Investigating reversed liquid crystalline mesophases

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Abstract

Reversed liquid crystalline mesophases, such as the reversed bicontinuous cubic, reversed hexagonal or reversed micellar cubic phase, are nowadays widely studied due to their potential applications in, for instance, the controlled delivery of functional molecules. In order to design the adequate mesophase delivery system for a specific application, the crystallographic structure of the phase has to be determined and related to the desired function. There are different analytical methods available for investigating the structural features of these mesophases. In this work we focus on the cryo-TEM method, which allows studying the crystallographic structure of single mesophase particles. Moreover, we are discussing the potential to use rheology as a unique technique to determine in a simple way both the crystallographic structure of the liquid crystalline phases as well as the dynamic processes occurring during relaxation of these mesophases. The dynamic characteristics are significantly determining and controlling the mechanism and kinetics by which guest molecules and active ingredients can be released from their host.

Keywords: Cubic phase; Hexagonal phase; Monoglyceride; Delivery; Cryo-TEM; Rheology

1. Introduction

Significant progress has been made in the characterization of reversed liquid crystalline mesophases in the last years since there is agreement on the fact that reversed self-assembled systems may allow creating new and/or better functionalities in food or pharmaceutical products. So far the best studied applications of reversed liquid crystalline mesophases are in the area of delivery and controlled release of active ingredients, material synthesis or structural biology [1,2•].

Controlled release is an issue in the delivery of aromas and drugs [3•,4]. The base for being able to control and modulate the release of molecules is built on the knowledge of the crystallographic structure of the mesophase delivery system. For example, it has been shown that the rate of release of drug molecules is much faster from a lamellar liquid crystalline phase than from a reversed bicontinuous cubic phase [5•]. The reversed bicontinuous cubic phase permits sustained release of both charged and uncharged hydrophilic and lipophilic molecules or peptides and proteins [4,5•]. These controlled release studies were all performed from a bulk (non-dispersed) reversed bicontinuous cubic phase. In these studies, unsaturated monoglycerides, such as glycerol-monooleate, were used to form the mesophase. In order to improve the utilization of liquid crystalline mesophases or to develop new application areas, it is crucial to have appropriate methods, which allow determining and following the evolution of the structural crystallographic properties of the mesophases.

Small angle X-ray scattering (SAXS) is the most recognized method to study the structural features of reversed mesophases, both in their bulk or dispersed form [6]. In general the position and intensity of the diffraction peaks can be used to identify a certain type of self-assembled structure and even to identify the corresponding space group. In some cases, reflection might be missing or of small intensity due to a vanishing form factor, inhomogeneity and strain. In addition, several different structures may coexist making the interpretation of the X-ray data difficult. Therefore, other methods are often necessary in order to fully determine the structural properties of the liquid crystalline mesophases.

Differential scanning calorimetry is a powerful method to study phase transitions and to analyze them in terms of their position on the temperature scale and of the associated energy [7]. Freeze fracture electron microscopy is also commonly used to study mesophase structures. This technique allows one to

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discriminate between different space groups by analyzing the fracture features [8●●]. For example, in the system formed of dioleoyl-phosphatidylcholine-dioleoylglycerol (DOPC/DOG), the analysis was compatible with the Fd3m but not with the Fd3 space group. Methods, such as SAXS, can not distinguish between the Fd3 and Fd3m space group. NMR or polarized microscopy [9] can be used to establish the presence of isotropic and anisotropic phases. Borné et al. [10] used deuterium single quantum NMR to establish the ternary phase diagram of D_2O-diolein-monoolein. Hughes et al. [11] showed that deuterium double quantum NMR is a useful technique to observe even small amounts of an anisotropic phase forming in an isotropic phase, such as the bicontinuous cubic phase, under conditions where the single quantum experiment would not allow detecting the presence of the anisotropic phase. Jeong et al. [12●] used Diffusion NMR to study the diffusion of single molecules within a specific liquid crystalline phase. The latter is a powerful method since the diffusion coefficient of water, emulsifier (such as monoglycerides) and guest molecules can be directly extracted.

