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Monoglyceride-based cubosomes stabilized by Laponite: Separating the effects of stabilizer, pH and temperature

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ABSTRACT

The use of colloidal stabilizers for liquid crystalline phases broadens the possible applications of cubosomes. However, in the case of Laponite XLG as the stabilizer, the pH is increased. This can lead to changes in the system. In this paper the effects of high pH and type of stabilizer on the liquid crystal structure of monoglyceride/water phases are separated and examined. Specifically, a comparison between the synthetic hectorite platelet (Laponite XLG) and Pluronic F127 triblock copolymer as the stabilizer of the dispersions is presented. At neutral pH a pure *Pn*3*m* phase is observed in the bulk phase, which transforms into a two-phase coexistence (*Pn*3*m* and *Im*3*m*) with high pH. In the dispersion the F127 triblock copolymer as stabilizer also promotes the coexistence of these two cubic phases, where *Im*3*m* phase dominates. Dispersions with Laponite exhibit only one cubic phase of type *Pn*3*m* in the same pH regime. The disruptive role of F127 on the internal structure of the particles is evidenced in lower melting temperatures than found in the bulk phase, whilst Laponite is found to be undisruptive and the phase behaviour is directly dictated by the bulk phase. Finally, the effect of temperature on Laponite-stabilized hexosomes, formed in-situ from cubosomes due to hydrolysis of the monoglyceride, was investigated.

1. Introduction

Larsson and co-workers introduced around 10 years ago the idea of mechanically breaking up liquid crystalline (LC) phases in the presence of a hydrophilic stabilizer to form aqueous dispersions [1–4]. Since then, many studies have been carried out leading to improvements in the fundamental understanding of these systems and in the design of useful and novel materials, using cubic, hexagonal or micro-emulsion as the internal phase. Particularly the use of a variety of internal components (mainly lipids), of types of stabilizers (surfactants, diblock and triblock polymers, proteins) and loading additives (such as oil) demonstrated the possibility to adjust the internal structure without destabilization of the dispersed particles [5–10]. These materials have received a generic name corresponding to their internal structure: cubosomes (internal cubic phases), hexosomes (internal hexagonal phase), and EMEs (emulsified micro-emulsion).

The family of cubosomes has attracted substantial attention during the last years because of their many practical applications ranging from cosmetics to food products [11,12]. These LC admit high interfacial area, hydrophilic and hydrophobic regions, which make them perfectly suitable in the area of delivery and controlled release of a wide range of active ingredients such as drugs, proteins, and vaccines [13,14]. In particular, the dispersions have an advantage on the binary lipid/water systems, which are in general rather viscous, making them difficult to handle and thus limited for many practical applications such as oral and intravenous delivery.

These smart systems have usually been made using organic stabilizers. Just recently the possible use of inorganic stabilizer was demonstrated in dispersion and stabilization of LC phases [15]. In this initial study, it was demonstrated that colloidal clay platelets (Laponite XLG, a synthetic Na hectorite, $R \approx 12–13$ nm, $h \approx 1$ nm) were able to physically stabilize monoglyceride/water and monoglyceride/tetradecane/water systems having an internal structure of L_2 (isotropic inverse micellar phase), *Fd*3*m* (micellar cubic phase), H_2 (inverse hexagonal), and *Pn*3*m* (bicontinuous cubic phase); despite evidence of chemical instability due to hydrolysis.

This initial attempt should be extended, at first, to study the effects of temperature on these new materials and to verify the difference arising from using colloidal stabilizers instead of organic stabilizers (for instance, Pluronic F127 triblock copolymer). We start by examining the case of reverse bicontinuous cubic phases (cubosomes when dispersed), which can be found at ambient temperature in the binary lipid–water systems. However, it is noteworthy that because of the high pH required when using Laponite a chemical instability can be observed in some lipids, rendering

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such systems rather complex. Indeed, in order to extract the specific effect of stabilizers, the pH effects should be separated even for reference systems, meaning the bulk phase and equivalent dispersions stabilized by an organic stabilizer.

In the present contribution, we focus on monoglyceride-based cubosomes, which hydrolyze with time; thus a system where the pH has a strong influence. As monoglyceride system, we used Dimodan U/J (DU), a commercially available product, which contains 96% distilled monoglycerides, of which 62% were linoleate and 25% oleate. For comparison, as another basic set of data, we could use of the temperature behaviour we recently obtained in the case of Laponite-stabilized phytantriol-based cubosomes [16]; phytantriol (PT; 3,7,11,15-tetramethylhexadecane-1,2,3-triol) being another lipid that is a common cosmetic ingredient which also forms reverse cubic phases at ambient temperature. PT cannot be hydrolyzed at the high pH required using Laponite as stabilizer.

We used Laponite at a concentration as low as 0.5 wt%. The residual salt and the initial pH (around 10) combined with the low concentration meant that we were initially restricted to the liquid phases of the phase diagram, more specifically to clear dispersions of discrete disc-like particles in water. A wide variety of non-equilibrium states, that undergo either phase separation or gel/glass formation with a very high viscosity, have been observed for Laponite and natural clays in water and in brine by several experimental groups [17–32]. Although, there is still considerable debate on the structure at the local scale in the non-equilibrium states, the liquid phases (with viscosity comparable to that of water) are rather well-defined. Complications arising from aggregated Laponite structures can thus be avoided by the initial conditions used in this study.

