

SMALL-ANGLE X-RAY DIFFRACTION STUDY OF MONOOLEIN UNDER PRESSURE: STABILITY AND ENERGETICS OF Pn3m AND Ia3d BICONTINUOUS CUBIC PHASES

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A bicontinuous cubic liquid crystalline phase is one of the most fascinating structures exhibited by water/amphiphile systems. Not only do the structure and dynamics of these cubic phases make them attractive as model soft matter systems, but they also have many practical applications. Small-angle X-ray scattering can reveal the symmetry as well as the topology of these structures.

Lipids are natural examples of amphiphilic molecules having a polar head and a non-polar tail. Because of the hydrophobic character of the large nonpolar tail, and other factors such as packing and steric constraints, van der Waals and electrostatic interactions, these molecules exhibit a variety of self-assembled states when mixed with water. Which state predominates depends on the concentration and thermodynamic parameters such as temperature and pressure. In neutral lipids, the polar head group bears no net charge under biological pH. Monooleoylglycol (monoolein) is one such lipid, which is well known for its mesomorphism in the hydrated state. It is a workhorse lipid involved in fat digestion. On the mesoscopic scale, monoolein molecules in water organise into a wide variety of structures of which bicontinuous cubic phases are well known. These cubic phases have found application in the crystallisation of a variety of bacterial membrane proteins. Understanding the relationship between microstructure and phase stability is important in the study of their biological function. The stability of bicontinuous cubic phases and the ability of a lipid membrane to bend are governed by the bilayer elastic curvature energy.

At normal pressures, the monoolein and water mixture shows a variety of lyotropic phases. These include a lamellar phase (L_α) in which molecules

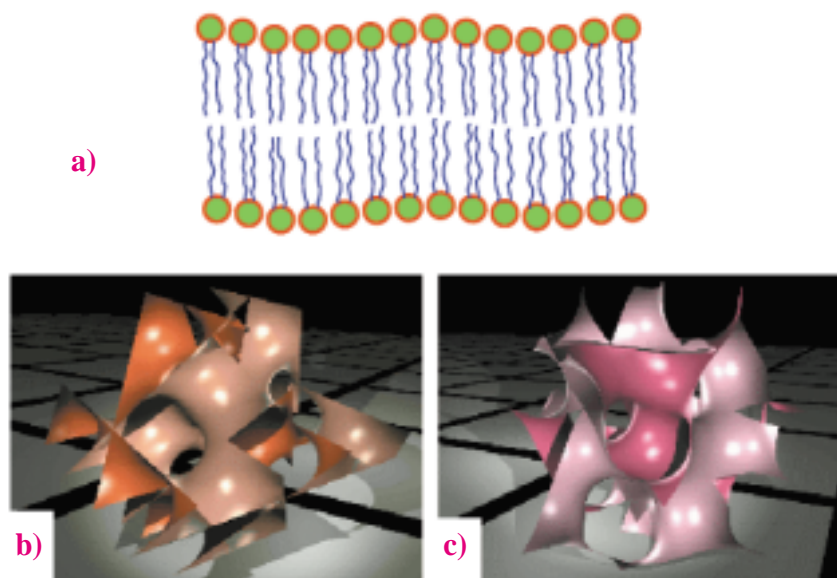


Fig. 1: (a) Schematic view of the cross-section of a lipid bilayer showing the hydrophilic head and hydrophobic tail of the lipid molecules. Computed periodic minimal surfaces corresponding to Pn3m (b) and Ia3d (c) cubic symmetries, respectively (courtesy of S. Hyde and S. Ramsden, Australian National University). In (b) and (c) each of the triply periodic minimal surfaces is formed by the lipid bilayer.

assemble into stacked sheets (as shown in Figure 1a), an inverted hexagonal phase H_{II} consisting of cylindrical structural elements packed on a 2-D hexagonal lattice, and two inverted cubic phases with space group symmetries Pn3m and Ia3d [1,2]. As depicted in Figure 1, the cubic structures are formed by mid-planes of bilayers satisfying the condition for periodic minimal surface (mean curvature $\rightarrow 0$) [3,4]. Consequently, this system is suitable for testing the

proposed curvature free energy model of lyotropic phases [3].

