

Hydration pressure and phase transitions of phospholipids

II. Thermotropic approach

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Abstract

It is widely known that dehydration increases the main phase transition temperature of phospholipids. A mathematical analysis now shows that hydration pressure can be calculated by the dehydration-induced shift of the phase transition temperature.

The hydration-dependent piezotropic and thermotropic phase transitions were determined by using calorimetry and FT-IR spectroscopy, and the application of our approach gives hydration pressure parameters that agree very well with the values obtained with the osmotic stress method.

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1. Introduction

There are several possibilities to use thermotropic phase transitions in lipid/water dispersions to obtain hydration pressure parameters. The approach of Ulrich et al. [1], demonstrated on DOPC, is based on the freezing point depression of the hydration water. Interestingly, the same approach was introduced by Bach et al. in 1982 [2], but instead of hydration pressure, the authors used the term “swelling pressure”. The hydration pressure as a function of water content is given by [1]:

$$P_h = \frac{\Delta H_{tr,w}}{V_w} \left(1 - \frac{T(R_w)}{T_0} \right) \quad (1)$$

where $\Delta H_{tr,w}$ is the molar enthalpy change of melting ice, V_w the molar volume of liquid water, $T(R_w)$ the hydration-dependent melting temperature and T_0 the melting temperature of pure water. Another approach uses the main phase

transition and it is based on the theory of water polarisation [3]:

$$\Delta T_m = \Delta T_m(0) \tanh \left(\frac{n_w V_w}{\lambda A} \right) \quad (2)$$

Here, $\Delta T_m(0)$ is the decrease in transition temperature, n_w the number of water molecules, λ a decay length and A the area per lipid molecule. A detailed inspection of the formula showed that there is a qualitative agreement but no quantitative agreement with experimental results [4]. It was concluded that calorimetric investigations of the thermotropic phase transition of the lipid might only give qualitative information on hydration processes.

Using a thermodynamic approach, we will here present a derivation that also gives quantitative information on the hydration pressure using the main phase transition temperature of lipids.

2. Theory

The derivation of the relationship between the main phase transition temperature and hydration pressure continues at the derivation for piezotropic transitions, presented in the accompanying paper [5]. The relationship between the

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differential shift of the piezotropic phase transitions, dP_{tr} , and the water potential, $d\Psi_w$, is given by:

$$dP_{tr} = -d\Psi_w \quad (3)$$

After a small shift of temperature, dT , there is a new equilibrium. The dependence of the transition pressure, P_{tr} , on the temperature is given by the equation of Clapeyron (Eq. (4)), which is also valid for main phase transitions of phospholipids [6]:

$$\frac{dT}{dP} = \frac{\Delta V_{L,tr}}{\Delta S_{L,tr}} \quad (4)$$

$\Delta V_{L,tr}$ and $\Delta S_{L,tr}$ are the volume change and the entropy change of the lipid. A comparison of Eqs. (3) and (4) shows that the dependence of water potential on the temperature is simply given by:

$$\frac{dT}{d\Psi_w} = -\frac{\Delta V_{L,tr}}{\Delta S_{L,tr}} \quad (5)$$

From Eq. (5), it follows that the determination of hydration pressure can be achieved by using calorimetry. Taking the definition of the pressure-induced temperature increase, α , given in:

$$\alpha = \frac{\Delta V_{L,tr}}{\Delta S_{L,tr}} \quad (6)$$

one can rewrite Eq. (5). Thus, the crucial point is the knowledge of the pressure-induced temperature increase α as a function of temperature and hydration. One obtains Eq. (7) which gives the relationship between phase transition temperature and water potential:

$$dT_{tr} = -\alpha d\Psi_w. \quad (7)$$

Thus, using the relationship between water potential and hydration pressure ($P_h = -\Psi_w$, accompanying paper [5]), one obtains:

$$dP_h = \frac{1}{\alpha} dT_{tr} \quad (8)$$

From Eq. (8) follows:

