Addition of n-Alcohols Induces a Variety of Liquid-Crystalline Structures in Surfactant-Rich Cores of Dispersed Block Copolymer/Surfactant Nanoparticles

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Supporting Information

ABSTRACT: Poly(acrylamide)-b-complex salts made from a symmetric poly(acrylate-b-acrylamide) block copolymer, where the acrylate charges are neutralized by cationic surfactant counterions, form kinetically stable aqueous dispersions of hierarchical aggregates with a liquid-crystalline complex salt core and a diffuse hydrated shell. By the addition of suitable amounts of long-chain alcohols, such as octanol or decanol, the structure of the internal phase can be varied, producing micellar cubic, hexagonal, lamellar, or reverse hexagonal liquid-crystalline phases. In addition, a disordered reverse micellar phase forms at the highest content of octanol. These core structures are the same as those previously obtained for macroscopic homopolymer poly(acrylate) complex salt/water/n-alcohol systems at the corresponding compositions. The poly(acrylamide)-b-complex salt dispersions are kinetically stable for several weeks, with their colloidal properties and internal structures remaining unchanged. The methodology described here establishes an easy and robust protocol for the preparation of colloidal nanoparticles with variable but controlled internal structures.

INTRODUCTION

Several studies of water-dispersible core–shell nanoparticles made from charged–neutral block copolymers and oppositely charged surfactants have been reported in the last years.1–10 The most studied systems involve alkyltrimethylammonium cationic surfactants and the block copolymer poly(acrylic acid-b-acrylamide) [P(AA-b-AAm)].1–5,6 When such complexes are prepared by mixing individual aqueous solutions of the charged–neutral block copolymer and the charged surfactant, the species associates into colloidal complexes in the form of core–shell nanoparticles. In addition, these solutions contain the original simple counterions of the charged polymer and the surfactant. The core of the nanoparticle contains densely packed surfactant micelles surrounded by polymer blocks. The outer part of the colloidal complex is a corona composed of neutral poly(acrylamide) blocks. Further studies revealed that the micelles in the core of the particles, prepared as described, were arranged in a disordered state, showing no long-range order.1,2,5,6

The use of stoichiometric polymer–surfactant ion “complex salts” (CSs), free of other ions, was proposed a few years ago as an alternative strategy to study the phase behavior of polymer/surfactant complexes. The principal advantage of this strategy is that the number of components is kept at a minimum, making it possible to study the effects of adding a single component to the system or replacing one by another, in isolation. This allowed the determination of truly binary phase diagrams for CS mixtures with water7–9 and the assessment of the effect of added n-alcohols or other organic solvents on their ternary phase behavior.10–13 On the basis of this methodology, our group has recently reported studies using poly(acrylamide)-b-CS block copolymers containing the hydrophilic block copolymer poly(acrylate-b-acrylamide), neutralized by dodecytrimethylammonium counterions, to produce core–shell nanoparticles dispersed in an aqueous medium.7 Notably, the dispersions produced from the block copolymers containing stoichiometric CSs invariably gave nanoparticle cores displaying a micellar cubic liquid-crystalline structure, agreeing with that previously observed for maximally water-swollen homopolymer dodecytrimethylammonium–polyacrylate CSs.8,9 Nanoparticles prepared by mixing individual aqueous solutions of the anionic–neutral block copolymer and the cationic surfactant, referred here as “conventionally prepared”, behave different from that obtained by dispersing poly(acrylamide)-b-CS in water, as will be described in the present report.

Nanoparticles with liquid-crystalline cores are known to be formed by different chemical systems. Examples include the cubosomes,14 which are dispersions of bicontinuous cubic phases studied for their potential for drug delivery. Other more recent examples are from studies reported by Lindman and co-
workers, and in the last few years, reports by Uchman have described internally structured particles using the complexation between surfactants and polyanions. During the past years, a series of studies have reported the formation of colloidal complexes made from oppositely charged surfactants and charged–neutral block copolymers. These liquid-crystalline nanoparticles have also been studied aiming at the control and change of their internal structures by specific triggers such as enzymes and near infrared light, to make them suitable for important practical applications such as drug delivery. It has previously been shown that, for example, differences in the liquid-crystalline structure can change the drug diffusion and hence release, with research progressing toward the use of in situ changes to control the drug release by these liquid-crystalline matrices.

In this work, we have expanded our studies of dispersions containing stoichiometric CSs of the symmetric block copolymer P(AA42-b-AAm42) and dodecyl- or hexadecyltrimethylammonium cationic surfactants (C12TA˚4 and C16TA˚4) with the aim to achieve additional internal structures by adding long-chain alcohols. This strategy is based on the previous experience of systems formed by the corresponding homopolymer CS, water, and various n-alcohols. Small angle X-ray scattering (SAXS) has been used to identify and characterize the liquid-crystalline interior of the aggregates. In addition, the average radius and the zeta potential of the colloidal nanoparticles have been investigated.

■ RESULTS

Internal Structure. Poly(acrylamide)-b-CS Nanoparticles Dispersed in Water. Figure 1 shows the SAXS patterns obtained for dispersions of PAAm42-b-C12TAPA42 nanoparticles displaying a cubic internal structure and PAAm42-b-C16TAPA42 nanoparticles with a hexagonal interior. Samples containing 1.0 wt % of poly(acrylamide)-b-CS, analyzed at 25 °C.

![Figure 1. SAXS patterns obtained for (A) PAAm42-b-C12TAPA42 nanoparticles displaying a cubic internal structure and (B) PAAm42-b-C16TAPA42 nanoparticles with a hexagonal interior. Samples containing 1.0 wt % of poly(acrylamide)-b-CS, analyzed at 25 °C.](image)