Hydrostatic pressure can be used to influence the structural properties and then to obtain an extended description of the phase behaviour, stability and energetics of cubic phases. Small-angle X-ray diffraction is a powerful tool for elucidation of the symmetry as well as the topology of these structures. Measurements were performed on the **ID2** beamline at the ESRF. Monoolein

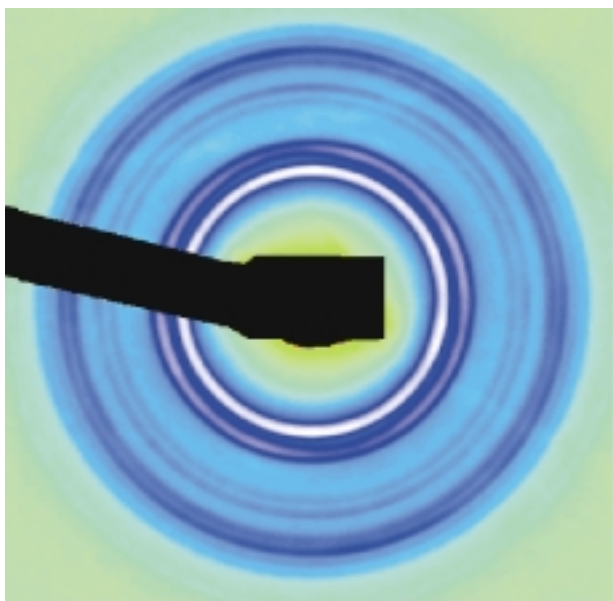


Fig. 2: Typical 2-D diffraction patterns from a cubic Ia3d phase at a pressure of 1.1 kbar and 28 wt.% of water.

samples were studied at different degrees of hydration (water content) as a function of pressure up to 3 kbar. A typical 2-D diffraction pattern from an Ia3d cubic phase is shown in Figure 2. Typical, azimuthally-averaged intensities as a function of scattering vector s ($= 2/\lambda \sin\theta$) at different pressures are depicted in Figure 3.

Results show that the pressure induces a transition from the Ia3d cubic to the lamellar L_α phase and then to the lamellar crystalline phase for a 30% hydrated sample. In the more hydrated

samples, a cubic-to-cubic phase transition (Pn3m to Ia3d) was observed. The crystalline phase is the only one that survives above 3 kbar.

While the crystalline phase is found to be incompressible, the unit cell dimensions of the cubic and the lamellar L_α phases increase as a function of pressure. The monolayer thickness, the cross-sectional area per molecule, and the mean and gaussian curvature [3], as a function of pressure and composition derived from these data, are summarised in Figure 4. The observed behaviour clearly indicates that the pressure induces a continuous change in the shape of monoolein molecules. In the cubic phases, these changes imply the stretching of the hydrocarbon chains and the consequent reduction of their cross-sectional area, resulting in the decrease in the mean curvature at the polar/apolar interface. These deformations are clearly involved in the associated curvature elastic

Fig. 3: Azimuthally averaged diffraction patterns at different pressures for a sample of 30 wt. % water.

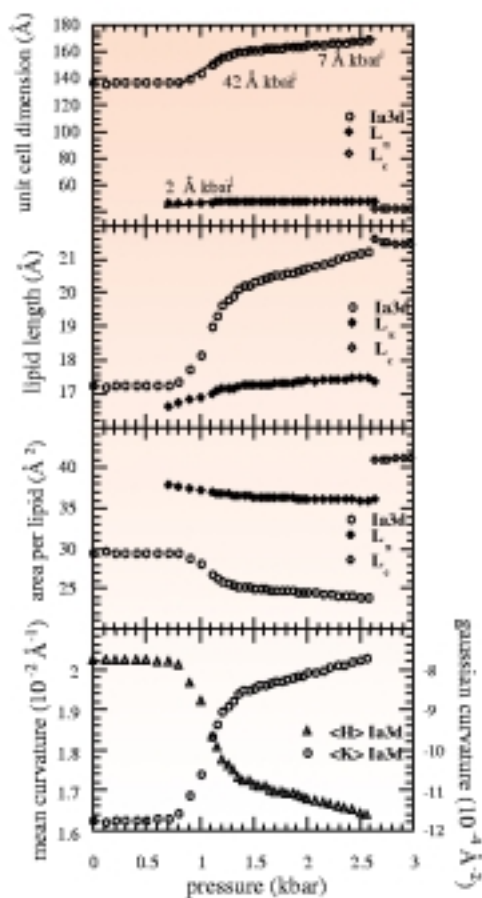
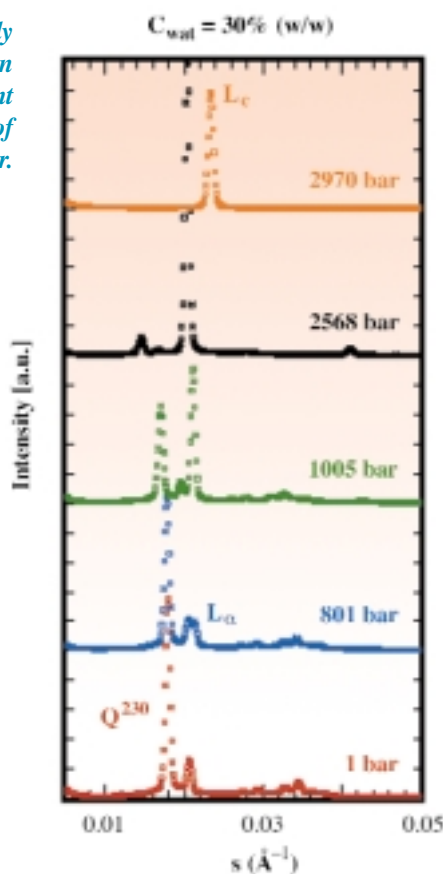


