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REVIEW

Cubic phases of ternary amphiphile–water systems

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Abstract The reversed cubic phases (Q_{II}) are a class of self-assembled amphiphile–water structures that are rich in diversity and structural complexity. These nanostructured liquid crystalline materials are generating much interest owing to their unique surface morphology, biological relevance and potential technological and medical applications. The structure of Q_{II} phases in binary amphiphile–water systems is affected by the molecular structure of surfactant, water content, temperature and pressure. The presence of additives also plays an important role. The structure and phase behaviour of ternary Q_{II} phases, which are comprised of two miscible amphiphiles and water, significantly differ from the binary system alone. The modulation of the phase behaviour through the addition of a second amphiphile offers an opportunity to control the size and shape of the nanostructures using a 'bottom-up' approach. In this mini-review, we discuss the structure of reversed cubic phases of amphiphile–water systems and highlight the modulation of cubic-phase structure in ternary-phase systems. We also extend this review to bulk cubic phases and the corresponding nanoscale dispersions, cubic-phase nanoparticles.

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Introduction

Surfactant molecules self-assemble in aqueous solution into a rich variety of well-defined, thermodynamically stable structures or 'phases' (Kaasgaard and Drummond 2006). Self-aggregation of surfactants (or amphiphiles) in water is a thermodynamically favoured process, which usually results in the orientation of the hydrophilic regions at the aqueous interface with a concomitant aggregation of the hydrophobic groups to minimise hydrogen-bonding interaction with bulk water molecules. The repulsive forces between water molecules and a non-polar hydrophobic moiety are greater than the net attractive forces between hydrophobic groups and this de-wetting assists in driving the self-assembly of amphiphiles in water (Caffrey 1987; Tanford 1978). Depending on the conditions, a wide array of structures with differing complexity emerges. These aggregates can have two-dimensional order, such as the classical monolayer and bilayer structures (lamellar phase), or higher complexity with three-dimensional architectural features (Fontell 1992; Seddon 1992), including the micellar (L), hexagonal (H) and cubic phases (Q) (Fig. 1).

Of the three-dimensional self-assembled structures, the cubic phase is of specific interest. These phases have been shown to be of particular importance to the normal operation of biological processes in cells, including membrane fusion and protein function (Hyde 1997; Luzzati et al. 1997; de Kruijff 1997). Pioneering work from Caffrey (2003) has also demonstrated the importance of cubic phase in protein crystallization. The incorporation of

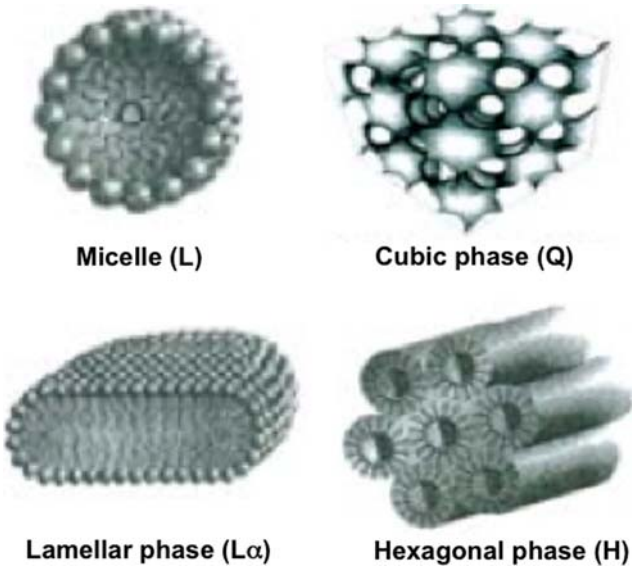

Fig. 1 Representative two- and three-dimensional structures of amphiphile self-assembled systems (adapted from Yang et al. 2004)

membrane proteins into Q phases has successfully resulted in the elucidation of the crystal structure of almost 40 integral membrane proteins (Cherezov et al. 2006). This technique is referred to as *in-meso* protein crystallization (Caffrey 2008), and cubic phases have been generated in the endoplasmic reticulum of mammalian tissue culture cells by controlled dimerisation of artificial membrane proteins (Lingwood et al. 2009).