Reversed liquid crystalline phases, such as the bicontinuous cubic phase, are rather viscous and, therefore, difficult to handle. For many practical applications these phases need to be dispersed into water in form of micron or sub-micron-sized particles. The procedure leading to the formation of stable dispersions of particles, which have a certain internal liquid crystalline structure, have been widely described in literature [13,14●●]. However, the determination of the self-assembled structure inside the particles is still a non-trivial task, since the dispersed particles are rather small and may have a non-uniform crystallographic structure. Moreover, their overall mass fraction can be below 2 wt.%. In this regard, cryo-TEM is a very powerful technique for the characterization of such dispersed mesophase particles, since it enables to determine the shape, morphology and crystallographic structure of individual particles.

It is the objective of the present work to describe in more details the progress made in the determination of the crystallographic structure and space group of individual mesophase particles, such as cubosomes or hexosomes, by means of cryo-TEM. Moreover, we will discuss the potential of using rheological methods for the determination of the dynamic properties of bulk liquid mesophases and the determination of static structural features. The use of rheological methods is interesting because it combines the study of both structural and dynamical properties in a single affordable technique.

2. Cryo-TEM

Cryo-TEM (cryo-Transmission Electron Microscopy) is an ideal tool to study routinely the morphology and structure of mesophase particles with a resolution down to about 2–3 nm. In particular, it allows one to obtain more detailed information on the crystallographic structure of particles. This information cannot be extracted by means of the other techniques mentioned before. Moreover, the shape of single particles can be observed and structural defects can be easily visualized giving important insight in the mechanism of phase transition or particle stabilization. Thus, TEM is the only technique allowing single par-

ticle analysis, which represents an important advantage over other analytical methods, especially when structural features of mesophase particles have to be determined in complex systems, such as foods.

In the following we will discuss how the crystallographic structure of single mesophase particles can be studied by cryo-TEM, and how more detailed information can be obtained on the shape, the defects or morphology of the particles. This information can be used for the proposition of mechanistic arguments explaining the stabilization of the particles against coalescence or aggregation under specific conditions.

Gustaffson et al. [13,14●●] were the first to characterize cubosomes (a dispersed reversed bicontinous cubic phase) by cryo-TEM. An extensive amount of information could be obtained on the particle morphology and the liquid crystalline structure present within the particles. The cryo-TEM image analysis revealed that cubosomes are always observed in co-existence with vesicles or vesicle-like structures. However, hexosomes (a dispersed reversed hexagonal phase) were not observed together with vesicular structures. These studies indicate that vesicles may play a role in the stabilization of cubosomes, but not in the stabilization of hexosomes. It has to be noted that it is much more tedious or even impossible to detect by means of SAXS the presence of vesicles in mesophase particle dispersions.

Fast Fourier transform (FFT) from cryo-TEM images can be used to determine more precisely the interplanar distances in the mesophase structure [10,15,16]. However, so far, cubosomes were always analyzed under conditions, where the electron beam was aligned either with the [100] direction or with the [111] direction. This analysis cannot discriminate between the primitive and the double diamond reversed bicontinuous cubic structure, since under such observation conditions, the observed patterns are compatible with both structures.

Recently, tilting experiments were used to obtain more detailed information on the space group of the mesophase particles [17●●]. It was possible to find conditions (i.e., a direction of observation where the {111} plane was also contributing) which enable to differentiate between the double diamond cubic and the primitive structure. The {111} reflection is present for the diamond structure but not for the primitive structure. In Fig. 1a–b, the electron beam direction is aligned with [111] and only the {110} planes are visible while in Fig. 1c–d, the electron beam is aligned with [112] and the {111} reflection is also visible which is not compatible with the primitive structure (space group Im3m) but with the diamond one (space group Pn3m). Cryo-TEM analysis can also discriminate between the presence of the gyroid structure and the two other inverted bicontinuous cubic structures.

