Structure and phase transitions of the multilamellar DMPC membranes in presence of the DMSO and DESO

Yu E Gorshkova[1], O I Ivankov[1,2,3]

[1] Joint Institute for Nuclear Research, Dubna, Russia
[2] Moscow Institute of Physics and Technology, Dolgoprudny, Russia
[3] Institute for Safety Problems of Nuclear Power Plants, Chornobyl, Ukraine

E-mail: gorshk@nf.jinr.ru

Abstract. The structure and phase transitions of the prepared and formed spontaneously multilamellar vesicles (MLVs) of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) in dimethyl sulfoxide (DMSO) and diethyl sulfoxide (DESO) were investigated using small angle neutron scattering (SANS). The both polar aprotic solvents increase the temperature of the main phase transition (T_m). The pre-transition does not observed at sulfoxides mole fraction $X = 0.2$. The transition of the MLVs DMPC in the presence DMSO from gel to liquid-crystalline phase occurs at lower temperature. The method of the MLVs preparation has directly effects on the temperature of the main phase transition and its structure. The value of T_m is higher with ~ 4.6 ºC in case of the spontaneous forming MLVs from extruded ULVs. The thickness of the solvent layer for prepared MLVs is less by 4.0 Å in gel phase and by 5.6 Å in liquid-crystalline phase than the thickness of the solvent layer for spontaneously formed MLVs.

1. Introduction

The lipid bilayer forms the structural framework of cell membranes, which play an important role in many life processes. The lipid bilayer of the phospholipid membranes is constructed from amphiphilic molecules like phosphatidylcholine (PC) having polar-head groups and two non-polar hydrocarbon tails. The presence of the ions, small molecules and solvents affect the structure and phase behaviour of the lipid bilayer as well as do the temperature, pressure, and pH. For example, anesthetics, short alcohols, and carbohydrates reduce the phase transition temperature .On the contrary, the extension of non-polar lipids chains, external pressure, calcium ions, long alcohols, and hydrocarbons increase the phase transition temperature [1].

It is well known that neutral PC membranes under the excess of water condition exhibit two thermotropic lamellar phase transitions [2]: a gel to gel ($L_{\beta'} \rightarrow P_{\beta'}$) pre-transition and a gel to liquid-crystalline ($P_{\beta'} \rightarrow L_{\alpha}$) main transition can be observed with temperature increase. The main phase transition is always accompanied by melting of the hydrocarbon tails. From this point of view, it is not a surprise that the thickness of the lipid bilayer becomes smaller. The detailed analysis of the structure parameters for pure 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) membranes in liquid-crystalline phase, using X-ray small-angle scattering, was done, for example, in [3]. Besides, a partial lipid volume (V_l) of DMPC bilayers is increased by 3 % in the main transition region as it was noticed from densimetric measurements (DM) [4]. At the same time, the enthalpy is modified as it was shown

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by differential scanning calorimetry (DSC) [5, 6]. The change in enthalpy and volume of the lipid molecules pointed out on the first-order phase transition.

In addition to the above mentioned methods, the density dilatometry [7, 8], electron spin resonance (ESR) [9, 10], permeability and fluorescence polarization [11], nuclear magnetic resonance (NMR) [12], small angle X-ray scattering (SAXS) [13] have been extensively used for identification of the lipid phases. However, diffraction methods have the advantage since they allow, in particular, to obtain information on the molecular packing [14].

In the current work, we present a small angle neutron scattering (SANS) investigation of the phase transition of the fully hydrated MLVs DMPC (2 wt %) dissolved in DMSO or DESO. The interest for these polar aprotic solvents is caused by several reasons. First, the distance between two planar lipid layers for uncharged PC membranes is determined by the superposition of the forces: long-range van der Waals attractive force, long-range undulation repulsion, and short-range repulsion («hydration») force [15]. In addition, the hydrophobic interactions can make a significant contribution to balance of the intermembrane interaction in the sulfoxides presence as it was proposed in [16]. Secondly, DMSO and DESO cause a fusion of the unilamellar vesicles (ULVs) DMPC as it was reported in previous work [17]. From this point of view, the above mentioned sulfoxides can be used as good agents for directional cell fusion *in vitro* with applications in biomedicine and biotechnology.

