Investigation of the phase behavior of monoolein/surfactant dispersions of different composition and preparation methods

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Colloidally dispersed cubic phases of glyceryl monooleate (MO, C18:1) in water have been proposed as potential carriers for the intravenous administration of peptide and protein drugs. For the preparation of such dispersions, e.g., high pressure homogenization of a predispersed cubic phase with the aid of an additional surfactant has been described. After homogenization, however, particles without cubic structure have been observed besides the desired particles with cubic structure [1]. The aim of this study was to investigate the influence of MO-grade, surfactant concentration, homogenization procedure and particle size on the phase behavior and structural parameters of the dispersed particles.

For the investigation of the influence of particle size and surfactant concentration, samples with different ratio of MO/PS80 (Polysorbate 80 concentrations from 25% to 45% (w/w) in steps of 5%, based on the sum of MO and PS80) were prepared. MO (GMOrphic-80, Eastman Chemical Company, consisting of ≥ 94% monoglycerides, min. 75% C18:1) and the required amount of PS80 were melted together at 60°C and mixed. The liquid mixture (approx. 40-50°C) was added dropwise to water at room temperature under stirring. The resulting MO/PS80-dispersions of 6.5% (w/w) were stored for approx. 12 h under stirring at room temperature for equilibration. To obtain dispersions of different particle sizes, portions of the stirred dispersions were treated by an ultraturrax (UT) only or subjected to ultraturrax treatment followed by high pressure homogenization (UT ML). High pressure homogenization, which led to much smaller average particle sizes as indicated by particle size measurement after preparation, was performed using a Micron Lab 40 (APV Gaulin, Lübeck, Germany), 3 cycles at 350 bar at room temperature.

Small angle X-ray diffraction data of selected dispersions were collected at 20°C, 37°C and 50°C to 90°C in steps of 10°C. The samples were equilibrated at the corresponding temperature for at least 5 to 10 minutes prior to the measurement (exposure time 5 to 10 minutes). Liquid crystalline phases were assigned by comparison of the diffraction patterns with the characteristic spacing ratios (table 1) of the expected phases. Lattice parameters were calculated from the first peak of the diffraction pattern.

Table 1: Characteristic spacing ratios of cubic liquid crystalline phases

phase	characteristic spacing ratios
Ia3d	√6 : √8 : √14 : √16 : √20 : √22 : ...
Pn3m	√2 : √3 : √4 : √6 : √8 : √9 : √10 : √11 : ...
Im3m	√2 : √4 : √6 : √8 : √10 : √12 : √14 : √16 : ...

None of the high pressure homogenized dispersions displays any reflections at room temperature. Of the non-homogenized samples only the dispersion with 25% PS80 has weak, not assignable reflections at room temperature. For dispersions with surfactant concentrations of 35% and above no liquid crystalline structures were observed at any of the temperatures. In the diffraction patterns of the 25% and 30% samples, reflections occur with increasing temperature. At temperatures above 37°C the reflections can be assigned to specific cubic symmetries. At 50°C and 60°C, all dispersions display reflections due to the Im3m phase as well as the Pn3m phase but in different ratios. The homogenized dispersions (smaller particle size) contain a higher amount of Im3m phase than their non-homogenized counterparts (figure 1), indicating that the phase behavior might be correlated with particle size (equilibration times were shown to be sufficiently long since, e.g., at 60°C there is no change in the diffraction pattern within at least 60 min.). With further heating, the Im3m-reflections disappear. The Pn3m-reflections can be observed until 80°C. The homogenized 30% sample was also investigated at 90°C and after recooling to 60°C. At 90°C no reflections were observed. At 60°C the Pn3m reflections reoccur but - at least within 15 min. - the Im3m reflections do not. This behavior may result from particle growth during heating to 90°C. The lattice parameter *a* of both cubic phases increases with the amount of surfactant from 25% to 30%, but is the same in homogenized dispersions compared to the corresponding non-homogenized samples.

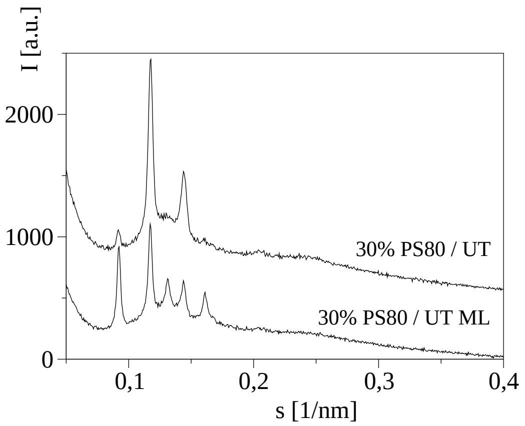

Figure 1: Small angle X-ray diffractograms of MO/PS80-dispersions for a homogenized and a non-homogenized MO/PS80 dispersion (curves are displaced along the ordinate)

To study the influence of MO grade and preparation method, different MO dispersions were prepared containing 7.4% (w/w, based on the sum of MO and P407) of Poloxamer 407 (P407) as the surfactant. The dispersions varied with respect to the type of MO (GMOrphic as described above versus RYLO MG 19, Danisco Cultor, Denmark, consisting of 98.7% monoglycerides, 90.3% C18:1) and to the preparation process. The GMOrphic containing system was prepared as described above (UT ML). The RYLO containing samples were not treated with an ultraturrax and high pressure homogenization (MF) was performed with a Microfluidizer M-110 (Microfluidics Inc., USA) at different pressures (350 to 700 bar), different homogenization times (5 to 20 min.) and different temperatures (40°C to 80°C) (similar to the process in [1]). For these dispersions data were collected at 20°C to 80°C in steps of 20°C and at 95°C.

In contrast to the dispersions prepared with PS80, P407 containing systems display reflections indicating the presence of Im3m symmetry already at 20°C. These reflections become more intense upon heating and can be observed clearly up to 60°C (in a few cases only the reflections were too weak to allow assignment of a specific symmetry). At 80°C, the diffraction patterns exhibit Pn3m-reflections, which disappear at 95°C. For the different dispersions at a given temperature there is no significant difference in the lattice parameters *a* (e.g., 14.2-14.7 at 20°C, figure 2), but *a* decreases with increasing temperature.

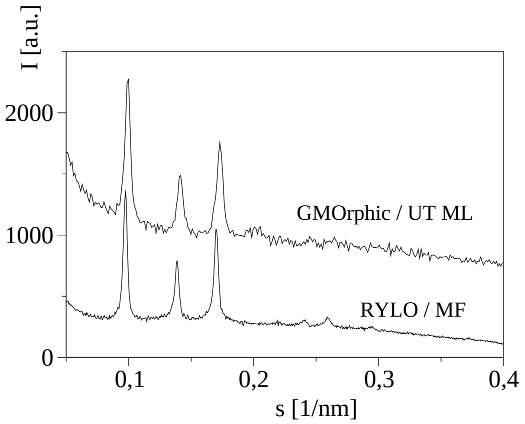

Figure 2: Small angle Xray diffractograms of MO/P407-dispersions with different MO grade and prepared by different homogenization processes (350bar, 40°C, 5 min. for RYLO) (curves are displaced along the ordinate)

References

[1] J. Gustafsson, H. Ljusberg-Wahren, M. Almgren, and K. Larsson, Langmuir 13, 6964-6971 (1997)