For increasing decanol contents in the aqueous dispersions of PAAm42-b-C12TAPA42, the internal structure of the aggregates changed in the following sequence: cubic, normal hexagonal, and lamellar. At a low decanol content (R = 0.256), the CS core displays a normal hexagonal phase with Bragg peak positions of 1:3/2:2 in the SAXS patterns (Figure 2A). When the decanol fraction is increased further (R = 0.613), SAXS peaks with a spacing ratio of 1:2:3 indicate the formation of a lamellar structure (Figure 2B). The same sequence of structures, as with decanol, was observed with added octanol (Figure 2C,D). SAXS patterns for additional decanol and octanol concentrations in PAAm42-b-C12TAPA42 dispersions (see Table 1), indicating hexagonal and lamellar structures, are presented in Figure S1. The repeat distances (d) between the bilayers in the lamellar structures in the core of PAAm42-b-C12TAPA42 nanoparticles was 4.72 nm when using decanol and d = 4.68 nm for octanol. PAAm42-b-C16TAPA42 Nanoparticles + Octanol or Decanol. Figure 3 shows the SAXS patterns obtained for PAAm42-b-C16TAPA42 nanoparticles with different amounts of decanol. At a low decanol content (R = 0.142), the SAXS pattern (Figure 3A) from the complex showed scattering peaks with a spacing ratio of 1:3/2:4/3:7:3, indicating a structure with hexagonal packing of cylindrical micelles, as for the alcohol-free sample (Figure 1B). However, some other peaks also appear. These peaks show a regular spacing ratio of 1:2:3, indicating a Pm space group for a lamellar structure being formed inside of the nanoparticles. Therefore, this SAXS pattern could be attributed to a mixture of two different structures of Pm lamellar and P6mm hexagonal. Further addition of decanol (R = 0.321) leads to the formation of a pure lamellar phase, with d = 4.34 nm (Figure 3B). SAXS patterns for other decanol compositions (see Table 1) indicating a lamellar structure, with d = 4.34 nm, are presented in Figure S2.

With increasing contents of octanol, the CS core of PAAm42-b-C16TAPA42 nanoparticles changed from normal to inverted structures. At low octanol contents (R = 0.818), the SAXS pattern revealed 1:2 Bragg reflections, indicating a lamellar structure, with d = 4.31 nm (Figure 4A). As more octanol was added (R = 4.067), the peak positions at 1:3/2:2 (Figure 4B) revealed that another hexagonal structure was achieved. There are two possible 2D hexagonal structures. One is the normal
Table 1. Liquid-Crystalline Structures and Their Lattice Parameters for Poly(acrylamide)-b-CS Nanoparticles at Varying n-Alcohol/CS Mass Ratios (R)

| poly(acrylamide)-b-CS | R (octanol) | R (decanol) | core structure | lattice parameter |
|------------------------|-------------|-------------|----------------|-------------------|
| PAAm_{42}-b-C_{12}TAPA_{42} | 0           | 0           | cubic          | a = 8.40 nm       |
| 0.256                  | hexagonal   | a = 4.53 nm |
| 0.262                  | hexagonal   | a = 4.56 nm |
| 0.613                  | lamellar    | d = 4.68 nm |
| 0.620                  | lamellar    | d = 4.68 nm |
| 0.256                  | hexagonal   | a = 4.42 nm |
| 0.262                  | hexagonal   | a = 4.52 nm |
| 0.613                  | lamellar    | d = 4.72 nm |
| 0.620                  | lamellar    | d = 4.72 nm |
| PAAm_{42}-b-C_{12}TAPA_{42} | 0           | 0           | hexagonal      | a = 4.84 nm       |
| 0.818                  | lamellar    | d = 4.31 nm |
| 4.067                  | reverse hexagonal | a = 3.86 nm |
| 8.540                  | reverse micellar | d = 4.10 nm |
| 0.142                  | hexagonal + lamellar | a = 5.28 nm, d = 4.34 nm |
| 0.321                  | lamellar    | d = 4.34 nm |
| 0.325                  | lamellar    | d = 4.34 nm |
| 0.335                  | lamellar    | d = 4.34 nm |

“Micelle–micelle correlation distance.

phase (also referred as H₂ phase), already discussed above, in which the hydrophobic alkyl chains of the surfactants are located inside of the cylinders with the hydrophilic polar headgroups facing the surface of the cylinder, surrounded by a continuous water domain containing the negatively charged polycrylate) chains. Another is the reverse phase (H₁ phase), where the water phase is located inside of the cylinders, with the hydrophobic surfactant chains facing toward a continuous alcohol domain surrounding the cylinders.

From a symmetry point of view, both structures (normal and reverse) are 2D hexagonal and possess peak position ratios of 1:3^{1/2}:2. In Figure 4B, the structure could be identified as reverse hexagonal, once the nanoparticles are in octanol. Still further addition of octanol (R = 8.540) leads to the formation of a reverse micellar phase, referred to as the L2 phase. This phase consists of an isotropic and disordered solution of reverse micelles. A SAXS pattern for the L2 phase, shown in Figure 4C, presents only a broad peak corresponding to the micelle–micelle correlation distance of around 4.1 nm.

Reversibility of n-Alcohol-Induced Structural Transitions. To confirm the reversibility of the structural transitions by the incorporation of n-alcohols to the nanoparticle cores, an aqueous dispersion of 1.0 wt% PAAm_{42}-b-C_{12}TAPA_{42} prepared by using decanol to promote the formation of a lamellar phase, was dialyzed against deionized water for 3 days. The reference sample containing no n-alcohol presented a cubic interior (Figure 5A), as already discussed above and the decanol-loaded nanoparticles at R = 0.613, a lamellar liquid-crystalline structure (Figure 5B). After 3 days of dialysis, the latter sample presented a SAXS pattern (Figure 5C) that was virtually identical to that from the initially n-alcohol-free reference sample (cubic Pm₃m with the same lattice parameter), confirming the full reversibility of this process.

Thermal Phase Behavior. It is well known that lamellar phases formed by lipid/surfactant liquid crystals present a characteristic thermotropic phase behavior. They undergo the gel-to-liquid-crystalline phase transition (L₀ → Lₙ) upon increasing the temperature. Below the transition temperature, Tₓ, the surfactant alkyl chains are in a solidlike state. Above Tₓ, the surfactant molecules are in the fluid liquid-crystalline state, in which the conformational disorder of alkyl chains predominates.\(^2\) Differential scanning calorimetry (DSC) was used to determine the thermal phase behavior in lamellar PAAm_{42}-b-C_{12}TAPA_{42} nanoparticles. In a similar manner, a lamellar sample prepared using the homopolymer CS C_{16}TAPA_{30}, prepared in the same way as in the earlier work,\(^3\) was also analyzed. Figure 6 compares the DSC thermograms obtained for the two samples that possess the same decanol/CS mass ratio.

By integrating the peaks in the thermograms, one can determine the enthalpy change (ΔH) associated with the phase transition. Table 2 shows the measured ΔH values of the gel-to-liquid crystalline transition as well as the transition temperature (Tₓ) and peak width (ΔTₓ/2) obtained from the DSC measurements. Again, the thermotropic data for the poly(acrylamide)-b-CS nanoparticles clearly agree with those found for the corresponding homopolymer CS, with the same n-alcohol content.

