Fusion of unilamellar DMPC vesicles in presence of the sulfoxides: effect of time and concentration

YU. E. GORSHKOVA*
Joint Institute for Nuclear Research, Dubna, Russia

The influence of the dimethyl sulfoxide (DMSO) and diethyl sulfoxide (DESO) on the structure of the unilamellar vesicles (ULVs) DMPC was investigated by small angle neutron scattering (SANS). The studied values of the DESO and DMSO concentrations covered a range $0.0 \le X \le 0.4$ (X – mole fraction of sulfoxide in sulfoxide/water mixture). It was shown that the ULVs are able to form multilamellar vesicles (MLVs) spontaneously in the presence of the sulfoxides without the application of external forces. Fusion of ULVs DMPC in presence of the sulfoxides depends on two factors: time and concentration. The ULVs exhibit long term stability for concentrations up to X=0.1. The spontaneous formation MLVs was observed at X = 0.2 in the presence of the both sulfoxides. However, DESO causes the fusion of the ULVs about 1/2 hour after samples preparation, while this process occurs within an hour in the presence of DMSO. Moreover, the investigation in short-term time scale shown that formation of the MLVs take place at X_{DESO} = 0.3 and X_{DMSO} = 0.4. Additionally, the dependence of lipid bilayer thickness on the mole fraction X_{DESO} in the gel and liquid-crystalline phases was determined. It turned out that lipid bilayer thickness decreases linearly with increasing of the DESO mole fraction up to 0.2 in both phases.

(Received August 17, 2015; accepted September 9, 2015)

Keywords: Unilamellar vesicles, Sulfoxides, SANS

1. Introduction

One of the most important functions of biological membranes is its ability to separate the cell from the extracellular environment. Violation of the integrity of the membrane leads to cell death. However, local changes in short-term time scale of membrane integrity leading to the creation of a new structure by fusion or dividing cells [1].

Fusion of the membranes plays an important role in physiological processes, such as exocytosis, secretion, formation of secondary lysosomes [2–5]. In addition, directional cell fusion by various fusion agents *in vitro* is widely used to solve a number of problems in biomedicine and biotechnology.

Fusion mechanism, caused by di- and trivalent cations (Ca^{2+}, La^{2+}), small organic molecules (n-Hexyl Bromide, Ethanol, Halothane) and a mixture of charged and neutral lipids was investigated on the model lipid membranes [6]. It has been shown that, the fusion's nature does not depend on the choice of the fusion agents. The fusion occurs by stages: close contact of the membranes (I), hemi-fusion (II) and fusion (III).

According to the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory [7] the fusion process of lipid membranes is a result of superposition of van der Waals attractive forces and electrostatic repulsive forces. Additionally, the hydrophobic interactions make a significant contribution to balance of the intermembrane interaction at short distances and maintain neighboring vesicles in equilibrium at the distance of ~ 2 nm [8].

Therefore, the reduction of the hydration repulsion leads to the membrane fusion (Stage I) [8]. Indeed, the decreasing of water reduces the repulsion of bound lipid bilayers and induces the steric contact of membranes [9].

Defects or fluctuations in the contact area, which are caused by the fusion agents action or by change of some physical parameters such as a temperature [10], a curvature of membranes [11] and a surface tension [10], play a key role in the fusion process (Stage II and III).

Dimethyl sulfoxide (DMSO) is one of the fusion agents. The fusion of the membrane in presence of DMSO is a result of the pore formation, thereby increasing permeability of the membranes [12], and reduction of the membrane rigidity [13]. Dimethyl sulfoxide is actively used in cell biology, cryobiology and medicine [14, 15]. DMSO, like most currently used cryoprotectant (glycerol, ethylene glycol, methanol, propylene glycol, etc.), is toxic to living cells. Degree of toxicity depends on the cryoprotectant concentration used for the freezing of plant or animal cells. This problem has been widely discussed in earlier works [16]. Diethyl sulfoxide (DESO) is less toxic than the DMSO and glycerol, for example, for *E. coli* [17]. The interactions of DESO and DMSO with the biological membranes are, probably, identical. By differential scanning calorimetry (DSC) method a partial dehydration of the lipid bilayer of DMPC liposomes and simultaneous change in the structure of water at low concentrations of sulfoxides (20 wt %) was shown [18]. The sulfoxides interact directly with the surface of the lipid membrane at high concentrations of DESO and DMSO ($\geq$ 40 wt %) [18]. Thus, it can be assumed that hydrophobic interactions play a crucial role in the intermembrane interaction in the presence of sulfoxides.

