

Reversible Phase Transitions in Emulsified Nanostructured Lipid Systems

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Aqueous submicron-sized dispersions of the binary monolinolein/water system, which are stabilized by means of a polymer, internally possess a distinct nanostructure. Taking this as our starting point, we were able to demonstrate for the first time that the internal structure of the dispersed particles can be tuned by temperature in a reversible way. Upon increasing the temperature, the internal structure undergoes a transition from cubic via hexagonal to fluid isotropic, the so-called L_2 phase, and vice versa. Intriguingly, in addition to the structural changes in topology, the particles expel (take up) water to (from) the aqueous continuous phase when increasing (decreasing) the temperature in a reversible way. At each temperature, the internal structure of the dispersed particles corresponds very well to the structure observed in nondispersed binary monolinolein with excess water. This agreement is independent of any thermal history (including phase transitions), which proves that the structures in the dispersed particles actually are in thermodynamic equilibrium with the surrounding water phase.

Introduction

In recent years, significant interest has been evinced toward formulating and characterizing complex lyotropic liquid crystalline phases (lamellar, hexagonal, and cubic structures) of surfactant-like lipids and their aqueous nanostructured dispersions.^{1–5} These unique systems are important in both scientific and industrial fields. Self-assembled structures of lipids build up biological membranes, and they play a very important role in cell structure and functions.⁶ In the last two decades, they have also attracted great attention because of their usability in various practical applications.^{7–11} These systems are important as membrane mimetic matrixes,¹² as vehicles for the solubilization of active ingredients^{12,13} (e.g., vitamins and enzymes), as unique microenvironments for the controlled release of additives^{8,9} (e.g., drug delivery) or the crystallization of membrane proteins,^{14–16} and also

as “nanoreactors” for generating active molecules.¹⁰ Especially Caffrey and co-workers have devoted considerable effort in investigating and characterizing the rich polymorphism of binary monoglyceride/water phases in great detail: these display, in general, a rich variety of assemblies, depending on concentration and temperature.^{17–20} Such phase diagrams often feature a fluid isotropic phase (so-called L_2), a lamellar phase (L_α), an inverted type of hexagonal phase (H_2), an inverted bicontinuous cubic (V_2) liquid crystalline phase, and, at low temperature, a lamellar crystalline phase (L_c). H_2 consists of water and hydrated headgroup aggregates arranged as cylinders in a continuous nonpolar matrix composed of the hydrocarbon chains.^{21,22} V_2 was shown to be a three-dimensional bicontinuous phase composed of bilayers^{1,7,23,24} which separate two aqueous channels giving an infinite periodic minimal surface (IPMS). Depending on the water content, two different types of bicontinuous cubic phases were reported:^{9,20,25} cubic assemblies with the $Ia3d$ (corresponding to the gyroid type of IPMS, C_G) and the $Pn3m$ (diamond type of IPMS, C_D) symmetry.

In the pioneering work of Larsson and co-workers,^{26–28} it was established that monoglycerides can be dispersed in water using different dispersing agents, whereby submicron-sized particles with preserved internal nano-

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structures are formed.^{4,5,26–33} The formulation of these aqueous dispersions requires stabilization by an additional emulsifier like a block copolymer. When the internal structure of the dispersed particles consisted of cubic phase, they were termed cubosomes,^{4,26–28} and when the internal structure was hexagonal (achieved by addition of a third component), they were termed hexosomes.^{4,27,30,33}

Various studies of lipid systems have been carried out aiming at getting stable colloidal dispersions of cubic and hexagonal phases, investigating their internal structure, and formulating drug-delivery systems.^{4,5,8–10,26–35} To better design such systems in the future, it is important to understand the internal structure of the formed dispersed particles. It is hypothesized that the nanostructural arrangement within the particle will significantly determine the delivery properties of the system.

The main goal of the present study is to investigate the phase transitions in such dispersions induced by temperature variations. The following two main questions will be answered: (a) does the internal structure of the dispersed particles change from cubic via hexagonal to L_2 upon heating as described in the literature for the nondispersed bulk phase of similar systems,²⁰ and (b) is the temperature-dependent formation of the self-assembled internal structure of the particles reversible, that is, are the dispersed particles in real thermodynamic equilibrium with the corresponding continuous aqueous phase? The used monoglyceride was a commercial monolinolein (MLO) sample. Due to its unsaturation (two double bonds), the monolinolein–water system forms all three major phases, that is, the reversed bicontinuous cubic, the reversed hexagonal, and the so-called L_2 phase, within the feasible experimental temperature range between 20 and 95 °C. To the best of our knowledge, no studies have been published so far in which the structural transitions induced by temperature are investigated in the diluted dispersed systems. We are also the first to report on the formation of what we termed an emulsified L_2 phase (ELP) at high temperatures. In analogy to ELP, for reasons of clarity (and possible future names), we found it more convenient to term the emulsified cubic phase ECP and the emulsified hexagonal phase EHP.

Materials and Methods

Materials. Monolinolein (emulsifier TS-PH 039, glycerol monolinoleate, MLO) was supplied by Danisco A/S (Brabrand, Denmark). This distilled monoglyceride has a purity of 93.8% monoglyceride (4.1% diglycerides). The fatty acids are composed of 91.8% linoleate, 6.8% oleate, and about 1% saturated fatty acids. Pluronic F127 (PEO₉₉–PPO₆₇–PEO₉₉) was a gift from BASF Corp. (Mount Olive, NJ). These ingredients were used without further purification. The water was double-distilled.

