# Volume constriction in a lipid-water liquid crystal using high-pressure x-ray diffraction

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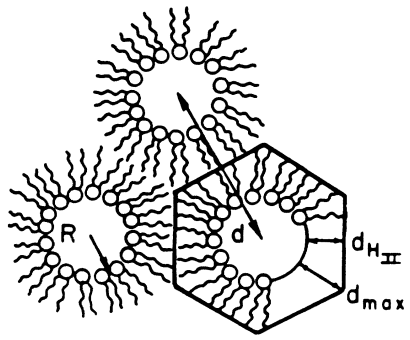
(Received 2 July 1990)

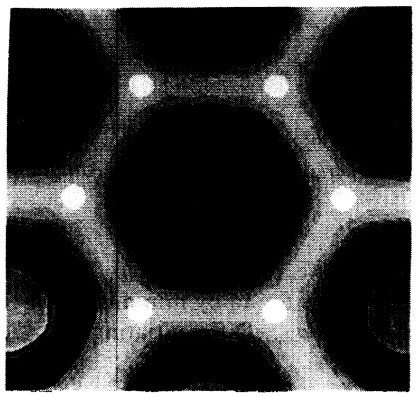
We report a measurement of the change in the total volume of a biological lipid-water system when water molecules are removed from the *fully hydrated* lyotropic liquid-crystal phase, where the water molecules are near a polar interface, to the surrounding bulk water. The commonly used assumption of linear additions of constituent volumes predicts that the water mole fractions of these lipid-water phases should be independent of hydrostatic pressure. The discrepancy between this prediction and our high-pressure x-ray-diffraction measurements is due to a decrease of 0.1 Å$^3$ in the total volume of the system per molecule of water incorporated into the fully hydrated lipid-water aggregate from the bulk water.

The study of water near surfaces is of considerable interest in many fields of science. Perhaps the most widely studied systems involving such restricted water are lyotropic liquid crystals formed by biological lipids and other amphiphilic molecules. A commonly used assumption in the field is that the transfer of water molecules from bulk water to the aggregate does not alter the total volume of the system, that is, the total volume of the system is the sum of the independent volumes of the lipid and the water constituents. This assumption of the linear addition of the specific volumes (henceforth called the linear approximation) has been used successfully for a long time as a means of measuring the internal dimensions of biological liquid-crystal phases.[1] The structural method was popularized by Luzzati and co-workers.[2] Several authors have noted that when almost all of the water is removed from the lipid surface by extensive drying, the remaining water molecules, which are tightly bound to the polar surfaces, have a smaller volume than that of molecules in the bulk water phase.[3] Present methods of volume measurement that are based on electron-density reconstruction from x-ray diffraction do not have a sufficiently high resolution to detect changes in volume when lipid systems are near *full hydration.* (Full hydration, or the excess water fraction, is defined as the point where the aggregate will take up no more water; that is, any water added beyond this point simply pools off as a bulk water phase.) Measurements of volume changes upon hydration have been somewhat controversial. Our measurements represent the first determination of the change in total volume upon removal of water from *fully hydrated* liquid-crystalline systems.

We report here the results of high-pressure x-ray diffraction studies using small-angle x-ray scattering (SAXS) on biological lipid-water systems that form nonbilayer lyotropic liquid-crystal phases (Fig. 1). Our results indicate that the mole fraction $n_w$ of water in the inverted hexagonal ($H_{II}$) lipid-water phase is more sensitive to hydrostatic pressure than for any lyotropic system that has been reported.[4] By applying standard thermodynamic relations, we demonstrate that the observed depen-




(a)



(b)