Colloidal Properties of the Nanoparticles. To complete the physicochemical characterization of the liquid-crystalline nanoparticles, particles in freshly prepared dispersions were also characterized by their hydrodynamic radii (Rdí) and zeta potentials (ζ). Table 3 summarizes the results for nanoparticles featuring all of the different internal structures. The average radius was around 120 nm for all nanoparticles. The polydispersity index (PDI) of the nanoparticles was consistently 0.1.

Comparing the nanoparticles prepared using pure PAAm_{42}-b-C_{12}TAPA_{42} or PAAm_{42}-b-C_{16}TAPA_{42} in water (no added n-alcohol), the former displayed zeta potential values that were slightly more negative (around −35 mV) than the latter (around −25 mV). For all combinations of block copolymer and alcohol, the absolute value of the nanoparticle zeta potential decreased with an increase in n-alcohol content (Table 3).

## DISCUSSION

Nature of the Colloidal Nanoparticles. A striking difference between ours and the previously reported poly(acrylate-b-acrylamide)/cationic surfactant nanoparticle disper-
sions is the occurrence or not of liquid-crystalline structures in the nanoparticle cores. Nanoparticles conventionally prepared were reported as possessing disordered cores.1,2,5,6 The strategy for preparing nanoparticles containing CS cores reported in the present work was chosen to allow a more accurate control of the phase behavior of the species and ensured the formation of nanoparticles with a liquid-crystalline interior and the evaluation of the effect of added n-alcohols on the structured nanoparticle cores.

It is questionable whether the small differences in composition (the presence or absence of low concentrations of inorganic ions), resulting from the two methods of preparation, could by itself be the cause of the difference in the nanoparticle core structure. The particle size could be more important: available data on particle size show that the nanoparticles prepared by dispersing poly(acrylamide)-b-CS display larger cores. By using our procedure, nanoparticles with an average radius slightly larger than 100 nm are produced. The size ratio core/lattice parameter ($n$) of the nanoparticles studied in this report was estimated by simply dividing the average particle diameter by the lattice parameter for each phase, as detailed in the Supporting Information. This ratio varied in the range $27 \pm 5$ for all internal structures (see Table S1). For conventionally prepared nanoparticles, the cores are much smaller and may be too small to accommodate a number of units sufficient to form a crystalline structure with long-range order. Taking into account the average size of conventionally prepared nanoparticles (~30 nm)$^7$ and the lattice parameter of a cubic phase (8.4 nm), for example, $n$ is around 3, which is

Figure 2. SAXS patterns for PAAm$_{42}$-b-C$_{12}$TAPA$_{42}$ nanoparticles with varying amounts of added decanol (A) $R = 0.256$; (B) $R = 0.613$; or octanol (C) $R = 0.256$; (D) $R = 0.613$ displaying hexagonal or lamellar structures. All samples containing 1.0 wt % of poly(acrylamide)-b-CS, analyzed at 25 °C.

Figure 3. SAXS patterns for decanol-loaded PAAm$_{42}$-b-C$_{16}$TAPA$_{42}$ nanoparticles displaying (A) coexistence of hexagonal (labeled with asterisks) and lamellar structures for $R = 0.142$ and (B) lamellar core structures for $R = 0.321$. Samples containing 1.0 wt % of poly(acrylamide)-b-CS, analyzed at 25 °C.

Figure 4. SAXS patterns for octanol-loaded PAAm$_{42}$-b-C$_{16}$TAPA$_{42}$ displaying (A) lamellar for $R = 0.818$, (B) reverse hexagonal for $R = 4.067$, and (C) reverse micellar core structures for $R = 8.540$. Samples containing 1.0 wt % of poly(acrylamide)-b-CS, analyzed at 25 °C.
complexes were reported as systems out of equilibrium.\textsuperscript{17} Polyelectrolyte complex dispersions formed by oppositely charged polymers in aqueous media were also described to vary in size, structure, and stability depending on the mixing conditions.\textsuperscript{23} Tarahovsky et al.\textsuperscript{26} described lipoplexes (DNA–lipid complexes) with phase preferences depending on the sample preparation. Nizri et al.\textsuperscript{27} reported the study [by means of cryo-transmission electron microscopy (TEM) and dynamic light scattering (DLS)] of liquid-crystalline nanoparticles formed by the direct mixing of C\textsubscript{16}TAB (x = 10–16) and sodium poly(acrylate) solutions, leading to different surfactant/polymer molar charge ratios (Z). They described a wide range of particle sizes depending on the Z values, the surfactant alkyl chain length, and the way the experiments were conducted. These findings are also valid in complex dispersions composed of positively charged polymers and negatively charged surfactants.\textsuperscript{28,29} The commonly encountered history dependence suggests that the nanoparticles studied in the present work are not equilibrium structures and that their size is process-dependent, which is consistent with the properties of colloidal dispersions. Nevertheless, we have established a method to produce, in a reproducible fashion, nanoparticles with colloidal properties that remain unchanged for several weeks. It is known that the shape of the internally structured nanoparticles may display changes depending on the core structure, giving rise to anisotropic geometries.\textsuperscript{30} However, such an investigation has not been conducted thoroughly yet and discussions about the shape of the produced nanoparticles are out of the scope of the present report.

Although the CSs were prepared at a charge molar ratio (polymer/surfactant) equal to 1, the produced nanoparticles possess a net negative charge, as indicated by their zeta potential values. This indicates that there is a dissociation of surfactant ions from the surface of the CS core of the nanoparticles, producing an excess of anionic acylate charges at the core surface. The nanoparticles containing the dodecyltrimethylammonium surfactant ion had a slightly more negative zeta potential than those with the hexadecyltrimethylammonium surfactant. This can be related to the difference in hydrophobicity and, hence, in aqueous solubility of the two surfactant ions, as reflected in their different critical micelle concentration (cmc) values,\textsuperscript{31} with the dodecyltrimethylammonium surfactant ion displaying more dissociation in the aqueous phase. The addition of n-alcohols promoted a decrease in the zeta potential of the liquid-crystalline nanoparticles. The incorporation of nonionic cosolutes into amphiphilic aggregates may induce a charge dilution, yielding nanoparticles with a lower surface charge. The higher the proportion of n-alcohol located in the surfactant aggregates, the higher the decrease in the zeta potential value.

