques have been described for the preparation of aqueous dispersions of cubic monoolein–water phases (Landh, 1991; Landh and Larsson, 1993; Gustafsson et al., 1996, 1997; Nakano et al., 2001; Spicer et al., 2001). Information about the characteristics of monoolein dispersions is, however, still limited, particularly with respect to pharmaceutically relevant properties. The aim of the present study was to evaluate the production of cubosome dispersions following a procedure based on the original compositions and process of Landh and Larsson (Landh, 1991; Landh and Larsson, 1993), starting from an equilibrated monoolein–water bulk phase which is fragmented with poloxamer 407 and soya PL. Dispersion procedures with high energy input such as ultrasonication and high-pressure homogenization were applied to obtain particle sizes suitable for parenteral administration. The resulting dispersions were investigated with respect to storage stability, temperature sensitivity and physicochemical properties. The central questions were, first, whether the cubic structure is preserved during the dispersion process, and, secondly, whether it is able to exist in discrete particles of colloidal dimension for a sufficiently long time after dispersion of the original cubic bulk phase by high shear forces during homogenization. The structure of these colloidally dispersed monoolein–water phases was investigated by transmission electron microscopy (TEM) and synchrotron radiation X-ray diffraction. The use of synchrotron radiation permits systematic, in particular temperature dependent X-ray studies of the native cubosome dispersions.

2. Experimental

2.1. Materials

Monoglycerides (MO) (TS-T 143) were obtained from Grindsted A/S (Brabrand, Denmark), the purified soya PL (Lipoid S 100) were supplied by Lipoid KG (Ludwigshafen, Germany), the non-ionic polyoxyethylene–polyoxypropylene triblock copolymer poloxamer 407 (Lutrol-F127) was a product of BASF (Ludwigshafen, Germany). Dispersions were prepared in bidistilled water using 0.01% (w/w) thimerosal (Sigma Chemicals Co., St. Louis, USA) as a preservative. Other chemicals used were reagent-grade, standard laboratory chemicals.

2.2. Preparation of monoolein dispersions

Cubic bulk phases (binary systems) were prepared by equilibrating 65% (w/w) MO with 35% (w/w) water at 42 °C, PL containing cubic bulk phases (ternary systems) by stirring the MO/water cubic phase in a dispersion of small unilamellar PL vesicles (SUV; prepared by sonication of PL in water) until the originally slightly turbid dispersion appeared optically clear. Dispersions of these binary (6.5% MO) and ternary mixtures (6.5% MO, 3.5% PL) were further processed by stirring in a poloxamer 407 solution (final conc. 1% w/w), predispersion by intermittent probe sonication (Soniprep 150, MSE, Crawley, UK, or Branson B-15, Branson Sonic Power Company, Danburg, USA) under cooling in a water bath for approximately 10–15 min (corresponding to 5–10 min effective sonication time) and final high pressure homogenization (Micron Lab 40, APV Gaulin, Lübeck, Germany, or Microfluidizer M-110, Microfluidics Inc., Newton, USA). In some cases the stirred dispersions were stored for a certain period of time (days to weeks) prior to further processing. The composition of the final dispersions was similar to the 'standard' dispersion described by Landh (1991).

2.3. Particle size determination

Particle size measurements were performed at 20–25 °C by photon correlation spectroscopy (PCS) using a Zetasizer 3 (Malvern Instruments, Malvern, UK). Samples were diluted in particle-free purified water to a scattering intensity of approximately 50–110 kcps. Scattered light was detected at 90°. The mean z-average diameter and polydispersity index (PDI) were obtained by cumulant analysis using the MALVERN software.

2.4. Polarized light microscopy

Samples were viewed between crossed polarizers and a λ-sheet in a Zeiss III light microscope (Zeiss, Oberkochen, Germany).

