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Influence of composition and preparation parameters on the properties of aqueous monoolein dispersions

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Abstract

Colloidal cubic phase particles formed in the monoolein/poloxamer/water system are being investigated as potential drug carriers for, e.g., intravenous administration. Preparation methods must, however, still be further developed to reliably yield monoolein dispersions with cubic particles in a size range acceptable for i.v. administration and adequate long-term stability. In this context, the influence of different composition and preparation parameters on the properties of monoolein dispersions prepared by high-pressure homogenization was studied. High pressure homogenization of coarse poloxamer 407-stabilized monoolein/water mixtures leads to dispersions with a large fraction of micrometer-sized particles at low poloxamer concentrations. Higher poloxamer concentrations lead to lower mean particle sizes but the fraction of cubic particles becomes smaller and vesicular particles are observed instead. A study of the characteristics of a dispersion with a standard composition indicated that the homogenization temperature has a much stronger influence on the dispersion properties than the homogenization pressure or the type of homogenizer used. Temperatures around 40–60 °C lead to the most favorable dispersion properties. The high temperature sensitivity of the preparation process appears to be at least partly correlated with the phase behavior of the dispersed particles determined by temperature-dependent X-ray diffraction.

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1. Introduction

Unsaturated long-chain monoglycerides such as monoolein form different lyotropic liquid crystalline phases with water which can be distinguished, e.g., by their characteristic small angle X-ray scattering patterns. At high water content (~20–40% for monoolein at room temperature), bicontinuous cubic mesophases are observed in which monoglyceride bilayers form a three dimensional network separating two identical water channels with a pore diameter of about 5 nm in the fully hydrated

state (Hyde et al., 1984; Briggs et al., 1996). These highly viscous cubic mesophases are interesting candidates for drug delivery because of the protective properties of the biodegradable lipid/water matrix and its ability to incorporate and slowly release drugs with different physicochemical properties (Shah et al., 2001).

The cubic mesophase formed at the highest water content is stable in excess water and can be fragmented into dispersions of cubic particles using adequate surfactants (Lindström et al., 1981; Larsson, 1989, 2000; Ljusberg-Wahren et al., 1996). In the pharmaceutical field, aqueous dispersions of cubic mesophase particles are particularly promising for parenteral drug delivery as they are syringeable and thus easier to administer than the very viscous cubic phase. For intravenous administration, dispersions of particulate material (e.g., oil in water emulsions used for intravenous nutrition) have to meet tight requirements, particularly concerning their particle size. Not only should the mean particle size be in the – preferably lower – colloidal size range (e.g., <500 nm) but it has also to be ensured that contami-

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nation with microparticles remains below low limits to exclude potentially serious side effects due to blockage of fine blood vessels after administration into the bloodstream.

Different methods have been described for the preparation of cubic phase nanoparticles which are either based on fragmentation of coarser particles using high-energy devices (Landh and Larsson, 1993; Gustafsson et al., 1997; Nakano et al., 2001; Siekmann et al., 2002; Esposito et al., 2003) or precipitation, e.g., from ethanol solutions by dilution with an aqueous phase (Spicer et al., 2001; Um et al., 2003) or from mixed micellar solutions by dialysis (Abraham et al., 2004). Due to their similarity to the well-established preparation technique for submicron lipid emulsions for parenteral nutrition and drug delivery (Lyons and Carter, 1997), the fragmentation techniques using high-pressure homogenization appear particularly promising for larger-scale pharmaceutical production. Typically, fragmentation is performed using poloxamer 407, a polyoxyethylene–polyoxypropylene–polyoxyethylene block copolymer as dispersing agent. Although it has been confirmed in many studies that the cubic phase can be preserved in colloidal dispersions in the monoolein/poloxamer/water system it has also been found that the ultrastructure of high-pressure homogenized nanodispersions is highly dependent on manufacturing parameters such as composition or dispersing conditions. These can affect relevant properties of the dispersions reflected in, e.g., a partial or complete loss of the cubic state of the particles and/or colloidal instability and the presence of unacceptably large particles (Gustafsson et al., 1997; Siekmann et al., 2002). Dispersions formed in the monoolein/poloxamer 407/water system thus seem to be very sensitive towards variations in preparative procedures. The present study was therefore aimed at investigating the influence of important preparative parameters on the structure and properties of high pressure homogenized monoolein dispersions in more detail. The study concentrates on variations in stabilizer concentration and homogenization parameters (pressure, temperature, type of equipment) taking the production of a dispersion of exclusively (small) submicron particles with cubic internal structure as ultimate goal. A particular focus was the reduction of microparticulate contaminations, a very important issue which has hitherto not been given sufficient attention.