Analysing defects and morphology also allows extracting information on the mesophase particle structure and on the stabilisation mechanisms. Hexosomes show often curved striations. Tilting experiments revealed in this case that the longitudinal axis of the cylinder forming the hexagonal structure can be bent since for the same particle, the observed pattern changed from hexagonal to curved striation when tilting the hexosome particle [17●●]. Notice that tilting experiments allow also determining the shape of the particles. Barauskas et al. [16]
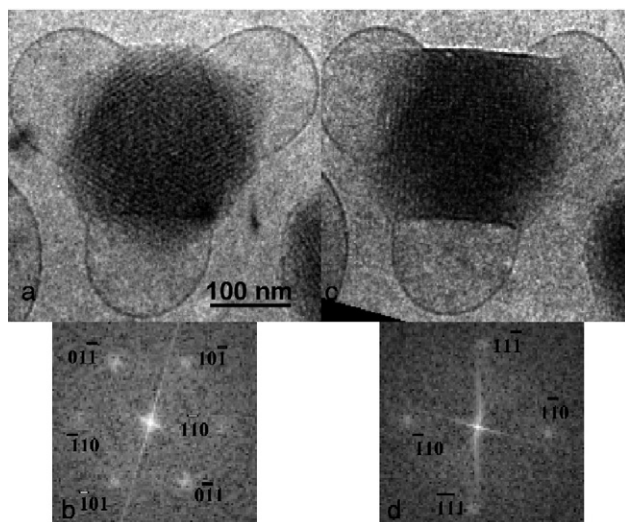
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Fig. 1. Tilting experiment which enables going from the [111] (a, b) axis of observation to the [112] (c, d) axis of observation of a particle having an inverted bicontinuous cubic structure. (a) and (c) are the original images and (b) and (d) are the FFT (intensity part). Notice that in (d) the (11−1) and (−1−11) reflections are present, which demonstrate that the space group is Pn3m and not Im3m.

described hexosomes which were plate-like having a width which is about 5 times larger than their thickness. From these results, two morphologies can be proposed for the hexosomes (Fig. 2c−f). In all the described cases, there is no direct contact between water inside the hexosomes and outside.

The mechanism of stabilization is specific for all different types of mesophase particles. For instance, Andersson et al. [18] proposed that for cubosomes (of space group Pn3m or Im3m), one of the water channel is open to the outside (Fig. 2b). However, in particles having a reversed micellar cubic structure (e.g., having a Fd3m space group and comprised of reversed micelles), all water is located inside the reversed micelles, and therefore, there is no direct contact between the water inside the particles and the outside continuous aqueous phase. As a consequence, such particles are stabilized in a different way than cubosomes.

The release of the water or hydrophilic molecules solubilized into the water domains of the mesophase depends very much on its internal structure. For instance, the release of hydrophilic molecules from a reversed micellar cubic phase is expected to be significantly different from the release of the same molecules from a reversed bicontinuous cubic phase. While the hydrophilic molecules need to cross many lipophilic domains before they are released from a reversed micellar cubic phase into the external continuous aqueous phase, their release from a reversed hexagonal or reversed bicontinuous cubic phase necessitates the crossing of much less lipophilic domains. These structural differences are thought to directly influence delivery properties, such as the rate of release of encapsulated molecules.

