A 3-D hexagonal inverse micellar lyotropic phase

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Lipids are ubiquitous in nature; together with proteins they constitute the plasma membranes of cells, and take part in a plethora of biological processes, ranging from cell signalling[1] and actin assembly[2] through to endo- and exo-cytosis[3]. More recently, lipids and other amphiphiles have been used as vehicles for drug delivery[4]. Understanding lipid polymorphism is the key to understanding biochemical control at membranes and to the development of intracellular delivery systems.

When mixed with a polar solvent, amphiphiles self-assemble into fluid interfacial structures where polar headgroups at the interface shield hydrocarbon chains from contact with the solvent. Most biological amphiphiles form 'inverse' liquid-crystalline phases where the interface curves towards the polar region. Conversely, most simple surfactants form 'normal' phases, where the interface bends away from the polar solvent and towards the chain region. Those lipids with a tendency for curvature play important roles in regulating the dynamics and function of membrane proteins, and are essential for enabling lipid based drug/gene delivery systems to fuse with the cell membrane. Those lipids with the greatest tendency for inverse interfacial curvature form spherical structural elements, where a core of water is surrounded by a monolayer of lipid. In some cases such inverse micelles are found to self-assemble into a close packing of cubic symmetry. Although only one such packing, of space group *Fd3m*, has so far been identified[5], we have sought to find different inverse micellar packings, of primitive, body-centred or face-centred cubic symmetry, mirroring the more diverse range of normal micellar cubic phases found in surfactant systems[6,7]. Here we report only the second liquid crystalline structure based on a close packing of inverse micelles: a 3-D hexagonal inverse micellar phase, of space group $P6_3/mmc$.

Seddon *et al.*[8] previously reported the formation of an *Fd3m* phase in excess water for a mixture of two typical lipids, dioleoylphosphatidylcholine (DOPC) and dioleoylglycerol (DOG). They also observed a different phase at lower water concentrations, but were unable to give an unambiguous phase determination, although electrical conductivity measurements and freeze-fracture electron microscopy suggested that this phase also consisted of packed inverse micelles. The *Fd3m* cubic phase was also found in a mixture of egg phosphatidylcholine, egg phosphatidylethanolamine, 1,2-diacylglycerol (derived from egg phosphatidylcholine) and cholesterol, at 1,2-diacylglycerol concentrations in excess of 40 mol%[9]. For the present study, we prepared ternary lipid mixtures comprising DOPC, DOG and cholesterol, of molar ratios 1:2:1 and 1:2:2, in excess water. Small-angle X-ray scattering (SAXS) experiments were carried out at the European Synchrotron Radiation Facility, Grenoble, France. Detailed information regarding the experimental procedure can be found in the Supporting Information.

The phase behaviour of both ternary mixtures was found to be very similar, and over the temperature range 16 – 52 °C a series of diffraction rings were obtained (Figure 1), which did not conform to any currently recognised inverse liquid-crystalline phase. This phase was also found to exist over a large range of pressures (between 1 – 3000 bar), and has been seen without excess cholesterol crystals. Although the positions of the Bragg peaks varied as a function of temperature and pressure within the unknown phase region, their ratios remained invariant showing that the diffraction peaks were all produced by a single phase.

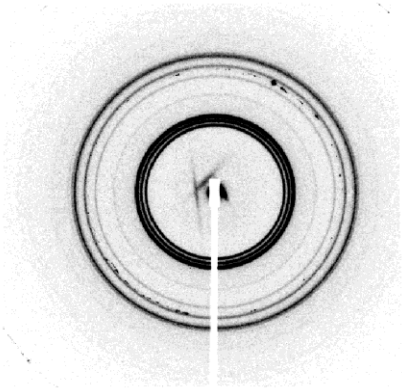


Figure 1. The SAXS diffraction pattern of the 3-D hexagonal close-packed inverse micellar phase formed with DOPC/DOG/cholesterol 1:2:1 in excess water at 36.6 °C and 600 bar pressure, where the crossed lines visible close to the beamstop are Kossel lines from the diamond windows of the sample cell, and the intense spots near the sixth ring are due to excess cholesterol crystals.