2. Materials and methods

2.1. Materials

A batch of Rylo MG 19 (Danisco Coultor, DK-Grindsted) consisting according to the manufacturer's certificate of analysis of about 99% monoglycerides with ~90% of the fatty acid residues being oleic acid, ~5% saturated and 6% polyunsaturated fatty acids, was used as monoolein for sample preparation. As the other materials – poloxamer 407 (Pluronic F127, BASF, D-Ludwigshafen) and thiomersal (Synopharm, D-Barsbüttel) – the monoolein was used for preparation as received from the manufacturer. Purified water was prepared by filtration and deionization/reverse osmosis (Milli RX 20, Millipore, D-Schwalbach) of drinking water.

Table 1
Composition of the dispersions

Name	Related to the sum MO + P407		Related to overall dispersion Composition (% w/w)
	P407 (%, w/w)	P407 (%, mol/mol)	
4% P407	4	0.12	4.8% MO, 0.2% P407, 95% water
6% P407	6	0.18	4.7% MO, 0.3% P407, 95% water
7.4% P407	7.4	0.23	4.63% MO, 0.37% P407, 95% water
8% P407	8	0.25	4.6% MO, 0.4% P407, 95% water
10% P407	10	0.32	4.5% MO, 0.5% P407, 95% water
12% P407	12	0.39	4.4% MO, 0.6% P407, 95% water

2.2. Dispersion preparation

The preparation process of the dispersions (which had the compositions given in Table 1) followed a procedure described by Gustafsson et al. (1997) with slight modifications. Different ratios of monoolein and poloxamer were mixed in the melt and the molten mixture was added dropwise to water under stirring at room temperature. The resulting coarse dispersion was equilibrated for at least about 1 day at room temperature before homogenization in a Microfluidizer M110S (Microfluidics, USA, equipped with a water bath to thermostatize interaction chamber and heat exchanger) at 350 bar for 15 min at 40 °C (“standard procedure”), unless otherwise specified. Alternatively, homogenization was performed with a piston-gap-homogenizer (Micron Lab 40, ML) with five cycles at 350 bar at an initial temperature of 20 °C or 60 °C. The dispersions were filled in glass vials, flushed with nitrogen and stored at 23 °C protected from light.

2.3. Characterization

2.3.1. Visual inspection

About 1 week after preparation, the dispersions were visually assessed for optical appearance (i.e., color, turbidity, homogeneity, presence of macroscopic particles and deposition of material on the glass surface of the vial at the air-dispersion-glass contact line—referred to as “ring formation” in the following).

2.3.2. Particle size analysis by laser diffraction

The particle size of the dispersions was measured with a Coulter LS 230 (Beckman Coulter, D-Krefeld) combining information from simple light scattering (LS) and polarization intensity differential scattering (PIDS). For data evaluation, an optical model based on the Mie theory was created using the instrumental software assuming 1.45 as the real and 0 as the imaginary part of the refractive index of the particles. Results given are the mean of 5 successive measurements of 120 s each.

2.3.3. Light microscopy

A Leica DMRXP microscope (Leica, D-Wetzlar) calibrated with a micrometer slide was used with differential interference contrast or polarized light at magnifications between 100 and 1000×.

2.3.4. Small angle X-ray diffraction

Samples were measured with a Kratky camera (Hecus Braun X-ray systems, A-Graz) on a conventional X-ray source (generator ID3003, Rich. Seifert, D-Ahrensburg) using a position sensitive detector (PSD-50 M, M. Braun, D-Garching) and an exposure time of 1–2 h at 25 °C. Alternatively, measurements were performed with 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. The camera was equipped with a position sensitive linear detector, exposure time was 5–10 min. Standard data acquisition and evaluation systems were used (Boulin et al., 1986, 1988). Sample cells were thermostated with a water bath (Huber Ministat) equilibrating the samples for 10 min prior to each measurement during temperature-dependent investigations. Reflections were assigned to the different lyotropic liquid crystalline phases using the characteristic spacing ratios (cubic type P ($Im3m$): $\sqrt{2} : \sqrt{4} : \sqrt{6} : \sqrt{8} : \sqrt{10} : \sqrt{12} : \sqrt{14} : \sqrt{16} : \dots$; cubic type D ($Pn3m$): $\sqrt{2} : \sqrt{3} : \sqrt{4} : \sqrt{6} : \sqrt{8} : \sqrt{9} : \sqrt{10} : \sqrt{11} : \dots$; hexagonal: $1 : \sqrt{3} : \sqrt{4} : \sqrt{7} : \sqrt{9} : \sqrt{12} : \sqrt{13} : \sqrt{16} : \dots$). The lattice constant a of the respective cubic phase was calculated from the spacing d of the first reflection observed by $a = d\sqrt{2}$.