The first results related to the structural and phase transition investigations of the model phospholipid

membrane (≥ 20 wt %) in the DESO presence were done by DSC [18] and X-Ray [19]. It was shown that the repeat distance of the multilamellar vesicles (MLVs) decreased and the temperature of the main phase transition increased with increasing of the diethyl sulfoxide concentration.

The goal of the present work was investigation of the influence of the DMSO and DESO on the structure of the fully hydrated unilamellar vesicles (ULVs) DMPC (2 wt %). The SANS is widely used technique for such systems studies.

2. Materials and methods

2.1 Sample preparation

14:0 PC (DMPC) – 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine ($C_{36}H_{72}NO_8P$) and 14:0 PC-d54 ($DMPC_{d54}$) – 1,2-dimyristoyl-d54-*sn*-glycero-3-phosphocholine ($C_{36}H_{18}NO_8PD_{54}$) 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% pure were purchased from the Reachim (Moscow, Russia). Heavy water (99.8 % D_2O) was from Isotope (St. Petersburg, Russia).

For neutron experiments, multilamellar vesicles (MLVs) were prepared in the following way. The lipids were added to DMSO/D_2O and DESO/D_2O solutions at predetermined mole fractions X_{DMSO} and X_{DESO}, mixed by a shaker. A homogeneous solution was obtained by the freezing–thawing membranes in the range from – 80ºC to + 55ºC. The procedure was repeated several times.

Large unilamellar vesicles (ULVs) were prepared by passing the MLVs of DMPC through polycarbonate filters 100 nm in diameter (Hamilton, Reno, Nevada, United States) of an extruder (Avanti, USA). After 25 cycles we obtained single bilayer vesicles with max diameter determined by the size of the filter. The vesicles in pure D_2O were stable for a sufficiently long time, fusion of the vesicles did not happen within a few days.

2.2 Experimental Technique

The influence of the sulfoxides DMSO and DESO on the structure and phase transitions of phospholipid membranes 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 [20] in the q-range of 0.007 – 0.4 $Å^{-1}$. The raw data treatment was done by the SAS program [21]. The final small-angle neutron scattering curves are presented in the absolute scale with background subtraction [22].

The studied samples were placed in 1 mm thick quartz cells (Hellma, Germany). During data collection the samples were in the temperature controlled holder (± 0.2 ºC) connected to the liquid thermostat (Lauda, Germany) at 12º C or 55 ºC. Standard data acquisition time per sample was 30 min.

2.3 SANS data analysis for ULVs and MLVs

In general case the SANS intensity for dispersions of monodisperse centrosymmetric particles is given by

$$I(q)=N_p\,|F(q)|^2\,S(q) \qquad (1)$$

where $q = 4\pi \sin\theta/\lambda$ is the scattering vector (2θ is the scattering angle and λ is the neutron wavelength), N_p – the number density of particles, $F(q)$ – their form factor and $S(q)$ – the interparticle structure factor. For dilute and weakly interacting particles the interparticle structure factor $S(q)$ is approximately equal to 1, what is a good approximation for unilamellar vesicles at phospholipid concentrations less than < 2 wt % [23, 24]. The model of weakly interacting particles describes well the ULVs prepared by extrusion [25]. A typical SANS curve of ULVs prepared by extrusion is shown in Fig. 2 *A*. For ULVs the intensity is determined as:

$$I(q) = I_0\ q^{-2} \exp[-R_t^2 q^2] \qquad (2)$$

where I_0 is the intensity at q = 0, and R_t is the radius of gyration.