2. Materials and methods

2.1. Materials

14:0 PC (DMPC) – 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine ($C_{36}H_{72}NO_8P$) were purchased from the Avanti (Birmingham, England). All lipids were used without further purification. DMSO ($(CH_3)_2SO$) and DESO ($(C_2H_5)_2SO$) over 99 % purity, were purchased from the JSC “Reachim” (Moscow, Russia). Heavy water (99.8 % D_2O) was from JSC “Isotop” (St. Petersburg, Russia).

2.2. Sample preparation

2.2.1. MLVs preparation. The lipid was dissolved in DMSO/D_2O or DESO/D_2O solution at predetermined mole fractions X_{DMSO} or X_{DESO} (X is mole fraction of sulfoxide in sulfoxide/water mixture) and mixed by a shaker. A homogeneous multilamellar vesicles (MLVs) were obtained by the freezing-thawing membranes in the range from – 80 ºC to + 55 ºC. The procedure was repeated several times. The final lipid concentration (C_l) for all prepared samples was 2 wt %.

2.2.2. Spontaneously formed MLVs. Large unilamellar vesicles (ULVs) were prepared by passing the MLVs of DMPC through two polycarbonate filters with 100 nm pores in diameter (Hamilton, Reno, Nevada, United States) of an extruder (Avanti, USA). The extrusion of the suspension was performed at the temperature above the temperature of the main phase transition (T_m). After 25 cycles we obtained unilamellar vesicles with average diameter determined by the size of the filter pores. An odd number of passages was chosen to avoid the MLVs, which might not penetrate through the filters, in the samples.

The vesicles in pure D_2O were stable for a sufficiently long time. The spontaneous MLVs formation at $X = 0.2$ was observed according to [17].

2.3. SANS technique

The phase transitions of the fully hydrated phospholipid membranes in the DMSO and DESO presence was investigated using small angle neutron scattering. The experiments were performed at YuMO time-of-flight spectrometer at the IBR-2 pulsed reactor (Dubna, Moscow region, Russia). The data were collected in two-detector configuration [18]. It gives a possibility to measure samples, twice faster, in the q-range of 0.007 - 0.4 $Å^{-1}$.

The raw data treatment was done using the SAS program [19]. The final small-angle neutron scattering curves are presented in the absolute scale with the background subtraction [20].

The studied samples were placed in 1 mm thick quartz cells (Hellma, Germany). During data collection, the samples were put in the temperature controlled holder (±0.2 ºC) connected to the liquid thermostat (Lauda, Germany) in the range of the temperature from 10 ºC to 60 ºC. The waiting time between two temperature points was 15 min. The standard data acquisition time per sample was 30 min. The measurements were performed 12 hours after samples preparation.

3. Results and discussion

The first structural and phase transition investigations of the model phospholipid membrane (≥ 20 wt %) in the DESO presence were done by DSC [16] and X-Ray [21]. It was shown that the repeat distance (D) of the MLVs decreases and the temperature of the main phase transition (T_m) grows with increasing of the sulfoxide concentration. The similar effect was observed for MLVs DMPC at smaller lipid concentration (C_l).

In this paper we present the results for sulfoxides mole fraction, X = 0.2, which is nonrandom value. First, the dramatic changes in the structure of the PC membranes in excess solution take place at smaller DMSO mole fraction as it was shown in our previous work [22]. At the same time, at this concentration the fusion of the ULVs has been observed [17].