Structurally, the cubic phase is an optically isotropic, viscous, liquid crystalline material. It is a bicontinuous structure that consists of continuous curved bilayers, which partition two separate, non-intersecting aqueous domains (Luzzati et al. 1997). By virtue of its structure, the cubic phase exhibits a large internal surface area ($\sim$400 m^2/g) (Garg et al. 2007), and this property can be exploited to solubilise a high loading of chemical or biological substrates (Drummond and Fong 1999). The thermodynamic stability of the cubic phase results in an aggregate system that is resistant to a micellar phase transition at high dilutions. This property is particularly attractive for medical applications, such as drug delivery, where the low concentrations of the lipid system are required in vivo and internal cubic-phase structure permits a high loading of a particular drug (Efrat et al. 2009).

An interesting property of cubic phases is the ability to disperse the material in aqueous solutions. The resulting dispersions have a significantly reduced viscosity relative to the bulk material and retain the cubic nanostructure of the liquid crystalline phase. The dispersed materials are sub-micron-sized particles and referred to as 'cubosomes' or 'cubic-phase nanoparticles' (CPnPs). These properties make cubic phases and CPnPs amenable to a range of other technologically relevant applications including biosensors (Barauskas et al. 2003), bioscaffolds (Lester et al. 2001), drug-delivery vehicles (Bender et al. 2005; Malmsten 2007), food science (Spicer 2005), and biomedicine (Jin et al. 2006). In such applications, complex mixtures of amphiphile, water and substrate often arise. These additives, including other lipids, proteins, carbohydrates and enzymes, can have a pronounced influence on the structure of a cubic phase and, therefore, the performance of phases in a particular application. In this review, we describe the structure of bicontinuous cubic-phase systems, in particular the reversed bicontinuous cubic (Q_{II}) phase, and report the influence of a second amphiphile on the phase behaviour and structure of binary cubic phases. The structural properties CPnPs comprised of ternary amphiphile mixtures are also considered. Although this review is not intended to be an exhaustive overview of ternary cubic-phase systems, we highlight key papers describing the phase behaviour and structure of ternary Q_{II} phase systems and critically evaluate where particularly interesting effects were observed.

Bicontinuous cubic phases and structure

The reversed bicontinuous cubic phase is comprised of a family of closely related mesomorphic structures which display continuous periodicity over three dimensions. The first general structure for the cubic phase was proposed by Luzzati et al. (1968). A refined structure was subsequently proposed based on extensive studies of the monoglyceride–water system (Lindblom et al. 1979). In this study, the authors propose a cubic phase which consisted of a single continuous curved lipid bilayer that forms a complex, symmetrical three-dimensional cubic matrix separating two continuous, non-intersecting water channels. This inherent feature differentiates these structures from other discontinuous cubic phases such as those formed by close-packed arrays of spherical micelles. The internal topography of a bicontinuous phase is commonly described as an infinite periodical minimal surface (IPMS) with zero mean curvature and a constant negative Gaussian curvature (Hyde et al. 1984). That is, all points on the bilayer surface are as concave as they are convex. The minimal surface is defined as the middle of the bilayer where the terminal methyl groups of the hydrophobic tails intersect (Larsson and Tiberg 2005). The cubic-phase structure also exhibits crystallographic periodicity, with three types of IPMS structures, the gyroid (G), diamond (D) and primitive (P) surfaces, corresponding to the crystallographic space groups *Ia3d*, *Pn3m* and *Im3m* respectively (Fig. 2). These structures have been verified using X-ray scattering techniques such as small-angle X-ray scattering (SAXS) (Tenchov et al. 1998) in addition