2.5. Synchrotron radiation X-ray diffraction

Small (SAXS) and wide angle (WAXS) synchrotron radiation X-ray scattering measurements were performed on the double focusing monochromator mirror camera X33 (Koch and Bordas, 1983) of the EMBL in HASYLAB on the storage ring DORIS III of the Deutsches Elektronen Synchrotron (DESY) at Hamburg, Germany. Two linear delay line readout detectors were connected in series to simultaneously monitor the small and wide angle diffraction patterns (Rapp et al., 1995) using standard data acquisition and evaluation systems (Boulin et al., 1986, 1988). The small angle patterns were measured over the range $0.05 \leq s \leq 0.65$ nm^{-1}, where $s = 2\sin\Theta/\lambda$ with 2Θ the scattering angle and λ (0.15 nm) the wavelength. The wide angle patterns covered the range $1.4 \leq s \leq 3.1$ nm^{-1}. Data reduction was done following standard procedures (Koch, 1991) using the program SAPOKO (Svergun and Koch, unpublished). Sample cells were thermostatized with a waterbath (Huber Ministat). The scattering of water was subtracted from the diffraction pattern of the dispersions. Selected bulk and dispersion samples were investigated successively at 25, 20, 15, 10, 5 °C and after reheating to 20 or 25 °C. Prior to the measurements, the samples were equilibrated for at least 10 min after cooling to a new temperature.

2.6. Transmission electron microscopy (TEM)

Samples were freeze-fractured at 173 K in a freeze-fracturing apparatus (BAF 400, Balzers AG, Liechtenstein). Fast freezing was accomplished by jet freezing in propane (JFD 030, Balzers AG, Liechtenstein). The samples were shadowed with platinum/carbon (layer thickness, 2 nm) at 45° and with pure carbon at 90° for replica preparation. Replicas were cleaned with dilute sulfuric acid and subsequently with water. Replicas on uncoated grids were viewed with a transmission electron microscope (EM 300, Philips, Kassel, Germany).

3. Results and discussion

3.1. Manufacturing procedure

Manufacturing of monoolein dispersions by fragmentation of cubic bulk phases is a time-consuming procedure involving multiple equilibration steps. Different preparation regimes were tested on a standard composition of 6.5% (w/w) MO, 3.5% PL and 1% poloxamer 407 to evaluate if the production process could be significantly shortened. This is only possible to a limited extent without influencing product stability and homogeneity. Equilibration of the cubic phase cannot be significantly reduced below 24–48 h without risking the presence of anisotropic (liquid) crystalline phases as demonstrated by polarizing light microscopy. The best macroscopic homogeneity and short-term storage stability was obtained by the following preparation schedule: (1) equilibration of MO/water cubic phase (24–48 h), (2) stirring with SUV dispersion (48 h), (3) addition of P407 solution and continued stirring (24–48 h), (4) predispersing by probe sonication (approximately 5–10 min), (5) high pressure homogenization.

The uptake of PL from vesicles into the cubic phase can be monitored macroscopically by clearing of the slightly turbid SUV dispersion and a color change of the cubic phase from clear to yellowish. PL from SUV appear to be completely taken up into the cubic phase without disturbing its macroscopic integrity. The use of multilamellar vesicle (MLV) dispersions yielded less homogenous and reproducible products, possibly due to the reduced PL uptake from MLV into the structure of the cubic phase within 48 h. Simultaneous addition of SUV and P407 solution can reduce the production time but at the expense of the macroscopic homogeneity and, hence, quality of the product. This may also be caused by a less effective PL incorporation into the cubic phase due to interactions of PL with P407. Addition of P407 was necessary since PL are not able to stabilize the dispersed state of MO/water phases. Dispersions could also be prepared from binary cubic bulk phases without using PL.

The preparation of colloidal monoolein dispersions of relatively low viscosity from predispersions obtained by the above scheme requires only moderate energy input independently of the homogenization equipment. Between two and five homogenization cycles at 300–400 bar were sufficient to obtain submicron-sized particles. Whereas, the cubic bulk phases were always homogenous, clear and isotropic, dispersions of cubic phases varied considerably in their macroscopic appearance after sonication or homogenization despite similar composition. Almost transparent dispersions, slightly turbid systems, opaque dispersions as well as milky emulsion-like systems were observed depending on preparation parameters (Table 1).

