Control of Nanomaterial Self-Assembly in Ultrasonically Levitated Droplets

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Supporting Information

ABSTRACT: We demonstrate that acoustic trapping can be used to levitate and manipulate droplets of soft matter, in particular, lyotropic mesophases formed from self-assembly of different surfactants and lipids, which can be analyzed in a contact-less manner by X-ray scattering in a controlled gas-phase environment. On the macroscopic length scale, the dimensions and the orientation of the particle are shaped by the ultrasonic field, while on the microscopic length scale the nanostructure can be controlled by varying the humidity of the atmosphere around the droplet. We demonstrate levitation and in situ phase transitions of micellar, hexagonal, bicontinuous cubic, and lamellar phases. The technique opens up a wide range of new experimental approaches of fundamental importance for environmental, biological, and chemical research.

Evaporation from aqueous droplets changes the composition droplet surface is subject to a uniform air liquid interface. Consequently, the droplet surface is subjected to a uniform air–liquid interface. Evaporation from aqueous droplets changes the composition

We introduce a controlled gas-phase environment and show that the relative humidity (RH) can be used to control condensation and evaporation to and from the droplet. In this way we can access a desired phase in soft matter and furthermore induce phase transitions. Manipulation of soft matter nanostructure by changing composition through humidity control has not hitherto been carried out in a contactless manner. Recently, humidity control of lyotropic liquid crystalline phases has been applied to soft crystals and thin films, in both cases supported on solid substrates potentially introducing surface effects. The application of ultrasonic levitation to soft matter droplets has, to our knowledge, not been previously reported.

A further advantage of the contact-less sample environment offered by ultrasonic levitation is the ready application of different analytical techniques. It has been combined with methods such as FTIR, Raman spectroscopy, and microscopy as well as UV–visible spectroscopy. There are few examples of the coupling of ultrasonic levitation with small-angle X-ray scattering (SAXS); it has been applied to the study of the growth mechanism of inorganic and organic crystals, and the structures of glassy materials. It is reasonably straightforward to combine

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several techniques with ultrasonic levitation to analyze the same sample simultaneously. Here we combine synchrotron SAXS with in situ optical imaging of the levitated droplet.

We describe the use of ultrasonic levitation to investigate lyotropic liquid crystals. These viscous self-assembled materials comprise a range of 2D and 3D nanostructures, and they are important from a wide variety of biological, commercial, and nanotechnological perspectives. “Type I” liquid crystals are typically formed by self-assembly of commercial surfactants and detergents into spherical or cylindrical micelles surrounded by water (“normal topology” phases) or into stacks of bilayers (“lamellar” phases). Biological lipid molecules can also form lamellar phases; the constituent lipid bilayers form the structural basis of cell membranes. In addition, lipid molecules adopt structures representing a variety of nonlamellar inverted analogues of their type I counterparts (“inverse topology” or “type II” phases). These contain spheres, cylinders, or 3D networks of water surrounded by lipid in the inverse micellar (IνII), inverse hexagonal (HνII), and inverse bicontinuous cubic (QνII) phases, respectively. Self-assembly of biological lipid crystalline phases is of importance from the perspective both of fundamental knowledge of the cell membrane and in applications for biological research; the QνII phase has been shown to be an excellent host matrix for the growth of cell membranes, so neither form of either lipid could be injected directly into the levitator. Furthermore, in an open environment, the lipids only form QνII and QνD phases at very high relative humidities, so glycerol was added as a humectant. Mixtures of phytantriol/glycerol (80:20 w/w) and Rylo/glycerol (68:32 w/w) prepared as ethanolic solutions (each containing 50% ethanol by weight) were injected into the levitator as ~5 μL droplets; the ethanol evaporated to leave a droplet of lipid/glycerol mixture that took up water from the humidified environment to adopt the different Qν phases. The sodium oleate/oleic acid/brine system was a liquid of sufficiently low viscosity that it could be injected directly into the levitator. Brij-S6 is a waxy solid at room temperature, small grains of which could be placed directly into the levitator, and because it is very hygroscopic it then also took up water from the surrounding humidified environment to undergo phase transitions. Alternatively, a suspension of 20% w/w Brij-S6 in water forms a micellar phase, which is also sufficiently liquid to be injected directly. This then dehydrated to form hexagonal and lamellar lyotropic phases.

Levitated droplets were in most cases found to be of ellipsoidal shape with typical droplet dimensions 0.5 to 1 mm vertically and 1.8 to 2 mm horizontally. The aspect ratio could be altered by adjusting the transducer—reflector distance or the sound pressure. In addition, we could levitate smaller particles, several hundred microns in diameter, which tended to be more spherical. Furthermore, vertical strings of droplets trapped in different nodes could be levitated. Some representative
examples are shown in Figure S1 in the Supporting Information.

