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Hydration pressure and phase transitions of phospholipids I. Piezotropic approach

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Abstract

Dehydration reduces the main phase transition pressure of phospholipids. An analysis based on the Gibbs–Duhem equation shows how the shift of the transition pressure is correlated to the hydration pressure.

By using Fourier transform infrared (FT-IR) spectroscopy we determined the hydration-dependent phase transition pressure. The application of our new approach gives hydration pressure values which agree with the values obtained with the osmotic stress method.

Keywords: Phospholipid; Hydration pressure; Hydrostatic pressure; Infrared; Thermodynamics; Lyotropic

1. Introduction

Surface forces are important in colloidal systems and there is one dominating force at low hydration which exceeds the predictions given by traditional interaction theories (electrostatic, undulatory and van der Waals interactions [1–3]). The force is called hydration force and it is usually described by an exponential function versus the surface distance, d_W (Eq. (1)):

$$F_h = F_{h,0}\exp\left(-\frac{d_W}{d_{W,0}}\right) \quad (1)$$

The parameters $F_{h,0}$ and $d_{W,0}$ represent the hydration force at zero hydration and the exponential decay constant, respectively. However, the surface distance is not well defined because of the rough nature of the surface of phospholipids. Therefore, in the present paper we consequently use the water content in order to quantify hydration.

Instead of hydration force, one may also consider hydration pressure. Hydration pressure is defined as the hydrostatic pressure which is required to maintain the chemical equilibrium of the water phase with a water phase existing at reference conditions [4]. The following equation gives the empirical description:

$$P_h = P_{h,0}\exp\left(-\frac{R_W}{R_{W,0}}\right) \quad (2)$$

where $P_{h,0}$ and $R_{W,0}$ are the hydration pressure at zero hydration and the decay constant, respectively. The water content between the phospholipid surfaces, R_W, is usually expressed by the molar ratio of water and amphiphile:

$$R_W = \frac{n_W}{n_A} \quad (3)$$

It must be stressed that the water content gives the macroscopic water-amphiphile composition. One may not automatically assume that the water is always situated between the bilayers. Some water can also be trapped in diffusionally restricted or sealed micro-volumes such as water-filled pockets. However, at reduced water contents ($R_W < 15$) this effect is negligible (see Ref. [5] and references therein).

Hydration pressure plays a role in lipids, surfactants, DNA, proteins, polyelectrolytes and polysaccharides [6]. It is of biological importance for the approach of biological surfaces (e.g. stress on cartilage, cell fusion) as well as for osmotic dehydration, such as in cryobiology (stress on biomembrane components due to freezing induced dehydration, [7]). One of the fundamental methods to determine

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hydration pressure is the "osmotic stress method". This method replaces piezotropic measurements by experiments at isopiestic conditions. The basic idea is the assumed equivalence of isopiestic sorption experiments and piezotropic experiments [8]. According to the osmotic stress method, lipids are placed in an arrangement where the water activity, a_W, is adjusted by a humidity-control system determining the amount of water vapour or by an osmotic solution separated from the lipids by a semipermeable membrane. The hydration pressure is given by:

$$P_h = -\frac{RT}{V_W}\ln a_W = -\Psi_W \quad (4)$$

where R is the gas constant, T the absolute temperature and V_W the molar volume of water [9]; Ψ_W is the water potential [10,11]. If one determines the hydration parameter, such as the water content, R_W, and/or the water layer thickness, d_W, one obtains functional pairs of hydration pressure and hydration.

It was successfully proposed to use the melting temperature of the hydration water to determine the hydration pressure [12,13]. However, the calculation of hydration pressure parameters by parameters of lipid phase transitions is, to the best of our knowledge, only qualitatively possible [14]. The present paper will offer a first quantitative approach to the problem. Furthermore, due to the pure thermodynamical character of the derivations, the approach is also applicable to other substances such as surfactants, DNA, proteins, polyelectrolytes and polysaccharides.

2. Theory

The presented approach gives a simple relationship between hydration pressure and main phase transition pressure. Let us consider a phospholipid/water dispersion existing in a two-state phase equilibrium (Fig. 1, liquid crystalline phase, L_α–gel phase, L_β). Water and lipid are considered as two components. According to the equilibrium condition, the chemical potential of the lipid in the liquid crystalline phase must be equal to the chemical potential of the gel phase.