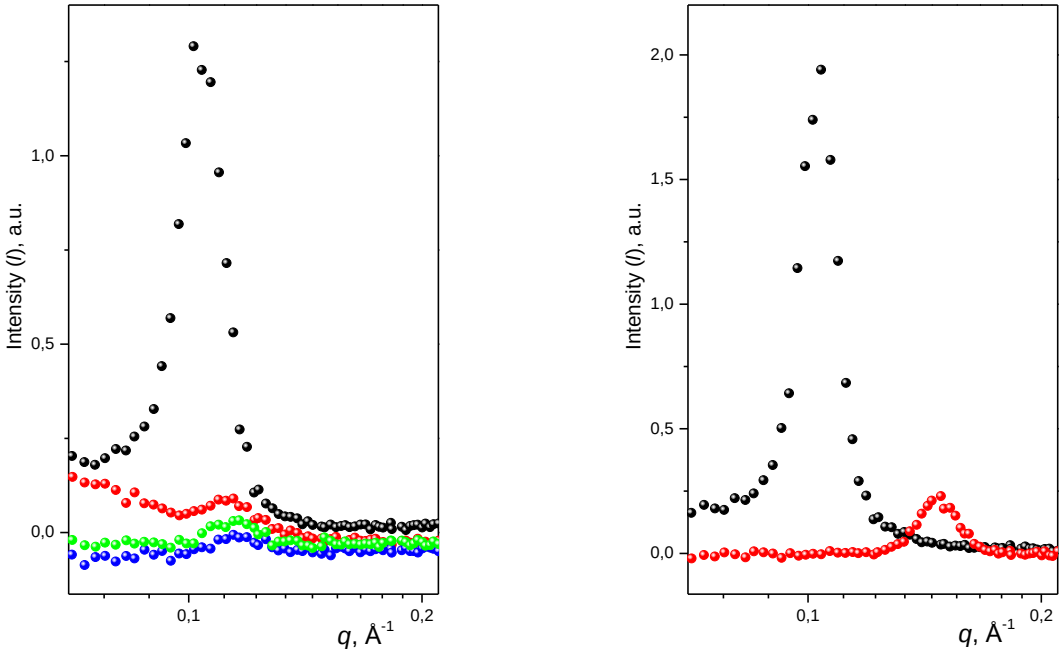


Figure 1. SANS curves for MLVs DMPC (2 wt %) in DESO/D_2O solution at different DESO mole fraction: X_{DESO} = 0.0 (black), 0.2 (red), 0.4 (blue) and 0.8 (green) in gel phase at T = 14 ºC (a) and in liquid phase at T = 64 ºC (b).

The investigations of the influence of the DESO mole fraction on the MLVs DMPC in excess solution condition in gel and liquid-crystal phases are presented in Figure 1. The decrease in the SANS curve intensity is caused by the reduction of the deuterium in the DESO/D_2O solution with X_{DESO} increase. Nevertheless, the obtaining of the D-values from the SANS spectra is possible. The repeat distance is calculated from the position of the diffraction peak q_0 at the intensity maximum using a relationship $D = 2\pi/q_0$. The D-value is a sum of two terms: a thickness of the lipid bilayer (D_b) and a thickness of the solvent layer – distance between two planar lipid layers (D_s). The parameter D is

collected in Table 1 for MLVs DMPC (2 wt %) at the DESO mole fraction from 0.0 to 0.8. It is clear seen that the considerable decreasing of the repeat distance for MLVs DMPC from 60.6 ± 0.1 Å in pure D_2O to 56.9 ± 0.2 Å at X_{DESO} = 0.2 are observed in gel phase. At the same region of DESO mole fraction the repeat distance decreases more significantly from 60.9 ± 0.1 Å to 42.6 ± 0.1 Å in the liquid-crystalline phase. The future increasing of the DESO mole fraction in DESO/D_2O solution in the region $0.3 \geq X_{DESO} \geq 0.8$ does not effect on the *D*-value in gel phase.