3.2. Particle size and stability on storage

Homogenized dispersions which differed in their macroscopic appearance also revealed differences in particle size. The transparent, slightly turbid systems with a macroscopically homogeneous appearance typically yielded mean z-average diameters in the range of 80–130 nm and PDI values usually below 0.2–0.25. The mean z-average diameters of the opalescent and opaque dispersions were between 120–200 nm, the PDI values below 0.2–0.25. Emulsion-like systems were in the same size range or above (up to more than 350 nm). The milky dispersions typically had PDI values above 0.25 indicative of broad size distributions (it should be noted that PCS is not very sensitive to particles in the upper nanometer and micrometer size range). The properties of selected dispersions are summarized in Table 1.

The macroscopic appearance of monoolein dispersions stored at room temperature tends to change with time. Dispersions which were almost clear and transparent directly after preparation turned increasingly turbid with storage time. A number of dispersions, particularly those with high PDI, tended to cream, and macroscopically visible particles with sizes in the micrometer to millimeter range were formed during storage within days or weeks after preparation making these systems unsuitable for intravenous drug delivery. No clear relationship could be established between the macroscopic appearance and manufacturing parameters. This requires further investigation of the influence of production parameters including additional factors such as time and temperature protocols.

Storage of homogenized dispersions at refrigerator temperature results in the formation of white, semi-solid, ointment-like gels. This phenomenon is correlated with the crystallization of the colloidally dispersed MO as indicated by temperature-dependent X-ray studies. Cooling a binary cubic MO/water bulk phase from room temperature to 5 °C leads to rapid crystallization of MO as illustrated in Fig. 1a. A phase change also occurs upon cooling in a corresponding dispersion (Fig. 1c). The formation of the single SAXS reflection around 0.2 nm^{-1} and two wide angle reflections that are also observed in crystalline MO raw material indicates that MO also crystallizes under these conditions. These observations are in agreement with the equilibrium phase behavior of MO/water bulk systems (Qiu and Caffrey, 2000).

Crystallization in the ternary MO/PL/water bulk and dispersion system seems to be retarded and could not be observed on the time scale of the cooling program (about 15–20 min at 5 °C, Fig. 1b and d). Addition of PL obviously modifies the crystallization behavior of MO, possibly due to a kinetic effect in the MO/water system, which exhibits a high tendency for supercooling at sub-ambient temperatures (Qiu and Caffrey, 2000).

3.3. Structural investigations

To prepare dispersions of cubic nanoparticles, the cubic MO/water bulk phase is dispersed into particles of colloidal dimension. Given the small particle size, the correspondingly large surface-to-volume ratio and the problem of surface stabilization of the bicontinuous cubic phase in dispersion (i.e. shielding of the a polar bilayer regions at terminated sites of the cubic structure) two basic questions arise: is the cubic structure of the present systems preserved during homogenization despite the high energy input and shear forces, and is it able to exist in the colloidally dispersed state over sufficiently long periods of time? These aspects were addressed using synchrotron radiation X-ray diffraction and TEM.

Table 1
Properties of selected dispersions

System	*z*-ave (nm)	PDI	Macroscopic appearance	X-ray
bUS_f	131	0.18	Opalescent	Diffuse
bML_f	196	0.33	Milky with top layer	SAXS reflections
bMF_s	121	0.12	Opaque	Diffuse
tUS_f	143	0.15	Opalescent	Diffuse
tML_f	134	0.31	Milky with top layer	SAXS reflections
tMF_s	166	0.12	Opalescent/opaque	Diffuse

Abbreviations: b, binary; t, ternary; US, sonicated; ML, Micron Lab, three cycles at 400 bar; MF, Microfluidizer, 2 min at approximately 350–450 bar; f, freshly prepared; s, stored for approximately 1 week; z-ave, z-average diameter (mean of 5); PDI, polydispersity index (mean of 5). X-ray data of the dispersions bUS_f, tUS_f, bML_f and tML_f are given in Figs. 1–3.