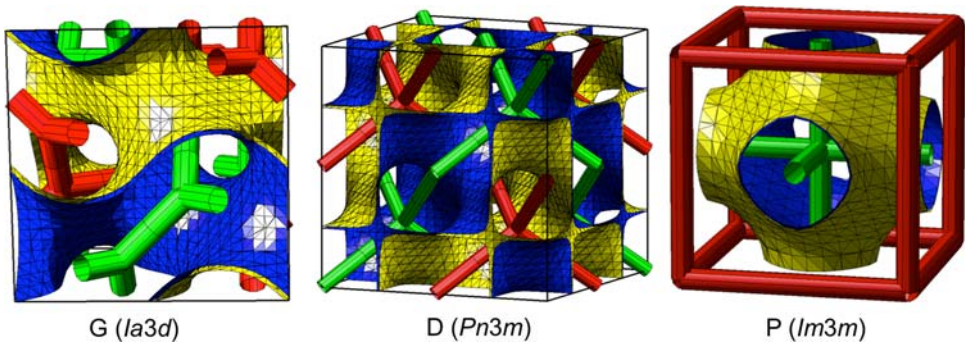

Fig. 2 Approximate mathematical models of the common inverse cubic-phase unit cells: *Ia3d* [gyroid (*G*)], *Pn3m* [diamond (*D*)] and *Im3m* [primitive (*P*)]. The IPMS is shown in *yellow* and *blue*, and the two non-intersecting water channels are represented as *red* and *green cylinders* (adapted from Squires et al. 2005)

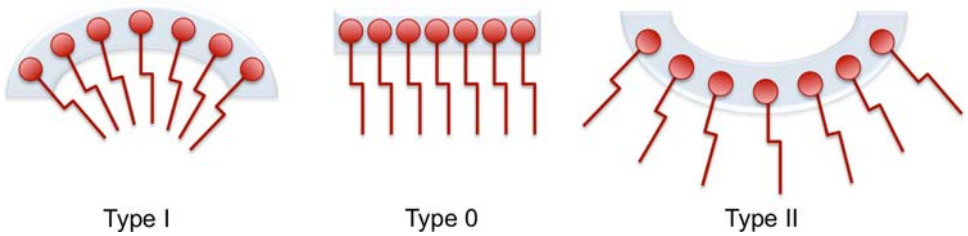

Fig. 3 The classification of liquid crystalline phases of amphiphile–water systems based on interfacial curvature. Negative interfacial curvature increases from *left* to *right*. The surfactant head groups are shown as *red circles*

to mathematical modelling (Chung and Caffrey 1995; Hyde et al. 1984; Squires et al. 2005; Templer 1998).

Interestingly, the interconversion of cubic phases readily occurs. Squires et al. (2002) report that reversed cubic phases comprised of lauric acid–diauroyl-phosphatidylcholine–water undergo phase transitions from the Q_{II}^{D} to the Q_{II}^{G}, that is, cubic-phase transition from diamond (D)-type minimal surface to one with a gyroid (G) minimal surface (Fig. 2). They determined that the kinetics of interconversion were rapid in comparison to the transition from Q_{II}^{D} to a reversed hexagonal phase (H_{II}). A similar structural progression occurs in the monoolein–water system where two or more cubic structures, the Q_{II}^{D} and Q_{II}^{G} phases, are observed in equilibrium in microenvironments of varied water content within a bulk cubic phase (Chung and Caffrey 1994, 1995).

Bicontinuous cubic phases may be considered to be normal or inverse phases depending on the relative spontaneous curvature of the hydrophobic phase at the aqueous interface. As illustrated in Fig. 3, the assignment of phase is type I (normal phase) or type II (reversed phase). Thus the conventional notation for normal and reversed cubic phases is Q_I and Q_{II} respectively. The propensity toward type II phase behaviour is strongly influenced by the geometry of individual surfactant molecules. The molecular shape of an amphiphile is governed by the cross-sectional area of the hydrophilic head group as well as the volume occupied by the hydrophobic tail region (Israelachvili and Mitchell 1975; Mitchell and Ninham 1981). This relationship is described by the shape factor, often referred to as the critical packing parameter (CPP), and is given by the following equation:

$$\mathrm{CPP} = v/a_0 l_c$$

In this relationship, v is the hydrophobic chain volume and a_0 and l_c are the optimal head-group area and critical chain length of the hydrophobic chain respectively. The critical chain length is defined, somewhat imprecisely, as the maximum chain length in which the hydrocarbon chains remain in a fluid-like state (Israelachvili 1991). The optimal head-group area is defined as maximum cross-sectional area of a fully hydrated head group at the hydrocarbon-water interface. Typically, normal-type phases occur when $v/a_0 l_c < 1/2$, lamellar phases when $1/2 < v/a_0 l_c < 1$, and inverse-type phases occur when $v/a/a_0 l_c > 1$. Notably, inverse behaviour is observed for related phases such as the hexagonal phase (Caffrey and Cheng 1995), and these have been shown to have significant biological relevance (Shalaev and Steponkus 1999; Kent et al. 2009; Wolfe and Bryant 1999).

