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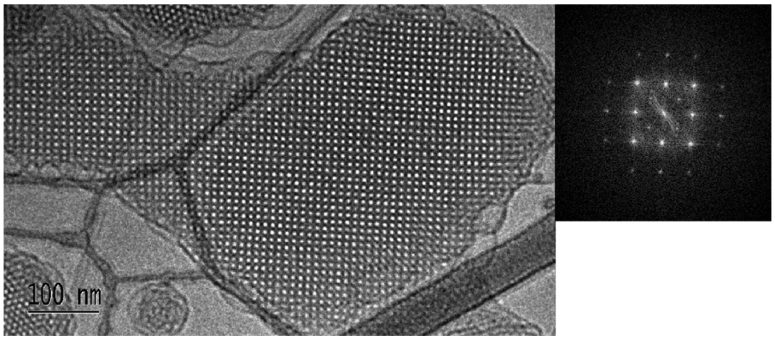

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# Cubic Phases and Cubic Phase Dispersions in a Phospholipid-Based System

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Bicontinuous cubic liquid crystalline (LC) phases formed in mixtures of lipids and water represent thermodynamically stable 3D bilayer structures forming an infinite periodical minimal surface (IPMS).[1,2] Unsaturated monoglycerides (uMGs), most noteworthy, glycerol monooleate (GMO), are particularly interesting as they exhibits two cubic phases, one of which coexists with a water phase in the dilute region of the phase diagram.[1−3] The exceptional physical chemical behavior of uMGs has stimulated their use as biomembrane models in a broad range of structural and functional investigations of proteins,[4] peptides,[5] and other biomolecules[6] entrapped in the uMG cubic phase. The uMG cubic phases have also been used in practical/technical applications such as bioelectrode[7] and biosensor construction[8] and protein[9,10] and metal nanoparticle[11] crystallization. Because of the 3D nanostructure with hydrophobic and hydrophilic domains, cubic liquid crystalline phases have also found application in pharmaceutical drug delivery applications.[12,13]

In many applications, and in particular for parenteral drug delivery, it would be of substantial value to construct cubic phases composed of lipids other than uMGs. This is due to the fact that uMGs exhibit toxic properties when injected in vivo at high concentrations. Here we present a cubic LC phase forming system based on the phospholipid dioleoylphosphatidylethanolamine (DOPE) which is fortified with small amounts of PEGylated (poly(ethylene) glycol) glycerol monooleate (PEG(660)-GMO). The cubic phase formed by the DOPE/PEG(660)-GMO/water system coexists with water in the dilute part of the phase diagram and can be fragmented into colloidal size particles (Cubosome[14]).

DOPE is a phospholipid forming a reversed hexagonal LC phase in water above ca. 10 °C, and its aqueous phase behavior is well characterized.[15] To promote the formation of cubic phases in DOPE-based systems, it is necessary to add a surfactant/lipid component that drives the phase behavior toward increased (less negative) monolayer curvature. In addition, this component must mix well with DOPE, facilitate dispersion formation (fractionation agent), and also act as a dispersion stabilizing agent. In this respect, PEGylated surfactants and/or lipids seem to possess the required properties.[16,17]

To determine the cubic phase regions of interest in the ternary DOPE/PEG(660)-GMO/water system, X-ray diffraction (XRD) was performed on equilibrated samples. The relevant regions were found in the DOPE/PEG(660)-GMO ratio range of 95/5−85/15 (wt/wt). Figure 1 shows two identified cubic phases at a constant DOPE/PEG(660)-GMO weight ratio of 89:11, at 45 and 50 wt % $H_2O$, respectively.

The Bragg peaks of the cubic lattice for the stiff isotropic sample with 45 wt % $H_2O$ were spaced in the ratios $\sqrt{3}:\sqrt{4}:\sqrt{7}:\sqrt{8}:\sqrt{10}:\sqrt{11}$, consistent with the formation of a $Q^{230}$ cubic phase with the space group $Ia3d$ (Figure 1). In terms of IPMS description of bicontinuous cubic phases[1,2] the $Ia3d$ phase corresponds to the cubic phase of gyroid type (G-surface or $C_G$). At 50 wt % $H_2O$, the Bragg peaks of the cubic lattice were spaced in the ratios $\sqrt{2}:\sqrt{3}:\sqrt{4}:\sqrt{6}:\sqrt{8}:\sqrt{9}:\sqrt{10}$, corresponding to the $Q^{224}$ phase with the space group $Pn3m$ (D-surface or $C_D$) (Figure 1).