2.3.5. Cryoelectron microscopy

A drop of dispersion was placed on a bare copper grid and plunge frozen in liquid ethane at approximately 100 K after removal of excess liquid. Excess ethane was removed and the sample was transferred into a cryoelectron microscope (CEM902a, Zeiss, D-Oberkochen) operated at 80 kV. Samples were viewed under low-dose conditions at a constant temperature around 77–100 K. Images were acquired by a Dage SIT low intensity TV camera system and processed by a Kontron IBAS image processing system.

3. Results and discussion

3.1. Influence of poloxamer 407 (P407) concentration

Depending on water content binary monoolein/water systems form a lamellar liquid crystalline phase or bicontinuous cubic phases (gyroid (G)-type cubic phase (Q^{230} , $Ia3d$) and diamond (D)-type cubic phase (Q^{224} , $Pn3m$)) at room temperature (Hyde et al., 1984). The D-type cubic phase is stable in excess water which separates from the liquid crystalline structure after its maximum swelling capacity is reached at about 40% (w/w) water. The admixture of P407 to monoolein/water further complicates the phase behavior. The occurrence of the so-called P-type cubic phase (Q^{229} , $Im3m$), which is also stable in excess water, at certain poloxamer concentrations is particularly important in the case of the nanodispersions under investigation here (Landh, 1994). Since an influence of poloxamer concentration is already observed in the bulk, some effect might also be expected for the dispersions. Therefore, the potential influence of poloxamer 407 concentration was studied on dispersions prepared with 4–12% of this surfactant (related to the sum of monoolein and poloxamer) according to the standard procedure (Table 1).

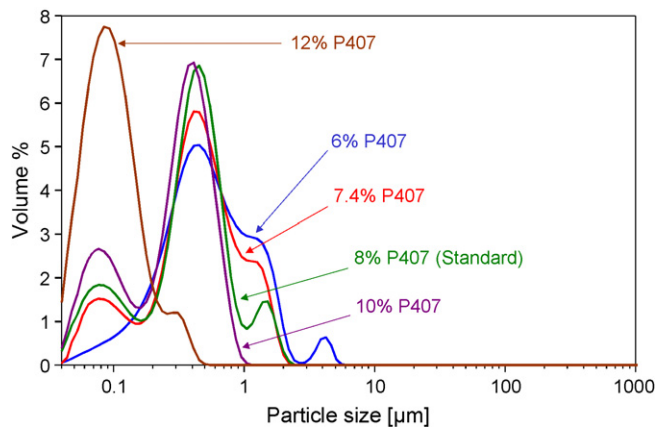


Fig. 1. Particle size distributions of the dispersions with different P407 concentration.

The particle size of the dispersions with 4% P407 could not be meaningfully investigated with laser diffraction due to the presence of very large particles accompanied by strong “ring formation” on the vial surface. Starting from a P407 concentration of 6% the dispersions displayed a lower tendency towards “ring formation” as well as a decreasing fraction of particles in the micrometer size range (Fig. 1) with increasing P407 concentration. At 10% P407 all particle sizes were below $\sim 1.3 \mu\text{m}$ according to LS-PIDS analysis. While the fraction of particles with sizes between about 0.2 and $1 \mu\text{m}$ changed only slightly up to a P407 concentration of 10%, it was distinctly reduced in the sample containing 12% P407. This dispersion contained almost exclusively particles with sizes around $0.1 \mu\text{m}$. The observed reduction in particle size with increasing P407 concentration is in good agreement with the results of other studies (Gustafsson et al., 1997; Nakano et al., 2001) which did, however, not address the microparticulate fraction. The formation of micrometer sized particles at low P407 concentrations may point to a lack of hydrophilic surfactant in these dispersions which does not allow to stabilize the large specific surface area of exclusively colloidal particles. Particles in the micrometer range (typically up to about $5\text{--}10 \mu\text{m}$) were also detected by microscopy in all dispersions under investigation, even in the one with 12% P407 (the dispersion with 4% P407 was not investigated due to its poor overall quality).