Preparation of Binary MLO/Water Nondispersed Systems (Bulk Phase). The samples in our study were prepared in Pyrex tubes by weighing the appropriate amounts of MLO with water, heating them by using an air gun, and homogenizing by vigorous agitation with a Vortex. They were then left to cool

at room temperature. To ensure thermodynamic equilibrium of the samples, they were frozen for at least 1 h prior to small-angle X-ray scattering (SAXS) investigations²¹ and then equilibrated for some hours at room temperature. Some samples were also prepared by cycles of only centrifuging and freezing. These did not show differences in the phase behavior, which reconfirms that the heating procedure did not damage the temperature-sensitive double bonds of MLO significantly. The relative error of the water content due to inhomogeneities in the sample is estimated to be less than 3%.

Formulation of MLO Aqueous Dispersions. Less than a gram of MLO (viscous liquid) was weighed into a 20 mL vial. F127 and then water, or a 1 wt % F127 solution, was added to have 10 g in total, and the sample containing all three ingredients was dispersed by ultrasonication for 20 min, resulting in a milky solution. Ultrasonication was carried out using a high-intensity ultrasonic processor (SY-LAB G.m.b.H, Pukersdorf, Austria), at 30% of the maximum power, and 0.5 s pulses interrupted by 0.5 s breaks. There was no external sample cooling applied.

Small-Angle X-ray Scattering Measurements. The SAXS equipment was a new SAXSess camera³⁶ (Anton-Paar, Graz, Austria) using an X-ray generator (Philips, PW 1730/10) operating at 40 kV and 50 mA with a sealed-tube Cu anode. A Göbel mirror is used to convert the divergent polychromatic X-ray beam into a focused line-shaped beam of Cu K_α radiation ($\lambda = 0.154$ nm). The 2D scattering pattern is recorded by an imaging-plate detector (model Fuji BAS1800 from Raytest, Straubenhardt, Germany) and integrated to the one-dimensional scattering function $I(q)$ using SAXSQuant software (Anton Paar), where q is the length of the scattering vector, defined by $q = (4\pi/\lambda) \sin \theta/2$, λ being the wavelength and θ the scattering angle. For indexing the peaks of the different mesophases, the reflection laws are presented in the Supporting Information. These comprise the space groups that have been determined for cubic and hexagonal phases containing membrane lipids.²⁴ The interplanar distance d between two reflecting planes is given by $d = 2\pi/q$, which enables us to calculate the corresponding mean lattice parameter a (see the Supporting Information). The scattering profiles of the so-called L_2 phase show only one broad peak, for which the position of the observed maximum is shifted to lower q -values due to the “smearing effects” of the line-shaped primary beam. These profiles were desmeared by fitting these data with the generalized indirect Fourier transformation method.³⁷ For the L_2 phase, d is called characteristic distance.

The sample was filled at room temperature into the sample holder (capillary in a metal block, temperature controlled by a Peltier element, ± 0.1 °C) and equilibrated at each experimental temperature for at least 10 min. All temperature scans were performed in the heating direction.

Cryogenic Transmission Electron Microscopy (Cryo-TEM). A laboratory-built controlled environment vitrification system for cryo-TEM (very similar to the one described by Egelhaaf et al.³⁸) was used. Humidity in the environment chamber was about 95% at 25 °C and 100% at 55 °C. Prior to the experiments performed at 55 °C, 1 mL of dispersion was left for 1 h at 70 °C and was then transferred into the environmental chamber at 55 °C. After at least 30 min of equilibration in the environmental chamber at the respective temperature, a droplet of 5 μ L of dispersion was deposited onto a 200 mesh copper grid (25 °C, Quantifoil R2/2; 55 °C, Quantifoil S7/2; both Jena, Germany) that was covered with a carbon film containing holes. It was left between two filter papers 595 (Schleicher & Schuell, Dassel, Germany) for about 2 s before being propelled into liquid ethane. Frozen grids were stored in liquid nitrogen and transferred into a cryo-holder (Gatan 6556, Pleasanton, U.S.) that was kept at -180 °C. Sample analysis was performed in a Philips CM12 TEM (Philips, Eindhoven, Netherlands) at a voltage of 80 kV. Low dose procedures were applied to minimize beam damage. The images were recorded with a slow scan digital camera Gatan 694 (Pleasanton, U.S.).