$$\mu_{L,liq} = \mu_{L,gel} \quad (5)$$

From this follows:

$$d\mu_{L,liq} = d\mu_{L,gel} \quad (6)$$

The relationship between the change of the chemical potentials of lipid and water, $d\mu_L$ and $d\mu_W$, is given by the Gibbs–Duhem relation,

$$0 = n_L d\mu_L + n_W d\mu_W \quad (7)$$

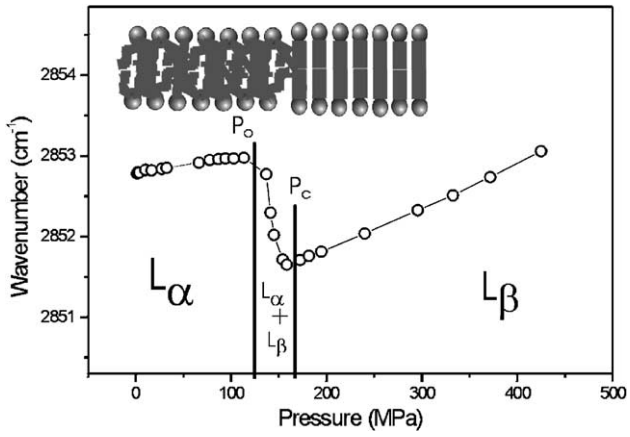

Fig. 1. Main phase transition of POPC at 25 °C at full hydration (RH = 100%), detected by using the symmetric stretching vibrations, ν_{sym} (CH_2), depending on hydrostatic pressure. The coexistence region between liquid crystalline (L_α) and gel-phase (L_β) starts at the onset pressure P_o and stops at the completion pressure P_c.

which is applied at both the left and the right side of Eq. (6):

$$R_{W,liq} d\mu_{L,liq} = R_{W,gel} d\mu_{L,gel} \quad (8)$$

The change of the chemical potential can be expressed by the second terms of the corresponding Taylor series,

$$d\mu = \left(\frac{\partial \mu}{\partial p}\right)_a dp + \left(\frac{\partial \mu}{\partial a}\right)_p da \quad (9)$$

where a is the activity of the water or the lipid. The derivative of the chemical potential with respect to pressure is the molar volume, V_W, and the derivative with respect to the activity can be obtained from the well-known relationship, $\mu_W = \mu_{W,0} + RT \times d\ln a_W$, which links the activity to the chemical potential.

$$d\mu = V_W dp + RT \times d\ln a_W \quad (10)$$

A combination with Eq. (8) gives:

$$R_{W,liq}(V_{W,liq} dp + V_{W,liq} d\Psi_W) = R_{W,gel}(V_{W,gel} dp + V_{W,gel} d\Psi_W) \quad (11)$$

where Ψ_W is the isopiestic water potential (see Eq. (4)). Rearrangement leads to:

$$(R_{W,gel} V_{W,gel} - R_{W,liq} V_{W,liq}) dp = -(R_{W,gel} V_{W,gel} - R_{W,liq} V_{W,liq}) d\Psi_W \quad (12)$$

From this it follows easily:

$$dp = -d\Psi_W \quad (13)$$

The absolute values of the differential shift of the transition pressure and the isopiestic water potential are thus numerically equal:

$$\int_{P_{re}(a_W=1)}^{P_{tr}} dp = -\int_0^{\Psi_W} d\Psi_W \quad (14)$$

Integration of Eq. (14) gives the relationship between the isopiestic water potential and the shift of the piezotropic phase transition pressure:

$$P_{tr} - P_{tr,0} = \Delta P_{tr} = -\Psi_W \quad (15)$$

where $P_{tr,0}$ is the transition pressure at full hydration ($a_W = 1$). Given the relationship between hydration pressure and isopiestic water potential (Eq. (4)), one can conclude that the hydration pressure, P_h, is equal to the dehydration-induced shift of the main phase transition pressure, ΔP_{tr}:

$$P_h = \Delta P_{tr} \quad (16)$$

3. Materials and methods

The test of our piezotropic approach requires the determination of the piezotropic phase transitions of phospholipids at a low and defined degree of hydration. To the best of our knowledge, such measurements have not been reported before.

Two unsaturated phospholipids (POPC and DOPC) were used and the phase transition parameters were determined by Fourier transform infrared (FT-IR) spectroscopy. POPC was hydrated, vortexed and annealed in a sample tube. The water content, R_W, was determined by weighing. After this, the paste-like sample was directly mounted into the sample holder of the diamond anvil cell (DAC).

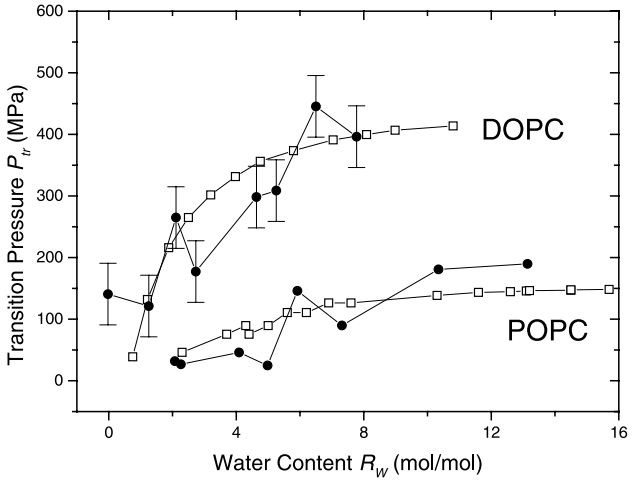

Fig. 2. Transition pressure (circles) at 25 °C versus water content. The transition pressure predicted by Eq. (17) is given by the open squares.