Membrane thickness d_b can be obtained from the radius of gyration R_t of ULVs using the Kratky-Porod approximation for the scattering intensity [26, 27] in q region where $q\ R_t \leq 1$ with accuracy about 1 Å according to the relationship between d_b and R_t:

$$d_b \approx \sqrt{12} R_t \qquad (3)$$

The exact value of the membrane thickness d_b can be calculated according to the procedure described in [28].

For MLVs spontaneously formed from ULVs after DMSO injection into the DMPC/water dispersion we observed a diffraction peak that indicates structural changes of ULVs DMPC. A typical SANS curve of MLVs is shown in Fig. 2 *B*. The observed peak indicates the multilamellar structural organization of lipid bilayers.

In such case the interparticle structure factor $S(q)$ is not equal to 1. The interaction peak can be approximated by Gaussian distribution function [29] and the structure factor can be determined as

$$S(q) = 1 + A\exp[-\frac{(q-q_0)^2}{2\sigma^2}] \qquad (4)$$

where q_0 is the position of maximum of the diffraction peak corresponding to the amount of multilamellar structures with repeat distance d. A and σ are the amplitude and the width of the Gaussian peak, respectively.

Then d can be calculated from the position of the maximum of the diffraction peak q_0 according to the equation

$$d = 2\pi / q_0 \qquad (5)$$

Taking in an account all mentioned above, the intensity for spontaneously formed MLVs can be determined as:

$$I(q) = I_0 q^{-2} \exp[-R_g^2 q^2] \bullet (1 + A\exp[-\frac{(q-q_0)^2}{2\sigma^2}]) \qquad (6)$$

where the scattering from ULVs and MLVs is described by this combined function as it has been done in [30].

3. Results and discussion

The spontaneous formation of the MLVs PC membranes from extruded ULVs in DMSO presence has been discussed in previous work [31]. It turned out that the ULVs fusion is caused by two factors: time and increasing of the DMSO concentration.

In current paper are presented the results of the time and DESO concentration influence on the spontaneous formation of the MLVs DMPC under excess solution condition.

DESO is a small organic molecule with polar hydrophilic S=O group and two hydrophobic groups as a DMSO, but has two additional CH_2 groups (Fig.1).

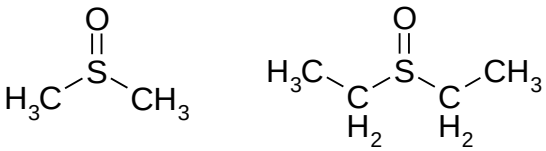

Fig.1. DMSO (left) and DESO (right) structures.

Taking in an account, at firstly, that DESO strongly interact with water, even stronger than DMSO [32] and, secondary, that DESO, more than DMSO, is able to penetrate living tissues without causing significant damage [17], we can propose that hydrophobic interaction plays more crucial role in the intermembrane interactions in the DESO presence.

3.1 Spontaneous fusion of the unilamellar vesicles: effect of time

Fully hydrated ULVs DMPC (2 wt %) were prepared according to the procedure described above with DMSO and DESO molar concentration (X_{DMSO} and X_{DESO}) ranging from 0.0 to 0.4 in DMSO/D_2O and DESO/D_2O mixtures. The measurements were carried out four times for each sample: 0, 1/2, 1 and 24 hours after sample preparation in gel ($L_{\beta'}$) and liquid-crystalline (L_α) phases.

It turns out that the ULVs DMPC in DMSO/$D_2$0 or DESO/$D_2$0 solutions are stable for a long time only for mole fraction of the DMSO and DESO $\leq$ 0.1. ULVs DMPC in DMSO/$D_2$0 formed MLVs spontaneously about 1 hour after preparation. Significant changes of the SANS curves with an appearance of the diffraction peak in q-region of about 0.1 $Å^{-1}$ as presented in Fig. 2 *B* directly point to the structural transition. For better visualization the data are shown in Kratky-Porod presentation *ln I(q)*q^2 *vs.* q^2 for ULVs (Fig. 2*A*) and in *ln I(q) vs. ln q* scale for MLVs (Fig. 2*B*).