Figure 2 (right-hand column) shows representative 2D images obtained from SAXS experiments on acoustically levitated liquid crystals. The corresponding 1D integrated plots are shown in the left-hand column. This demonstrates the range of easily accessible phases that can be stably levitated.

Lattice parameters for levitated droplets of the QII$^D$ phases formed by phytantriol and Rylo with glycerol in high humidity are comparable to those measured previously on thin films using GI-SAXS (see Table 1), which themselves agreed with literature values for lipid/water mixtures under excess water conditions. In that experiment, it was not possible to separate effects from the two different interfaces, with the vapor phase and the substrate lying above and below the QII$^D$ phase films. In levitated droplets, because there are no other interfaces, we can rule out substrate interface effects. However, we must take into account additional effects from the two different interfaces with the vapor phase and the substrate lying above and below the QII$^D$ phase films. In levitated droplets, because there are no other interfaces, we can rule out substrate interface effects. However, we must take into account additional effects from the two different interfaces, with the vapor phase and the substrate lying above and below the QII$^D$ phase films. In levitated droplets, because there are no other interfaces, we can rule out substrate interface effects. However, we must take into account additional effects from the two different interfaces, with the vapor phase and the substrate lying above and below the QII$^D$ phase films. In levitated droplets, because there are no other interfaces, we can rule out substrate interface effects. However, we must take into account additional effects from the two different interfaces, with the vapor phase and the substrate lying above and below the QII$^D$ phase films. In levitated droplets, because there are no other interfaces, we can rule out substrate interface effects. However, we must take into account additional effects from the two different interfaces

Figure 2. Integrated and 2D SAXS data taken from levitated droplets showing a range of lyotropic phases measured: (a, b) lamellar phase from Brij-56 (RH = 67%); (c, d) hexagonal phase from 1:1 oleic acid/sodium oleate initially 3 wt % in 1 wt % NaCl solution (RH = 84%); (e, f) QII$^D$ phase from Rylo/glycerol (RH > 98%); and (g, h) QII$^C$ phase from phytantriol/glycerol (RH = 83%).

**Table 1. Lattice Parameters Obtained through SAXS from Ultrasonically Levitated Droplets Compared with Literature Values from Thin Films**

<table>
<thead>
<tr>
<th>sample</th>
<th>phase</th>
<th>lattice parameter (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>phytantriol/glycerol</td>
<td>QII$^D$</td>
<td>67.5 ± 0.2 (this work)</td>
</tr>
<tr>
<td>(80:20 w/w), &gt;95% RH</td>
<td></td>
<td>67 ± 1 (Richardson et al., 2015)$^{15}$</td>
</tr>
<tr>
<td>Rylo/glycerol</td>
<td>QII$^D$</td>
<td>80.0 ± 0.3 (this work)</td>
</tr>
<tr>
<td>(68:32 w/w), &gt;95% RH</td>
<td></td>
<td>81–86 (Rittman et al., 2013)$^{15}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>87 ± 1 (Richardson et al., 2015)$^{15}$</td>
</tr>
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</table>

By controlling the relative humidity in the chamber, we can effectively change the water content of the levitated droplet in equilibrium with the surrounding water vapor and therefore control the lyotropic phase adopted, and we can induce phase transitions that can be monitored in real time using time-resolved SAXS. An example of this is shown in Figure 3, where a transformation from a lamellar to a micellar phase of the type I surfactant Brij-56 (C$_{16}$EO$_{10}$) is induced by changing the surrounding relative humidity from 67 to 95%. Similar transformations between L$_{II}$ and QII$^D$ phases in type II systems are shown in the Supporting Information (Figure S2).

We exploited the fact that the X-ray beam dimensions (0.3 × 0.3 mm) were smaller than those of the droplet (up to 2 × 1 mm), allowing scans horizontally and vertically through the levitated sample. Variations in droplet composition could be seen, in particular, when using larger droplets or relatively rapid changes in RH. On increasing RH for type II lipids, it was common to observe the development of a core–shell structure comprising a micellar phase on which a shell of QII$^D$ phase would gradually form, as illustrated in Figure 4. The sharp QII$^D$ reflections are present throughout because the X-ray beam always interacts with the shell of the droplet, whether it is passing through the front and back of the sample (when aligned with the center) or glancing through one edge; however, the broad inverse micellar peak becomes more intense as the beam trajectory approaches the center of the droplet (Figure 4).

Interestingly, when the X-ray beam passes through the top or bottom edge of a levitated droplet with such a QII$^D$ shell, a high degree of alignment can often be seen in 2D SAXS images, as shown in Figure 4. These highly oriented patterns in every case demonstrated an approximately vertical [111] direction, that is, normal to the cubic phase/vapor interface, as shown by the more intense on-axis (vertical) reflection in the second ring. (See Figure 4a,c.)