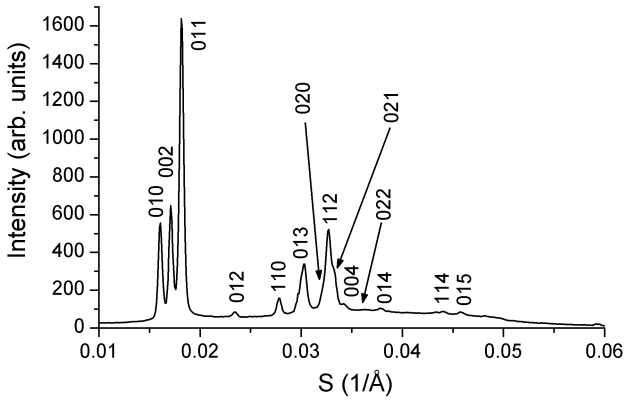


Figure 2. The densitometer scan showing the intensities of each of the Bragg peaks, together with their assigned Miller indices.

We found that the observed reflections (Figure 2) were not compatible with any phase of cubic symmetry, but noted that there was a systematic absence of *hhl* and *00l* reflections with odd l indices (in both cases). These reflection conditions correspond to the extinction symbol *P--c*, for which there are five possible space groups. Indexing of the reflections was achieved assuming a 3-D hexagonal unit cell (Table 1), where the d-spacing (d_{hkl}) is related to the two lattice parameters, *a* and *c*, as well as their ratio ($R = c/a$) by the equation:

$$\frac{1}{d_{hkl}^2} = \frac{1}{a^2}\left(\frac{4}{3}\,h^2 + k^2 + hk\; + \frac{1}{R^2}l^2\right)$$

The lattice parameters were found to be a = 71.5 Å and c = 116.5 Å, with a c/a ratio of 1.629, which is extremely close to the ratio of 1.633 found for a 3-D hexagonal close-packing of spheres (spacegroup $P6_3/mmc$).

Table 1. Indexing of the powder diffraction data of the 3-D hexagonal close-packed inverse micellar phase, where $s = a/d_{hkl}$.

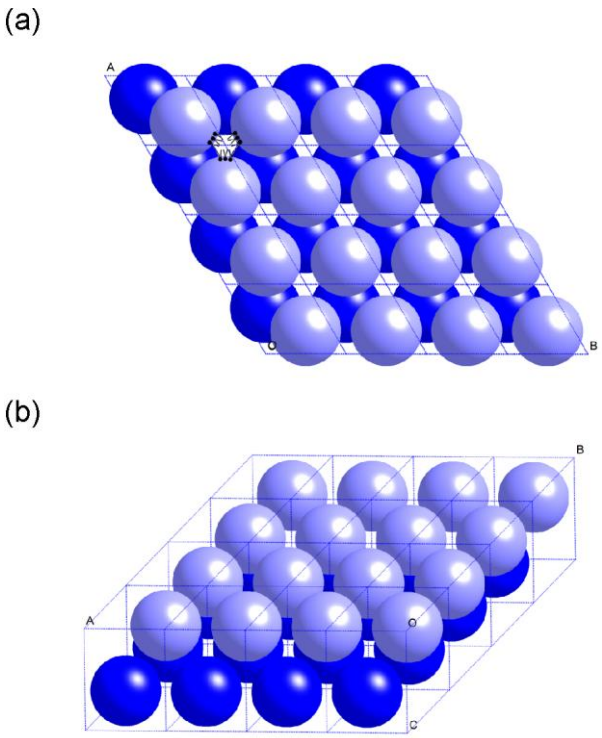
Experimental d-spacing (Å)	s^2	Index (hkl)	Calculated d-spacing (Å)
62.0	4/3	010	61.9
58.2	$4/R^2$	002	58.3
54.7	$4/3 + 1/R^2$	011	54.7
42.5	$4/3 + 4/R^2$	012	42.4
35.8	4	110	35.8
32.9	$4/3 + 9/R^2$	013	32.9
30.8	16/3	020	31.0
30.5	$4 + 4/R^2$	112	30.5
30.0	$16/3 + 1/R^2$	021	29.9
29.2	$16/R^2$	004	29.1
27.4	$16/3 + 4/R^2$	022	27.3
26.3	$4/3 + 16/R^2$	014	26.4
22.6	$4 + 16/R^2$	114	22.6
21.8	$4/3 + 25/R^2$	015	21.8