3. Shear rheology

Shear rheology is extensively applied to investigate polymeric bulky liquid crystalline mesophases to reveal order–order (OOT)
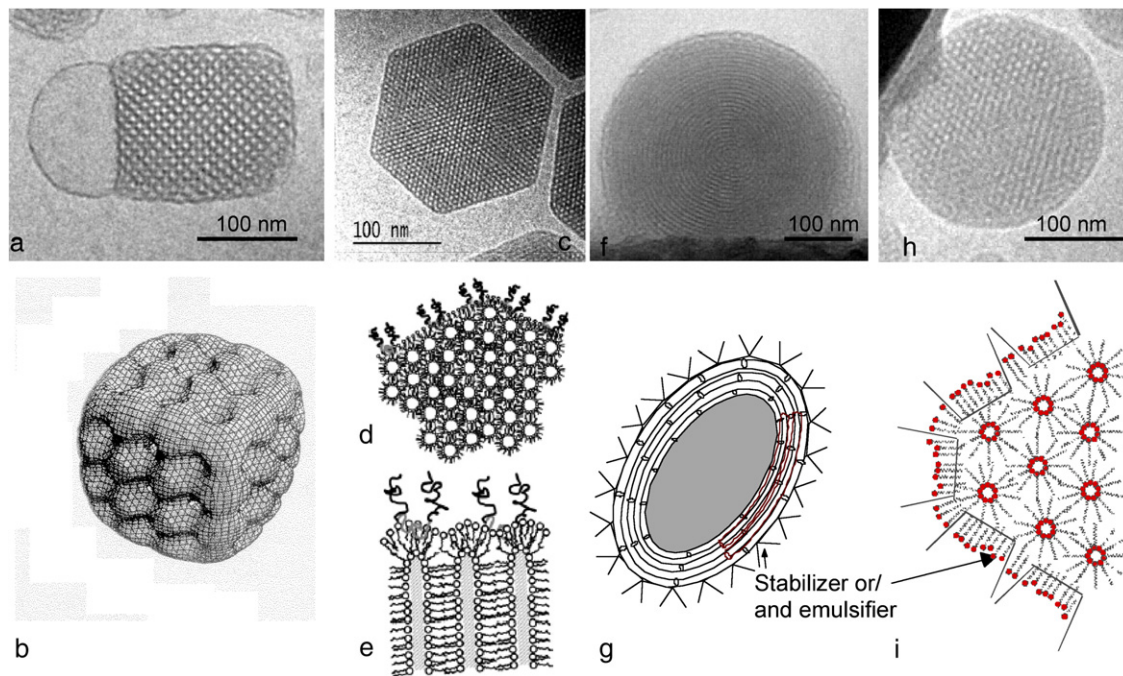

Fig. 2. Experimental images of various self-assembled particles and proposed mechanism of stabilisation. The drawings are qualitative and do not show the exact position of each molecule. (a) Experimental image of a cubosome having Im3m as space group, adapted from [17●●]. (b) Surface of a stabilized cubosome with Im3m space group. Adapted with permission from [14●●]. Copyright (1997) American Chemical Society. (c-d-e) Hexosomes with no curved striation, adapted with permission from [19●]. Copyright (2005) American Chemical Society. (c) is the experimental image and (d−e) represent the proposed stabilisation mechanism. (f) Experimental image of an hexosomes with curved longitudinal axis. Adapted with permission from [15]. Copyright (2004) American Chemical Society. (g) Morphology corresponding to (f), from [17●●]. Contrary to case (c-d-e), only one surface type needs to be stabilized. (h) Experimental images of a micellar cubosome (space group Fd3m). (i) Proposed stabilisation mechanism for micellar structures such as L2 or inverse micellar cubic (Fd3m). To simplify the situation, the micelles are circular and only one type of micelle is represented.

and order–disorder transitions (ODT) and to describe dynamic phenomena [20–22]. However, the rheological characterization of self-assembled low-molecular weight surfactant liquid crystalline mesophases has been only recently gaining growing attention. The uncommon feature with these systems is the virtual absence of entanglements between hydrophobic chains, which makes the relaxation phenomena to occur in short time scales, typically of the order of seconds. Since this happens to be exactly in the typical time scale of the tasting and release process, rheology appears to be one of the most relevant techniques that allows to obtain an insight in the perception or release features of liquid crystalline mesophase materials [23].

When dealing with the rheology of liquid crystalline phases a first distinction has to be made depending on the viscoelastic regime considered. Whereas no systematic study has been carried out at large deformations for lamellar and hexagonal phases, in the non-linear viscoelastic regime, models based on the presence of crystalline slip planes within which surfactant molecules can cooperatively diffuse have been proposed for cubic phases [24•] and applied successfully to primitive (Im3m) [24•] and micellar cubic phases [25]. Although most of the studies focused on the linear-viscoelastic regime, interpretation of rheological phenomena of liquid crystalline phases has principally been tacked by studying the storage, G', and loss moduli, G''. Changes in the slope of G' and G'' versus temperature and composition have been shown to be a valuable way to detect OOT and ODT [25–28]. Cubic-to-hexagonal, cubic-to-cubic and hexagonal-to-isotropic fluid transitions have been observed by this procedure, taking advantage of the differences in the intrinsic rigidity of the different phases, such as the reversed hexagonal, Ia3d and Pn3m bicontinuous cubic phase. For the Ia3d–Pn3m phase transition in monoglyceride-based liquid crystalline mesophases, some authors have also reported a step in the plateau modulus G_0 [29]. Nevertheless, such discontinuity in rigidity may be system-sensitive since it appears to be virtually absent in other systems.