FIG. 1. (a) Schematic illustration of the $H_{II}$ phase of lipid-water dispersions. (b) An electron-density reconstruction of the x-ray diffraction on DOPE water, showing the Wigner-Seitz cell. Some of the parameters of the unit cell that can be determined by electron-density reconstruction from x-ray-diffraction data are the (i) the distance $d$ between the centers of the water cores, (ii) the radius $R$ of the water-lipid interface, and (iii) the water volume fraction $\phi_w = 2\pi R^2/\sqrt{3}d^2$. The number density $n_w$ is related to $\phi_w$ according to $\phi_w = n_w v_w/(v_L + n_w v_w)$, where $v_L$ and $v_w$ are the specific volumes of lipid and water, respectively (Refs. 1, 7, and 13). At 25 °C, 1 bar fully hydrated DOPE-water samples have values of $d \simeq 75$ Å, $R \simeq 21$ Å.

dence of $n_w$ on pressure directly measures the deviations from the linear approximation. In conjunction with some recent measurements on the effect of osmotic pressure on the lamellar and nonlamellar phases, our data show that the total volume of the system increases by 0.1 Å$^3$ for every water molecule transferred from the lipid-water aggregate to the bulk water at constant pressure. Since the interaction of water with molecularly rough surfaces is regularly encountered in a variety of biological and nonbiological systems, we expect our measurements to have an impact in many fields.

We start with a demonstration that the linear approximation implies that the excess water point is insensitive to pressure. A physical picture will supplement a rigorous thermodynamic argument given later. We start with a (conserved) quantity of lipid and water, where the water fraction is much greater than the excess water fraction of the lipid-water aggregate that is formed. In the linear approximation, the specific volume of a phase is taken to be the *sum* of the specific volumes of its lipid and water components. This means that the total volume of the system is independent of the amount of water partitioned into the lipid aggregate. Since increasing pressure will only result in shifting the equilibrium of the system to states with lower total volume, the free-energy difference between states of differing hydration is independent of pressure. Since the excess water point represents the state of the system with the lowest free energy, it too will be independent of pressure. It is important to note that constriction of the "first few" tightly bound water molecules does not change the result. It is the rate of change in the total volume upon removal of a water molecule *at the excess water point* that will determine the shift in the excess water point with pressure. Conversely, a measurement of the shift in the excess water point with hydrostatic pressure may therefore be used to measure volume changes upon removal of water.

In order to quantitatively estimate the deviation from the linear approximation, consider a system of a lipid in coexistence with water at pressure $P$. We assume that the excess water exists as pools of bulk water with no lipid in it. This is justified because the partitioning of dual-chain lipids into water is very low.[5] Let $G(P,n_w)$ be the total Gibbs free energy of a system consisting of $N_L$ lipid and $N_W$ water molecules, where $n_w$ is the number of water molecules associated with each lipid molecule inside the lipid-water aggregate phase ($N_W > N_L n_w$). At a pressure $P_0$, the excess water point $n_w^e(P_0)$ is given by the value of $n_w$ which minimizes $G(P_0,n_w)$. If the pressure is increased to $P_0+\delta P$, the change in $G$ is given by

$$G(P_0+\delta P,n_w)=G(P_0,n_w)+V(n_w)\delta P\ , \tag{1}$$

where $V(n_w)$ is the total volume of the system. Define $\delta n_w^e = n_w^e(P_0+\delta P)-n_w^e(P_0)$ as the change in the excess water point with pressure, and $\delta G(\delta P,\delta n_w)$ as $G(P_0+\delta P,n_w^e(P_0)+\delta n_w)-G(P_0,n_w^e(P_0))$. For small $\delta P$ and $\delta n_w$, retaining only terms relevant to the subsequent discussion, this has the expansion

$$\delta G(\delta P,\delta n_w)=\frac{1}{2}(\delta n_w)^2\left.\frac{\partial^2 G(P_0,n_w)}{\partial n_w^2}\right|_{n_w=n_w^e(P_0)}+\delta P\delta n_w\left.\frac{\partial V(P_0,n_w)}{\partial n_w}\right|_{n_w=n_w^e(P_0)} \tag{2a}$$

$$=\frac{N_L}{2\rho_w}\frac{\partial\Pi}{\partial n_w}(\delta n_w)^2+\frac{\partial V(P_0,n_w)}{\partial n_w}\delta P\,\delta n_w\ , \tag{2b}$$

where $\Pi$ is the osmotic pressure and $\rho_w$ is the number density of water. $\delta n_w^e$ is found by minimizing this expression as a function of $\delta n_w$. Differentiating Eq. (2b) leads to the relation

$$\frac{1}{N_L}\left.\frac{\partial V(P_0,n_w)}{\partial n_w}\right|_{n_w=n_w^e(P_0)} = -\left.\frac{dn_w^e}{dP}\right|_{P=P_0}\left[\rho_w\left.\frac{dn_w}{d\Pi}\right|_{\substack{P=P_0\\ \Pi=0}}\right]^{-1}\ . \tag{3}$$