Investigation of selected dispersions by cryo-TEM (Fig. 2) revealed ultrastructural parameters which were in good agreement with the particle sizing results, although no quantitative information about particle size could be obtained by this method for the dispersions under investigation: In the dispersion with 7.4% P407 particle sizes reached several hundred nm whereas they were distinctly smaller in the dispersion with 10% P407. The 12% P407 dispersion contained almost exclusively particles with sizes below $0.1 \mu\text{m}$, without detectable fraction of microparticles. The micrographs also revealed that large particles predominantly had a regularly ordered internal structure whereas particles around $0.1 \mu\text{m}$ or smaller either had no internal structure (vesicles) or were irregularly structured. Similar results on the ultrastructure of monoolein dispersions with different P407 concentrations were previously reported by Gustafsson et

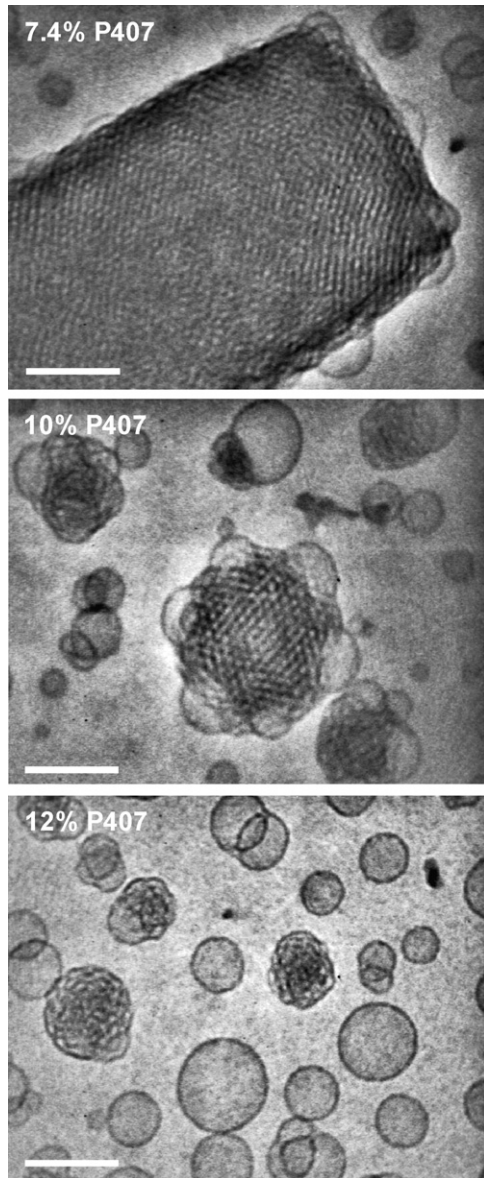


Fig. 2. Cryoelectron micrographs of dispersions with different P407 concentration (bars represent 100 nm).

al. (1997). Moreover, the results were confirmed for an 8% and a 12% P407 dispersion in a recent study dealing with temperature effects (Wörle et al., 2006a).

In X-ray diffraction measurements, the dispersion with 12% P407 which contained almost exclusively particles without regular internal structure according to the electron microscopic investigations did not display assignable reflections even with synchrotron radiation. This dispersion is thus devoid of a detectable fraction of cubic phase particles. In contrast, all dispersions with lower P407 concentration displayed small angle X-ray reflections characteristic of a P-type cubic phase with very similar lattice constant (ca. 14.1–14.6 nm, independently of P407 concentration). The X-ray diffraction results are thus in good agreement with those reported by Gustafsson et al. (1997) except those obtained on the dispersion with 4% P407. A fraction of

D-type cubic phase might be expected in dispersions with lower P407 concentration according to previous investigations on dispersions of cubic phase particles (Gustafsson et al., 1997) as well as to results obtained for the behavior of the bulk phases (Landh, 1994). Small angle X-ray reflections corresponding to a D-type cubic phase could, however, not be detected for any of our dispersions at room temperature which may tentatively be attributed to a slightly different MO/P407 ratio and/or distinct differences in homogenization temperature. Only after increasing the temperature to 60 °C did the dispersion with 6% P407 give rise to additional reflections that indicate the presence of D-type cubic phase (Fig. 3). These additional reflections apparently already started to form at 40 °C as the two smaller reflections of the P-type cubic phase were broadened at this temperature. In contrast, reflections indicating the presence of D-type cubic phase could not be detected for the dispersion with 8% P407. Transferred to the situation during homogenization (performed at 40 °C) it may thus be assumed that the dispersion with 6% P407 might contain a non-negligible fraction of D-type cubic phase under these conditions. This suggests that the presence of D-type cubic phase has some relevance for the poorer colloidal quality (presence of macroscopically visible particles, stronger ring-formation) of the 6% P407 dispersion compared to the one prepared with 8% P407. Accordingly, the transient formation of a D-type cubic phase during the homogenization step might also contribute to the even worse quality of the dispersion with 4% P407. Unfortunately, this assumption could not be proven experimentally since the low stability of this dispersion precluded any meaningful temperature-resolved X-ray investigation.