Colloidal nanoparticles have a tendency to agglomerate as a result of van der Waals attractive forces. Two modes of preventing the agglomeration and stabilizing the nanoparticle dispersions kinetically are via electrostatic and steric stabilization, and the resultant dispersion stability is a balance between repulsive and attractive forces between the particles. The electrostatic stabilization results from the repulsion between the nanoparticles caused by the dissociated counterions in the electrostatic double layer. The steric repulsion between nanoparticles surface-covered with polymer chains may also contribute to the kinetic stabilization of the nanoparticles.\textsuperscript{32} It is believed that the poly(acrylamide) neutral chains composing the shell may contribute to the stabilization of the colloidal

### Table 2. Transition Temperatures (T\textsubscript{m}), Peak Width (\(\Delta T_{1/2}\)), and Transition Enthalpies (\(\Delta H\)), with Standard Deviation, for the Gel-to-Liquid-Crystalline Phase Transition for 1.0 wt % PAAm\textsubscript{42}-b-C\textsubscript{16}TAPA\textsubscript{42} Dispersion and C\textsubscript{16}TAPA\textsubscript{30}/Water/Decanol System

| complex salt       | T\textsubscript{m} (°C) | \(\Delta T_{1/2}\) (°C) | \(\Delta H\) (kJ·mol\textsuperscript{-1}) | ±         |
|--------------------|-------------------------|--------------------------|-----------------|-----------|
| PAAm\textsubscript{42}-b-C\textsubscript{16}TAPA\textsubscript{42} | 28.7                    | 3.7                       | 36 ± 1         |           |
| C\textsubscript{16}TAPA\textsubscript{30} | 28.9                    | 3.6                       | 35 ± 1         |           |

*\(R = 0.325\) for both samples. \(\Delta H\) expressed as kJ per mole of alkyl chains (surfactant + n-alcohol).*

Figure 5. SAXS patterns for a 1.0 wt % dispersion of PAAm\textsubscript{42}-b-C\textsubscript{16}TAPA\textsubscript{42} showing the reversibility of alcohol-induced structural changes: (A) cubic structure in the n-alcohol-free reference dispersion; (B) lamellar structure with added decanol at R = 0.613; (C) return to cubic structure after 3 days of dialysis of the latter sample against deionized water. All measurements were performed at 25 °C.

Figure 6. DSC thermograms for (A) 46.7 wt % C\textsubscript{16}TAPA\textsubscript{30} 19.1 wt % water, 15.2 wt % decanol system and (B) an aqueous dispersion containing 1.0 wt % PAAm\textsubscript{42}-b-C\textsubscript{16}TAPA\textsubscript{42} and 0.8 wt % decanol. R = 0.325 for both samples.

around 10-fold lower than what is described in this paper and most likely not enough to constitute a liquid-crystalline domain. Similar arguments were used, for example, by Guillot et al.\textsuperscript{24} to explain the absence of a liquid-crystalline order in hexosomes (dispersions of reverse hexagonal lipid-based phases) below 30 nm (the lattice parameter being \(a = 5.82\) nm).

Colloidal complexes similar to those studied here are known to display properties that depend on the sample preparation procedures. Cationic lipid–polyelectrolyte (DOTAP/PAA)
Table 3. Hydrodynamic Radii ($R_h$), Polydispersity Index (PDI), and Zeta Potential ($\zeta$) for Poly(acrylamide)-b-CS Nanoparticles at Varying n-Alcohol/CS Mass Ratios ($R_n$) (av ± SD, Triplet of Independent Preparations)

| poly(acrylamide)-b-CS | $R_\text{octanol}$ (nm) | $R_\text{decanol}$ (nm) | $R_h$ ± SD (nm) | PDI | $\zeta$ ± SD (mV) |
|------------------------|-------------------------|-------------------------|-----------------|-----|-------------------|
| PAAm$_{42}$-b-C$_{12}$TAPA$_{30}$ | 0 | 0 | 110 ± 10 | 0.12 | −35 ± 5 |
|                          | 0.256 | 115 ± 10 | 0.14 | 30 ± 5 |
|                          | 0.262 | 115 ± 10 | 0.13 | 28 ± 4 |
|                          | 0.613 | 115 ± 10 | 0.15 | 25 ± 5 |
|                          | 0.620 | 115 ± 10 | 0.13 | 22 ± 5 |
| PAAm$_{42}$-b-C$_{10}$TAPA$_{30}$ | 0 | 0 | 115 ± 10 | 0.11 | 25 ± 5 |
|                          | 0.818 | 120 ± 10 | 0.13 | 22 ± 5 |
|                          | 4.067 | 120 ± 10 | 0.12 | 20 ± 2 |
|                          | 8.540 | 130 ± 10 | 0.10 | 14 ± 5 |
|                          | 0.142 | 120 ± 10 | 0.13 | 25 ± 5 |
|                          | 0.321 | 120 ± 10 | 0.12 | 20 ± 5 |
|                          | 0.325 | 118 ± 10 | 0.15 | 20 ± 5 |
|                          | 0.335 | 115 ± 10 | 0.12 | 18 ± 5 |

nanoparticles, together with the surface charge, through both steric and electrostatic stabilization mechanisms. It has been observed that the colloidal dispersions are kinetically stable with no sign of macroscopic phase separation for several weeks. The observed variations in the zeta potential values could have consequences for the long-term colloidal stability of the nanoparticle dispersions; however, the latter topic is beyond the scope of the present study.

**Internal Structure of the Nanoparticles.** Previously reported ternary phase diagrams$^{10,12}$ have shown that CS/n-alcohol mixtures, such as pure CSs, display a finite maximum water uptake when equilibrated against excess water. With added decanol or octanol, the maximum water uptake of the CS/n-alcohol mixtures varied in the range 35–55 wt %, depending on the specific choices of CSs and n-alcohol and their mixing ratio. An obvious question is then whether the maximally water-swollen cores of poly(acrylamide)-b-CS nanoparticles obey the same phase diagrams, that is, if the same structure is obtained, at a given n-alcohol/surfactant ion ratio, in the nanoparticle core as for the corresponding homopolymer CS/alcohol mixture. This direct comparison was made and is presented in Figure 7 below.

Because of a non-negligible fraction of the alcohol residing in the water phase of the very dilute dispersions of poly-(acrylamide)-b-CS studied here, previously reported$^{31}$ partition coefficients of the n-alcohols between docetyl- and hexadecyltrimethylammonium bromide micelles and water were used to calculate the true mole fractions of alcohol, $X_p$, of the mixed surfactant aggregates in the cores, based on overall sample compositions. The points in Figure 7 indicate the thus calculated compositions and the determined structures of the cores of the copolymer nanoparticles. As a background in Figure 7, reference phase boundaries shown have been calculated from data for maximally swollen C$_{12}$TAPA$_{30}$/n-alcohol mixtures, taken from refs 10 and 12. Clearly, there is an exact correspondence, within the resolution of the two studies, between the two types of maximally swollen CS/n-alcohol mixtures. Because the C$_{12}$TAPA$_{30}$/octanol system was not included in the previous studies, the reference phase diagram for these mixtures is lacking in Figure 7D.