Fig. 4: Structural parameters deduced from the diffraction data as a function of pressure for a sample containing 30 wt.% of water.

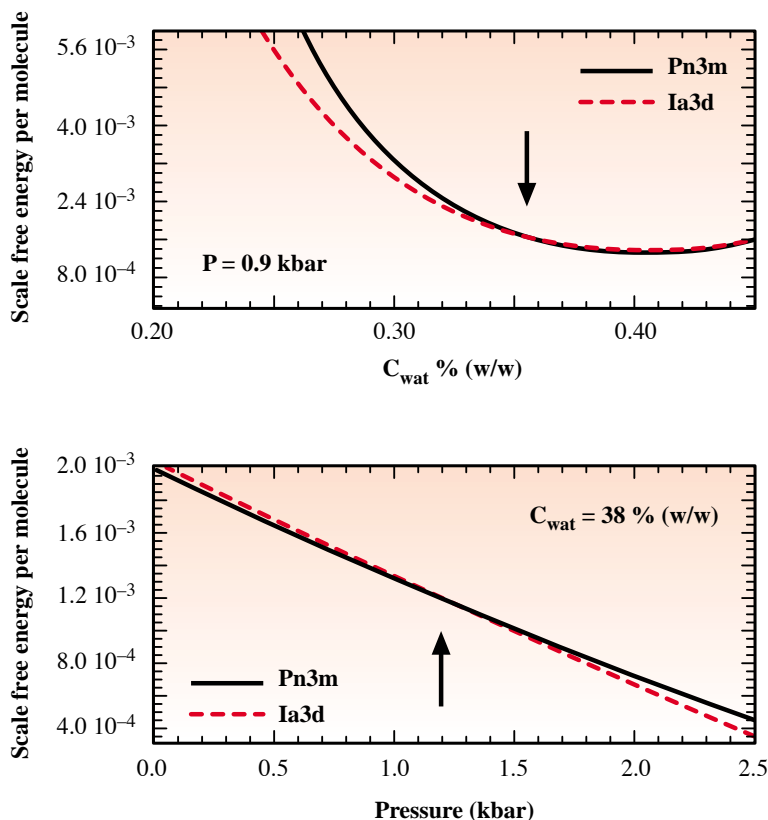


Fig. 5: Scaled free energy for the Pn3m and the Ia3d cubic phases as a function of concentration at constant pressure ($P = 0.9$ kbar) and as a function of pressure at constant concentration (38% w/w of water).

energy contributions to the cubic phase free energy which explains the observed phase behaviour.

Starting from the Helfrich description [3,5] for the curvature elastic energy, the ratio of the monolayer gaussian bending constant to mean-curvature bending constant k_G/k and the spontaneous curvature H_0 (the curvature that the monolayer would assume if it were free from all other constraints) were determined in the Pn3m and Ia3d cubic phases by a numerical fit of structural data obtained at different pressures. The pressure dependence of the spontaneous curvature and of the ratio k_G/k have been determined for the first time. Despite ignoring (or assuming only a minimal change across the phase transition at constant pressure) the chain packing and other contributions like interlamellar interactions, the curvature model provides the proper location and stability of each cubic phases with respect to the others, both as a function of pressure and concentration. For the sake of illustration, the recalculated free energy of Pn3m and Ia3d cubic phases as a function of concentration at constant pressure and as a function of

pressure at constant concentration are shown in Figure 5. The arrow indicates the point at which the phase transition was observed experimentally. The good agreement demonstrates how well this type of experiment can provide a quantitative test of theories on stability and energetic of lyotropic phases. ■

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