The number of amphiphiles known to be capable of forming Q_{II} phases is steadily growing. A recent review by Kaasgaard and Drummond highlighted the association between amphiphile structure and resulting phase behaviour (Kaasgaard and Drummond 2006), and the reader is referred to this paper for a detailed review of the topic. However, the amphiphile most commonly used to study reversed cubic-phase behaviour is the monoacylglycerol, monoolein or glyceryl monooleate (GMO) (Fig. 4) (Ericsson et al. 1983). The *cis*-double bond in the acyl chain increases the effective volume (v) occupied by the tail region, thereby favouring the formation and stability of reversed phases. In terms of phase behaviour, an increase in water content at a fixed temperature initially results in a

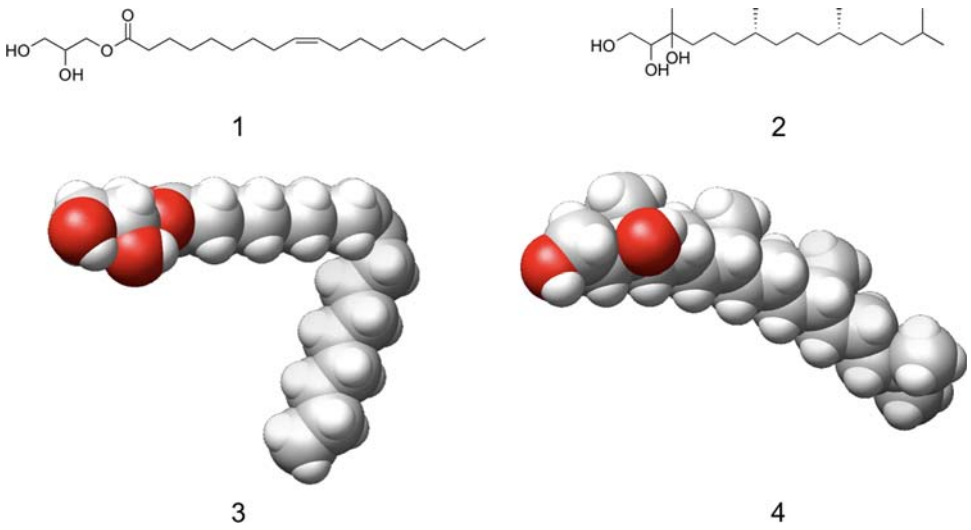

Fig. 4 The chemical structure of *1* glycerol monooleate (GMO) and *2* phytantriol (3,7,11,15-tetramethyl-1,2,3-hexadecanetriol). The three-dimensional representations of the minimised structures for *3* GMO and *4* phytantriol are shown

phase transition from the lamellar phase (L_α) to the Q_{II}^G phase and a further increase in water content favours an inter-cubic phase transition from the Q_{II}^G phase to Q_{II}^D phase (Qiu and Caffrey 2000).

Another amphiphile that readily forms cubic phases and is, therefore, generating considerable interest, is the aliphatic alcohol 3,7,11,15,-tetramethyl-1,2,3,-hexadecanetriol, or 'phytantriol' (Fig. 4). This amphiphile differs greatly in structure from GMO. Phytantriol is comprised of an isoprenoid hydrophobic tail rather than an oleoyl group. The large volume of the tail is derived from the curvature of the isoprenoid group (Fig. 4), and the Q_{II} phase appears at ambient temperatures (20–40°C) when the water content exceeds 15% (Barauskas and Landh 2003). Furthermore, the attachment of the triol head group to the hydrophobic tail is through a C–C bond rather than an ester linkage, which confers greater chemical stability relative to GMO.