As shown above, it was possible to reproduce the structures found in homopolymer CS/water/n-alcohol ternary mixtures in the cores of the corresponding poly(acrylamide)-b-CS nanoparticles dispersed in an aqueous medium. In addition, the lattice parameters were also very similar, based on the data of refs 10 and 12. The close correspondence found is not self-evident because one would expect some influence of the poly(acrylamide) chains of the block copolymer on the surfactant aggregate arrangement. A comparison of the effective radii of the nanoparticles, approximately 100 nm, with the 11 nm length of a fully stretched block of 42 acrylamide units
implies that a fraction of the poly(acrylamide) blocks must necessarily reside among the surfactant aggregates in the cores. However, apparently, they affect neither the mesophase structure nor the aggregate-to-aggregate distance represented by the lattice parameters, for any of the structures investigated.

Moreover, it was a priori expected that the core−shell interface and the consequent confinement of these structures could impose restrictions on the lattice parameters of 2D or 3D internal structures, but such effects were not seen. Confinement effects on the arrangement of the liquid-crystalline structures in the inner part have previously been reported. Golan and Talmon 17 described lamellar structures in the form of onion-like aggregates formed in complexes between cationic lipids and oppositely charged polymers. The same arrangement of bilayered structures was found in colloidal complexes formed by the electrostatic association between ionic lipids and an oligonucleotide.13 Cubosomes 34 and hexosomes 35 with distorted structures were also reported elsewhere.

In some samples, two different internal structures were found to coexist in the liquid-crystalline nanoparticle dispersions. The systems must then feature either particles with two phases coresiding in the same core or a mixture of two kinds of particles featuring single-phase cores of two very different structures that also differ substantially in composition. The average n-alcohol mole fractions in the particle cores are in the same range as for the corresponding bulk systems belonging to a three-phase region (hexagonal, lamellar, and excess isotropic solution). Hence, again, the structural data agree with those for the homopolymer CSs. From the corresponding homopolymer CS phase diagrams (see Figure 7), it can be inferred that there is a large difference in the alcohol content between the two coexisting phases in the hexagonal/lamellar two-phase area, for example. Guillot et al. 24 have also reported oil-loaded liquid-crystalline nanoparticles, in which the SAXS data indicated phase coexistence, depending on the oil content. In their interpretation, the possibility to have a mixture of nanoparticles having different internal liquid-crystalline structures was rejected because the oil would be transferred from oil-rich nanoparticles to the oil-poor ones. On similar grounds, we favor the interpretation that mixed-phase dispersions of the systems studied here contain nanoparticles with the two phases coresiding in the same core. However, the present results do not allow us to distinguish these two cases.

The n-alcohols, when incorporated in surfactant aggregates, can give two different effects commonly known as cosurfactant and cosolvent effects, leading to changes in the structure of the aggregate.10,12 Decanol acts as a cosurfactant, being incorporated with the surfactant ions in the mixed aggregates, where the hydroxyl groups are located at the interface.12 This effect results in an increase in the critical packing parameter, favoring the formation of structures with low and even inverted curvatures.12 Reverse structures, such as the reverse hexagonal and micellar phases, are seen to form only for octanol and at high octanol mole fractions. From this, one can conclude that octanol functions as a cosolvent better than decanol forming an octanol-continuous domain that can dissolve the reverse CS aggregates, leading to the formation of an isotropic L1 phase at the highest octanol contents. This is in accordance with previous findings, for homopolymer CSs, that the efficiency as a cosolvent increases for shorter alcohols.10,12,13 The estimated solubility of the homopolymer CS C16TAPA30 is 10 and 40% in decanol and octanol, respectively.10

In contrast to the nanoparticles containing normal ordered phases, there are few reports on liquid-crystalline nanoparticles with reverse structures; the latter reports concern essentially lipid−water dispersions.37−39 In the present study, two different reverse phases were found: a disordered micellar and a hexagonal phase. The reverse hexagonal phase is composed of cylindrical micelles, in which the polymer/aqueous phase is located inside of the cylinders with the surfactant alkyl chains facing the outside of the oil phase. Bernardes et al.15 studied reverse micelles in bulk systems formed by poly(acrylate)−hexadecyltrimethylammonium CSs, water, and different alcohols (octanol, hexanol, and butanol). Their results indicated the formation of alcohol-soluble aggregates, where each aggregate had a core of one polyion chain in water, surrounded by neutralizing surfactant ions. Such an aggregate was described as a reverse micelle with a spine.

A few findings of nanoparticles with reverse internal structures have been reported in the literature, such as the aqueous tetradecane-loaded monolinolein particles, which can display cubic, hexagonal, discontinuous cubic, and micellar reverse structures, depending on the oil content.38 Nakano et al.37 and Tran et al.38 have also reported reverse particles occurring in monoolein-based aqueous dispersions, when a fatty acid (oleic or decanoic) is added to the system. More recently, citrem (citric acid ester of glycerides) nanoparticles dispersed in an aqueous medium were also shown to comprise the internal reverse micellar (Lα) structure when mixed with lipids.40 However, our study is the first report on nonlipid nanoparticles displaying reverse liquid-crystalline structures at room temperature.

Depending on the n-alcohol fraction incorporated into the core, the nanoparticles may display a lamellar interior structure. Bilayers in the gel phase are highly ordered and packed structures.25 In some cases, the cooling of confined bilayers below the Tm does not result in gel phase formation, which is attributed to the presence of an effective area constraint. It was also shown that the curvature of the bilayers affects its properties compared to the planar lamellar state. For vesicles of both lipids and surfactants, the transition temperature is found to decrease gradually with decreasing vesicle size for aggregates smaller than 70 nm in diameter.21,22 In the limit of high curvature, for very small vesicles, the formation of gel domains is strongly suppressed, which is directly related to the increase in the bending modulus of gel bilayers with respect to bilayers in the fluid state.43

In view of these previous findings, the quantitative agreement between dispersed poly(acrylamide)-b-CSs and nondispersed homopolymer CSs regarding both Tm and ΔH for the thermally induced Lα−Lβ phase transition is quite remarkable. It indicates that, apparently, there is no significant curvature or confinement effect on the lamellar structures composing the core of n-alcohol-loaded dispersed nanoparticles. This result thus follows the general pattern found here and in one previous3 study that the dispersed poly(acrylamide)-b-CS systems reproduce the phase behavior of bulk homopolymer CSs.