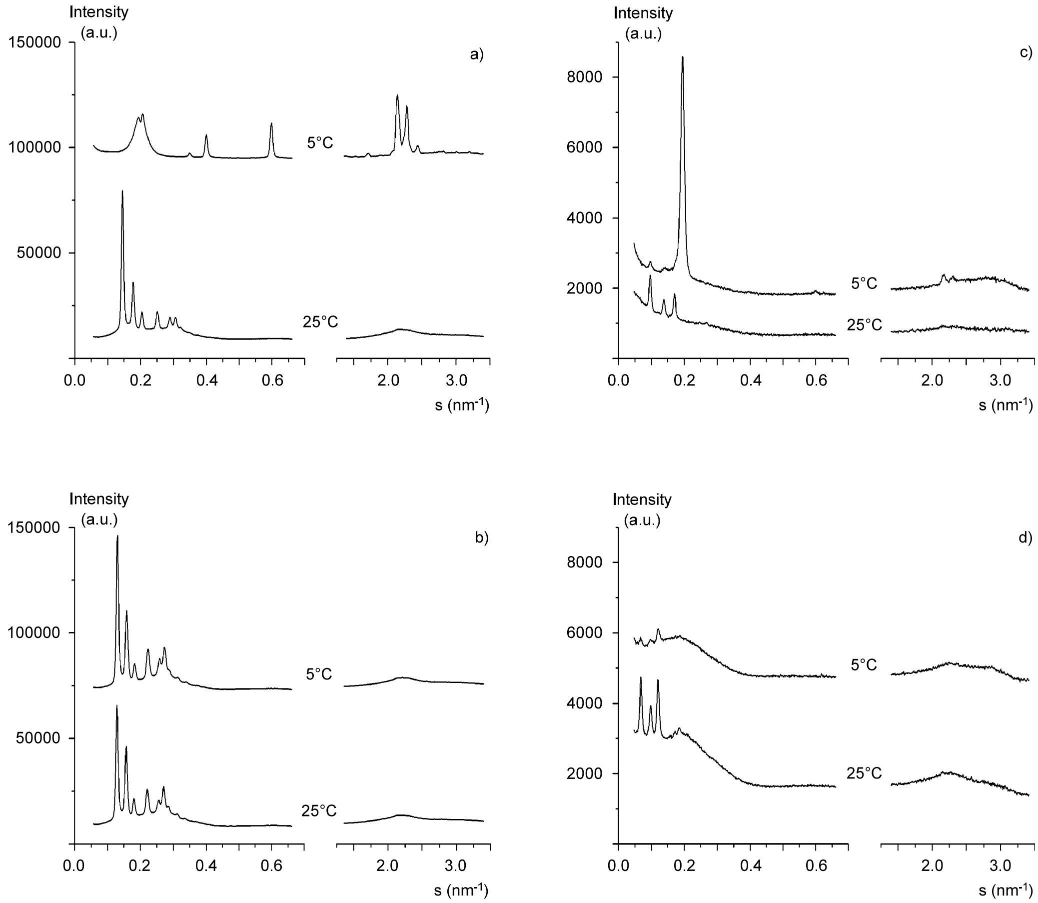

Fig. 1. X-ray diffractograms at 25 °C and after cooling to 5 °C: (a), binary bulk phase (bBU); (b), ternary bulk phase (tBU); (c), dispersion from binary bulk phase (bML; stirred, sonicated and homogenized (Micron Lab) three cycles at 400 bar); (d), dispersion from ternary bulk phase (tML; stirred, sonicated and homogenized (Micron Lab) three cycles at 400 bar). Note that in (b) and (d) there are no reflections at 0.2 nm and in the wide angle range at 5 °C. The low intensity of the cubic reflections at 5 °C in (d) is due to creaming of the particles, which could not be avoided in the X-ray measurements.

