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Changes in the Area per Lipid Molecule by P–V–T and SANS Investigations

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Summary: This paper describes the study of lipid DPPC, carried out on setup for simultaneous investigations by P–V–T and SANS methods. The data allowed us to calculate in area per lipid molecule changes during the lipid phase transition from ripple phase to liquid-crystalline phase.

Keywords: phase transition; phospholipids; SANS

Introduction

For the lipid systems investigations on small angle neutron scattering spectrometer YuMO[1,2] at IBR-2M reactor (Dubna, Russia) we created setup for simultaneously P–V–T and small angle neutron scattering (SANS) studies.[3] This setup allowed us to obtain the structural parameters of the samples, and the thermodynamic characteristics in the same time. Here we would like to present the calculation of value change of lipid membrane surface area based on our experiments.

Experimental Part

We studied the fluid system DPPC/D_2O (10% wt) at 50°C in the pressure range (10÷400) bar (Figure 1). As a result we obtained small angle scattering curves and P–V–T data of DPPC/D_2O system.

Fitting of curves[4] allowed us to obtain pressure dependences of DPPC/D_2O repetition period and isothermal compressibility (Figure 2). Change of the repetition period and the isothermal compressibility of the system allows us to determine the pressure at which lipid phase transition from ripple to liquid crystalline phase performs.

Calculations

To calculate the changes of the area per one lipid molecule during phase transition from ripple-phase to liquid crystalline phase, we consider the model of lipid molecule which is shown in Figure 3. In this model we assume the volume occupied by lipid molecule as a cylinder.

Within this model, the volume per one molecule of lipid, can be written as

$$V_{mol} = S_i \cdot L_i, \qquad (1)$$

where S_i - area per one molecule in the lateral direction, L_i - height of lipid molecule in the i phase.

Denote V_{mol1} volume per one molecule in ripple phase, a V_{mol2} – in the liquid crystalline phase.

Then the difference of volumes can be written as:

$$V_{mol2} - V_{mol1} = S_2 \cdot L_2 - S_1 \cdot L_1 \qquad (2)$$

Thus, the length of the lipid tails in the liquid crystalline phase is less than in

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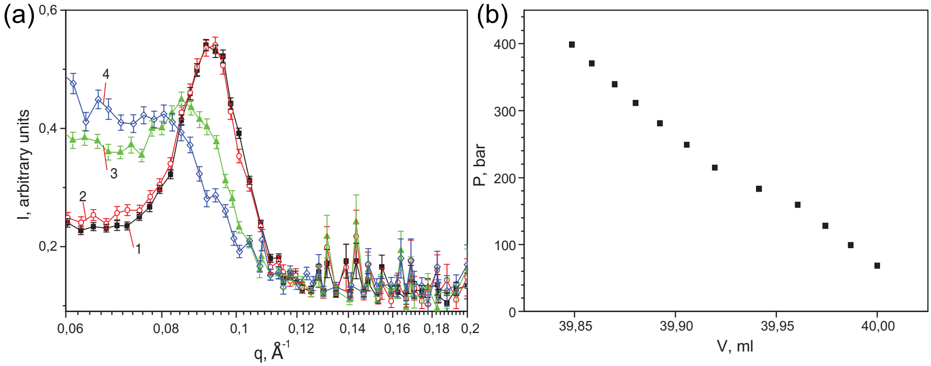

Figure 1.
Simultaneously P–V–T and SANS investigations of liquid system DPPC/D_2O at 50°C: a) small-angle neutron scattering under pressure 40bar (1), 100bar (2), 190bar (3), 340bar (4); b) pressure as a function of volume.

ripple-phase:

$$L_1 > L_2 \tag{3}$$

Transform the equation (2) as:

$$\frac{V_2 - V_1}{N} = S_2 \cdot L_2 - S_1 \cdot L_1, \tag{4}$$

where N - number of lipid molecules contained in the investigated sample volume, V_1,V_2 - volume of lipid in the ripple-phase and in the liquid crystalline phase, respectively.

To the right side of the equation and add subtract expression $S_1 \cdot L_2$

$$\begin{aligned} & S_2 \cdot L_2 - S_1 \cdot L_1 \\ & = S_2 \cdot L_2 - S_1 \cdot L_2 - S_1 \cdot L_1 + S_1 \cdot L_2 \\ & = L_2(S_2 - S_1) - S_1(L_1 - L_2) \end{aligned} \tag{5}$$

In[5] the authors showed that the transition from the gel phase to ripple-phase doesn't change volume per one molecule of

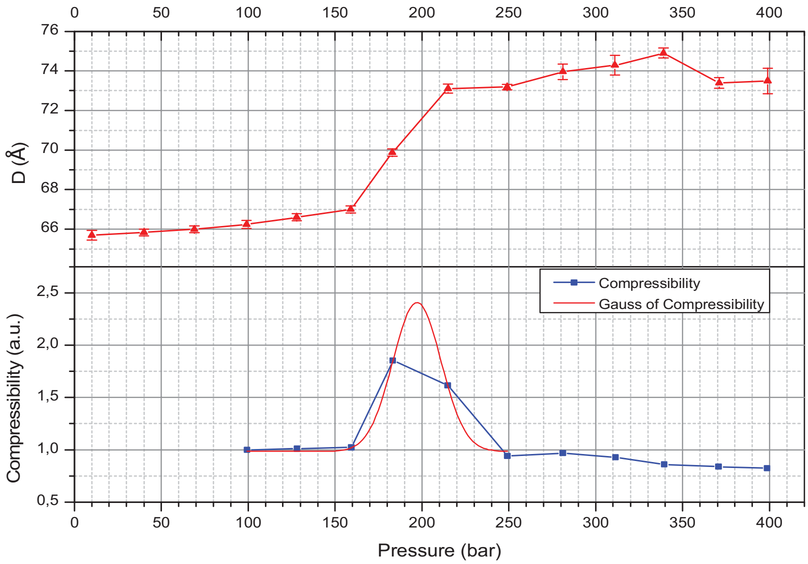

Figure 2.
The DPPC main phase transition at 50°C and pressure from 400 to 10 bar range. a) repetition period vs pressure and b) Isothermal compressibility as the function of pressure.