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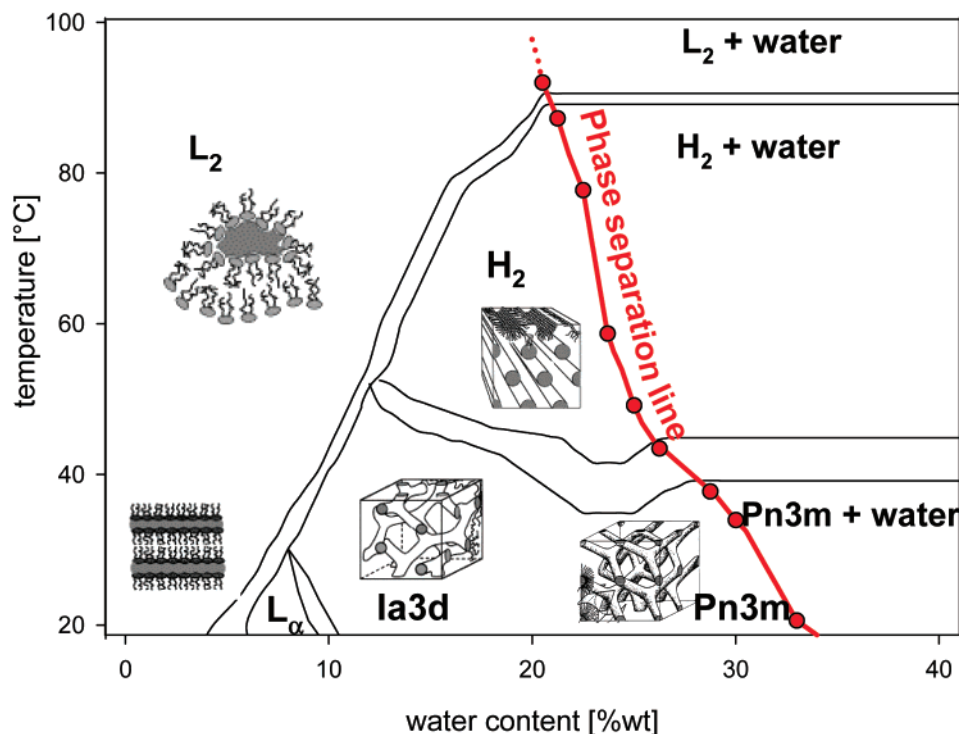


Figure 1. Identity and location in the temperature–composition phase diagram of MLO/water as determined by SAXS in the heating direction between 20 and 94 °C. The black lines are guides for the eye to see phase transitions and regions of coexistence between the mesophases. The red line corresponds to the phase separation line between the single-phase regions (left) and the excess water region (right). Schematic representations of the phases are taken from refs 20 and 22. Reprinted with permission; copyright 2000 and 1990 by Elsevier.

Dynamic Light Scattering (DLS). The DLS instrument used is a laboratory-built goniometer equipped with a diode laser (Coherent Verdi V5, $\lambda = 532$ nm, $P_{\max} = 5$ W) with single-mode fiber detection optics (OZ from GMP, Zürich, Switzerland), an ALV/SO-SIPD/DUAL photomultiplier with pseudo cross correlation, and an ALV 5000/E correlator with fast expansion (ALV, Langen, Germany). The measurements were carried out at a scattering angle of $\theta = 90^\circ$ at different temperatures, using an effective laser power in the sample of $P = 200$ mW. Data were collected in repeated measurements (approximately 10–25) of 10 s each, until a total of 10 million counts were detected. The intensity autocorrelation functions, which were affected least by intrinsically appearing dust particles, were averaged. From these functions, the average diffusion coefficient D was obtained by means of a second-order cumulant analysis of the intensity autocorrelation function.³⁹ The hydrodynamic radius R_H was calculated using the Stokes Einstein relation:

$$R_H = \frac{k_B T}{6\pi\eta D} \quad (1)$$

where k_B is the Boltzmann constant, T is the absolute temperature, and η is the viscosity of the continuous medium at a given temperature (see the Supporting Information).

Optical Microscopy. Polarized light microscopy (PLM) was used to observe the mesophases. For using this technique, a 3 cm long capillary (Camlab VD/3530-050) was used. It was closed on both ends by silicone paste to avoid any water loss during heating. It was then placed on the furnace of a Linkam THMS 600 heating chamber (Linkam Scientific Instrument, Waterfield, U.K.). The sample was then heated from room temperature to 90 °C at a heating rate of 1 °C per minute. Images were recorded using polarized light (cross Nichols) on a microscope Zeiss Axioplan 2 (Zeiss, Germany). Heating could be stopped so that the phase transformation is complete.

Results and Discussion

The main aspect of the present work deals with the impact of temperature on the internal nanostructure of

kinetically stabilized aqueous dispersions based on MLO. To better understand such systems, it is important to compare the internal structure of the dispersions to that of the corresponding bulk (nondispersed) binary MLO/water systems. Therefore, this chapter is organized in the following way: we start with reporting on the phase behavior and the temperature/composition-dependent structures in the bulk (nondispersed) binary MLO/water system and then turn to the investigation of the temperature effect on the nanostructured aqueous dispersions.

The Temperature-Dependent Binary MLO/Water Phase Diagram. The binary phase diagram of MLO/water up to 40 wt % water and in a temperature range between 20 and 94 °C is presented in Figure 1: a variety of mesophases is formed depending on temperature and water content (polymorphism). Left of the phase separation line (red line in Figure 1), the samples appear macroscopically as one rather transparent phase. Between the single-phase regions, there are small areas of two different mesophases in narrow temperature and composition intervals. Right of the phase separation line, the samples appear to be completely white (heterogeneous): the system separates into the mesophase and excess water (excess water region). The bicontinuous cubic mesophase can contain considerably more water than the reversed hexagonal and L_2 phase formed at higher temperatures. At 20 °C, MLO can solubilize up to 33 wt % water, while at 94 °C it can solubilize only 20.3 wt % water, so the phase separation line has a negative slope. The observed general pattern of the phase behavior of this system is similar to that of the monoolein (MO)–water system.^{17,20} However, as expected, the transition temperatures are lowered because MLO has two double bonds, while MO