3.3.1. Synchrotron radiation X-ray diffraction

Structural investigations on selected dispersions and their bulk phases were initiated using synchrotron X-ray diffraction which permits X-ray studies in the native dispersions without separation of the individual phases. The different cubic phases (G, D and P-type) can be distinguished by their characteristic X-ray spacing ratios (Table 2).

The X-ray diffractograms of the bulk phases displayed SAXS reflections corresponding to the D-type cubic phase, indicating that the cubic structure is retained upon incorporation of phospholipid (Figs. 1 and 2). Two types of dispersions were found, those displaying exclusively diffuse SAXS scattering and those with additional SAXS reflections (Fig. 2). These two types of dispersions can also be distinguished by their PDI (Table 1) with the high polydispersity dispersions displaying SAXS reflections. The creaming tendency of the dispersions with high PDI impaired especially temperature-dependent X-ray studies since the dispersions became increasingly inhomogenous during the measurements.

With the exception of the crude stirred dispersion, which displayed the same spacing ratio as the bulk phase corresponding to the D-type cubic

Table 2
Characteristic X-ray spacing ratios of the cubic G-, D- and P-type phases according to Lindblom and Rilfors (1989)

Cubic phase	Space group	Characteristic spacing ratio
G-type (Q^{230})	*Ia*3*d*	$\sqrt{3}:\sqrt{4}:\sqrt{7}:\sqrt{8}:\sqrt{10}:\sqrt{11}:\sqrt{12}:\sqrt{13}$
D-type (Q^{224})	*Pn*3*m*	$\sqrt{2}:\sqrt{3}:\sqrt{4}:\sqrt{6}:\sqrt{8}:\sqrt{9}:\sqrt{10}:\sqrt{11}$
P-type (Q^{229})	*Im*3*m*	$\sqrt{2}:\sqrt{4}:\sqrt{6}:\sqrt{8}:\sqrt{10}:\sqrt{12}:\sqrt{14}$

phase, the reflections observed with the homogenized dispersions are consistent with the P-type cubic phase as postulated by Landh (1991). The homogenized systems are, thus, not dispersions of the original cubic bulk phase. This may be due to the surfactant used for fragmentation which can induce formation of a P-type phase in bulk systems (Landh, 1994). The generally small number of reflections in the patterns of the dispersions does not allow unambiguous assignment of structures. For the homogenized ‘ternary’ systems the smaller d-spacings of the stored dispersion (tML_s) compared with the freshly prepared one (tML_f) (Fig. 2) suggest that there is a subsequent contraction of the cubic lattice due to annealing.

The exclusively diffuse SAX scattering observed in a number of dispersions (Table 1 and Fig. 2) indicates that there is no ordered cubic structure in these dispersions after sonication or high pressure homogenization. The diffuse SAX scattering