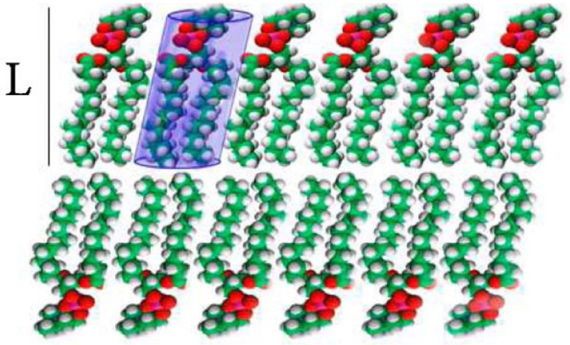

Figure 3.
Cylindrical model of lipid molecule.

lipid. Also during this transition conformational changes of lipid tails is negligible. So we make the assumption that the area per one lipid molecule in lateral direction does not change. In[6] the method of molecular dynamics allowed authors to estimate area per one lipid molecule DPPC in the gel phase. This value was $S_0 = 65 Å^2$. We'll use this value for further calculations.

Substituting (5) in (6), and writing S_0 instead of S_1, we get the expression for the difference of squares per one lipid molecule in ripple-phase and in the liquid crystalline phase:

$$S_2 - S_1 = \frac{\frac{V_2 - V_1}{N} + S_0(L_1 - L_2)}{L_2} \tag{6}$$

Lipid mass in our experiment was $m = 0{,}3\,g$ and lipid molar mass was $M = 734,043 \frac{g}{mol}$. So, we can calculate the number of molecules in our sample:

$$N = \frac{m}{M} \cdot N_A = \frac{0,3 \cdot 6,02 \cdot 10^{24}}{734,043} = 2,46 \cdot 10^{21}. \tag{7}$$

For the calculation of the lipid molecule length we'll use the Guinie approach for flat particles.[7] To do this we construct the dependence $\ln(q^2 I(q))$ on q^2. In the range of small q this dependence is described by a linear function:

$$\ln(q^2 I(q)) = \ln(I(0)) - q^2 R_t^2, \tag{8}$$

To find the lipid molecule length we'll use the formula for flat particles:

$$L = \frac{1}{2}\sqrt{12 R_t^2}. \tag{9}$$

Coefficient $\frac{1}{2}$ appeared because lipid bilayer consists of two lipid monolayers, and its thickness is twice the length of the lipid.

Performing construction (8) for SANS curves at pressures 30 bar and 510 bar, calculate lipid molecule lengths in the liquid crystalline phase (L_2) and ripple phase (L_1):

$$\begin{aligned} L_1 &= 25,8 \pm 0,4\mathring{A} \\ L_2 &= 23,6 \pm 0,4\mathring{A} \end{aligned}.$$

Analysis of the curve at Figure 1b shows that the inflection point on the curve is associated with changes in the lipid volume, because D_2O pressure dependence of the volume in this pressure range has no inflection points. Therefore, to find the volume of lipid in ripple-phase and in the liquid crystalline phase, we must differentiate curve at Figure 1b by volume (Figure 4).

Substitute all the values into formula (6):

$$\begin{aligned} S_2 - S_1 &= \frac{\frac{V_2 - V_1}{N} + S_0(L_1 - L_2)}{L_2} \\ &= \frac{\frac{(39,963323 - 39,917477)\cdot 10^{24}\mathring{A}^3}{2,46\cdot 10^{21}} + 65\mathring{A}^2(25,8\mathring{A} - 23,6\mathring{A})}{23,6\mathring{A}} \\ &\approx 6\mathring{A}^2 \end{aligned}$$

Therefore, the phase transition in DPPC/D_2O liquid system from ripple-phase to liquid crystalline phase increases the

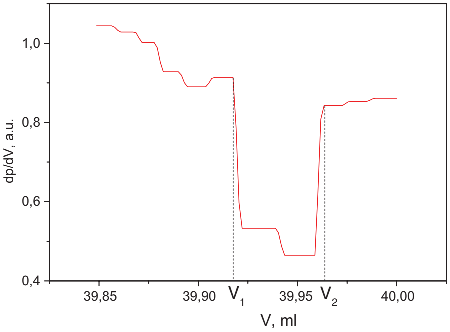

Figure 4.
dp/dV as a function of volume for liquid system DPPC/D_2O at 50°C. Volume $V_1 = 39,917477 \pm 0,000002\,ml$ corresponds to the volume of lipid in the ripple phase, and $V_2 = 39,963323 \pm 0,000002\,ml$ - in liquid crystalline phase.

area per one lipid molecule by $6\,\text{Å}^2$. Interesting that the molecular dynamic computer simulation[8] of DPPC lipid bilayer at 52°C (liquid crystalline phase) gives us the value of the area per lipid molecule $59\,\text{Å}^2$. We used in our calculations the area per lipid molecule in ripple-phase $S = 65\text{Å}^2$[6] and got the decreasing in the area equal to $6\,\text{Å}^2$, that's why results of our calculations are consistent with the computer simulation.[8]

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