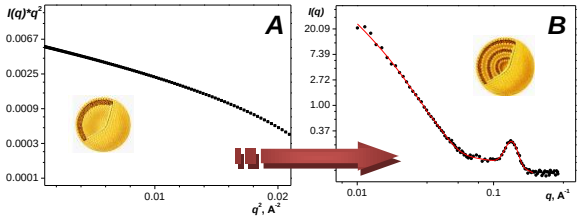

Fig. 2. SANS curves on ULVs DMPC (2 wt %) in DMSO/$D_2$0 mixture at X_{DMSO} = 0.2 in liquid- crystalline phase at T = 55 ºC after preparation (A) and 1 hour later (B).

The data are well fitted by equation 6 (Fig. 2*B*, red line) as well in small q-region related to the small-angle contribution and corresponds to the Kratky-Porod approximation of the scattering on ULVs as in q-region corresponding to the MLVs with d = 46.5 ± 0.4 Å. This value is in a good agreement with repeat distance for prepared MLVs. For example, d = 48.2 ± 0.3 Å for MLVs $DMPC_{d54}$ (2 *wt* %) in $DMSO_{d6}/H_2$0 at T = 55 ºC [31].

The spontaneously formed MLVs were determined for extruded ULVs DMPC in DESO/$D_2$0 mixture. The well ordered multilamellar system was observed through 30 min after sample preparation.

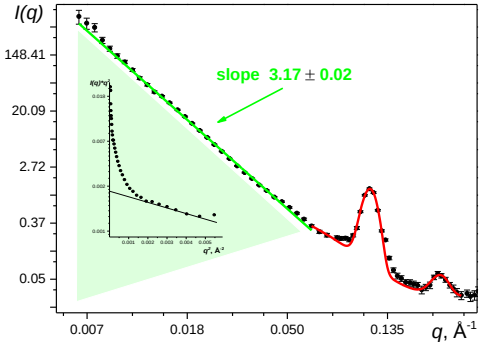

Fig. 3. SANS curves on ULVs $DMPC_{d54}$ (2 wt %) in DESO/$H_2$0 (40 %)/$D_2$0 (60 %) mixture at X_{DESO} = 0.2 in gel phase at T = 10 ºC.

Additionally, the structure change has been observed in long-term time scale. For this purpose the fully deuterated DMPC lipids and water isotopic substitution were used as was proposed in [33]. The SANS curve for spontaneously formed MLVs $DMPC_{d54}$ (2 wt %) in DESO/water has been measured four hours later (Fig. 3). Two diffraction peaks (first and second orders) were observed in this case and well approximated by Gaussian distribution function (Fig.3, red line). The repeat distance for spontaneous formed MLVs $DMPC_{d54}$ according to Eq. 5 is equal to 55.36 ± 0.37 Å (for first order peak) and 54.97 ± 0.35 Å (for second order peak). However, the presented model (Eq. 6) does not approximate well the

scattering curve at small q values (Fig. 3, insert). The aggregation is one of the possible reasons for it. On the other hand, the scattering curve has slope m = 3.17 in double log scale (ln $I(q)$ vs. ln q), what corresponds to the structure with surface fractal distributions ($3 \le m \le 4$) of membrane components. In this case the scattering intensity is determined by a power law:

$$I(q) \sim q^{-m} \qquad (7)$$

where m is a power-law exponent related to a fractal dimension as $D = 6 - m$.

The observed effect can be explained taking in an account a theoretical prediction of the lateral transport processes in biomembranes [34] based on the Cohen-Turnbull free volume theory [35]. The theoretical model for lateral diffusion mechanism of lipids in bilayer systems predicted three steps of the diffusion: (1.) pore formation in the solvent by lateral density fluctuations, (2.) a molecular jump into this pore, and (3.) the resulting void is filled by solvent molecules [34].

Thus, the experimental data presented here confirm that the mechanism of the membrane fusion is the same in the presence of DESO and DMSO: the fusion of the membrane in presence of DESO is a result of the pore formation as it was mentioned for DMSO.