Ternary amphiphile–water Q_{II} phase systems

The modification of cubic-phase structure may be desirable where a 'swelled' structure or a structure of greater porosity is required for the entrapment of large substrates (for example, proteins). A Q_{II} phase with contracted aqueous channels or reduced porosity may be preferential for the controlled transport of substrates in applications such as drug delivery. The addition of a lipid to the cubic phase provides a straightforward method to control the phase behaviour and, therefore, structure of these systems. In the GMO–water or the phytantriol–water binary systems, the addition of a lipid has a pronounced effect on the Q_{II} phase behaviour and structure for each binary mixture. The added lipid may influence a change in the CPP depending on the structure of the added lipid. For example, Popescu et al. (2007) investigated bulk liquid crystalline phases of the GMO–glycerol monooleyl ether (GME)–water system. Using SAXS, polarised light microscopy and nuclear magnetic resonance (NMR), they determined that an increase in the weight fraction of GME at constant water content and temperature effects a reduction in the stability of the Q_{II} phase relative to the binary GMO–water system. From the resulting phase diagrams, a reversed cubic to reversed hexagonal ($Q_{II} \rightarrow H_{II}$) phase transition was readily observed at a lower temperature relative to the binary GMO–water system (Fig. 5). The destabilisation of the Q_{II} region is rationalised by considering the higher comparative hydrophobicity of GME, relative to GMO. The absence of an ester group in GME forces the head-group deeper into the GMO lipid bilayer when the two lipids are admixed. This acts to increase the chain volume (v) and leads to an increase in the CPP, resulting in a greater negative interfacial curvature than the binary system. A similar destabilisation of the GMO Q_{II} phase was observed in GMO–diglycerol–MO–water systems (Pitzalis et al. 2000) and when phytantriol was added to GMO (Wadsten-Hindrichsen et al. 2007). In the latter case, it was demonstrated that the addition of phytantriol influenced a contraction of the *Pn*3*m* cubic phase of GMO. The lattice parameter (a_0, unit cell size of the *Pn*3*m* crystallographic space group) is smaller for phytantriol (6.8 nm) compared to GMO (9.3 nm), and SAXS measurements showed that the lattice parameter for the mixed systems fell between these two values. The contraction of the phase is attributed to an increase in the interfacial curvature of the GMO phase, which is derived from an increase in the CPP.

The addition of certain lipids to GMO can swell the cubic phase (Engblom et al. 2000; Gustafsson et al. 1998, 1999; Lindell et al. 1997). These effects have been observed with the addition of ionic lipids or lipids with a large effective head-group area (a_0). For example, the addition of the anionic lipid, distearoylphosphatidylglycerol to

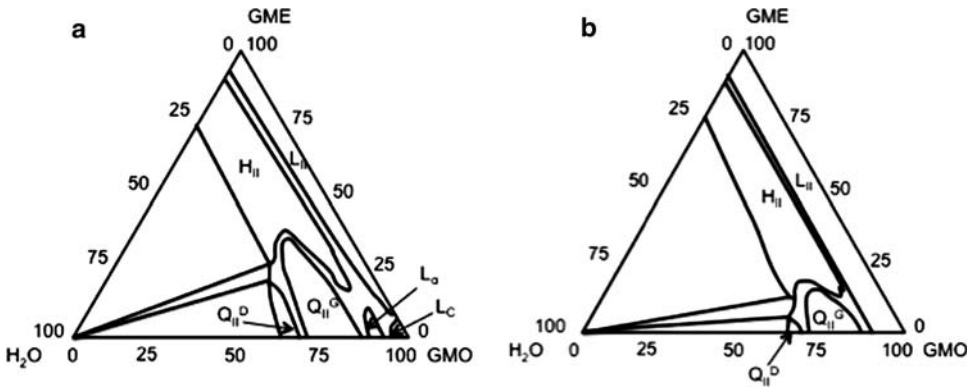

Fig. 5 Phase diagrams of the GMO–GME–water system at (**a**) 25°C and (**b**) 40°C (both adapted from Popescu et al. 2007). Phases are symbolised as L_C (liquid crystalline), L_α (lamellar), L_{II} (reversed micellar), H_{II} (reversed hexagonal), Q_{II} (reversed cubic)