$$P_h = \frac{1}{\alpha} \Delta T_{tr} \quad (9)$$

where ΔT_{tr} is the shift of the transition temperature with respect to the fully hydrated state. Fortunately, the slope α is obviously not a function of hydration because its value is invariant even if one replaces water by different polar solvents [7]. Furthermore, it is interesting to know that α is nearly constant (about 0.2 K/MPa [6,7]) for almost all phospholipids, because it is mainly determined by the ratio of the volume and entropy change per CH_2 -segment in the aliphatic chain. This even enables us to calculate an

estimate of hydration pressure from the temperature shift ($P_h \approx 5\Delta T_{tr}$; [P_h]=MPa, [T]=K).

Finally, the comparison of the present approach with the conventional isothermal osmotic stress method requires knowledge on the temperature dependence of hydration pressure. However, Simon et al. [8] found that hydration pressure hardly varies with temperature. This enables us to make a direct comparison between isothermal and thermotropic methods.

3. Materials and methods

The phospholipids POPC, DPPC and DMPC were purchased at Avanti Polar Lipids, Inc. (Birmingham, AL) and used as obtained. Impurities were less than 1% according to thin layer chromatography. Apart from POPC from Avanti Polar Lipids (sample 1), additional POPC was used (sample 2) which was synthesised at the Institut für Physikalische Chemie/Technische Universität Graz (Austria). All lipids were dried under high vacuum ($p=10^{-2}$ Pa) for 24 h. Subsequently, water was added in appropriate amounts and the samples were homogenised by vortexing and annealing. The accuracy of the water concentration was better than ± 1 water molecule per lipid molecule.

The differential scanning calorimetry (DSC) experiments were performed using a Perkin-Elmer DSC-7 calorimeter. The substances were filled in aluminium pans, mechanically sealed to prevent dehydration during the measurement. Variations of the heating rate and a comparison of heating and cooling scans showed an excellent reproducibility of the data sets.

The determination of the main phase transition temperature was also done by using a high-pressure differential thermal analysis (DTA)-calorimeter. In general, the heating rate was 1 K/min and the accuracy of temperature determi-

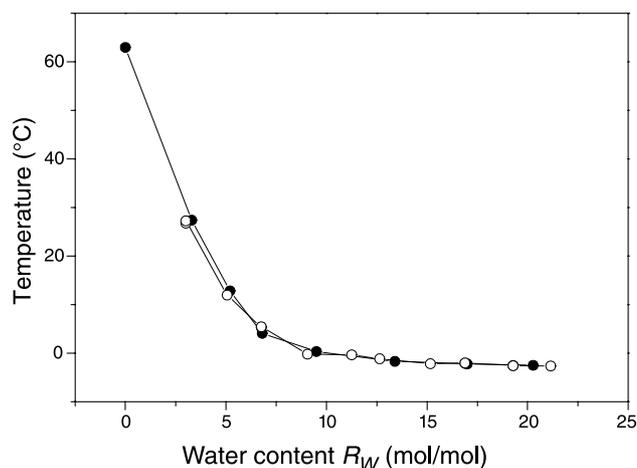


Fig. 1. Extrapolated onset temperature of different samples of POPC (sample 1 — open circles, sample 2 — solid circles) as a function of water content at normal pressure.