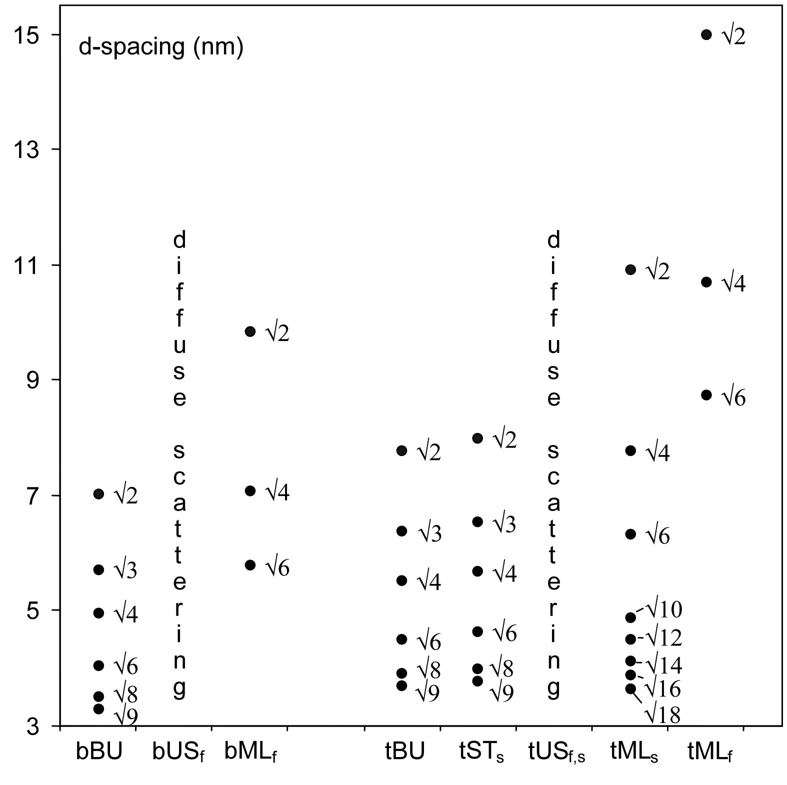

Fig. 2. X-ray spacings in binary and ternary bulk cubic phases and dispersions prepared thereof (b, binary; t, ternary; ST, stirred (magnetic stirrer); US, sonicated; ML, Micron Lab; f, freshly prepared; s, stored at room temperature; e.g. tST_s is a ternary stirred system stored at room temperature. Storage time was approximately 6 weeks for tUS_s and tML_s, and approximately 10 weeks for tST_s). (Note that the results presented for tML_s and tML_f were not obtained on the same sample (tML_s homogenized three cycles at 800 bar, tML_f homogenized three cycles at 400 bar.)) The diffraction pattern of bBU is given in Fig. 1a, of tBU in Fig. 1b, of bML_f in Fig. 1c, and of tML_f in Fig. 1d. The spacings marked with $\sqrt{16}$ and $\sqrt{18}$ in the system tML_s are consistent with the assignment to a P-type cubic phase (Hahn, 1989).

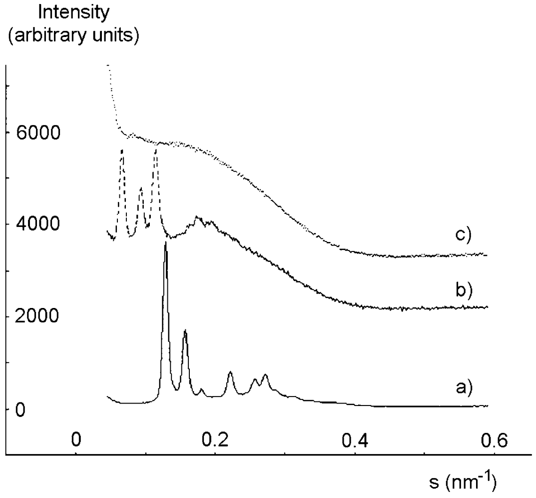

Fig. 3. SAX-diffractograms of (a), ternary bulk phase (tBU); (b), tML_f and (c), tUS_f (curves for dispersions and bulk not on same scale).

in the dispersions may result from (single) bilayer structures without long-range order, such as MO/(PL) vesicles or a dispersed L_3 phase (i.e. a bicontinuous isotropic phase of lipid bilayers closely related to cubic phases but without long-range order (Anderson et al., 1989). The presence of micellar structures may also contribute to the diffuse scattering.

The spacing ratios suggest that the crude stirred system is a mechanical dispersion of the original cubic bulk phase (Fig. 2). Sonication of the crude cubic dispersion obviously destroys the original cubic structure and particles without long-range order are formed. In some homogenized systems, a new cubic structure differing from the original cubic bulk phase structure can be observed (Fig. 2). The X-ray diffractograms display a diffuse halo underlying the SAXS reflections (Fig. 3b), and, thus, indicate that cubic particles coexist with other, non-ordered structures, e.g. single bilayers. The coexistence of cubic and vesicular structures in dispersions of MO/water systems has been observed by others using cryoelectron microscopy (Gustafsson et al., 1996, 1997; Spicer et al., 2001). It is noteworthy that particle growth was predominantly observed in the dispersions which displayed SAXS reflections (i.e. those containing particles with a cubic structure).