GMO–water, induces a *Pn*3*m* → *Im*3*m* phase transition (Engblom et al. 2000; Lindell et al. 1997). The interfacial curvature of the polar region for different cubic phases (Q) decreases in moving from *Ia*3*d* → *Pn*3*m* → *Im*3*m* (Engblom et al. 2000; Lindell et al. 1997), which suggests that the added phospholipid effects a reduction in the CPP. The cationic head group increases the effective head-group area (a_0) of the amphiphiles through electrostatic interaction and perhaps due to an increase in hydration of the charged groups. A similar *Pn*3*m* → *Im*3*m* inter-cubic phase transition has been observed in the phytantriol–water system with the addition of a glycolipid (Polyzos et al. 2007), and a concomitant stabilisation of the Q_{II} phase region was also observed. In these studies, the Q_{II} → H_{II} phase transition temperature was increased by at least 20°C with the addition of the glycolipid G_{M1}. Pure phytantriol adopts a cubic phase with a *Pn*3*m* space group in the presence of excess water (Barauskas and Landh 2003). However, in excess water and at room temperature, the addition of 10–20% G_{M1} to pure phytantriol induces a phase transition to the *Im*3*m* space group. In this case, the lattice parameters for the *Im*3*m* cubic phases were 10.71 and 13.73 nm for 10% G_{M1} phytantriol and 20% G_{M1}-phytantriol respectively compared to ~6.5 nm in the absence of G_{M1} (Barauskas and Landh 2003).

Cubic-phase nanoparticles (CPnPs)

As we discussed earlier, Q_{II} phases may be dispersed in aqueous solutions as sub-micron-sized particles (Yang et al. 2004). It is possible to create stabilised nanoparticle dispersions of cubic phases (CPnPs or cubosomes) with retention of bulk properties and microstructure of the parent bulk Q_{II} phase (Fig. 6). As such, CPnPs hold promise for applications in medically relevant areas, including biosensing, bioscaffolds and protein crystallization, and have drawn considerable interest in pharmaceutical applications (Gin et al. 2008) particularly in drug delivery (Boyd 2003; Drummond and Fong 1999; Esposito et al. 2005; Johnsson et al. 2006; Yaghmur and Glatter 2009). The separate hydrophilic and hydrophobic regions within the CPnP structure permit incorporation of both hydrophilic and hydrophobic bioactive molecules (Sagar et al. 2007). The tortuosity of the aqueous channel architecture within CPnP allows for the possibility of diffusion controlled or sustained release of a drug within the therapeutic concentration range (Barauskas et al. 2005; Clogston and Caffrey 2005; Stoltze 1995).

The preparation of CPnPs is usually achieved by high-pressure homogenisation or ultrasonication of the viscous bulk phase (Yang et al. 2004). This processing yields sub-micron-sized particles following the high-energy fragmentation of the viscous bulk phase. The addition of a stabilising agent is often employed to avoid agglomeration of the dispersed particles. Although colloidal stability is achieved using a number of different stabilising agents, the most common stabilising agent is the surfactant poloxamer 407, commonly known as Pluronic (Alexandridis 1997). Pluronic is a block copolymer comprised of a central polypropylene oxide (PPO) block and adjacent polyethylene glycol (PEG) blocks with the general structure $(PEG)_x(PPO)_n(PEG)_y$, i.e., $HO(C_2H_4O)_x(C_3H_6O)_n(C_2H_4O)_yH$. The polymer is available commercially in a range of sizes and is generally non-toxic (Singh-Joy and McLain 2008).

Pluronic confers steric stability by forming a brush-type layer at the CPnP surface. It is generally accepted that stabilisation is mediated through the insertion or adsorption of the PPO region onto the surface bilayer of the CPnP. The PEG regions presumably extend into the water phase creating a steric barrier that prevents aggregation (Sagalowicz et al. 2006). The concentration of Pluronic used in the preparation of CPnPs ranges from 0 to 20% (w/w) if a high energy (e.g. high-pressure homogenization, ultrasonication) or a low energy (e.g. dilutions with organic solvent, simple mixtures) method of preparation is employed (Garg et al. 2007).

Alternative techniques of CPnP manufacture have been developed, with an emphasis on reduced-energy processes.