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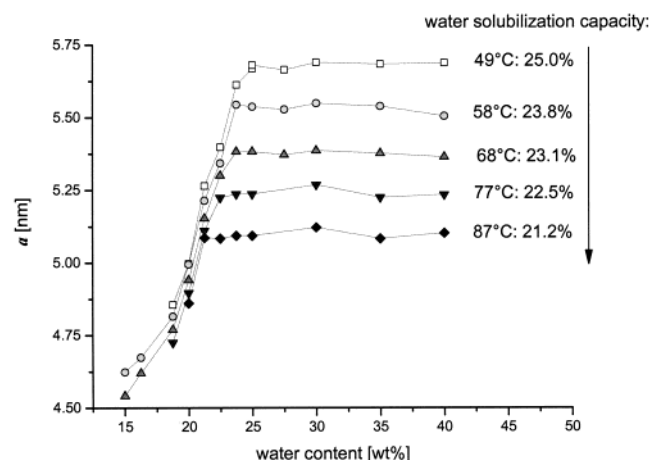


Figure 2. Composition dependence of the lattice parameters a [nm] in the H_2 phase at different temperatures in the range of 49–87 °C. Also given: respective water solubilization capacities of the mesophases in equilibrium with excess water.

has only one double bond. A more detailed description of the phase diagram and the measured samples is given in the Supporting Information.

The calculated lattice parameters for the distinct symmetries in dependence on temperature and water content show that MLO exhibits the same typical liquid crystal thermal and composition expansivities as does, for example, MO:^{17,20} generally, the lattice parameter of all mesophases decreases with increasing temperature, and the lattice parameter of all single phases increases with sample hydration. The temperature-induced shrinkage within the single-phase regions was attributed mainly to an increase of kink states in the lipid acyl chains.⁴¹ The molecular wedge shape increases with increasing temperature, resulting in an increased curvature of the lipid bilayers and thus in a reduction of the unit cell (see the Supporting Information). As mentioned above, hydration swells the pure phases (see Figure 2). As soon as the water solubilization capacity is exceeded, that is, reaching the excess water region, the lattice parameters coincide within experimental error: for example, at 58 °C, H_2 swells starting from 15 wt % water (4.62 ± 0.05 nm) to 23.8 wt % water (5.54 ± 0.05 nm) initial water content (single phase); above this point, the solubilization capacity is exceeded, and any additional water is being expelled from the mesophase (excess water), in which the lattice parameter stays constant (quasi horizontal tie-lines). At 58 °C, the MLO–water hexagonal phase can solubilize almost 23.8 wt % water, while at 87 °C, the solubilization capacity decreases to 21.3 wt % water. As a consequence, the lattice parameter of this mesophase in the excess water region decreases from 5.54 to 5.10 nm. The temperature-induced shrinking of the lattice parameter for samples in the excess water region is generally more pronounced than in the single-phase regions, since it is caused not only by the usual thermal expansivities but additionally by the expulsion of water. The most interesting of the bulk samples is the one which is already in equilibrium with excess water (prepared with 40 wt % water) at room temperature, because this is the sample that can best be compared to the aqueous dispersions. The temperature-dependent scattering curves of this sample are shown in Figure 3, together with the actual water content in the

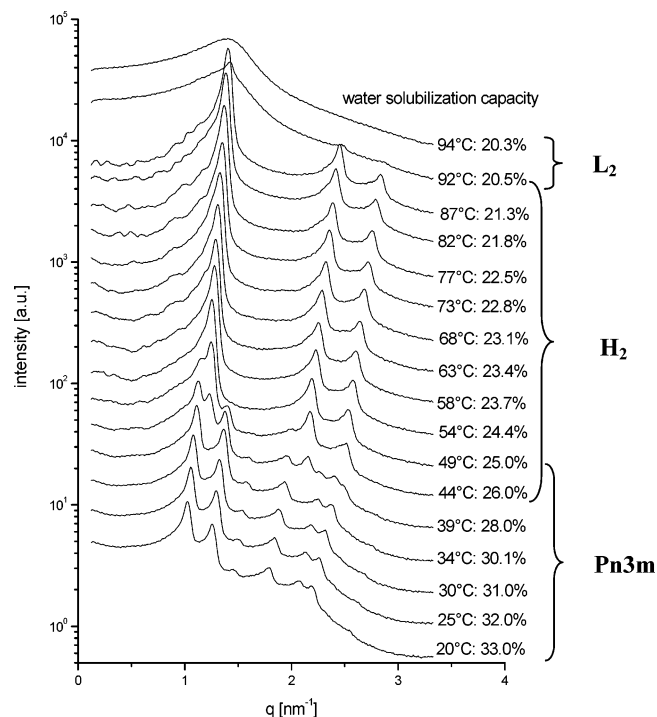


Figure 3. Temperature dependence of scattering curves for a nondispersed sample of MLO with excess water (40 wt % water). Also given: respective water solubilization capacities of the mesophases.

mesophase as determined by the phase diagram (Figure 1). Increasing temperature induces a phase transition from $Pn3m$ via H_2 to L_2 , whereby increasing amounts of water are expelled (from 20 to 94 °C, 38% of the initial water content is expelled).