At the same time, Laponite is known to be chemically unstable at $pH < 9–10$, leading to destabilization of their dispersions. However, as previously reported [15,16], the decrease of pH, due to hydrolysis of DU chains, from a value of 10–8.2 within 100 days and the variation of temperature (for PT-based cubosomes) appears not to affect the physical stabilization of cubosomes by Laponite; namely the chemical degradation of Laponite and/or increase of temperature seems not to destabilize them, in contrast with when in dispersion.

We start by investigating the influence of high pH on DU/Water bulk phase and DU-based cubosomes stabilized with F127 as opposed to its effects on the cubosomes stabilized with Laponite. This is followed by the effects of temperature on the Laponite-stabilized particles and comparison with dispersions stabilized by F127. This investigation was made using freshly prepared samples to minimize the effects of hydrolysis. We extended the study to hexosomes formed in-situ from cubosomes due to hydrolysis of the monoglyceride.

2. Experimental section

2.1. Materials

Dimodan U/J (DU) was supplied by DANISCO A/S (Braband, Denmark). It contains 96% distilled monoglycerides, of which 62% were linoleate and 25% oleate. The clay particles used are Laponite XLG (Southern Clay Products, Inc., Gonzales, TX), which are a form of Laponite RD further characterized for use in the cosmetics industry. Laponite XLG clays are synthetic Na hectorite belonging to the smectite family. They are disc-like particles with a mean radius of $R_{XLG} = 12.5$ nm and a thickness of $h = 1$ nm. Their surface area is 370 $m^2 g^{-1}$ and the pH of aqueous suspensions below 2 wt% is around 9–10, which indicates a repulsive potential between particles. It contains in majority 59.5% SiO_2 and 27.5% MgO with traces of LiO_2 and Na_2O of about 0.8% and 2.8%, respectively. As a reference, the triblock copolymer used is Pluronic F127, a gift from BASF Corporation (Mount Olive, NJ). Pluronic F127 consists of polymer chains as $PEO_{99}–PPO_{67}–PEO_{99}$. All the materials were used without any further purification. The water used in the preparations was double distilled.

2.2. Sample preparation

The samples were prepared using the following consistent procedure. The dispersions were prepared by weighing the DU, the stabilizer (Laponite XLG or Pluronic F127) and water into vials. The raw mixture was ultrasonicated (SY-Lab GmbH, Pukersdorf, Austria), without external cooling, for 20 min at 30% of maximum power in pulse mode (0.5 s on and 1.5 s off). The samples were then sealed and left at room temperature for equilibration. The weight fraction of the DU bulk was kept constant at 0.05 $g\,g^{-1}$ (5 wt%) and the used stabilizer weight fraction at 0.005 $g\,g^{-1}$ (0.5 wt%), meaning that the stabilizer to DU weight ratio was constant at a value of 0.1. The bulk samples were prepared by adding water at pH 10 to a molten DU solution and the samples were sealed in a Pyrex tube and treated according to de Campo et al. [5] before re-equilibration at room temperature.

The hydrolysis of DU chains occurs in time in all samples at pH 10. Because of this, we define the origin of time at the end of the preparation and in order to perform accurate comparisons, all types of samples (Laponite, F127 stabilizers, and bulk phase) have been measured at the same time after preparation. In particular, it is noted that the pH varies from 10 to 8.2 after 18 days.

2.3. Small angle X-ray scattering

The SAXS equipment consists of a slit-geometry camera with high flux and low background (SAXSess, Anton-Paar, Austria), connected to an X-ray generator (Philips, PW1730/10) operating at 40 kV and 50 mA with a sealed-tube Cu anode ($\lambda = 0.154$ nm). A CCD camera, from Princeton instruments, which is a division of Roper Scientifics (Trenton, NJ, USA), records the 2D scattering pattern. The images are then integrated into the one dimensional scattering function $I(q)$. The temperature of the capillary in the metallic sample holder is controlled by a Peltier element. Measurements at ambient temperature correspond to 25 °C and a thermal equilibration time of 45 min was used before each SAXS measurement. Measurements at different temperatures were made either in the heating direction or cooling direction as indicated in the text, in both cases a 45 min equilibration time was used before measurements. The measuring times were 3×10 min for all dispersions. This allows for the proper subtraction of cosmic rays that happen when using a CCD camera. The scattering of the water solvent, $I_w(q)$, was measured for similar times and further subtracted to $I(q)$ giving the scattering intensity from the particles. Data are smeared with the beam profile (slit profile). However, the influence of this effect is minimized by the different normalizations used for the data interpretation and because liquid crystalline structures are studied.

2.4. Scattering data treatment and analysis

We previously reported that the scattering of the Laponite particles, even at 0.5 wt%, contributes strongly to the total scattering of DU-based system. As a consequence, we find that the best way to emphasize the diverse possible contributions is expressing the excess of intensity compared to pure Laponite at 0.5 wt% since this latest contribution corresponds only to the form factor of the particles. Therefore $I_{EXC}(q)$ is defined as:

$$I_{EXC}(q) = \frac{I_{DU:TC/Lapo}(q) - I_w(q)}{I_{Lapo}(q) - I_w(q)} = \frac{I_{DU:TC/Lapo}(q) - I_w(q)}{kP_{Lapo}(q)}, \qquad (1)$$