3.2 Spontaneous fusion of the unilamellar vesicles: sulfoxides concentration effect

The influence of the DMSO and DESO molar concentration (X_{DMSO} and X_{DESO}) on the structure of the fully hydrated DMPC lipids in gel and ($L_{\beta'}$) liquid-crystalline (L_α) phases was examined. For this purpose the ULVs DMPC (2 wt %) were prepared according to the procedure described above. SANS curves of the unilamellar vesicles in DESO/D_2O prepared by extrusion are shown in Fig.4 and Fig.5 for X_{DESO} = 0.0 (■); 0.1 (●); 0.2 (▲) and 0.3 (▼) in $L_{\beta'}$ phase at 12 °C and in L_α phase at 55 °C, respectively. The measurements have been done immediately after preparation of the ULVs DMPC. As it can be seen from SANS curves the ULVs are stable only at molar concentrations X_{DESO} up to 0.2. We observed a diffraction peaks at X_{DESO} = 0.3 in both gel and liquid-crystalline phases. In inserts *B* in Fig.4 and Fig.5 the SANS curves (blue symbols) with best fits (red line) in diffraction peak region are presented. Exponential function was used as a base line (blue (for $L_{\beta'}$) and green (for L_α) lines). It was found one diffraction peak with q_o = 0.074±0.001 Å^{-1} in gel phase. The detailed analysis of the scattering curve in q-region 0.05 Å $\le q \le$ 0.25 Å revealed the three diffraction peaks with q_o = 0.081±0.002 Å^{-1}, 0.133±0.001 Å^{-1} and 0.206±0.004 Å^{-1} in liquid-crystalline phase.

The appearing of the diffraction peaks means that increasing of the DESO molar concentration up to 0.3 leads to the spontaneous formation of the MLVs. However, the liquid-crystalline phase data is difficult for interpretation. The one of the possible reason is the insufficient equilibrium of the system after the sample preparation.

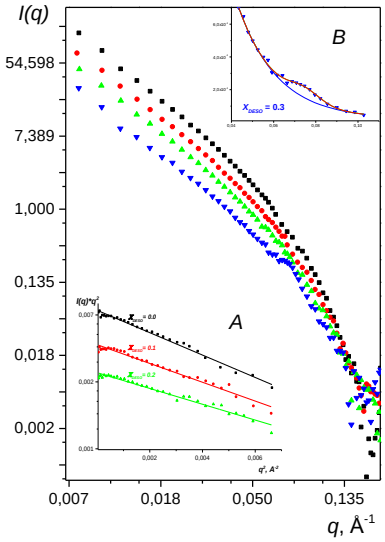

Fig.4. SANS curves on ULVs DMPC (2 wt %) in $L_{\beta'}$ phase at X_{DESO}= 0.0 (■); 0.1 (●); 0.2 (▲) and 0.3 (▼)

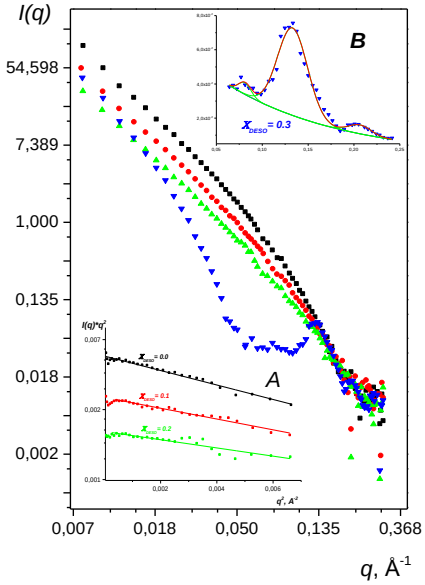

Fig.5. SANS curves on ULVs DMPC (2 wt %) in L_α phases at X_{DESO}= 0.0 (■); 0.1 (●); 0.2 (▲) and 0.3 (▼)

Nevertheless the position of the biggest peak (blue symbols) coincides with the peak position in the scattering curve measured one day later at the same temperature (black symbols) as presented in Fig. 6. The approximation of the diffraction peaks by Gaussian distribution function (red and green lines) gives the repeat distance for spontaneously formed MLVs DMPC at X_{DESO} = 0.3 in liquid-crystalline phase d = 47.2±0.2 Å and d = 47.7±0.3 Å after ½ hour and 1 day after sample preparation, respectively.