The predominance of ordered cubic structures or non-ordered lipid bilayers in the dispersions seems to depend on production parameters. In most colloidal dispersions investigated here non-ordered lipid bilayers appear to be the prevailing structures. Particles with cubic structures were observed in crude predispersions and in dispersions with high polydispersity which tended to cream and form macroscopic particles. These may result from the continuous transformation of non-ordered bilayers to cubic structures and growth of the latter during storage, finally leading to the formation of large particles with a cubic liquid crystalline structure.

3.3.2. Transmission electron microscopy

Further structural investigations were performed by TEM to study the shape and structure of particles in selected ternary monoolein dispersions (stirred, sonicated and homogenized).

Transmission electron micrographs of replicas of the freeze-fractured dispersions revealed predominantly spherical particles with a low fracturing tendency similar to SUV (Fig. 4), which impeded differentiation between particles of cubic structure and MO/(PL) vesicles. Faceted particles as reported previously (Engström, 1990b; Gustafsson et al., 1996, 1997) could not be detected, although there were some polyhedrally deformed particles. The particle dimensions in the fracture plane of the predominantly spherical particles are in the size range between approximately 35 and 150 nm in the homogenized dispersions, in agreement with PCS measurements (although the latter yield somewhat larger diameters due to differences in the underlying analytical principles). The size of particles in the stirred predispersion is much larger ($\gg$ 1 μm). In sonicated or homogenized dispersions which were freeze-fractured directly after preparation a population of extremely small particles (diameters in the fracture plane below 10–20 nm) was found which was not detected by PCS. These extremely small particles were also observed in a crude stirred dispersion indicating that they do not originate from larger particles as a result of high shear forces during sonication or homogenization. They may have formed spontaneously without need for high energy input, or represent the excess SUV added to the dispersion during preparation (step 2, cf. Section 3.1).

3.3.3. Structure of monoolein dispersions

The macroscopic appearance of dispersions of cubic monoolein–water phases may vary considerably and reflects differences in the microstructure. The X-ray studies demonstrated that only a small number of our dispersions contain cubic structures. The dispersions displaying X-ray reflections characteristic of cubic phases were those with an emulsion-like milky appearance, a high polydispersity and considerable particle growth during storage. The dispersions revealing cubic structures which had been sonicated and homogenized had a different cubic structure (reflections consistent with P-type) than the original cubic bulk phase (reflections consistent with D-type). This indicates that the original cubic D-phase is disrupted during sonication and homogenization, and that another cubic phase is formed in the dispersions. This may be related to the presence of the dispersing agents (poloxamer, PL) since the occurrence of a P-type phase has also been reported for bulk systems containing these substances (Landh, 1991, 1994). The diffuse scattering underlying the SAXS reflections observed with those dispersions may result from coexisting MO/(PL) vesicles or the L_3 phase which could be precursors of the cubic structure and may form during sonication or homogenization.

The macroscopically opalescent or opaque dispersions with a low polydispersity displayed exclusively diffuse X-ray scattering without any sign of ordered cubic structures. TEM of freshly prepared opalescent dispersions revealed predominantly spherical particles with a low fracturing tendency indicating that the structures displaying diffuse X-ray scattering are discrete vesicles of MO, PL and probably P407. The coexistence of minor amounts of cubic structures cannot completely be excluded although they were not detected by TEM or X-ray scattering.