By dialysis, it was possible to achieve the initial liquid-crystalline structure of the nanoparticles by removing the n-alcohol incorporated in the core of the nanoparticles. This highlights the possibility to switch the internal structure of the poly(acrylamide)-b-CS nanoparticles from water-continuous to oil-continuous, or vice versa, by simply adding or removing the alcohol.
CONCLUSIONS AND OUTLOOK

In this work, we were able to reproduce the liquid-crystalline phases of the bulk systems in the cores of poly(acrylamide)-b-CS nanoparticles. The various phases were achieved by the incorporation of n-alcohols in the ordered cores, acting as cosurfactants, inducing variation in the packing parameter of the surfactant aggregates, and for n-octanol, as a cosolvent in reverse structures. The SAXS data revealed the following liquid-crystalline phases, depending on the nature and concentration of n-alcohol: cubic, hexagonal, lamellar, reverse hexagonal, and reverse micellar phase. Apparently, the confinement of the phases into nanometric cores does not change their structural properties. DLS measurements revealed nanoparticles with an average radius of about 100 nm. These dispersions are kinetically stable, most likely due to steric and electrostatic repulsion arising from PAAm surface chains and negative zeta potentials, respectively. The colloidal and structural properties of the dispersions are dependent on the mixing pathways, but by using our procedure to prepare poly(acrylamide)-b-CS nanoparticles, we were able to obtain liquid-crystalline core nanoparticles with reproducible colloidal and structural features.

In summary, the investigated poly(acrylamide)-b-CS dispersions present a rich structural variety in the cores of the nanoparticles, which can be controlled in a systematic way by the addition or removal of n-alcohols. This is interesting as a versatile system for the purposes of delivering active agents and stimuli-responsive systems, for example. The present study also contributes to a better understanding of the nature of polymer/ oppositely charged surfactant interactions and the mapping of ordered liquid-crystalline structures confined in colloidal domains. Over the years, lipid-based liquid-crystalline nanoparticles have become the subject of many scientific studies pointing out their applications as drug-delivery systems, surface coatings, and environmentally responsive colloids and their use as versatile model particles for soft matter studies, for example. We present here a chemically different way to produce these internally structured nanoparticles, with the advantage of possessing a potential switchable mechanism that allows a precise control of the internal phase. The knowledge of liquid-crystalline structures is fundamental in the design of colloidal materials with desired properties and functions, as evidenced by the huge research effort on various applications of polymer/surfactant dispersions. Thus, the information reported in the present study is encouraging for future work involving cryo-TEM, aiming at the determination of the aggregates shape and arrangement of the confined liquid-crystalline structures and to elucidate the possible phase coexistence in the nanoparticle core and how the coexisting phases are arranged.

EXPERIMENTAL SECTION

Chemicals. The acid form of the symmetric block copolymer P(AA42-b-AAm42) was synthesized using controlled radical polymerization as described in a previous work of our group.3 Dodecyltrimethylammonium bromide and hexadecyltrimethylammonium bromide, (C12TABr and C16TABr, respectively) of 99% purity were purchased from Sigma-Aldrich (USA). The alcohols used were n-octanol (Sigma, USA) and n-decanol (Fluka, USA); both were of the highest purity available (99%) and used as received. Deionized water with a resistivity above 18.2 MΩ cm⁻¹ as obtained by a Milli-Q system was used in all experiments.

Preparation of Poly(acrylamide)-b-CS Block Copolymers. All poly(acrylamide)-b-CS block copolymers were prepared by titrating the hydroxide form of the surfactants (C12TA and C16TA) obtained by an ion-exchange step, with aqueous solutions of the acid P(AA42-b-AAm42) until the equivalence point, following the general procedure described earlier for CSs prepared in our laboratories.6,8,9,12 The equivalence point at pH 8.6–8.9 was found to be the same as that determined using the homopolymers.6–9 The mixture was left overnight at 4 °C and its pH was adjusted to the equivalence point when necessary, with P(AA42-b-AAm42) solution. The poly(acrylamide)-b-CS block copolymers were freeze-dried to obtain their powders, which were kept in a desiccator. The final products were named PAAm42-b-C12TABr and PAAm42-b-C16TABr according to the alkyl chain length of the surfactants used in their preparation.

Sample Preparation. The dispersions were prepared by vortexing the appropriate amounts of CSs and water for approximately 1 min to achieve the final poly(acrylamide)-b-CS concentration of 1.0 wt %. The samples containing n-alcohols were prepared in the same way, with the addition of desired amounts of octanol or decanol to the solids before adding water and vortexing.

Techniques. SAXS. SAXS measurements were taken to identify the liquid-crystalline structures formed in the nanoparticle cores. The measurements were taken at the SANS1 beamline of the Brazilian Synchrotron Light Laboratory, LNLS, in Campinas, Brazil. The samples were positioned in a cell with two flat mica windows, and a thermal bath connected to the sample holder was used for temperature control. The X-ray wavelength was 1.608 Å, and the sample-to-detector distance was around 0.6 m, as calibrated using silver behenate. The obtained charge-coupled device (CCD) images were integrated and treated with Fit2D software14 to obtain the scattering function I(q), where q = (4π/λ) sin(θ/2), with λ being the wavelength and θ being the scattering angle. The relative diffraction peak positions were used to identify the mesophase structures of the liquid-crystalline nanoparticles. The interplanar distance (d) between two reflecting planes is given by d = 2π/q, which enables us to calculate the corresponding mean lattice parameter (a). The scattering pattern of the so-called L2 (micellar reverse) phase shows only one broad correlation peak, corresponding to the micelle–micelle correlation distance. All measurements were recorded at 25 °C.

DSC. DSC measurements of the poly(acrylamide)-b-CS block copolymer dispersions were taken using a Microcal VP high sensitivity differential scanning calorimeter (Microcal Inc. Northampton, MA, USA) equipped with 0.542 mL twin total-fill cells. Scanning was performed at a heating rate of 60 °C h⁻¹ from 10 to 80 °C. For the concentrated homopolymer CS samples, the DSC measurements were taken using a Q1000 differential scanning calorimeter, TA Instruments. Around 5 mg of sample was hermetically sealed into an aluminum pan. Measurements were taken at a heating rate of 90 °C h⁻¹ from 10 to 80 °C. In all cases, the analyses were made with null prescan, producing fully reproducible results when comparing consecutive runs. Transition enthalpies were obtained by integrating the area under the thermograms along the temperature range. The calorimetric analyses were made in triplicate.