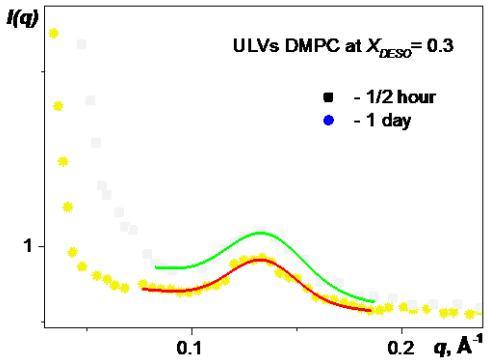

Fig. 6. SANS curves on ULVs DMPC (2 wt %) at X_{DESO} = 0.3 in L_α phase in time scale ½ hour and 1 day after sample preparation.

The spontaneously formed MLVs with increasing of the sulfoxide concentration has been discussed in our previous work [31]. It turns out that ULVs $DMPC_{d54}$ (2 wt %) in $DMSO_{d6}/H_20$ are stable up to the DMSO mole fraction 0.3 (*Fig.7A*). However, a spontaneous formation of the MLVs $DMPC_{d54}$ has been observed at X_{DMSO} = 0.4 (*Fig.7B*). This argument is valid only for a short period of time. As it was shown above, the ULVs are stable for a long time, only at mole fraction of both sulfoxides X = 0.1.

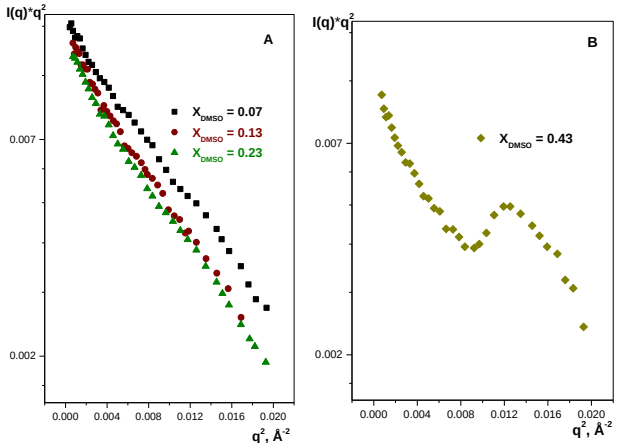

Fig.7. ULVs $DMPCd_{54}$ (2 wt %) in H_2O/DMSO in liquid phase (T=55 ºC) at different X_{DMSO}

3.3 Membrane thickness

The thickness of the ULVs DMPC d_b was estimated according to equation (3). R_t can be obtained from the linear part of the Kratky-Porod plot *ln* $I(q)q^2$ *vs.* q^2 (insert *A* in Fig.4 and Fig.5) in *q*-region where $qR_t \leq 1$. The data are presented in Table 1 for ULVs in gel phase at T = 12 *°C* and in liquid-crystalline phase at T = 55 *°C.*

The increasing of the DESO mole fraction in range $0.0 \leq X_{DESO} \leq 0.2$ causes the essential decrease in the membrane thickness for both investigated phases: from 44.8 Å to 37.7 Å in $L_{\beta'}$ phase and from 35.2 Å to 26.5 Å in L_α phase. The experimental error for the gyration radius is less than 0.5 Å in all cases.

On the contrary, the thickness of the membranes in the DMSO presence changes slightly in the gel phase and is almost constant in the liquid-crystalline phase within the experimental errors. For example, d_b decreases from 48.8±0.2 Å in pure H_20 to 47.6±0.4 Å for X_{DMSO} = 0.2 for $DMPC_{d54}$ in $L_{\beta'}$ phase. The average d_b is equal to 44.15 for $DMPC_{d54}$ in L_α phase for $0 \leq X_{DMSO} \leq 0.3$. This result is in a good agreement with data for PC membranes published earlier [35, 36].

