

ОБЪЕДИНЕННЫЙ  
ИНСТИТУТ  
ЯДЕРНЫХ  
ИССЛЕДОВАНИЙ

Дубна

98-91

E14-98-91

M.A.Kiselev, P.Lesieur<sup>1</sup>, A.M.Kisselev,  
C.Grabielle-Madmond<sup>2</sup>, M.Ollivon<sup>2</sup>

DMSO-INDUCED DEHYDRATION  
OF DPPC MEMBRANES  
STUDIED BY X-RAY DIFFRACTION,  
SMALL-ANGLE NEUTRON SCATTERING  
AND CALORIMETRY

Submitted to the Conference «ISSRNS'98», June 15–20, 1998,  
Ustron-Jaszowiec, Poland

<sup>1</sup>LURE, Université Paris-Sud, Bat. 209-D, F91405 Orsay cedex, France

<sup>2</sup>Physico-Chimie des systèmes polyphasés, URA 1218 du CNRS,  
Faculté de Pharmacie, tour B, F – 92296, Chatenay Malabry, France

1998

## 1. INTRODUCTION

Dimethyl sulfoxide has two very important biological properties. The first DMSO property is its ability to protect a variety of cells from the damaging effects of freezing and storage at a very low temperature. The second one concerns the modification of X-ray induced damage in cells and whole animals when DMSO is presented before and during exposure to radiation [1].

The main purpose of cryobiology is to find an "optimal" way for cooling biological systems down to low temperatures (about the temperature of liquid nitrogen) and to prevent the crystallisation of water inside of the biological tissue. One of the problems here is a long time, compared with the time of ice formation, of freeze-induced dehydration (diffusion of water molecules through the membranes from the biological tissue to the conserving solvent). The mechanism of DMSO cryoprotection is still debated [2] and on the molecular level is not clear till now.

DMSO makes hydrogen bonds with water molecules. The structure of binary DMSO/water system has been investigated by means of the spin-lattice relaxation and chemical shift behavior of water and DMSO protons [3]. It was found that DMSO and water molecules have a tend to form hydrogen bonds in the relation 1/2 (mole DMSO fraction  $X_{\text{DMSO}} = 0.33$ ) or 1/3 ( $X_{\text{DMSO}} = 0.25$ ). The DMSO/water phase diagram is well-known [4]. The phase behavior of binary DPPC/water system has been investigated by calorimetry [5]. The influence of DMSO fraction on the pre-transition existence and on the repeat distance of DPPC multilamellar structure has been investigated [6] for the region of small DMSO mole fraction  $X_{\text{DMSO}} \leq 0.13$ . A conclusion about the decreasing of intermembrane solvent space with the increasing of DMSO concentration was made.

In present work the DMSO influence on 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine (DPPC) membranes was studied by X-ray diffraction, small-angle neutron scattering (SANS) and differential scanning calorimetry (DSC). Our purpose was to obtain information about the changes in the structure of DPPC in the present of DMSO at  $T=20^{\circ}\text{C}$  and about the ice formation (melting) under the cooling (resp. thawing). Calorimetric and X-ray diffraction measurements was performed in order to explore the main phase transition in the DPPC bilayers and the influence of DMSO on the membrane structure for mole DMSO fraction  $0 \leq X_{\text{DMSO}} \leq 1$ .

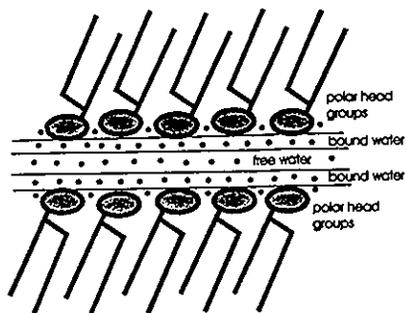


FIGURE 1. Model of phospholipid/water multilayers. Water molecules are divided into three fractions: the first one – free water molecules located into the center of intermembrane space, the second – bound water molecules located into the intermembrane space near the polar head of DPPC molecules and the third - bound water molecules located into the spatial region of DPPC head groups.