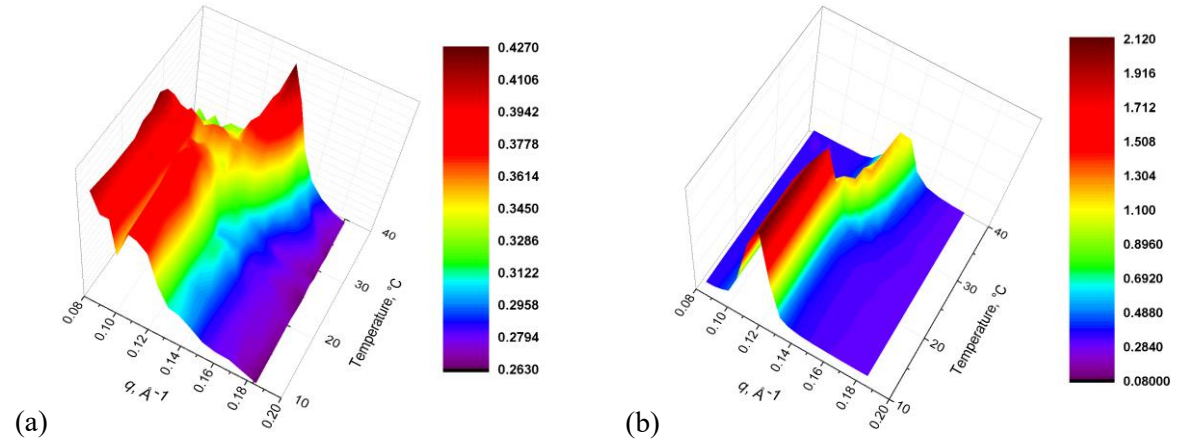
Table 1. The repeat distance (*D*) of the MLVs DMPC (2 wt %) in dependence on the DESO mole fraction (X_{DESO}) in gel phase at T = 14 °C.

X_{DESO}	0.0	0.2	0.3	0.4	0.5	0.7	0.8
***D* (Å)**	60.6±0.1	56.9±0.2	54.1±0.4	54.0±0.4	54.5±0.2	54.7±0.3	54.9±0.3

3.1. MLVs DMPC

The SANS spectra of the prepared MLVs DMPC (2 wt %) (a) and MLVs DMPC (20 wt %) (b) in DESO/D_2O solution at X_{DESO} = 0.2 are shown in Figure 2. The measurements were carried out in the temperature range 10 ºC ≤ T ≤ 40 ºC. The reduction in the lipid fraction of the samples resulted in a decrease in intensity of the scattering curves for MLVs DMPC (2 wt %). Nevertheless, the behaviour of the phase transitions is similar and two phase transitions were observed for both cases during heating at the same temperature.

Additionally, the changes on the membrane's structure during phase transitions can be observed using SANS technique. The dependences of the repeat distance on the temperature are presented in Figure 3 for MLVs DMPC (2 wt %) (a) and MLVs DMPC (20 wt %) (b) in DESO/D_2O at X_{DESO} = 0.2. Analysis of the data indicates that the *D*-value does not change in the gel phase and is equal to 55.5 ± 0.4 Å for C_l = 2 wt % and 53.8 ± 0.2 Å for C_l = 20 wt %. Further increase of the temperature led to decrease of the repeat distance for both lipid concentrations.



(a) (b)

Figure 2. SANS spectra of the prepared MLVs DMPC (2 wt %) (a) and MLVs DMPC (20 wt %) (b) in DESO/D_2O at X_{DESO} = 0.2 with scales of the intensities.

The T_m values were obtained in two ways. One of them is to use the Boltzmann function for fitting experimental data as shown in Figure 3. Besides, the first derivatives, d*D*/d*T* (Figure 3, insets) can be successfully used for determination of the temperature of the main phase transition. Without going into the details, the following results were obtained. The transition from gel to liquid-crystal phase is observed at T_m = 28.9 ºC for C_l = 2 wt %. Increasing the lipid concentration does not change the temperature of the main phase transition within the experimental errors. Thus, the transition is taking

place at T_m = 28.4 ºC for sample with C_l = 20 wt %. This is in a good agreement with DSC data for MLVs DMPC (20 wt %) [16].