Table 1. Radius of gyration for ULVs in gel ($L_{\beta'}$) and liquid-crystalline (L_α) phases.

Molar concentration, X	Radius of gyration R_t, Å	
	$L_{\beta'}$	L_α
ULVs DMPC in $DESO/D_20$		
0.0	12.9±0.3	10.2±0.2
0.1	11.8±0.1	8.8±0.2
0.2	10.9±0.1	7.7±0.1
ULVs $DMPC_{d54}$ in $DMSO_{d6}/H_20$*		
0.0	14.1±0.1	12.8±0.2
0.1	13.7±0.4	12.7±0.1
0.2	13.7±0.2	12.7±0.3
0.3	13.7±0.1	12.8±0.1

* all data adopted from [31].

The dependence of the ULVs DMPC thickness *vs.* DESO molar concentration is shown in Fig. 8. It's clear that d_b decreases monotonously in specified range of X_{DESO} for both phases. Nevertheless, more detailed analysis should be done for understanding of the DESO influence on the intermembrane interaction.

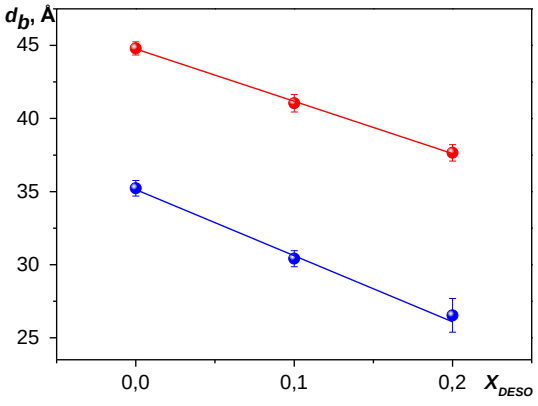

Fig.8. The dependence of the ULVs DMPC thickness vs. DESO mole fraction in $L_{\beta'}$ (red symbols) and L_α (blue symbols) phases.

4. Conclusions

The spontaneous formation of the MLVs in the sulfoxides presence was observed. The mechanism of the membrane fusion is the same in the presence of DESO and DMSO. Time and concentration play significant role in the fusion of the fully hydrated ULVs DMPC. The present work confirms the hypothesis about a crucial role of the hydrophobic interactions in the intermembrane interaction in the presence of sulfoxides. However, it should be noted that these hydrophobic interactions are stronger in the presence of DESO. At first, DESO causes the fusion of the ULVs about 1/2 hour after samples preparation, while this process occurs in an hour in the presence of DMSO. And at the second, the investigation in short-term time scale shown that formation of the MLVs take place at X_{DESO} = 0.3 and X_{DMSO} = 0.4.

Moreover, it was demonstrated that using of the fully deuterated DMPC lipids and water isotopic substitution may provide additional information about some important processes in biomembranes such as a lateral transport.

Acknowledgements

This work was supported by the Romania – JINR (Dubna) Programme 2014 – 2015. The author thanks A. Ivankov for experimental assistance and A. Islamov for useful discussions.

The paper contains results partially presented at the International Summer School and Workshop "Complex and Magnetic Soft Matter Systems: Physico-Mechanical Properties and Structure", CMSMS'14, 29 September-3 October 2014, Dubna, Russia.