The coexistence of H_2 , $Pn3m$, and water between 40 and 45 °C suggests that the monolinolein used in this study does not behave exactly as a pure material. The used MLO is a commercial sample consisting of different molecules (see Materials and Methods). At this point, it is difficult to determine to which extent the impurities influence the overall observed phase behavior.

The observed fluid isotropic phase can only be called L_2 phase in a broad sense. We have a strong indication that the characteristic distance grows linearly with increasing water content (“linear swelling”) in the temperature range between 20 and 94 °C (unpublished data), which would suggest a locally flat structure (probably consisting of bilayers), similar to the L_3 phase which can appear in water rich surfactant systems.²¹ At least at lower temperatures it might essentially be a disordered version of the bicontinuous cubic phases.

Aqueous Dispersions of MLO/Water Phases. To gain insight into the structure of the MLO/water system in its colloidal emulsified state, MLO was dispersed in large amounts of excess water by means of ultrasonication, using the block copolymer F127 as stabilizer. This led to the formation of submicron-sized particles with nanostructured interiors, which can be clearly observed in the cryo-TEM images of Figure 4.

Figure 5 shows the temperature-dependent scattering curves of this dispersion. It gives clear evidence that the internal structure of the dispersion undergoes a transition from $Pn3m$ (emulsified cubic phase ECP) via H_2 (emulsified hexagonal phase EHP) to the so-called L_2 phase (emulsified L_2 phase ELP), as the temperature is increased from 20 to 94 °C, in analogy to the investigated nondispersed sample in equilibrium with excess water (bulk

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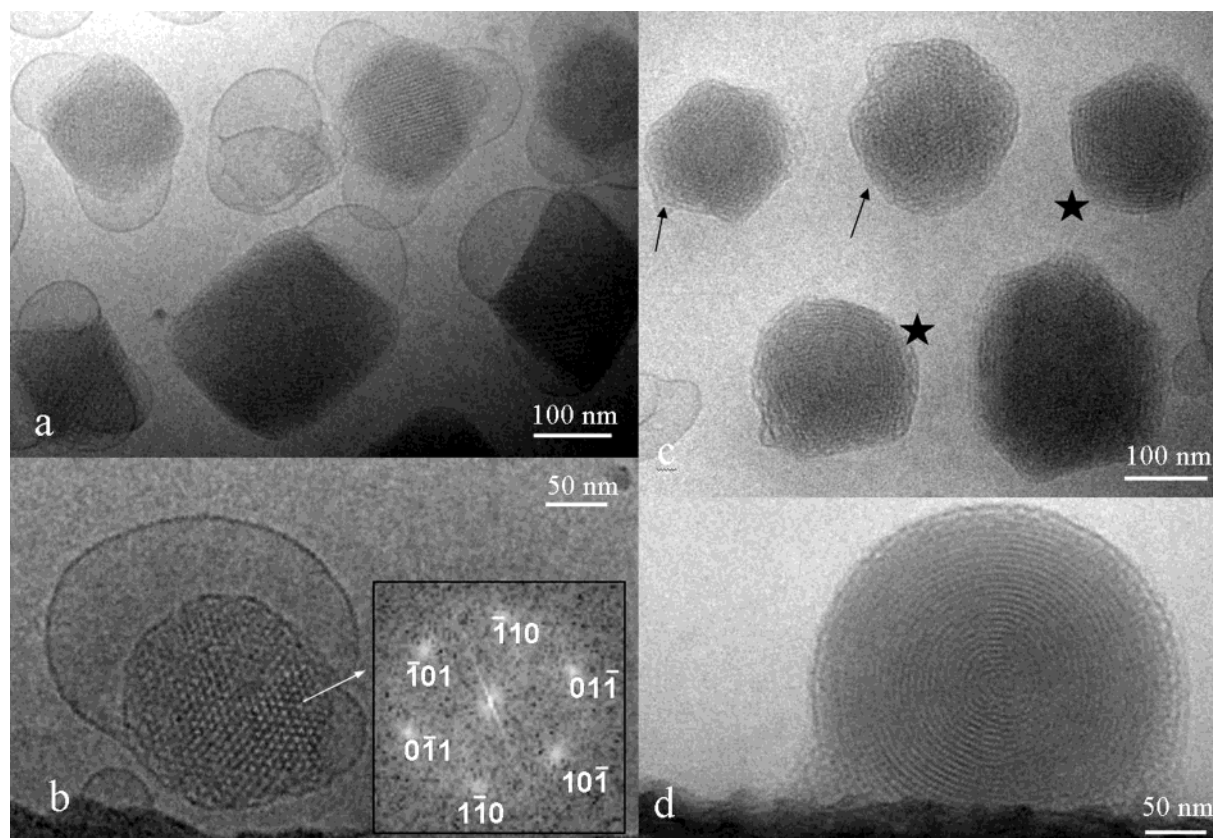


Figure 4. Cryo-TEM images of a dispersion consisting of 4.625 wt % MLO, 0.375 wt % F127, and 95.0 wt % water. At 25 °C (parts a and b), nanostructured particles together with some vesicles were observed. The Fourier transform of the internal structure of the particle (inset in part b) shows a hexagonal arrangement with interplanar distances d of about 6 nm. The internal structure is observed along the $[111]$ axis, and the crystallographic planes observed are of $\{110\}$ type. This is compatible with a cubic structure of $Pn3m$ symmetry with a lattice parameter a of 8.5 nm. At 55 °C (parts c and d), some of the nanostructured particles exhibit an internal hexagonal symmetry (arrows), others exhibit both hexagonal symmetry and curved striations (stars), and finally some exhibit only curved striations (part d).