Thus, we can conclude that the temperature of the main phase transition of the MLVs DMPC in the presence of DESO does not depend on the lipid concentration for $C_l \leq 20$ wt. The situation is similar as for PC membranes in excess water [23].

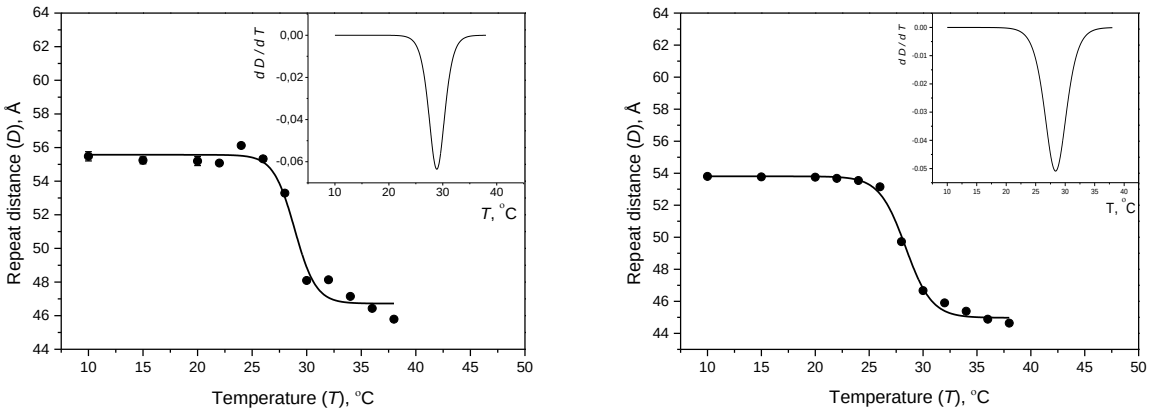


Figure 3. Repeat distance vs. temperature for prepared MLVs DMPC (2 wt %) (a) and MLVs DMPC (20 wt %) (b) in DESO/D_2O at X_{DESO} = 0.2. Dots are the experimental data and lines are the fits by the Boltzmann function.

3.2. Spontaneously formed MLVs

The MLVs can be formed spontaneously from ULVs in the sulfoxides presence [17]. Here, we present the results for MLVs DMPC (2 wt %) produced by extruded ULVs in sulfoxide/water solutions at X = 0.2. The SANS spectra with intensity scales are shown in Figure 4 for X_{DMSO} = 0.2 (a) and X_{DESO} = 0.2 (b). It is clear that both sulfoxides induce the increasing of the temperature of the phase transitions. The transitions $L_{\beta'} \rightarrow P_{\beta'}$ and $P_{\beta'} \rightarrow L_{\alpha}$ were observed for MLVs prepared in the DESO/D_2O solution. However, for the system DMPC/DMSO/D_2O the pre-transition from gel to ripple phase disappeared and MLVs have the abrupt transition $L_{\beta'} \rightarrow L_{\alpha}$ only, as can be clearly seen in Figure 4 (a).