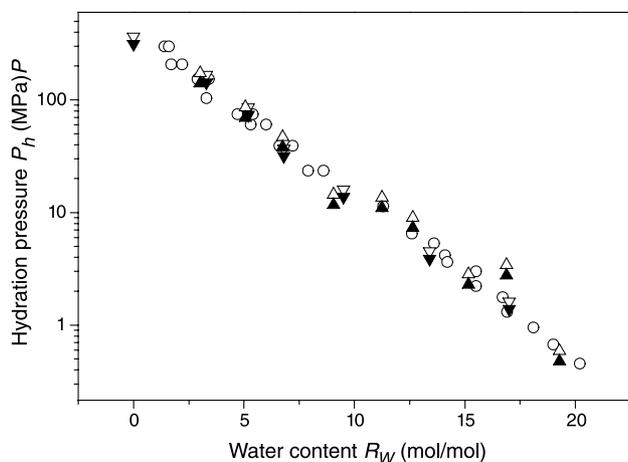


Fig. 2. Determination of the hydration pressure of POPC by Eq. (9) (triangles) and by the osmotic stress method (circles). The different triangles refer to different samples (up triangles — sample 1, down triangles — sample 2) as well as different pressure-induced temperature increases α (solid triangles $\alpha = 0.21$ K/MPa [6]; open triangles $\alpha = 0.18$ K/MPa [7]) which were obtained by different methods.

nation was about ± 0.1 K. The pressure was measured by a Heise Bourdan gauge and by a strain gauge (for the online registration) with an accuracy of ± 1 MPa. Further details are described in Landwehr and Winter [9].

The FT-IR experiments were done by using a Bio-Rad FTS-60a spectrometer, which was equipped with a deuterated triglycine-sulfate detector. The spectra were recorded by using the ATR-technique. Two hundred fifty-six interferograms with a resolution of 2 cm^{-1} have been co-added. The moisture cell for adjusting the corresponding humidities is described in Ref. [10].

4. Results and discussion

The hydration pressure (according to Eq. (9)) for different samples of POPC, calculated with the extrapolated onset temperatures (Fig. 1), is shown in Fig. 2. Additionally, to check the effect of the parameter α , we used two separate values of α which were obtained by different methods (SANS and DTA [6,7]). In any case, the results fit very well with those obtained by the isothermal osmotic stress method [5].

The results for the lipids POPC, DMPC and DPPC are summarised in Fig. 3. They confirm the validity of our thermotropic approach. All curves show exponential behaviour (in contrast to Eq. (2)) and the corresponding parameters, $P_{\text{hydr},0}$ and $R_{W,0}$, of hydration pressure ($P_h = P_{h,0} \exp(-R_W/R_{W,0})$; Ref. [5]) are summarised in Table 1. The values taken from literature show a sufficient agreement considering the fact that isothermal and thermotropic values are compared. The reference values of POPC are most accurate because all raw data were given [11]. The decay constants, $R_{W,0}$, for some reference values were derived from decay volumes, μ [12], by using the volume of one water molecule of $V_{\text{water}} = 30 \text{ \AA}^3$.

It is interesting that the decay constant of DPPC obtained by DSC is exactly the average value ($R_{W,0} = 2.6$) of the decay constants of gel and liquid-crystalline phase ($R_{W,0} = 1.8$ and 3.4 [12]). This seems to confirm that this approach gives average parameters of the liquid-crystalline and the gel phase. However, DMPC shows the opposite behaviour because the decay constant in the liquid-crystalline phase is smaller than our values obtained from the DSC measurements. This deviation is probably due to the fact that the value presented in literature was only determined with water contents from $R_W = 15$ to $R_W = 28$ [13]. The differences in