By studying the G' and G'' dependence on shear frequency, attempts have been made to establish rheological signatures of individual liquid crystalline phases [30–33•,34••]. Current opinions tend to agree that the bicontinuous cubic phases Ia3d, Pn3m or Im3m are the most rigid liquid crystalline phases, followed by the reversed hexagonal phase, which is a moderately viscoelastic fluid, and the lamellar phase which can been described as a plastic fluid undergoing yielding. Spectra of G' and G'' versus frequency, also allow extracting the longest relaxation time, τ_{MAX}, defined as the inverse of the frequency at which crossover of G' and G'' takes place. There is a general consensus on the physical meaning of τ_{MAX}, attributed to the characteristic diffusion time of the lipid molecules at the water–lipid interface. Alternatively, τ_{MAX} can be viewed as the time scale for relaxation to equilibrium configuration of the liquid crystalline water–lipid interface, perturbed by shear or orthogonal deformations. Therefore, τ_{MAX} provides a typical order of magnitude for many diffusion processes taking place in liquid crystalline phases, and can be used to parameterize the release kinetics of active molecules through the hydrophilic/hydrophobic interface. The study of τ_{MAX} variations with temperature and composition has been shown to be the most reliable rheological method to detect

OOT and ODT in liquid crystalline phases [32,33•,34••], as well as an alternative method to establish phase diagrams, since changes in the slope of τ_{MAX} versus temperature and composition capture all phase transitions, the regions of phase coexistence, and also OOT induced by the presence of host molecules in either the hydrophilic or hydrophobic phases [35•]. Nevertheless, as it was previously recognized in literature [24•], a single relaxation time cannot relistically account for the complex viscoelastic behavior of the whole ensemble of liquid crystalline phases, in particular bicontinuous cubic phases. Therefore, recent efforts have been devoted to model the complex rheological frequency response of liquid crystalline phases by multiple Maxwell spring/dashpot pairs, which is equivalent to introduce a multitude of relaxation times [32,34••]. The same concept of multiple relaxation times has been further expanded and formalized by introducing the time Laplace-transform of $G'(\omega)$, $G''(\omega)$, which yields the shear modulus time dependence starting from experimentally measured frequency scans of storage and loss moduli [34••]. By doing so, the weight of different relaxation times can also be obtained, and thus real relaxation spectra of individual liquid crystalline phases, which can be regarded in all respect as their rheological signature (see Fig. 3). In this scenario, while the dominating relaxation time in the spectra can still be attributed to τ_{MAX}, the residual times can account for secondary relaxation mechanisms, typical of confined hydrophobic and hydrophilic phases. Whether secondary relaxation mechanisms can be directly ascribed to constrained phase relaxation is still under debate, although these findings are in agreement with independent rheological measurements on sub-nanometer lamellar phases [36]. Thus, rheology can be viewed not only as a tool to quantify the relaxation of the lipid–water interface through which active ingredients are diffusing, but also as a technique to track the mobility of guest molecules within the confined liquid crystalline mesophases.
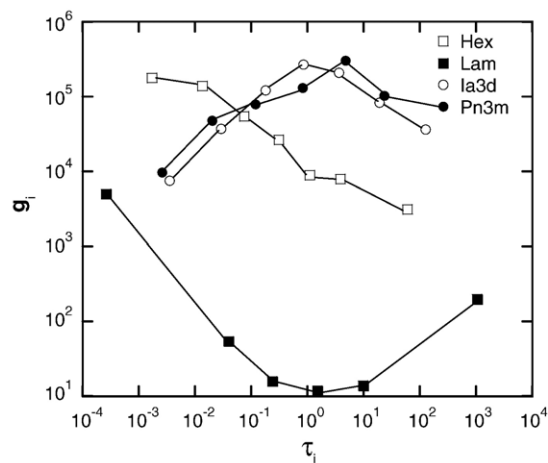

Fig. 3. Discrete relaxation spectra weights, for the main lipid–water liquid crystalline phases encountered in foods. The lamellar phase shows two dominating relaxation times, one of the order of 10^3 s, attributed to the relaxation of the water–lipid interface, and the other, very short of the order of 10^{-3} s, attributed to the viscoelasticity of water in confined lamellae. The hexagonal phase shows a monotonic distribution of relaxation times, while both the Ia3d and Pn3m inverted bicontinuous cubic phases, show, together with one dominating relaxation time, secondary multiple relaxation times capturing the complex rheological response of these materials (reproduced from [34••]).