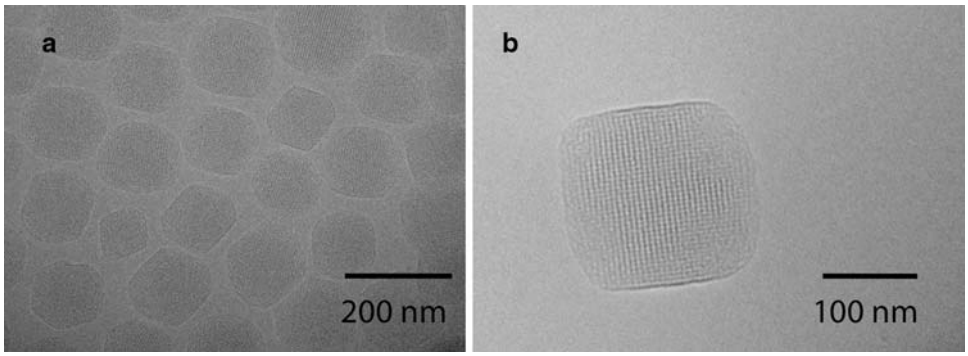

Fig. 6 A cryogenic transmission electron microscopy (Cryo-TEM) image of **a** phytantriol–Pluronic F-127–water CPnP prepared by high-pressure homogenization. **b** A magnified image of a representative CPnP. The internal structure is visible in this image

Almgren and Rangelov (2006) have reported a method whereby L_{II} or inverse micellar droplets are added to water at 80°C. Subsequent controlled cooling then promoted the nano-droplets to adopt a cubic structure and form CPnPs. Spicer et al. (2001) have used a spontaneous emulsification method based on precursor formulations where certain additives, such as PEG–lipid conjugates or ethanol, produce CPnPs without use of extra energy input beyond that of blending the two liquids. Other reported methods include use of dialysis, stirring and spray drying (Abraham et al. 2004, 2005; Spicer et al. 2002). Similar to the originating bulk phase, CPnPs undergo temperature-induced phase transitions (de Campo et al. 2004). A $Q_{II} \rightarrow H_{II}$ phase transition was observed at 45°C for Phytantriol–Pluronic F-127 CPnPs as shown by SAXS and cryogenic transmission electron microscopy (cryo-TEM). Interestingly, cubic symmetry was regained subsequent to cooling.

Similarly to bulk cubic phases, the addition of a third miscible component can be exploited to regulate the structure of CPnPs. One method of achieving structural control is through the polymeric stabiliser itself, where the added stabiliser may modulate phase behaviour of the binary system. Nakano et al. (2001) showed that increasing concentrations of Pluronic F-127 from 3 to 10% (w/w) in a GMO–water system, afforded an inter-cubic phase transition from Q_{II}^{D} to Q_{II}^{P}. Studies by Boyd and co-workers (Dong et al. 2006) investigated the effect of Pluronic F-127 and vitamin E acetate (VitEA) on CPnP comprised of phytantriol–water or GMO–water. The addition of larger weight fractions of Pluronic F-127 to the GMO–water CPnP system promoted a *Pn*3*m* → *Im*3*m* inter-cubic phase transition. Interestingly the addition of VitEA (5% w/w) was shown to reduce the $Q_{II} \rightarrow H_{II}$ phase transition temperature for the bulk phases and CPnP. Furthermore, these studies also showed a correlation between the mean particle size of CPnPs with additives. In the phytantriol system, the addition of 5% (w/w) Pluronic F-127 resulted in a mean particle size of approximately 270 nm, however, when the loading of polymer was increased to 30% (w/w), the average particle size was reduced to approximately 220 nm. Similarly, the addition of VitEA to the phytantriol CPnP reduced the mean particle diameter with higher loadings of the additive. Furthermore, the addition of a second amphiphile can similarly be employed to control the internal structure and particle size of CPnP. For example, Popescu et al. (2007) reported that CPnP comprised of GMO–GME and Pluronic F-127 yielded Q_{II}^{D} or Q_{II}^{G} phases, and the occurrence of these phases could be controlled by varying the ratio of GMO:GME.

Conclusion

In this paper, the structure of reversed bicontinuous cubic (Q_{II}) phases in binary and ternary amphiphile systems is described. We have highlighted research in which the modulation of cubic-phase structure occurs in ternary-phase systems for both bulk cubic phases and the corresponding nanoscale dispersions, CPnPs. Precise control of phase behaviour and, therefore, cubic-phase structure can readily be achieved through the simple addition of an amphiphile to a binary cubic-phase system. It is clear that the CPP and hence interfacial curvature can be modulated through the judicious choice of a secondary amphiphile having the requisite molecular geometry and hydrophobic–hydrophilic balance. Such control can also be extended to CPnPs where phase behaviour and particle size are modulated in ternary systems with potential biotechnological applications. The recent emergence of cubic phases in areas of technological and medical importance requires precise control of structure and ternary amphiphile systems provide the tools to achieve the requisite structural optimisation.

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