In contrast to our results, Nakano et al. (2001) found a D-type cubic phase, besides increasing fractions of P-type cubic phase,

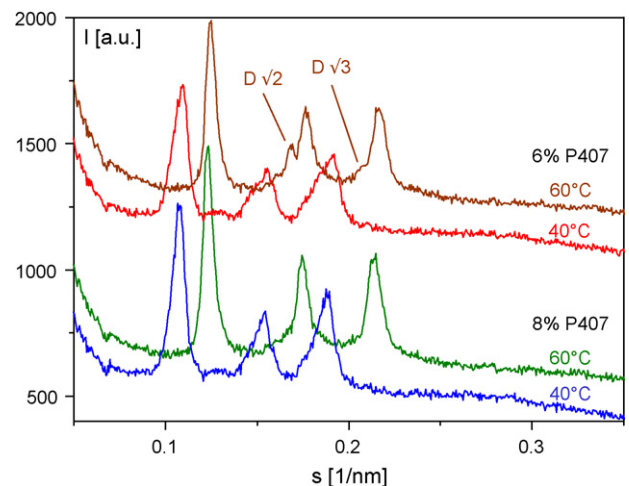


Fig. 3. Small angle X-ray diffractograms of selected dispersions in dependence on temperature. The three strong reflections of the sample with 8% P407 are indicative for the presence of P-type cubic phase. The shift of the reflections to larger s -values with increasing temperature indicates a decrease in lattice parameter under these conditions. The sample with 6% P407 displays two additional distinct reflections ($D\sqrt{2}$, $D\sqrt{3}$) that point to the presence of D-type cubic phase. A minor fraction of this phase has probably already formed at 40 °C in this sample as indicated by the broadening of the superimposed reflections of the P-type cubic phase.

up to quite high P407 concentrations without loss of cubic state at even higher P407 concentrations than those used in our study. These differences in the behavior of the dispersions are probably caused by differences in composition (different monoolein quality, buffered saline instead of water) and dispersion procedure (ultrasonication).

Although the use of more than 8% P407 leads to a better result of homogenization in terms of smaller particle sizes and reduced ring formation it does not improve the properties of the dispersions as the fraction of particles with cubic internal structure decreases and completely vanishes with 12% P407. The presence of predominantly vesicular structures at high poloxamer concentrations may be due to the formation of mixed monoolein/poloxamer bilayers which sterically stabilize the particles against their fusion into the cubic state as also pointed out by Gustafsson et al. (1996). Recent investigations have shown that the vesicles can be transformed into cubic phase particles by a special heat treatment procedure (Barauskas et al., 2005a; Wörle et al., 2006a, 2006b) and thus confirm that the cubic and not the vesicular structure is the equilibrium state even at these high P407 concentrations.

In summary, for the preparation conditions applied here, P407 concentrations between about 8 and 10% seem to yield dispersion properties that are closest to the desired submicron dispersions of cubic particles. The properties of the corresponding dispersions are, however, still not fully satisfactory as they both contain microparticulate contaminations (in particular the dispersion with 8% P407) and a considerable fraction of non-cubic particles (in particular the dispersion with 10% P407).

3.2. Influence of homogenization pressure

The influence of several important preparation parameters (homogenization pressure, temperature during homogenization and type of homogenizer), on the properties of the resulting dispersions were investigated using a standard formulation with 8% P407 which provides a reasonable compromise between small particle size and low content of non-cubic particles.

Comparison of the particle sizes obtained by preparation of three dispersions with 8% P407 according to the standard procedure (Microfluidizer, 40 °C, 15 min) but with different homogenization pressures (280, 350, 500 bar) revealed negligible differences between the dispersion prepared at 280 and 350 bar (Fig. 4). The expected higher disruptive power at higher homogenization pressures became, however, evident for the dispersion prepared at 500 bar: For this dispersion, the fraction of non-colloidal particles had almost disappeared. A shift to smaller particle sizes was observed, with a distinct increase of the particle fraction around 0.1 μm which might indicate the presence of a larger fraction of non-cubic particles according to the results reported above (cf. Section 3.1). No influence of homogenization pressure on ring formation could be detected. Investigations at higher homogenization pressure were precluded by the limited cooling capacity of the equipment which did not allow to obtain a constant homogenization temperature at higher pressures.