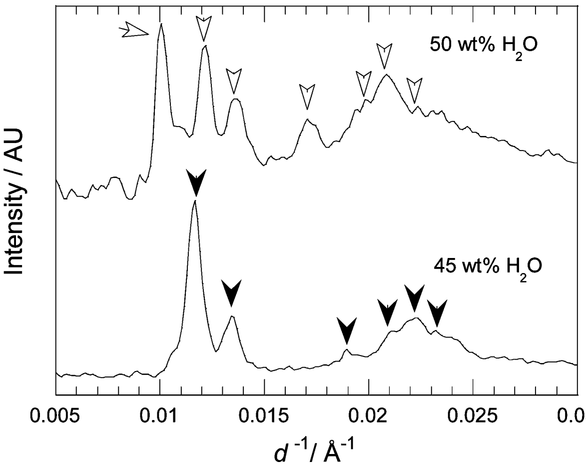

***Figure 1.*** X-ray diffractograms of DOPE/PEG(660)-GMO (89:11 wt/wt) mixtures. The black and white arrows denote the Bragg peaks associated with the $Q^{230}$ ($Ia3d$) cubic phase (45 wt % $H_2O$) and the $Q^{224}$ ($Pn3m$) cubic phase (50 wt % $H_2O$), respectively. $T = 25$ °C.

The lattice parameter ($a$) of the cubic phases was determined using the relation $d^{-1} = (a^{-1})(h^2 + k^2 + l^2)^{1/2}$ from linear fits of the plots of $d^{-1}$ versus $(h^2 + k^2 + l^2)^{1/2}$, where $d$ is the measured peak position and $h$, $k$, and $l$ are the Miller indices (Supporting Information). The lattice parameters of the $Ia3d$ and the $Pn3m$ cubic phases were 211.1 and 142.9 Å, respectively. The ratio between these parameters, 1.48, is close to the calculated ratio of 1.57, which is obtained theoretically by the Bonnet transformation of the $C_G$ to the $C_D$ phase with constant average and Gaussian curvature (isometric relationship).[18] In this respect it should be noted that the theoretically predicted value is derived at cubic phase coexistence, whereas the value obtained here is underestimated. This is because the $C_G$ phase is likely to accommodate more water before the phase transition to $C_D$ takes place, meaning that the maximum lattice parameter of the $C_G$ phase is higher than that measured above. Similarly, the $C_D$ phase likely exists at slightly lower water contents with a slightly lower lattice parameter.

At water contents greater than 50 wt % the $Pn3m$ phase coexisted with excess water, indicating the reversed nature of the cubic phase. The lattice parameter at 57 wt % $H_2O$ was determined to be 141.3 Å, i.e., within experimental error identical to the 50 wt % $H_2O$ sample.

The dispersion of the $Q^{224}$ ($Pn3m$) cubic phase was prepared by adding excess water to a fully equilibrated sample originally containing 50 wt % $H_2O$ and thereafter magnetically stirring the sample for 5 days. The resulting milky dispersion (2 wt % lipid) was examined using cryo-transmission electron microscopy (cryo-TEM). Figure 2 clearly shows that the particles retain their cubic

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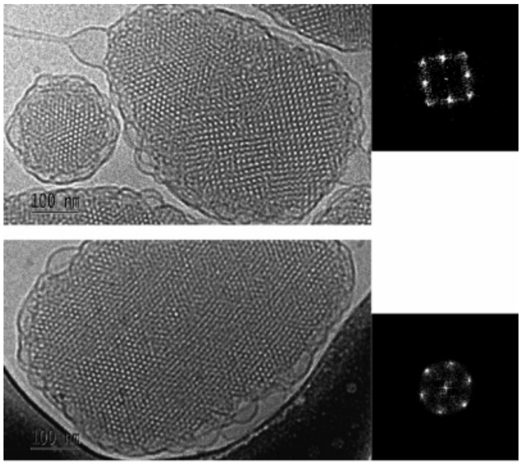

***Figure 2.*** Cryo-TEM images of dispersed cubic phase particles obtained at DOPE/PEG(660)-GMO = 89:11 wt/wt and at 2 wt % lipid. Fast Fourier transforms are shown on the right.