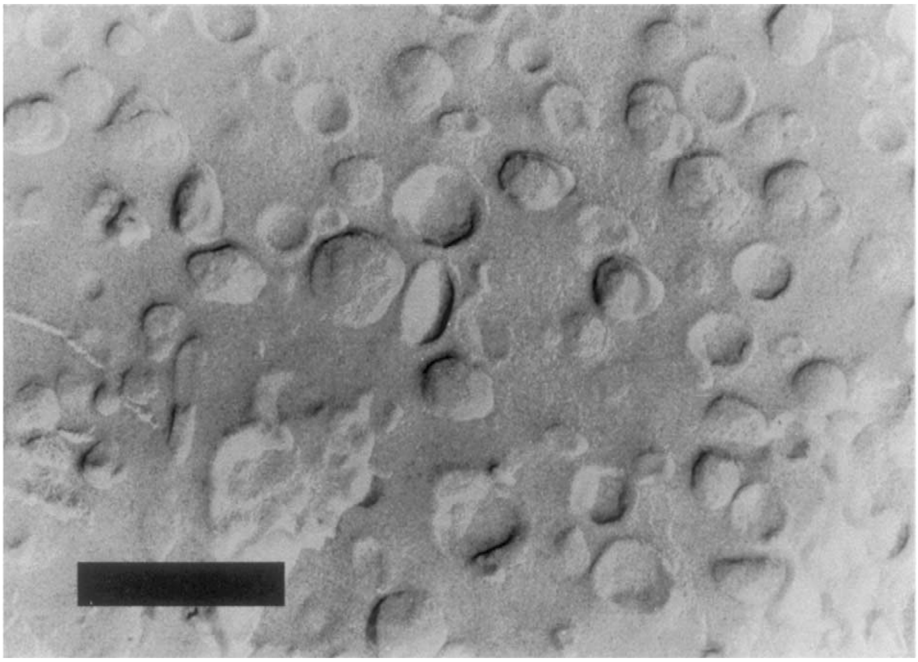

Fig. 4. TEM micrograph of a homogenized, slightly turbid 'ternary' dispersion (stirred, sonicated and homogenized (Microfluidizer) for 5 min at 530–645 bar). The bar corresponds to approximately 270 nm.

The above results indicate that systems prepared from cubic MO/water bulk phases display a complex behavior upon dispersion down to the colloidal scale. The original cubic phase of the D-type is obviously destroyed during high energy input (sonication, high pressure homogenization) in the presence of PL and P407, the lipids and P407 forming non-cubic structures such as vesicles. Whereas, the vesicle dispersions are homogeneous with a mean size around 100 nm, dispersions where an ordered cubic structure could be detected seem to contain much larger particles. Vesicles and particles with cubic structure may coexist in cruder dispersions, and there might be a transformation of MO vesicles into cubic phase particles upon storage, e.g. by aggregation and fusion of the vesicles, leading to the observed particle growth.

4. Conclusions

Dispersions prepared from bulk monoolein–water phases may vary considerably in their macroscopic appearance reflecting differences in the microstructure of the systems, and structural changes can occur both during storage and upon refrigeration. The observation that the structural changes upon refrigeration may be affected by the presence of addititves such as PL may be of more general relevance and important for the further development of these dispersions.

It was not possible to obtain unambiguous indications about the presence of cubic structures in the colloidal dispersions by TEM, whereas, X-ray diffraction studies demonstrated that cubic phases were present only in some of the dispersions. Dispersion of cubic MO/water phases down to colloidal size leads to very complex systems. In cruder colloidal dispersions particles of cubic and non-cubic structure (vesicles?) may coexist. In contrast, no ordered cubic structures could be detected in opaque or opalescent, homogenous colloidal dispersions. Many of the dispersions prepared as described were not suitable for intravenous administration due to the presence and/or formation of coarse particles, particularly those containing cubic structures.

The effect of manufacturing parameters, such as temperature and homogenization conditions, on the structure of the dispersions needs further clarification, and an optimization of the composition and manufacturing procedure is required to obtain colloidal cubic particles (‘cubosomes’) which are stable on storage for sufficiently long times in an adequate temperature range. Recently described manufacturing procedures that do not start from a cubic bulk phase (Gustafsson et al., 1996, 1997; Nakano et al., 2001; Spicer et al., 2001) may provide relevant contributions in this direction even though some important pharmaceutical aspects have not yet been fully explored.

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