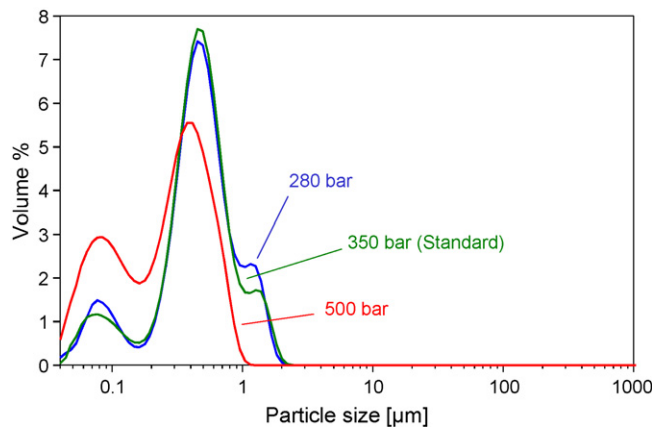


Fig. 4. Influence of homogenization pressure on dispersions prepared with 8% P407 (related to the sum of MO + P407).

3.3. Influence of temperature during homogenization

In order to investigate the influence of temperature during homogenization four dispersions were prepared according to the standard procedure (Microfluidizer, 350 bar, 15 min) but at different temperatures (20, 40, 60, 80 °C). No colloidal dispersions could be obtained at 20 and 80 °C. Homogenization at 20 °C led to a heavily foaming, rapidly creaming dispersion with a large number of visually detectable particles and heavy ring formation; large particles and aggregates were also observed microscopically. According to LS-PIDS analysis (Fig. 5), 50% of the particles were larger than 37 μm. During homogenization at 80 °C very large aggregates (several mm to cm) built up in the sample container of the homogenizer. These aggregates stuck to the walls and could not be brought back into the homogenization cycle by stirring. They remained in the storage container upon recovery of the homogenized dispersion which precluded any meaningful particle sizing. In contrast, homogenization at 60 °C led to a dispersion with smaller particle sizes than upon processing at the standard temperature of 40 °C (Fig. 5). In particular, there was no fraction of microparticles in the dispersion homogenized at 60 °C according to LS-PIDS. Ring formation was also distinctly reduced upon storage of this dispersion. The

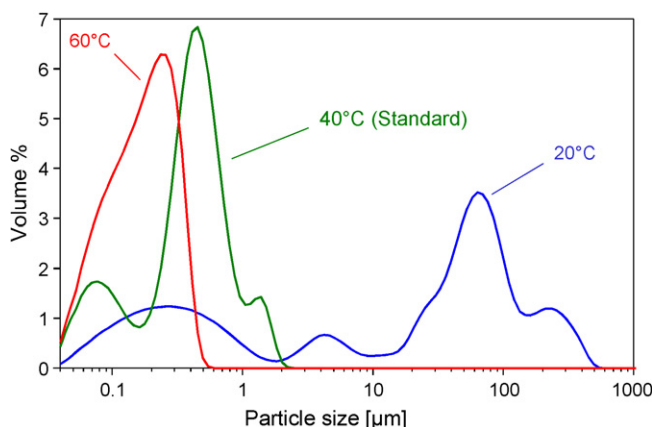


Fig. 5. Influence of homogenization temperature on dispersions prepared with 8% P407 (related to the sum of MO + P407).