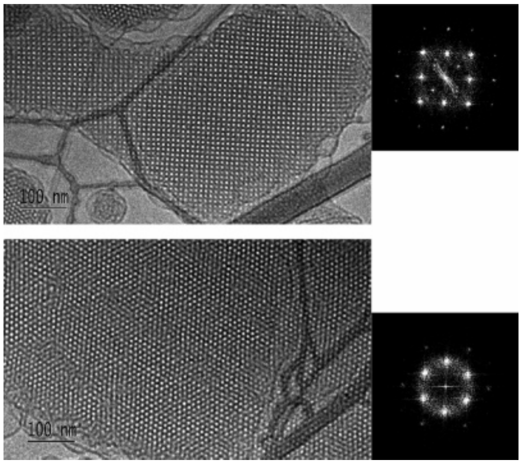

***Figure 3.*** Cryo-TEM images of dispersed cubic phase particles obtained at DOPE/PEG(660)-GMO = 85:15 wt/wt and at 2 wt % lipid. Fast Fourier transforms are shown on the right.

phase structure in dispersion (the [001] and [111] viewing directions of the cubic lattice can be observed).

From fast Fourier transforms (FFTs) of Figure 2 the {110} and {200} reflections were detected. FFT analysis of about 30 particles yielded a mean lattice parameter of 151 Å, in reasonable agreement with the XRD results given above for the bulk $Q^{224}$ cubic phase in slight excess of $H_2O$. Note that the accuracy of the lattice parameter determined from cryo-TEM is approximately ±10%.

By adding more of PEG(660)-GMO to the mixture, there is eventually a transition to a lamellar phase (not shown). However, at DOPE/PEG(660)-GMO = 85:15 wt/wt we still observed cubic phase particles with well-ordered inner structure in dispersions of the bulk phase as shown in Figure 3. As with the 89/11 (wt/wt) sample shown in Figure 2, cubic phase domains are viewed along the [001] and [111] directions.

The peaks of the cubic lattice obtained from the FFTs of Figure 3 were spaced in the ratios $\sqrt{1}:\sqrt{2}:\sqrt{4}:\sqrt{6}:\sqrt{8}:\sqrt{9}$ (143.7, 99.6, 71.9, 57.0, 50.8, 46.0 Å, respectively), indicating a simple cubic lattice of different type compared to the 89/11 DOPE/GMO-PEG-(660) weight ratio. In the case of the 85:15 sample, we also observed some liposomes (not shown). The latter observation probably reflects the fact that at the 85:15 weight ratio the dispersed sample is located in a three-phase region where the cubic phase coexists with a lamellar phase (giving liposomes in the dispersion) and a dilute water phase. A more detailed phase behavior study is ongoing to clarify this and to identify the exact nature of the cubic phase (space group) giving rise to the intriguing structures observed in Figure 3. Note, however, that the observed reflections from two viewing directions (Figure 3) prove the cubic nature of the particles.

The findings in the present study open up new exciting vistas for the understanding of the phospholipid phase behavior and the application of lipid-based cubic phase materials alluded to earlier. Because DOPE is a naturally occurring double-tailed phospholipid with negligible monomer activity, it is more suitable as a base for parenteral pharmaceutical applications compared to uMG-based cubic phase materials. In addition, a phospholipid-based cubic phase could potentially improve the performance of membrane protein crystallization.[19]

Finally, we note that the transition from the $C_G$ to the $C_D$ cubic phase appears to conform to the IPMS description of bicontinuous cubic phases.[1,2,18] Thus, the results presented provide further evidence in favor of the minimal surface model of cubic phase membranes.

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**Supporting Information Available:** Complete experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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