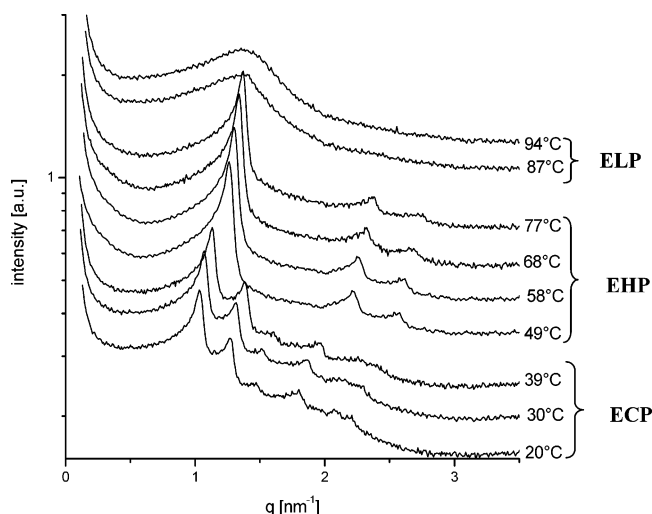


Figure 5. Temperature dependence of scattering curves for the same dispersion as described in Figure 4.

phase, see Figure 3). The formation of ELP is quite intriguing and has never before been described in the literature. The presented data clearly show that it is a dispersion consisting mainly of submicron-sized particles with a nanostructured, fluid isotropic water–MLO rich interior that has no long-range order.

At 25 °C, the ECP of symmetry $Pn3m$ has a mean lattice parameter of 8.47 ± 0.05 nm as determined by SAXS. Fast Fourier transforms (FFTs) of the internal structure of the particles observed by cryo-TEM (e.g., Figure 4a,b)

are compatible with the cubic $Pn3m$ symmetry and render a lattice parameter of about 8.5 nm, which is in very good agreement with the SAXS analysis. On almost all ECPs, attached vesicles can be observed (Figure 4a). These are quite large, but given their thin shell, only a small amount of material is used for their formation. This coexistence was observed also in all previous studies on dispersed monoglycerides, in which F127 was used as stabilizer.^{27,29} At 55 °C, the internal structure of the particles was determined by SAXS to be H_2 (EHP). This change in internal symmetry can also be seen in the respective cryo-TEM images (Figure 4c,d): the particles show internal arrangements of hexagonal symmetry (arrows in Figure 4c) and/or curved striations (stars in Figure 4c,d). The outer surface planes of some particles resemble a hexagon (e.g., top left particle in Figure 4c; the angles are close to 120°). The presence of a hexagonal internal symmetry is an indication for the presence of EHPs, and curved striations such as the ones observed in Figure 4d are characteristic for EHPs.²⁷

The vesicles attached to the ECPs are much smaller or not present in the EHP after heating the corresponding ECP. Also, Gustafsson et al. observed fewer vesicles in the EHPs which are based on mixtures of MO and a hydrophobic additive than in the ECPs which are based on MO.²⁷ They suggested that the presence of attached vesicles or, more precisely, of a lamellar phase in coexistence with the ECP was a prerequisite for the stabilization of the ECPs, which is probably not the case for the EHPs.

Figure 6 shows the scattering curves of the dispersions (thick lines) and the corresponding nondispersed binary