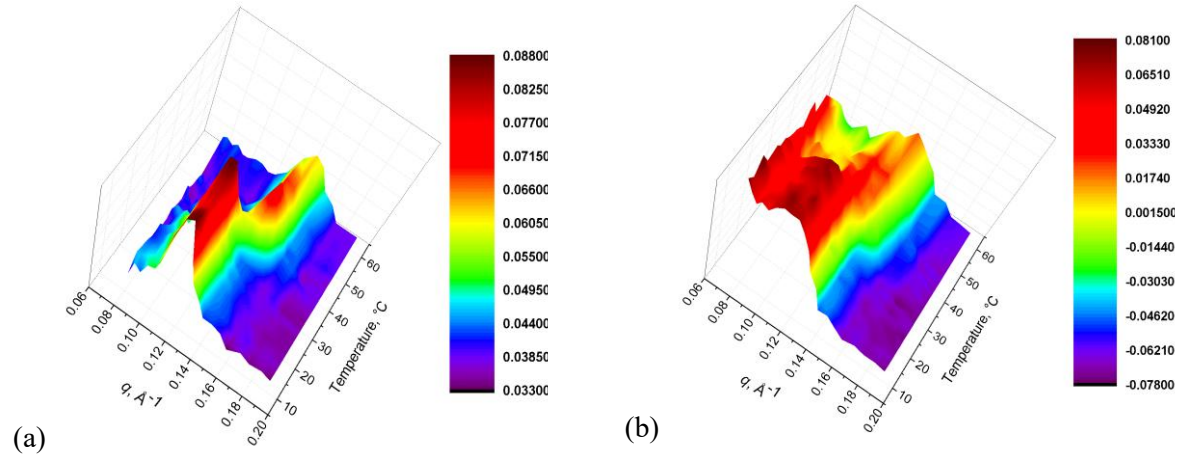


Figure 4. SANS spectra of the spontaneous MLVs DMPC (2 wt %) in DMSO/D_2O at X_{DMSO} = 0.2 (a) and in DESO/D_2O at X_{DESO} = 0.2 (b) with scales of the intensities.

The transition temperatures were calculated as it was described in the previous section. As a result we obtained the following values: T_m = 35.2 ºC for MLVs DMPC in DMSO/D_2O and T_m = 33.6 ºC for MLVs DMPC in DESO/D_2O. It should be noted that the temperatures of the main phase transitions are higher with ~ 4.6 ºC in case of the spontaneous forming MLVs from extruded ULVs.

Additionally, we estimated the region of the temperatures in which transition $L_{\beta'} \rightarrow L_{\alpha}$ was observed. With this purpose we analyzed the dependence of the first derivative, dD/dT *vs.* T (Figure 5, insets) with the background subtraction, dD/dT = 0. It turns out that the transition of the MLVs DMPC in the presence of DMSO from gel to liquid-crystal phase occurs in a narrow temperature range. It begins at T = 28 ºC and ends at T = 42 ºC, while for MLVs dissolved in DESO/D_2O, this transition is observed in the temperature range 20.6 ºC ≤ T ≤ 47.3 ºC.

The analysis of the dependence of the repeat distance *vs.* temperature for spontaneous MLVs DMPC in the sulfoxide/water solvent was done in the wide range of the T from 10 to 60 ºC. The experimental data (dots) with fits by the Boltzmann function (lines) are presented in Figure 5 for MLVs DMPC (2 wt %) in DMSO/D_2O at X_{DMSO} = 0.2 and in DESO/D_2O at X_{DESO} = 0.2. It is clear that the D-values are constant in the gel phase. The repeat distance is equal to 57.5 ± 0.4 Å for DMPC/DMSO/D_2O system. The average magnitude of D is 59.5 ± 0.3 Å for spontaneous MLVs DMPC dissolved in DESO/D_2O. The difference in the repeat distances in the liquid-crystalline phase is much greater. For example, the D = 45.0 ± 0.2 Å and D = 50.3 ± 0.1 Å at T = 50 ºC for MLVs DMPC in DMSO/D_2O and DESO/D_2O, respectively.