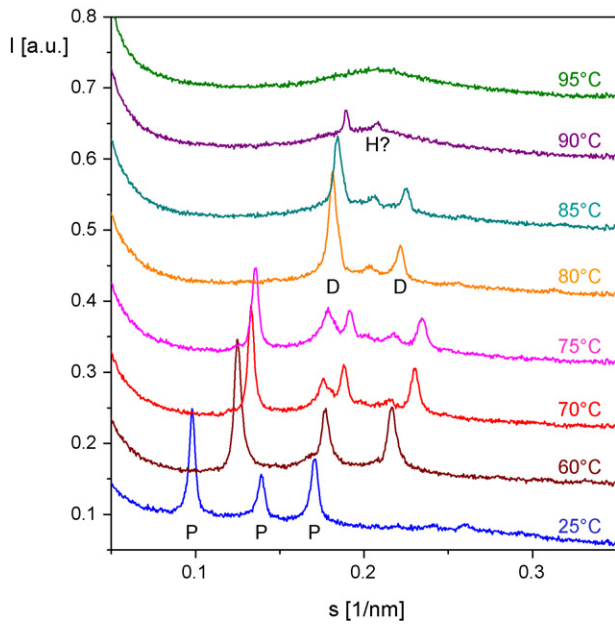


Fig. 6. Temperature-dependent small angle X-ray diffractograms of the standard dispersion (8% P407 related to the sum of MO + P407, homogenization at 350 bar and 40 °C for 15 min). The letters indicate the reflections of the P-type (P) and D-type (D) cubic as well as the assumed inverse hexagonal (H?) phase.

presence of cubic particles in this dispersion was confirmed by X-ray diffraction which revealed distinct reflections characteristic of the P-type cubic phase with about the same intensity and lattice constant as for the standard dispersion homogenized at 40 °C (the dispersions prepared at 20 and 80 °C were not investigated by X-ray diffraction because of their poor quality).

Homogenization of the standard formulation thus leads only to colloidal dispersions in a comparatively narrow temperature window between about 40 and 60 °C. The strong dependence on preparation temperature is probably related to the temperature-dependent phase behavior of the particles (Fig. 6). X-ray diffractograms of the standard dispersion revealed the exclusive presence of P-type cubic phase up to about 60 °C. Above about 70 °C an additional D-type cubic phase was detected and around 80–85 °C the reflections almost exclusively corresponded to this phase. Before the sample transformed into an isotropic state between 90 and 95 °C additional reflections became visible in addition to those of the D-type cubic phase, suggesting the presence of an additional hexagonal phase (such a phase was also observed by polarized light microscopy in a corresponding coarse, non-homogenized dispersion in this temperature range). The results of the temperature-dependent homogenization studies are a further indication that the D-type cubic phase (which would form during the homogenization step at 80 °C) cannot be processed into stable colloidal dispersions as already assumed above (cf. Section 3.1). This behavior might be specific for monoolein based dispersions since dispersions of D-type cubic particles phase have been described for the system monolinolein/poloxamer/water (de Campo et al., 2004). In contrast to our results, other authors have reported the formation of colloiddally dispersed particles with comparable monoolein/poloxamer/water compositions at 80 °C (Gustafsson

et al., 1997). This apparent contradiction cannot be explained at present. A possible explanation would be deviations in actual sample processing temperature between the two studies.

The unsatisfactory homogenization result at 20 °C cannot be explained by a negative influence of a D-type cubic phase as the particles should exist in the P-type cubic phase at this homogenization temperature. Whether it might be caused by a low diffusion coefficient of the large poloxamer molecules at this temperature leading to slow stabilization during homogenization, an unfavorable distribution coefficient towards the lipid bilayers, an increased viscosity of the cubic phase at 20 °C or other factors remains to be investigated. Barauskas et al. (2005b) reported the preparation of a homogeneous dispersion with mean particles sizes around 100 nm at 25 °C with a slightly higher poloxamer concentration and a modified preparation procedure. This dispersion contained, however, a large fraction of non-cubic particles and its transformation into a dispersion of predominantly cubic particles required heat treatment.

3.4. Influence of the type of homogenizer

Since the use of different types of homogenizers may lead to differences in particle size distribution, e.g., for liposomal or conventional emulsion systems (Brandl, 1998; Stang et al., 2001), the efficiency of the Microfluidizer used as standard equipment for dispersion preparation in our studies was compared to that of a piston-gap-homogenizer (Micron Lab 40) at two different homogenization temperatures, 20 and 60 °C. For processing, the coarse dispersions were filled into the homogenizers which had been preheated or precooled to the process temperature. Dispersions were homogenized in the Microfluidizer for 15 min at 350 bar (dispersions were identical to those described in Section 3.3) or with three immediately successive cycles at 350 bar in the Micron Lab. The process temperature was less well controlled with the Micron Lab: after homogenization at 20 °C the temperature of the dispersion had increased to 23 °C, whereas during homogenization around 60 °C the temperature of the dispersion fell to 52 °C until the end of the process although the equipment had been preheated to 65 °C before homogenization. In contrast, the temperature in the Microfluidizer remained essentially constant during the process as monitored for selected dispersions by a lab thermometer. At both temperatures, homogenization with the Micron Lab yielded, however, similar dispersion properties as processing with the Microfluidizer. Processing at 20 °C led to a heavily foaming, rapidly creaming dispersion with a large amount of visually detectable particles and strong ring formation; in the microscope, large numbers of large particles and aggregates were detected. According to LS-PIDS, 50% of the particles were larger than $\sim 110 \mu\text{m}$. In contrast, a homogenous, milky-white dispersion was obtained after homogenization at 60 °C which caused only very slight ring formation. The particle size distribution (50% $< 0.22 \mu\text{m}$) was shifted to slightly larger sizes compared to homogenization with the Microfluidizer (Fig. 7). According to these results, the type of homogenizer had only minor influence on the properties of the dispersions formed within the frame of experimental conditions studied.