DLS. The dispersions were evaluated using DLS at 25 °C, using a Malvern Nano Zetasizer instrument with a 632.8 nm laser and a detector positioned at 173°. From the apparent
diffusion coefficients, the hydrodynamic radii (R_d) of the nanoparticles were determined using the Stokes–Einstein relationship for translational diffusion. The zeta potential (ζ) was estimated based on the electrophoretic mobility measurements using the Smoluchowski model.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.6b00267.

SAXS patterns for n-alcohol-loaded poly(acrylamide)-b-CS nanoparticles with different values of R and size ratio, core/parameter for all investigated samples (PDF)

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All the experimental work was designed by the three authors, conducted by G.A.F. and the results analyzed and discussed by the three authors. All authors have contributed and given approval to the final version of the manuscript.

Notes
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REFERENCES

1 Berret, J.-F.; Cristobal, G.; Hervé, P.; Oberdiez, J.; Grillo, I. Structure of colloidal complexes obtained from neutral/poly-electrolyte copolymers and oppositely charged surfactants. Eur. Phys. J. E: Soft Matter Biol. Phys. 2002, 9, 301–311.

2 Berret, J.-F.; Hervé, P.; Aguierre-Chariol, O.; Oberdiez, J. Colloidal complexes obtained from charged block copolymers and surfactants: A comparison between small-angle neutron scattering, cryo-TEM, and simulations. J. Phys. Chem. B 2003, 107, 8111–8118.

3 Vitorazi, L.; Berret, J.-F.; Loh, W. Self-Assembly of Complex Salts of Cationic Surfactants and Anionic–Neutral Block Copolymers. Dispersions with Liquid-Crystalline Internal Structure. Langmuir 2013, 29, 14024–14033.

4 Uchman, M.; Stępaniak, M.; Prévost, S.; Angelov, B.; Bednár, J.; Appavou, M.-S.; Gradzielski, M.; Procházka, K. Coassembly of Poly(ethylene oxide)-block-poly(methacrylic acid) and N-Dodecylpyridinium Chloride in Aqueous Solutions Leading to Ordered Micellar Assemblies within Copolymer Aggregates. Macromolecules 2012, 45, 6471–6480.

5 Hervé, P.; Destarac, M.; Berret, J.-F.; Lal, J.; Oberdiez, J.; Grillo, I. Novel Core–Shell Structure for Colloids Made of Neutral/Polyelectrolyte Diblock Copolymers and Oppositely Charged Surfactants. Europhys. Lett. 2002, 58, 912–918.

6 Berret, J.-F.; Vigolo, B.; Eng, R.; Hervé, P.; Grillo, I.; Yang, L. Electrostatic Self-Assembly of Oppositely Charged Copolymers and Surfactants: A Light, Neutron, and X-Ray Scattering Study. Macromolecules 2004, 37, 4922–4930.

7 Piculell, L. Understanding and Exploiting the Phase Behavior of Mixtures of Oppositely Charged Polymers and Surfactants. Langmuir 2013, 29, 10313–10329.

8 Svensson, A.; Piculell, L.; Cabane, B.; Ilekiti, P. A New Approach to the Phase Behavior of Oppositely Charged Polymers and Surfactants. J. Phys. Chem. B 2002, 106, 1013–1018.

9 Svensson, A.; Normann, J.; Piculell, L. Phase Behavior of Polyion–Surfactant Ion Complex Salts: Effects of Surfactant Chain Length and Polyion Length. J. Phys. Chem. B 2006, 110, 10332–10340.

10 Bernardes, J. S.; Normann, J.; Piculell, L.; Loh, W. Complex Polyion–Surfactant Ion Salts in Equilibrium with Water: Changing Aggregate Shape and Size by Adding Oil. J. Phys. Chem. B 2006, 110, 23433–23442.

11 Bernardes, J. S.; Loh, W. Structure and phase equilibria of mixtures of the complex salt hexadecyltrimethylammonium poly(methacrylate), water and different oils. J. Colloid Interface Sci. 2008, 318, 411–420.

12 Bernardes, J. S.; Piculell, L.; Loh, W. Self-Assembly of Polyion–Surfactant Ion Complex Salts in Mixtures with Water and n-Alcohols. J. Phys. Chem. B 2011, 115, 9050–9058.

13 Bernardes, J. S.; da Silva, M. A.; Piculell, L.; Loh, W. Reverse micelles with spines: Lω phases of surfactant ion–polyion complex salts, n-alcohols and water investigated by rheology, NMR diffusion and SAXS measurements. Soft Matter 2010, 6, 144–153.

14 Spicer, P. T.; Hayden, K. L.; Lynch, M. L.; Ofori-Boateng, A.; Burns, J. L. Novel Process for Producing Cubic Liquid Crystalline Nanoparticles (Cubosomes). Langmuir 2001, 17, 5748–5756.

15 Rosa, M.; Infante, M. R.; Miguel, M. d. G.; Lindman, B. Spontaneous formation of vesicles and dispersed cubic and hexagonal particles in amino acid-based catanionic surfactant systems. Langmuir 2006, 22, 5588–5596.

16 Uchman, M.; Gradzielski, M.; Angelov, B.; Toiner, Z.; Oh, J.; Chang, T.; Stępánek, M.; Procházka, K. Thermodynamic and Kinetic Aspects of Coassembly of PEO–PAA Block Copolymer and DPCI Surfactants into Ordered Nanoparticles in Aqueous Solutions Studied by ITC, NMR, and Time-Resolved SAXS Techniques. Macromolecules 2013, 46, 2172–2181.

17 Golan, S.; Talmon, Y. Nanostructure of Complexes between Cationic Lipids and an Oppositely Charged Polyelectrolyte. Langmuir 2012, 28, 1668–1672.

18 Kang, K. J.; Patel, H.; Lindberg, S.; Hartley, P. G.; Knott, R.; Spicer, P. T.; Boyd, B. J. Controlling the Mesosstructure Formation within the Shell of Novel Cubic/Hexagonal Phase Cetyltrimethylammonium Bromide–Poly(acrylamide-acrylic acid) Capsules for pH Stimulated Release. ACS Appl. Mater. Interfaces 2015, 7, 24501–24509.

19 Wadsäter, M.; Barauskas, J.; Nylander, T.; Tiberg, F. Formation of Highly Structured Cubic Micellar Lipid Nanoparticles of Soy Phosphatidylcholine and Glycerol Dioleate and Their Degradation by Triacylglycerol Lipase. ACS Appl. Mater. Interfaces 2014, 6, 7063–7069.