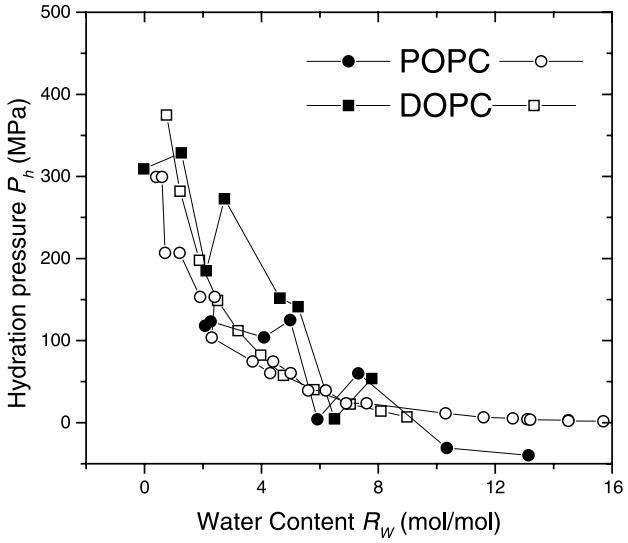

Fig. 3. Hydration pressure of POPC and DOPC at 25 °C (solid symbols) according to Eq. (16) versus water content. The hydration pressure calculated with to the osmotic stress method (Eq. (4)) is given by the open symbols.

An alternative hydration procedure (applied for DOPC) was the hydration in the sample holder by a humidity-control system. For this case, the sample holder of the DAC was filled with the aqueous lipid solution and the pressure sensor (powdered natural quartz). The water content was chosen so that it was suitable for an easy filling. Then, the water was removed using a dry stream of nitrogen gas [15]. In order to adjust the humidity, a special cell was developed. It enables the exposure of the sample holder to a defined humidity at controlled temperature. The cell consists of a beaker, which was sealed to prevent the exchange of water with the environment. The beaker was mounted in a box and temperature was adjusted by using a tube connected to a thermostat which was wrapped around the beaker. The beaker contains the sample holder of the DAC as well as another beaker with the saturated salt solution needed to adjust the required humidity. The information about the resulting water content was finally obtained from the usual sorption isotherms of POPC [16] and DOPC [17]. After the hydration process, the DAC was immediately sealed and mounted into the spectrometer. The water peaks for different exposure times gave information about the kinetics of hydration. For all exposing times, $t > 1$ h, there was no visible time-correlated change of the water peak. In general, the required equilibrium was reached after a few minutes at very low humidity up to 1 h at relative humidity (RH) = 92%.

In order to trace the piezotropic phase transitions we chose FT-IR spectroscopy. We used an FT-IR spectrometer (FT-IR Bruker, IFS 66) equipped with a DAC (Diacell Products, Leicester, UK). The thickness of the gasket was 25 μm and the diameter of the sample hole was 0.6 mm. The pressure was determined from the 695 cm^{-1} phonon band of natural quartz, as described in Refs. [18,19]. The accuracy of pressure determination is about 50 MPa in the present

configuration [20]. We found that the $BaSO_4$ method [21] is unsuited for pressure determination at low degree of hydration because of the hygroscopic nature of $BaSO_4$.

4. Results and discussion

A typical main phase transition detected by FT-IR spectroscopy is shown in Fig. 1. The discontinuous step of the symmetric stretching vibration of the CH_2 groups is correlated with the trans-gauche isomerization in the hydrocarbon chains of the lipid.

Fig. 2 shows the experimental transition pressure of the lipid versus water content. The experimental results are consistent with the predictions calculated according to Eq. (17), which combines Eqs. (4) and (16):

$$P_{tr} = P_{tr,0} + \frac{RT}{V_W} \ln a_W \quad (17)$$

The calculation of Eq. (17) required the knowledge of the RHs (a_W = RH/100) as a function of hydration, R_W, and these functional pairs were obtained from sorption isotherms [16,17].

Thus, the determination of the piezotropic transition pressure enables one to determine hydration pressure (Fig. 3). For this aim, one only measures the hydration-dependent transition pressure and calculates the difference with the transition pressure at full hydration. The transition pressure at full hydration (25 °C) is 150 ± 50 MPa for POPC and 450 ± 50 MPa for DOPC.

The present procedure establishes one of the most direct methods to determine hydration pressure. It is a piezotropic method and the model makes use of very few assumptions only. However, it has to be considered that hydration pressure parameters depend on the phase state of the lipid [22] and our method uses the parameters of phase equilibrium. Therefore, we measure averaged values of two phases.

Unfortunately, the accuracy of pressure determination in the DAC is very modest with respect to our requirements (about 50 MPa). This is due to uncertainties in the spectroscopic determination of pressure. This prevents further investigation and application of this method. An alternative would be the use of another pressure device. However, one will then lose also the advantages of the DAC such as the extraordinary low samples amounts and the extended pressure range.

In many cases, it is easier to measure thermotropic phase transitions instead of piezotropic phase transitions. Therefore, in an accompanying paper [23] it will be shown that it is possible to apply Eq. (13) to thermotropic measurements as well. The theory works in this case too, as will be shown by a number of phospholipids.

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