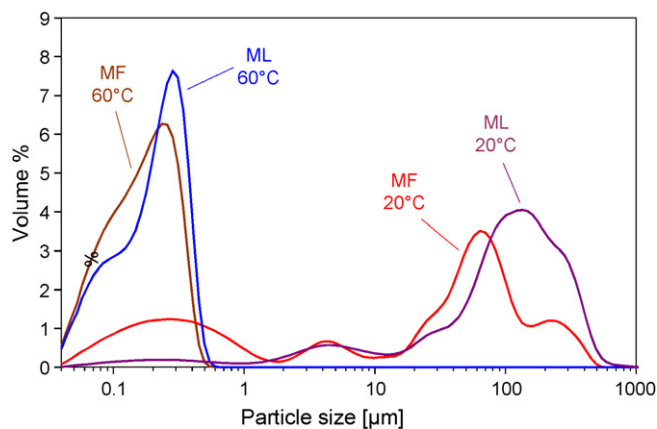


Fig. 7. Particle size distributions obtained after homogenization of dispersions with 8% P407 (related to the sum of MO-P407) at different temperatures in dependence on the type of homogenizer MF=Microfluidizer, ML=Micron Lab).

4. Conclusion

The results obtained with different poloxamer 407 concentrations underline the pronounced influence of composition on the properties of the resulting dispersions. Although comparatively high P407 concentrations are beneficial in terms of formation of smaller particles, they also promote formation of vesicular particles rather than of the desired particles of cubic structure. In addition to its simple particle surface stabilizing function the polymeric surfactant also acts in a different way in the monoolein dispersions under investigation here than in classical colloidal dispersions (e.g., emulsions of liquid oils). It becomes part of the structure of the particles and influences their phase behavior. In particular, the presence of a sufficient amount of P407 ensures the appearance of P-type cubic phase under the standard homogenization conditions which seems to promote the formation of a stable dispersion of colloidal cubic particles. In contrast, the presence of D-type cubic phase during the fragmentation step appears to induce formation of large, non-colloidal particles.

The study of the influence of process parameters shows that temperature during homogenization is of crucial importance for the final particle size distribution of the dispersions under investigation. Colloidal dispersions of cubic particles could only be obtained between 40 and 60 °C for the composition investigated here with homogenization at 60 °C leading to the smallest mean particle sizes. The poor quality of a dispersion prepared at 80 °C might again be correlated with the transient formation of D-type cubic structure during homogenization whereas the cause for the difficulties of dispersing the samples at 20 °C remains to be elucidated. The type of homogenization equipment did not influence the properties of the dispersion much, whereas an increase in homogenization pressure led to a slight reduction of the fraction of micrometer size particles compared to the usual pressure of 350 bar.

From a more general point of view, the results indicate that the development of optimal formulations for colloidal dispersions of cubic phase monoglyceride particles, characterized by the

absence of a large fraction of vesicular or micron size particles, requires careful optimization of the formulation parameters, including the composition, especially the fraction of surface active agent, and the homogenization temperature. It has to be stressed that the parameters investigated in the present study are not exhaustive and other factors may also play a role. For example, differences in the type of energy input (ultrasonication versus high pressure homogenization) or composition of the aqueous phase (saline buffer versus water) may be responsible for the different behavior of dispersions with similar compositions (Nakano et al., 2001) compared to that observed here. Factors like these remain to be identified and evaluated in more detail. Moreover, as several different parameters are relevant for the properties of the dispersions, their characteristics are very likely influenced by these parameters in a multifactorial way. It would thus be interesting to study potential mutual influences in a corresponding experimental study designed to obtain more detailed information concerning the way the characteristics of the dispersion can be optimized into the desired direction.

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