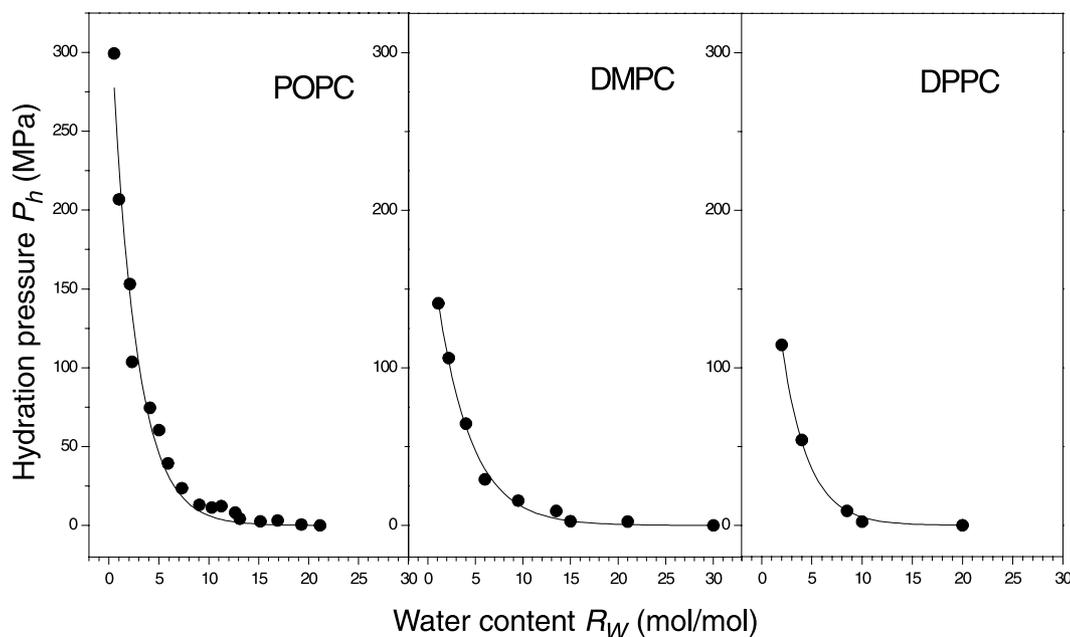


Fig. 3. Hydration pressure of different lipids determined by Eq. (9). The symbols are connected by the fitting line according to a single-order exponential decay.

Table 1

Parameter of hydration pressure determined by the isopiestic thermotropic method (DSC, Eq. (9)), by the osmotic stress method (OSM) and by nuclear magnetic resonance spectroscopy (NMR); (a) [6], (b) [11], (c) [12], (d) [4], (e) [1], (f) [15], (g) [16]

Lipid	dT/dp (K/MPa)	$\ln P_{h,0}$ (MPa)	$R_{W,0}$
POPC	0.175 ± 0.01	19.64 _(DSC)	2.5 ± 0.2 _(DSC)
		19.56 _(OSM, b)	$2.5 \pm$
		18.82 _(2H-NMR, f)	$0.2(L_{m} + L'_{m}, 25 \text{ }^\circ\text{C}, b)$ 4.2 ± 0.4 _(NMR, f)
DMPC	0.201 ± 0.01	19.08 _(DSC)	3.0 _(L'_{m}, OSM, 27 \text{ }^\circ\text{C}, c)
DPPC	0.22 ± 0.1 _(a)	18.56 _(DSC)	2.6 ± 0.1 _(DSC)
		19.87 _(OSM, 25 \text{ }^\circ\text{C}, b)	1.8 _(L'_{m}, OSM, 25 \text{ }^\circ\text{C}, c)
		20.21 _(OSM, 50 \text{ }^\circ\text{C}, b)	3.4 _(L'_{m}, OSM, 50 \text{ }^\circ\text{C}, c)
DOPC	0.10 _(a)	19.30 _(DSC)	3.6 ± 0.4 _(DSC)
		19.52 _(OSM, g)	3.3 _(OSM, g)
		19.00 _(c)	4.0 _(c)
		19.61 _(DSC, e)	2.8 ± 0.4 _(DSC, e)
DPPE-Me ₂	0.20 _(estimation)	19.38 _(2H-NMR, f)	4.5 ± 0.2 _(2H-NMR, f)
		19.41 _(DSC)	3.0 ± 0.1 _(DSC)
		19.70 _(OSM, 20 \text{ }^\circ\text{C}, d)	

The second column gives the pressure-induced temperature increase used for Eq. (9).

decay constants teach us that there is a principal difference between the isothermal and the thermotropic method. Further work is required to understand and interpret these differences in terms of the hydration states in phase equilibria.

Phospholipid phase transitions broaden during dehydration. This effect is not explicitly taken into consideration in our approach. The broadening is sometimes even accompanied by the occurrence of multiple overlapping transition peaks and this question was already raised by Simon et al. [4]. However, this broadening has only little effect on the results because it is small compared to the range of the hydration-induced temperature shift.