Equation (3) gives the variation in the total volume of the entire system as a function of water concentration at the excess water point in terms of a ratio of two quantities that can be experimentally determined. In the linear approximation, $\partial V/\partial n_w=0$ by definition, so $\partial n_w/\partial P$ must be zero. [Even if we were to relax the assumption that no lipid molecules are present in the bulk water, this result is not altered, although Eq. (3) is modified slightly. This is as would be expected from the argument in the previous paragraph.] The point is that, since $dn_w^e/dP$ and $dn_w/d\Pi$ can be measured, the small volume change $\partial V/\partial n_w$ can be easily determined *at full hydration,* representing the departure from the linear approximation.

X-ray diffraction data were taken on dispersions of the lipid DOPE (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine from Avanti Polar Lipids, Birmingham, AL) in water. The data were taken using a recently constructed beryllium high-pressure cell on a Rigaku rotating anode x-ray machine equipped with an image-intensified silicon intensified target television (SIT TV) detector that is specifically designed for small-angle x-ray diffraction.[6] The excess-water point was determined as a function of pressure by two different techniques: In the first, samples with known mole fractions of water (measured gravimetrically) were examined, and their unit-cell spacings were determined. Beyond the excess-water point the unit-cell spacing no longer increases with increasing water concentration. A second, less tedious method was used over a wider range in pres-

sure. The water volume fraction was derived from an electron-density reconstruction of the unit cell of the two-dimensional liquid-crystalline $H_{II}$ phase from the x-ray-diffraction peak intensities using a recently developed method.[7] It has been shown that this method gives results in good agreement with the gravimetric method. Figure 1 shows an electron-density reconstruction of the $H_{II}$ phase from data taken under high pressure. The data indicate that the change in water volume fraction with pressure is

$$\frac{d\phi_w^e}{dP} = (3\pm1)\times10^{-11}\ \text{dyn}^{-1}\,\text{cm}^2\ , \tag{4}$$

which can be converted to water mole fractions using standard tables[8] of the densities of water and lipid respectively, to yield

$$\frac{dn_w^e}{dP} = (3\pm1)\times10^{-9}\ \text{dyn}^{-1}\ \text{cm}^2\ . \tag{5}$$

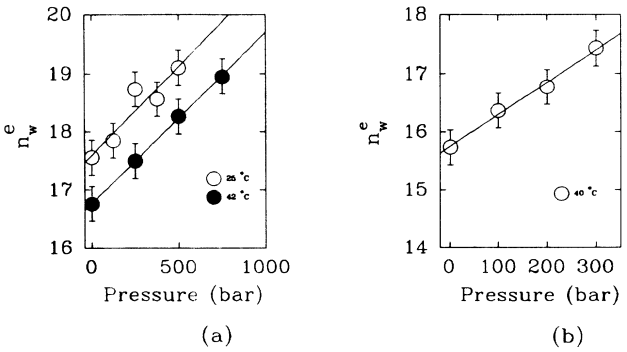
Figure 2(a) shows a plot of $n_w^e$ as a function of pressure. The major source of systematic error introduced in this conversion arises from the uncertainty in the differential compressibility of lipid and water. The magnitude of this error is much smaller than the effect we are trying to measure, as can be seen from the fact that at fixed water concentrations below excess the change in lattice spacing as a function of pressure is an order of magnitude smaller than at the excess water point.[9] Also, Fig. 2(b) shows a plot of $n_w^e$ measured by the gravimetric technique, which agrees with the results in Fig. 2(a) to within the errors of our measurements. We note that the gravimetric technique involves no assumptions about differential compressibility of lipid and water.