Calculations$^{37}$ have shown (111) to be the most thermodynamically stable facet for a QII$^D$ phase. This has also been observed from grazing-incidence SAXS data from thin films.$^{14}$ In that experiment, it was not possible to separate effects from the two different interfaces, with the vapor phase and the substrate lying above and below the QII$^D$ phase films. In levitated droplets, because there are no other interfaces, we can rule out substrate interface effects. However, we must take into account additional effects due to the ultrasonic field itself, which may also orient a particular axis in the vertical direction. To study these two competing effects,
we carried out horizontal scans to investigate the sides of the droplets. If the orientation shown in Figure 4 was solely due to interfacial effects, we would expect the sides of the droplet to show a pattern that was rotated by 90° compared with that obtained at the top or bottom; conversely, if the orientation was solely due to the (vertical) acoustic field, the pattern would remain unchanged. In fact, neither was observed, and instead the sides showed reduced alignment (Figure S3). This suggests that both the acoustic field and the interfacial effects contribute to the alignment at the top and bottom, whereas these effects act in opposition at the sides.

In summary, we have demonstrated for the first time that (i) droplets of soft nanomaterials can be acoustically levitated and analyzed in a contact-less manner and (ii) we have successfully developed an experimental setup allowing simultaneous SAXS/ acoustic levitation in a controlled gas-phase environment. Our study shows that ultrasonic levitation can be applied to a range of different lyotropic liquid crystalline phases formed by different lipids and surfactant molecules, and that the nanostructure can be controlled by changing the humidity of the surrounding atmosphere. We observed a number of new phenomena for lyotropic phases, including shape deformation caused by the ultrasonic field, high degrees of interfacial orientation, and a core–shell morphology reflecting variations in sample hydration. The technique opens up new avenues in experimental approaches of fundamental importance for environmental, biological, and chemical research.

**EXPERIMENTAL METHODS**

All compounds were used as received. Phytantriol (3,7,11,15-tetramethyl-1,2,3-hexadecanetriol) was obtained from Adina Cosmetic Ingredients (U.K.). Rylo MG-19 (Danisco, Copenhagen, Denmark, approximate purity 90%) is an industrial form of monoolein (2,3-dihydroxypropyl (Z)-octadec-9-enoate), which we refer to simply as Rylo in this paper. Glycerol (propane-1,2,3-triol), Brij-56 (C16H33(OCH2CH2)10OH, also known as polyoxyethylene (10) cetyl ether, C16EO10, or Brij C10), oleic acid, and sodium oleate were purchased from Sigma-Aldrich (U.K.). All compounds were used as received. Phytantriol (3,7,11,15-tetramethyl-1,2,3-hexadecanetriol) was obtained from Adina Cosmetic Ingredients (U.K.). Rylo MG-19 (Danisco, Copenhagen, Denmark, approximate purity 90%) is an industrial form of monoolein (2,3-dihydroxypropyl (Z)-octadec-9-enoate), which we refer to simply as Rylo in this paper. Glycerol (propane-1,2,3-triol), Brij-56 (C16H33(OCH2CH2)10OH, also known as polyoxyethylene (10) cetyl ether, C16EO10, or Brij C10), oleic acid, and sodium oleate were purchased from Sigma-Aldrich (U.K.). Our experimental setup is based on a modified commercial levitator (tec5, Oberursel, Germany) with a fixed transducer frequency of 100 kHz and a variable HF power of 0.65 to 5 W. A concave reflector was mounted on a micrometer screw for adjustment of the reflector–transducer distance. The distance between the transducer front face and the reflector was set to ~26 mm with a maximum distance variation of ±6 mm. The levitator was enclosed in a custom-built flow-through Pyrex environmental chamber fitted with X-ray transparent windows and access ports for relative humidity and temperature measurements as well as gas supply and removal. (See Figure 1.) This chamber was placed in the sample position of beamline I911-4 at MAX-lab. The desired relative humidity was achieved by controlling the ratios of flows of dry and H2O-saturated O2 from a gas cylinder. The liquid samples were introduced by means of a microliter syringe (Hamilton). The droplets were detached from the tip of the needle of the syringe by altering the reflector–transducer distance and simultaneously adjusting the sound pressure to stabilize the levitated droplets. The levitator was mounted on an x-, y-, z-stage for precise alignment of X-ray beam and levitation zone. SAXS experiments were carried out using a beam size of 0.3 × 0.3 mm full width at half-maximum. The wavelength was 0.91 Å and data were collected over a q range of 0.006 to 0.18 Å⁻¹. Exposure times were typically 30 to 60 s for a typical trapped droplet size of 0.5 to 1 mm vertically and 1.8 to 2 mm horizontally. Data were analyzed using an in-house developed macro.38

**ASSOCIATED CONTENT**

3 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpclett.6b00449.

Photographs showing typical examples of levitated droplets; additional SAXS data showing phase transitions; and 2D SAXS images from the sides of a droplet. (PDF)

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Notes

The authors declare no competing financial interest.

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