References

[1] Yu. A. Chismadzhev, Soros Educ. J. **7**, 4 (2001).
[2] J. A. Lucy, Nature **227**, 814 (1970).
[3] J. Meldolesi, N. Borgese, P. De Camilli, B. Ceccarelli, Cell Surface Reviews, North-Holland Biomedical Press, Amsterdam **5**, 510 (1978).
[4] R. Bischoff, Cell Surface Reviews, North-Holland Biomedical Press, Amsterdam **5**, 127 (1978).
[5] Q.F. Ahkong, D. Fisher, W. Tampion, J.A. Lucy, Nature **253**, 194 (1975).
[6] S. Mondal and M. Sarkar, J. of Lipids **2011**, Article ID 528784 (2011).
[7] W. Helfrich, Z. Naturforsch. A **33A**, 305 (1978).
[8] R. P. Rand and V. A. Parsegian, BBA **988**, 351 (1989).
[9] G. Cevc, J. M. Seddon and D. Marsh, BBA **814**, 141 (1985).
[10] G. Cevc and H. Richardsen, Advanced Drug Delivery Reviews **38 (3)**, 207 (1999).
[11] S. Nir, J. Wilschut and J. Bentz, Biochim. et Biophys. Acta **688 (1)**, 275 (1982).
[12] R. Notman; M. Noro; B. O'Malley, J.J. Anwar, Am. Chem. Soc. **128**, 13982 (2006).
[13] A.A. Gurtovenko and A. Jamshed, J. Phys. Chem. B **111 (35)**, 10453 (2007).
[14] J.E. Lovelock and M.W.H. Bishop, Nature **183**, 1394 (1959)
[15] J. Ali and J.N Shelton, J. Reprod. Fertil. **99**, 471 (1993).
[16] http://www.benbest.com/cryonics/viable.html
[17] S.A. Markarian; S. Bonora, K.A. Bagramyan, V.B. Arakelyan, Cryobiology **49(1)**, 1 (2004).
[18] S. Bonora, S.A. Markarian, A. Trinchero, K.R Grigorian, Thermochim. Acta **433**, 19 (2005).
[19] Yu.E. Gorshkova, O. I. Ivankov, A.I. Kuklin, V.I. Gordeliy, J. of Physics: conf. series **351**, 1 (2012).
[20] A.I. Kuklin, A.Kh. Islamov and V.I. Gordeliy. Neutron News **16 (3)**, 16 (2005).
[21] A.G. Soloviev, T.N. Murugova, A.Kh. Islamov and A.I. Kuklin, J. Phys. Conf. Ser. **351**, 012027 (2012).
[22] Yu.M. Ostanevich, Makromol. Chem. Macromol. Symp. **15**, 91 (1988).
[23] W. Knoll, J. Haas, H.B. Sturhrmann, H.H. Fuldner, H. Vogel and E. Sackmann, J. Appl. Cryst. **14,** 191 (1981).
[24] T. Nawroth, H. Conrad and K. Dose, Physica B **156&157**, 477 (1989).
[25] P. Balgavý, M. Dubničková, D. Uhríková, S. Yaradaikin, M. Kiselev and V. Gordeliy, Acta Physica Slovaca **48,** 509 (1998).
[26] L.A. Feigin and D.I. Svergun, Plenum Press, New York and London (1987).
[27] V.I. Gordeliy, V.G. Cherezov and J. Teixeira, J. Mol. Struct. **383**, 117 (1996).
[28] V.I. Gordeliy, L.V. Golubchikova, A.I. Kuklin, A.G. Syrykh and A. Watts, Prog. Colloid Polym. Sci., **93,** 252 (1993).
[29] D. Uhríková, N. Kučerka, J. Teixeira, V. Gordeliy, and P. Balgavý, Chem. Phys. Lipids. **155**, 80 (2008).
[30] V.I. Gordeliy, V. Cherezov and J. Teixeira, Phys. Rev. E **72**, 1 (2005).
[31] J.E. Gorshkova, V.I. Gordeliy, Crystallography Reports **52(3)**, 535 (2007).
[32] S.A. Markarian, A.L. Zatikyan, S. Bonora, C. Fagnano, J. Mol. Struct. **665**, 285 (2003).
[33] V.I. Gordeliy, Physica B **180&181**, 750(1992).
[34] T.F. Monnenmacher, Eur. Biophys. J. **16**, 375 (1989).
[35] V.I. Gordeliy, M.A. Kiselev, P. Lesieur, A.V. Pole and J. Teixeira, Biophys. J. **75**, 2343 (1998).
[36] D.R.V. McIntosh, S.A. Simon, Biochem. **28**, 7904 (1989).

*Corresponding author: gorshk@nf.jinr.ru