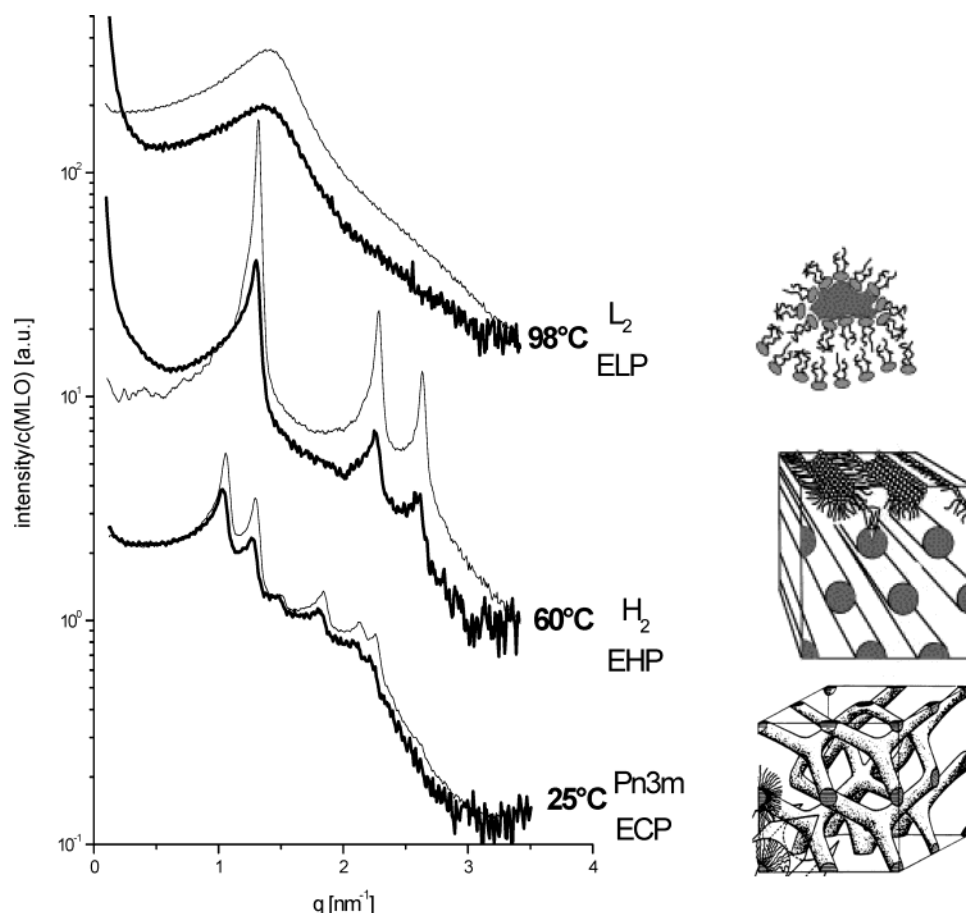


Figure 6. Comparison of scattering curves from the same dispersion with those from the nondispersed bulk sample with excess water (40 wt % water) at different temperatures: the thick lines correspond to the dispersion, and the thin lines to the bulk phase. The intensities were normalized by the respective MLO concentration. Schematic representations of the phases are taken from refs 20 and 22. Reprinted with permission; copyright 2000 and 1990 by Elsevier.

bulk phase with excess water (thin lines) at three different temperatures: 25, 58, and 94 °C; the intensities of all samples were normalized by the respective MLO content. The resemblance of the corresponding scattering curves at each temperature is remarkable: the peak positions for both the bulk and the dispersion practically coincide. The peaks of the dispersions are just broadened and lowered in the scattered intensity. In the ECP six peaks and in the EHP three peaks are observed. This means that the interior of the dispersed particles is still highly ordered and only slightly affected by the dispersion procedure. The lower intensity and the slight broadening of the peaks from the dispersion with respect to those from the bulk phase can be attributed to an interplay of the following effects: (1) a limited size of the coherently scattering subvolume (limited by the size of the dispersed droplets, also indicated by the upturn of the scattering curves at low q -values), (2) a lower degree of order especially near the particle surface caused by an incorporation of F127, and (3) the formation of vesicles²⁷ of small parts of MLO with F127 or possibly also small aggregates in coexistence with the dispersed particles with highly ordered internal phase.

A comparison of the temperature-dependent lattice parameters of the internal phase (and for the L_2 phase the characteristic distance) in the dispersion to the parameters of the nondispersed bulk phase with excess water is provided in Figure 7. For all investigated temperatures, the symmetries of the internal phase were preserved (only the internal phase transition from H_2 to L_2 takes place already at slightly lower temperature in

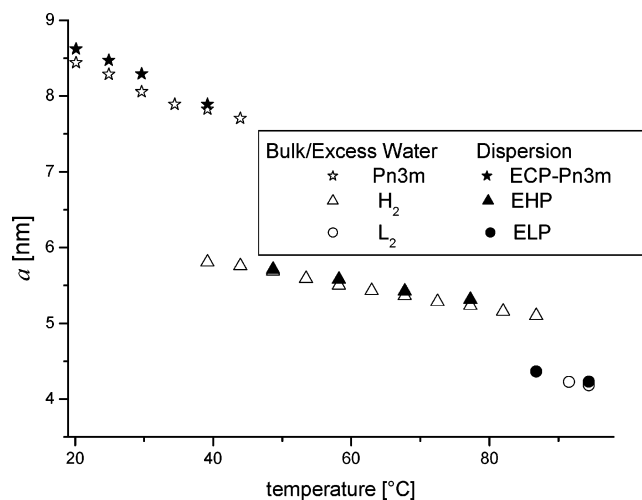


Figure 7. Temperature dependence of the internal lattice parameters a [nm] (for $Pn3m$, H_2 , and L_2) for the same dispersion (full symbols) compared to the nondispersed bulk sample with excess water (40 wt % water) (open symbols).

the dispersion). Moreover, the lattice parameters were practically the same, given the broadened peaks of the dispersions. This good agreement clearly shows that the internal structure of the dispersions at each temperature corresponds to the equilibrium structure of MLO with excess water. If at all, only a small part of F127 is incorporated into the internal structure of the particles.

To prove that the temperature-induced structural changes are reversible, a dispersion was measured at three