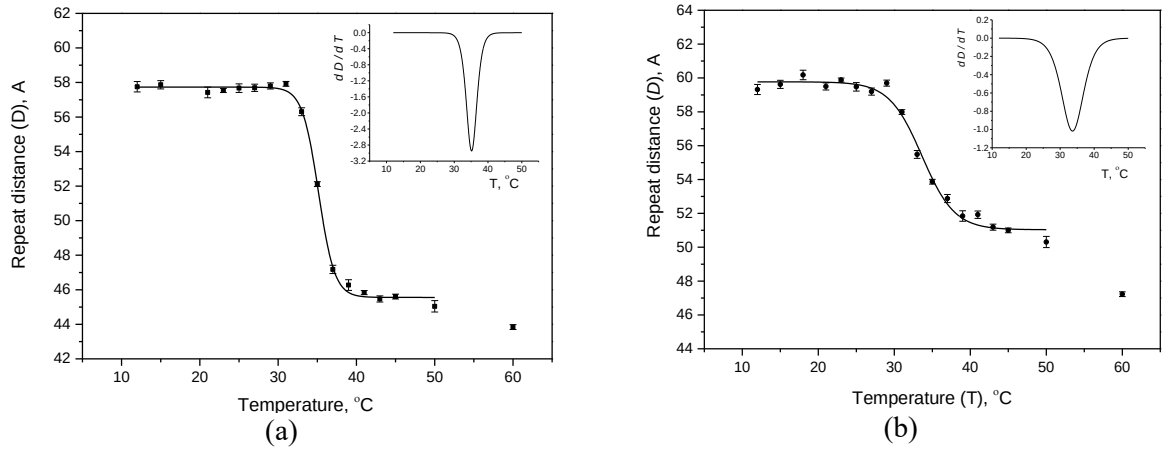


Figure 5. Repeat distance vs. temperature for spontaneous MLVs DMPC (2 wt %) in DMSO/D_2O at X_{DMSO} = 0.2 (a) and in DESO/D_2O at X_{DESO} = 0.2 (b). Dots are the experimental data and lines are the fits by the Boltzmann function.

3.3. Distance between two planar layers

It is clear that the conditions of the sample preparation affect significantly the structure and phase behavior of the lipid membranes. The T_m is shifted by 4.6 ºC and the repeat distance also depends on the type of the samples. Thus, the three regions in D-value can be selected for MLVs DMPC (2 wt %) in DESO/D_2O solution in the range of the investigated temperatures as presented in Figure 6. The region I (yellow) corresponds to the gel phase for both systems. The repeat distances are constant for prepared and spontaneously formed MLVs DMPC in this case. Nevertheless, the difference of 4 Å in D-values was calculated for these samples. The second region (II) represents the transition area between $L_{\beta'}$ and L_{α} phases. Here, the structure of the lipid molecules dramatically changes. The melting of the hydrocarbon tails led to reduction of the repeat distance of the DMPC membranes from 59.5 ± 0.2 Å to 53.1 ± 0.1 Å and from 55.5 ± 0.1 Å to 47.6 ± 0.1 Å for spontaneous formed and

prepared MLVs, respectively. Finally, the region III (green) matches the liquid-crystalline phase. All experimental points are parallel shifted by 5.6 Å as it is clearly seen in Figure 6. Thus, we obtain the D = 41.6 Å for prepared MLVs using extrapolation (blue line) at T = 55 ºC.