20 Fong, W.-K.; Hanley, T. L.; Thierry, B.; Kirby, N.; Waddington, L. J.; Boyd, B. J. Controlling the Nanostructure of Gold Nanorods–Lyticotropic Liquid-Crystalline Hybrid Materials Using Near-Infrared Laser Irradiation. Langmuir 2012, 28, 14450–14460.

21 Lee, K. W. Y.; Nguyen, T.-H.; Hanley, T.; Boyd, B. J. Nanostructure of liquid crystalline matrix determines in vitro sustained release and in vivo oral absorption kinetics for hydrophilic model drugs. Int. J. Pharm. 2009, 365, 190–199.

22 Zhou, S.; Liang, D.; Burger, C.; Yeh, F.; Chu, B. Nanostructures of Complexes Formed by Calf Thymus DNA Interacting with Cationic Surfactants. Biomacromolecules 2004, 5, 1256–1261.

23 Ferreira, G. A.; Loh, W. Structural parameters of lamellar phases formed by the self-assembly of dialkyl(dimethylammonium) bromides in aqueous solution. J. Braz. Chem. Soc. 2016, 27, 392–401.

24 Guillot, S.; Salentining, S.; Chemelli, A.; Sagalowicz, L.; Leser, M. E.; Glatter, O. Influence of stabilizer concentration on the internal
liquid crystalline order and the size of oil-loaded monolinolein-based dispersions. *Langmuir* 2010, 26, 6222–6229.

(25) Buchhammer, H.-M.; Mende, M.; Oelmann, M. Formation of mono-sized polyelectrolyte complex dispersions: Effects of polymer structure, concentration and mixing conditions. *Colloids Surf., A* 2003, 218, 151–159.

(26) Tarahovsky, Y. S.; Koynova, R.; MacDonald, R. C. DNA Release from Lipoplexes by Anionic Lipids: Correlation with Lipid Mesomorphism, Interfacial Curvature, and Membrane Fusion. *Biophys. J.* 2004, 87, 1054–1064.

(27) Nizri, G.; Makarsky, A.; Magdassi, S.; Talmon, Y. Nanostructures formed by self-assembly of negatively charged polymer and cationic surfactants. *Langmuir* 2009, 25, 1980–1985.

(28) Nizri, G.; Magdassi, S.; Schimdt, J.; Cohen, Y.; Talmon, Y. Microstructural Characterization of Micro- and Nanoparticles Formed by Polymer–Surfactant Interactions. *Langmuir* 2004, 20, 4380–4385.

(29) Pojžák, K.; Fegyver, E.; Mészáros, R. Effect of Linear Nonionic Polymer Additives on the Kinetic Stability of Dispersions of Poly(diallyldimethylammonium chloride)/Sodium Dodecylsulfate Nanoparticles. *Langmuir* 2013, 29, 10077–10086.

(30) Kulkarni, C. V. Lipid crystallization: From self-assembly to hierarchical and biological ordering. *Nanoscale* 2012, 4, 5779–5791.

(31) Abu-Hamdiyyah, M.; Kumari, K. Partitioning of amphiphilic additives between the micelles of n-alkyltrimethylammonium bromides and the surrounding aqueous solution as a function of surfactant chain length. *J. Phys. Chem.* 1990, 94, 2518–2523.

(32) Wiese, G. R.; Healy, T. W. Effect of particle size on colloid stability. *Trans. Faraday Soc.* 1970, 66, 490–499.

(33) Weisman, S.; Hirsch-Lerner, D.; Barenholz, Y.; Talmon, Y. Nanostructure of cationic lipid–oligonucleotide complexes. *Biophys. J.* 2004, 87, 609–614.

(34) Angelov, B.; Angelova, A.; Drechsler, M.; Garamus, V. M.; Mutatchieva, R.; Lesieur, S. Identification of large channels in cationic PEGylated cubosome nanoparticles by synchrotron radiation SAXS and cryo-TEM imaging. *Soft Matter* 2015, 11, 3686–3692.

(35) Sagalowicz, L.; Michel, M.; Adrian, M.; Frossard, P.; Rouvet, M.; Wätzke, H. J.; Yaghmur, A.; de Campo, L.; Glatter, O.; Leser, M. E. Cryo-Micrography of dispersed liquid crystalline phases studied by cryo-transmission electron microscopy. *J. Microsc.* 2006, 221, 110–121.

(36) Teixeira, C. V.; Itri, R.; do Amaral, L. Q. Decanol Effect on Micellar Structure and Phase Transitions. *Langmuir* 1999, 15, 936–939.

(37) Nakano, M.; Teshigawara, T.; Sugita, A.; Leesajakul, W.; Taniguchi, A.; Kamo, T.; Matsuoka, H.; Handa, T. Dispersions of Liquid Crystalline Phases of the Monoolein/Oleic Acid/Pluronic F127 System. *Langmuir* 2002, 18, 9283–9288.

(38) Yaghmur, A.; de Campo, L.; Salentinig, S.; Sagalowicz, L.; Leser, M. E.; Glatter, O. Oil-loaded monolinolein-based particles with confined inverse discontinuous cubic structure (Fd3m). *Langmuir* 2006, 22, 517–521.

(39) Tran, N.; Mulet, X.; Hawley, A. M.; Hinton, T. M.; Mudie, S. T.; Muir, B. W.; Giakoumatos, E. C.; Waddington, L. J.; Kirby, N. M.; Drummond, C. J. Nanostructure and cytotoxicity of self-assembled monoolein–capric acid lyotropic liquid crystalline nanoparticles. *RSC Adv.* 2015, 5, 26785–26795.

(40) Hedegaard, S. F.; Nilsson, C.; Laurinmäki, P.; Butcher, S.; Urtti, A.; Yaghmur, A. Nanostructured aqueous dispersions of citrem interacting with lipids and PEGylated lipids. *RSC Adv.* 2013, 3, 24576–24585.

(41) Biltonen, R. L.; Lichtenberg, D. The use of differential scanning calorimetry as a tool to characterize liposome preparations. *Chem. Phys. Lipids* 1993, 64, 129–142.

(42) Koynova, R.; Caffrey, M. Phases and phase transitions of the phosphatidylcholines. *Biochim. Biophys. Acta, Rev. Biomembr.* 1998, 1376, 91–145.

(43) Risselada, H. J.; Marrink, S. J. The freezing process of small lipid vesicles at molecular resolution. *Soft Matter* 2009, 5, 4531–4541.

(44) Hammersley, A. P. FIT2D: An Introduction and Overview. *European Synchrotron Radiation Facility Internal Report*, 1997.