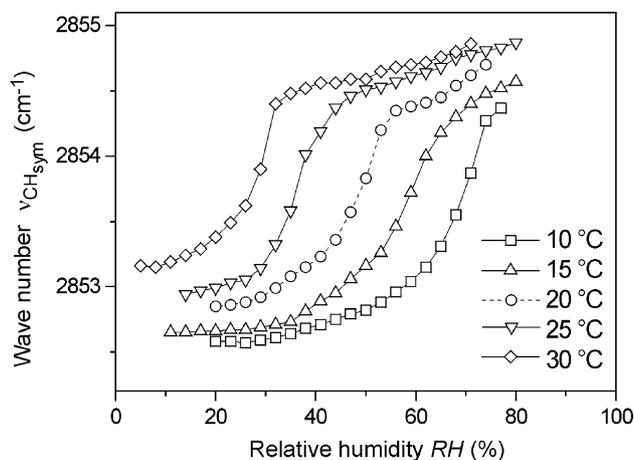


Fig. 4. Lyotropic main phase transition of POPC at normal pressure as a function of humidity and temperature determined by FT-IR spectroscopy.

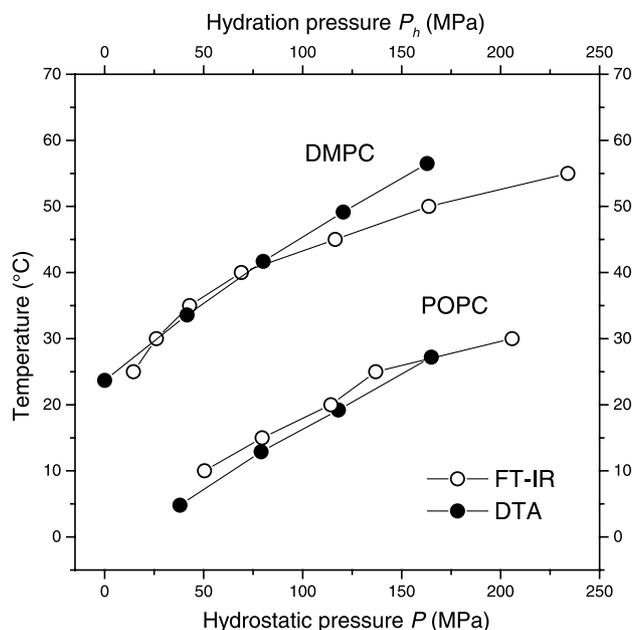


Fig. 5. Phase transition temperature of DMPC and POPC as a function of hydration pressure (open circles) and hydrostatic pressure (solid circles).

For a further test of Eq. (9), FT-IR and DTA measurements were performed. By using FT-IR spectroscopy, the lyotropic phase transitions of POPC and DMPC were recorded as a function of humidity at isothermal conditions and at different constant temperatures (Fig. 4). Using the humidity, RH, one can easily calculate the hydration pressure: $P_h = -RT/V_W \ln(RH/100)$ [5], where R is the gas constant, T the absolute temperature and V_W the molar volume of water. The thermotropic transitions as a function of hydrostatic pressure were determined by DTA by using fully hydrated dispersions of POPC and DMPC. Thus, the main phase transition temperature depending on hydrostatic pressure and hydration pressure is given by Fig. 5. The slope shows that the pressure-induced temperature increase α is the same for hydrostatic pressure and for hydration pressure. Furthermore, the results correspond to the behaviour predicted by Eq. (9). Only at very high hydration pressure (>120 MPa), the results for DMPC start to deviate. At this hydration pressure, the water content is about $R_W = 1$ and then, the molar water volume starts to deviate substantially from the bulk property [14]. In this case, the water potential is overestimated. The same occurs for POPC, but at higher hydration pressure (≈ 200 MPa $\leftrightarrow R_W \approx 1$). This deviation establishes the limit of the new method. However, for water contents of $R_W > 1$, the present approach yields true hydration pressure parameters.

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