4. Consequences for application

The progress made in the description of reversed liquid crystalline mesophases leads also to a better elucidation of the various applications described in literature [1,2[•],3–5[•],37,38]. In the following section we will discuss two examples showing how the characteristics of the formed liquid crystalline mesophases can be used to create a desired functionality.

One major limitation of using reversed bicontinuous cubic phases for application is that they are very rigid and 'solid-like' materials. In other words, reversed bicontinuous cubic phases have a storage modulus which is significantly higher than the modulus of many products, and, therefore, their use as a possible building block can be complicated due to the mismatch in rigidity with the main product matrix. A nice way to overcome this problem has been described by Engström et al. [39], making use of the fact that the reversed bicontinuous cubic phase can be easily transformed into a liquid crystalline lamellar phase $L\alpha$, which is a much more fluid mesophase, by changing temperature and the water content in the system. Upon heating from room temperature to body temperature or swelling with water, the $L\alpha$ transforms to the reversed bicontinuous cubic phase. Since the $L\alpha$ is much more fluid than the bicontinuous cubic phase, it can be easily introduced/added to certain place of choice. After putting the $L\alpha$ to the right place of action, it can be changed into the rigid bicontinuous cubic phase by adjusting the local environmental conditions, such as temperature and water content according to the respective phase diagram, and the transformation into the cubic phase can now be used to induce a certain sustained release of an active molecule into the environment.

Another striking application of self-assembled mesophases is their ability to increase Maillard reaction yield [40]. The formation of, for instance, 2-furfurylthiol (FFT) from the model reaction between L-cysteine and furfural was shown to be particularly efficient when the reaction was performed in the L2 microemulsion or reverse bicontinuous cubic phase compared to reaction efficiency in the respective aqueous system [40]. Comparing the reaction yield obtained in the two different self-assembly phases, the reaction was even more efficient when performed in the reverse bicontinuous cubic phase than in the L2 phase. This indicates that the characteristics of the formed self-assembly system significantly determines the functionality of the system. A similar conclusion was described in a recent study [41], in which the Maillard reaction between xylose and glycine was investigated in self-assembled structures and compared to the reaction in the respective water system. For this system, the production of 4 hydroxy-5-methyl-3(2H) furanone (norfuraneol) was very dependent on the presence of a particular liquid crystalline structure. At 70 °C, the production of norfuraneol was about two times higher when performed in the reversed hexagaonal phase than in the reversed bicontinuous cubic phase. Again, the characteristics of the liquid crystalline mesophase significantly influenced the macroscopic outcome. This is another example which illustrates that knowing and controlling the formation of the self-assembled structure using suitable analytical methods is a crucial requirement when designing new and appropriate applications.

5. Concluding remarks

We have discussed standard and emerging techniques for studying reversed liquid crystalline phases of interest to pharmaceutical, cosmetic or food applications. Cryo-TEM has shown to be an excellent tool to study the crystallographic structure of single liquid crystalline mesophase particles as well as the mechanism of their stabilization against aggregation and coalescence. Bulk rheology, on the other hand, allows coupling to the intrinsic crystallographic structure of bulk liquid crystalline phases, their relaxation properties, which are directly affecting diffusion properties in heterogeneous mesophases. Both the crystallographic structure and the dynamic properties of the mesophase account and control the release pattern and rate of guest molecules from a given mesophase. While cryo-TEM is the technique to be preferred in order to study dispersed mesophase particles, because of the capability of revealing internal structure of individual dispersed mesophases, rheology can be used to exploit essentially the corresponding bulk samples. The careful synergistic use of experimental methods such as those described in this paper will trigger the development of a broader range of new applications, in particular in the fields of food technology and nutrition.

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