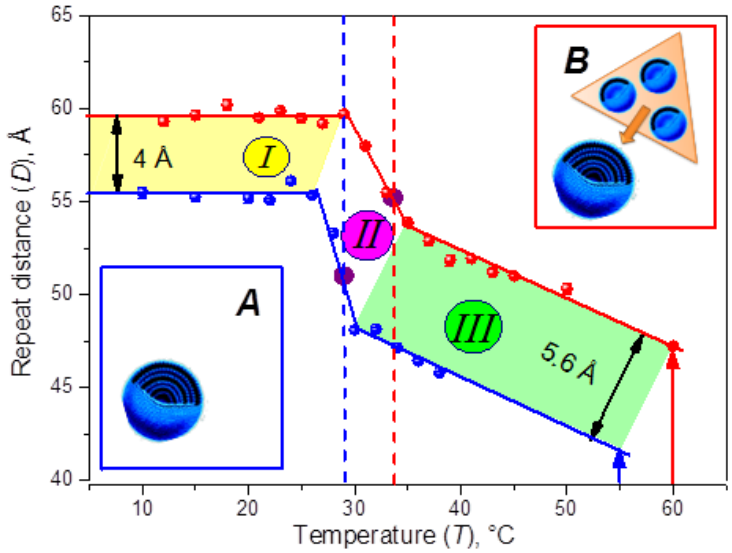


Figure 6. Repeat distance vs. temperature for MLVs DMPC (2 wt %) in DESO/D_2O at X_{DMSO} = 0.2. The blue dots correspond to prepared MLVs (schematically presented in inset A) and red dots correspond to spontaneously formed MLVs from ULVs (schematically presented in inset B). The red and blue vertical dashed lines indicate the phase transition temperatures for the corresponding samples.

Taking into account that the bilayer thicknesses (D_b) of the DMPC (2 wt %) are the same for prepared and spontaneously formed MLVs and equal to 37.7 Å in $L_{\beta'}$ phase at T = 12 °C and 26.5 Å in L_α phase at T = 55 °C [17], we calculated the distance between two planar layers (thickness of the solvent D_s) using relationship $D_s = D - D_b$. The results are presented in Table 2.

The data analysis has shown that the thickness of the solvent layer decreases with increasing of the temperature. However, it should be noted, that the reduction of the D_s-value is significant for the prepared MLVs. The distance between two planar layers decreases by 2.7 Å, while the changing of this value equal to 1.1 Å for spontaneously formed MLVs. It is important to note that the thickness of the solvent layer for the prepared MLVs is less by 4.0 Å in $L_{\beta'}$ phase and by 5.6 Å in L_α phase. This fact can be explained by the different interactions of the DESO molecules with polar heads of the lipid membranes. We can suppose that the number of the DESO molecules binding with D_2O molecules is higher in the case when the MLVs DMPC are spontaneously formed. However, this hypothesis should be proved in future investigations using contrast variation method.

Table 2. Structure parameters for the prepared and spontaneously formed MLVs DMPC (2 wt %) at X_{DESO} = 0.2 in gel $L_{\beta'}$ phase at T = 12 °C and in liquid-crystalline L_α phase at T = 55 °C.

	Structure parameters in $L_{\beta'}$ phase			Structure parameters in L_α phase		
	D(Å)	D_b (Å)	D_s (Å)	D (Å)	D_b (Å)	D_s (Å)
Prepared MLVs	55.5±0.1	37.7±0.3	17.8±0.2	41.6±0.1	26.5±0.3	15.1±0.2
Spontaneously formed MLVs	59.5±0.2	37.7±0.3	21.8±0.3	47.2±0.1	26.5±0.3	20.7±0.2

4. Conclusions

We can conclude that the intermembrane interaction of the PC membranes in the presence of DESO is similar as in case of the DMSO. First, the both polar aprotic solvents increase the temperature of the main phase transition. Nevertheless, the transition of the MLVs DMPC in the presence of DMSO from gel to liquid-crystalline phase occurs at lower temperature. Secondly, the dramatic changes in the structure of the PC membranes in excess solution take place at smaller DMSO and DESO mole fraction. The increasing of the temperature beyond T_m led to a linear decreasing of the repeat distance of the MLVs in investigated range of the temperatures. The study of the samples with different lipid concentration pointed out on non-dependent PC membrane – DESO interaction for the $C_l \leq 20$ wt %.

The method of the MLVs preparation directly affects the temperature of the main phase transition and its structure. The temperature of the main phase transition is higher with ~ 4.6 ºC in case of the spontaneous forming MLVs from extruded ULVs. The thickness of the solvent layer for prepared MLVs is less by 4.0 Å in gel phase and by 5.6 Å in liquid-crystalline phase than the thickness of the solvent layer for spontaneously formed MLVs.

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