Gruner, Parsegian, and Rand[10] have reported an x-ray-diffraction study of DOPE-water dispersions as a function of osmotic pressure at 20 °C. Their data yield

$$\frac{dn_w}{d\Pi} = -1.0\times10^{-6}\ \text{dyn}^{-1}\,\text{cm}^2\ . \tag{6}$$

Combining Eqs. (3), (5), and (6) we find that the loss in volume of the entire system per water molecule moved from the bulk to the aggregate is 0.1 Å$^3$. The error in this value is estimated to be about 30%.[11]

There are additional methods that one might use to perform studies on volume changes. Direct volume measurements could in principle be made sufficiently accurate to determine such small changes in volume upon mixing. An extension of our method to below excess may be carried out using the method of Gruner, Parsegian, and Rand.[10] However, the constriction of water in the presence of the solute used to generate the osmotic stress must be determined to an accuracy better than the precision of the measurement reported here.




(a) (b)

FIG. 2. (a) Plot of $n_w^e$ as a function of pressure at 25 and 42 °C, for DOPE-water dispersions calculated through electron-density reconstruction. The slope of the straight lines is $3\pm1\times10^{-9}$ dyn$^{-1}$ cm$^2$. In the linear approximation, the slope of this line should be zero. (b) Plot of $n_w^e$ as a function of pressure at 42 °C calculated gravimetrically. The slope is $5\pm2\times10^{-9}$ dyn$^{-1}$ cm$^2$, which is the same as in (a), to within the errors of our measurements. The uncertainty in determining the slope in this graph is larger owing to the smaller range in pressure that we have covered.

The small change in volume of the system upon the addition of one water molecule to the lipid phase suggests that to a first approximation, the linear approximation is indeed correct; the volumes of lipid and water can be assumed to add simply without sacrificing much accuracy. However, our observations have implications for models that attempt to analyze the interaction of water molecules with polar surfaces in detail. The observed volume constriction is consistent with a recent estimate suggesting that the constriction is in the range 0–0.2 Å$^3$ per water molecule.[12] Furthermore, as we have seen, it would be inconsistent to use the linear approximation in any models that seek to explain the variations with pressure observed in these systems.

Our method is applicable not just to lipid-water systems but also to many systems in which the interaction with water is of interest, such as silicate clays in geology and to protein and nucleic acid crystals in biology. Subtle changes in the nature of water bound to biomolecules at full hydration are expected to influence the structure and dynamics of such complex molecules, and the mechanisms for such an influence are a topic of great current interest.

This work was supported in part by Office of Naval Research Contract No. N00014-86-K-0396P00001, U.S. Department of Energy Grant No. DE-FG02-87ER60522 and National Institutes of Health Grant No. GM32614. E.S. would like to thank Professor W.B. Daniels for his advice on the construction of the high-pressure cell.

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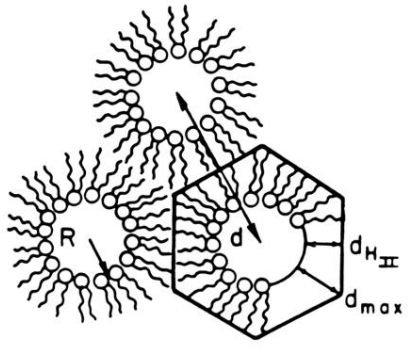
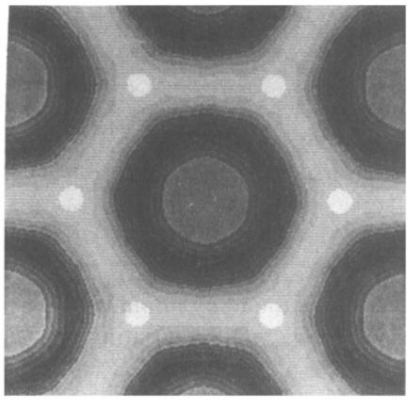



(a)



(b)

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