As with the structure determination of the 'normal' micellar $P6_3/mmc$ phase[7], from the assumptions both of a hexagonal close-packing (hcp) and of the highest symmetry[10], we have deduced that the space group for the new inverse micellar phase is $P6_3/mmc$. The structure consists of an hcp packing of identical, spherical inverse micelles (Figures 3(a) and (b)).

The intensities for each of the observed reflections were also found to be similar to those obtained by Zeng *et al*[7] for the 3-D hexagonal $P6_3/mmc$ phase of nonionic surfactant micelles in water. For this system, identical spherical micelles were found to pack into an hcp arrangement, with a continuous water region filling the gaps between micelles. From Babinet's principle, the inverse situation i.e. discrete spherical polar cores surrounded by a continuous hydrophobic region, should produce a similar diffraction pattern. The similarity in the two intensity profiles corroborates our conclusion that the $P6_3/mmc$ phase reported here consists of an hcp packing of spherical inverse micelles.

It is unclear why the cubic *Fd3m* phase is much more prevalent for inverse lyotropic systems than the 3-D hexagonal $P6_3/mmc$ phase reported here. The packing fraction, defined as the percentage of the unit cell volume occupied by close-packed spherical inverse micelles, is 0.74 for the $P6_3/mmc$ phase but only 0.71 for the *Fd3m* phase, which would suggest a higher degree of hydrocarbon chain packing frustration for the latter phase.

This is the first new inverse lyotropic liquid-crystalline phase to be reported for two decades, and is the only known phase whose structure consists of a close packing of identical inverse micelles.



Figures 3(a) and (b). Plan and perspective views of the schematic structure of the 3-D hexagonal inverse micellar phase. The spheres represent the polar regions (water cores plus lipid headgroups), and the remaining fluid volume is filled by the hydrophobic regions of the lipid molecules. The different colour shading of the two identical layers of spheres is purely for clarity.

Such a phase, stable in excess aqueous solution, should have numerous applications, such as the storage and controlled release of drugs, as nanoreactors and for templating.

Acknowledgements. We thank the ESRF for provision of the synchrotron facilities, and Professor R. Winter, Dortmund University, for use of the X-ray pressure cell. This work was supported by ESPRC Platform grant GR/S77721 and by an EPSRC Doctoral Training Centre Studentship from the Chemical Biology Centre (Imperial College London) awarded to AIIT.

Supporting Information Available: Experimental procedures. This material is free of charge via the Internet at http://pubs.acs.org.

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Abstract

Lipids that are found in cell membranes form a variety of self-assembled phases in the presence of water. Many of these structures are liquid-crystalline with structural motifs mirrored in cells and organelles and can be exploited in the delivery of drugs and genes. We report the discovery of a lyotropic liquid crystalline phase based on a 3-D hexagonal close-packed arrangement of inverse micelles, of space group $P6_3/mmc$. This is the first new inverse lyotropic liquid-crystalline phase to be reported for two decades, and is the only known lyotropic phase whose structure consists of a close packing of identical inverse micelles.

Table of Contents Graphic