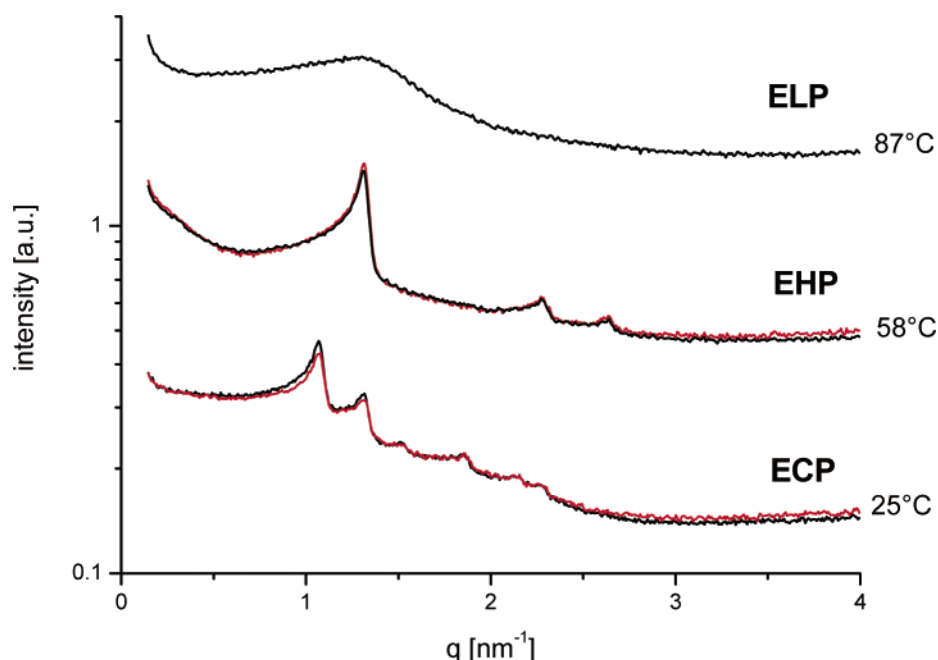


Figure 8. Reversibility: scattering curves of a dispersion, containing 8 wt % MLO in a 1 wt % F127 solution, that were measured in the heating direction at 25, 58, and 87 °C (black lines) and then in the cooling direction again at 58 and 25 °C (red lines).

different temperatures in a heating–cooling cycle (see Figure 8). At 25 °C, the dispersed particles are ECP with the internal structure of $Pn3m$, after heating to 58 °C they have transformed to EHP, and upon further heating to 87 °C the transition to ELP had taken place. Cooling the dispersion back to 58 °C, we found EHP again, and at 25 °C ECP. This means that upon cooling the melted entrapped L_2 phase in the kinetically stabilized droplets of our dispersions (ELP) recrystallizes back to the original H_2 and finally $Pn3m$ at room temperature (reversible structural transformation). The respective scattering curves of the dispersion at 25 °C (ECP) and 58 °C (EHP) coincide. This shows that the internal structure of the dispersed droplets depends only on the current temperature, irrespective of whether it was reached by heating or cooling. This proves that the internal structure of the dispersed particles is a thermodynamic equilibrium structure. This observation has an interesting consequence: since the internal structures follow the phase separation line in Figure 1, the particles expel water upon heating (deswelling) and take up water again upon cooling (swelling) in a reversible way. We termed this effect “breathing mode”. For dispersions based on MLO, the internal structure of the particles contains 33% water at 20 °C, while it contains only 20% water at 94 °C (see Figure 3), in analogy to that of the nondispersed bulk phase in equilibrium with excess water (see Figure 1).

The slope of the phase separation line was observed to be negative for many investigated monoglyceride–water systems,^{17–21,42} but in some systems, it was observed to be positive (e.g., monolaurin⁴²), which suggests that also a reverse breathing mode of dispersed monoglyceride particles is feasible.

DLS allowed us to investigate the overall size of the emulsified particles. It can be tuned within a certain range by, for example, changing the amount of added stabilizer.³² For the particles in the dispersion, whose scattering curves are shown in Figure 5, we observed that the average hydrodynamic radius was approximately 140 nm with a size distribution width of about 25% (rather polydisperse)

at each measured temperature in the range between 24.2 and 75.0 °C. This means that the mean size of the dispersed particles remains unchanged within significance upon temperature increase (see the Supporting Information). There was also no significant change observed when the sample was remeasured after cooling from the highest investigated temperature (75 °C). This demonstrates that the kinetic stability of the dispersion is remarkably high. Note that the overall radius is expected to shrink by only 3.5% between the lowest and highest measured temperature (breathing mode), which is beyond the resolution of DLS experiments on samples with this high polydispersity.

Conclusions

The present study proves that the internal structures of emulsified particles based on monolinolein–water mixtures are thermodynamic equilibrium structures. Upon heating (cooling), the dispersions change from emulsified cubic phase (ECP) via emulsified hexagonal phase (EHP) to emulsified L_2 phase (ELP) (and vice versa), in analogy to the binary MLO samples in equilibrium with excess water. A fascinating consequence of this observation is what we have termed the “breathing mode”: upon heating (cooling), the dispersed particles expel (take up) water according to the negative slope of the phase separation line between mesophases and mesophases with excess water as shown in Figure 1.

These nanostructured aqueous dispersions offer a useful approach in the development of novel food and pharmaceuticals applications, such as drug delivery, solubilization of active molecules, selective molecular transport, biosensors, solubilization and crystallization of proteins, or utilization of these systems as nanoreactors for water-sensitive reactions and so forth. They are interesting for many reasons, since they give, for example, the possibility to create hierarchically structured particles by using for instance ultrasonication (easy to prepare and to control) and the possibility to tune their confined structures reversibly in a dispersion that is formed with kinetic stability.

(42) Lutton, E. S. *J. Am. Oil Chem. Soc.* **1965**, *42*, 1068–1070.

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Supporting Information Available: A table of reflection laws for indexing the peaks of the different mesophases, a phase diagram of the binary MLO–water system, a figure showing the temperature dependence of the lattice parameters, and a table of the temperature dependence of overall average particle size determined by DLS. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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