Phospholipid/water multilayer system can be represented as described at Fig. 1. It is possible to divide the water molecules into two fractions; the first fraction – molecules bonded to polar head groups of phospholipid molecules and located in or near the region of DPPC head groups, so-called bound water, the second – so-called free water molecules, which exist

between lipid bilayers without any interaction with membrane surface. The bound water, in turn, can be divided into the fraction of molecules embedded into the spatial region of polar head groups and the fraction located into the intermembrane space near the polar head groups. The bound water is in the interaction with the head groups of DPPC. The numbers of bound water molecules located into the region of polar head groups and into the intermembrane space are equal to 2.2 and 8.6 per one DPPC molecule respectively, the number of free water molecules per one DPPC molecule equals 4 [7, 5].

## 2. MATERIALS

1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine (DPPC, over 99% purity) was obtained from Sigma-Aldrich corporation (Paris, France). Spectrophotometric grade Dimethyl sulfoxide (DMSO, over 99% purity) was produced by Aldrich (Paris, France). Water (18 MΩ·cm) was obtained by means of Millipore.

## 3. SAMPLE PREPARATION

The samples were prepared either in eppendorfs for calorimetry or in capillaries for X-ray diffraction. The X-ray quartz capillaries (1.5 mm diameter and 0.01 mm wall thickness) were purchased from GLAS company (W.Muller, Berlin, Germany). Desired amount of lipid and DMSO/water solution was placed in the containers. Both eppendorfs and capillaries were hermetically sealed. The samples were heated up to a temperature above the main phase transition temperature, sonicated (slightly, in order to prevent cracking of the capillaries) for 5 min in the Branson sonicator, stored at this temperature about 3 hours and then cooled down to a room temperature. The process was repeated 4 times in order to obtain homogeneous DPPC multilamellar suspension. For the differential scanning calorimetry measurements, one drop of sample was placed in the sealed aluminium platelets. The sample weight in the platelets was about 10 to 15 mg. The weight ratio between DPPC and solvent was chosen to keep constant the number of solvent molecules per DPPC molecule. Let us call  $N_{DMSO}$ ,  $N_w$ ,  $N_{lip}$  the number of DMSO, water and DPPC molecules respectively. For DPPC in pure water the ratio  $N_w/N_{lip} = 40.8$  corresponds to a 1/1 weight ratio. For DPPC in DMSO/water mixture the ratio  $(N_{DMSO} + N_w)/N_{lip}$  was kept equal to 40.8. It means that if  $W_{sol}$  and  $W_{DPPC}$  are weights of solvent and DPPC respectively then the solvent concentration in the sample  $W_{sol}/(W_{sol} + W_{DPPC})$  is larger than 0.5 for all DMSO concentrations. In the following, the mole fraction of DMSO  $X_{DMSO}$  will be defined as the mole fraction of DMSO in the solvent mixture  $N_{DMSO}/(N_{DMSO} + N_w)$ . All measurements were performed in the excess of solvent.

For SANS study we used large unilamellar vesicles which were prepared by extrusion of a heated (50-60°C) 1% (w/w) DPPC dispersion through Nuclepore membrane filters (pores with 2000 Å in diameter) [8].

## 4. EXPERIMENTAL TECHNIQUES

X-ray diffraction patterns were measured at LURE on D22 beam lines of DCI synchrotron ring. The period  $d$  (repeat distance) of the layered structure was determined from the position of the diffraction peak by using Bragg equation:  $2d \sin\theta = n\lambda$ , where  $\theta$  is a half of the scattering angle and  $n$  is order of diffraction peak,  $n=1$  in our experiment.

SANS measurements were performed at the YuMO time-of-flight small-angle spectrometer (pulse neutron source IBR-2) in Frank Laboratory of Neutron Physics (FLNP). Guinier approximation of the small-angle scattering cross-section was used to interpret the

neutron small-angle scattering spectra [9, 10]. For extended unilamellar structures (sheets or vesicles) with a surface area  $S$ , a thickness  $d_l$  of the layer (or radius of gyration  $R_g$ ) much smaller than the radii of curvature of the surface (or the lateral dimensions of the layer) the approximation, valid for scattering vectors  $q$  in the domain  $2\pi/\sqrt{S} < q < 1/R_g$ , is given by equation (1)

$$\frac{d\sigma}{d\Omega} = \frac{2\pi S(\Delta\rho d_l)^2}{q^2} \exp[-q^2 R_g^2], \quad (1)$$

where  $\Delta\rho$  is scattering length density relative to the solvent. For an homogeneous membrane, its thickness  $d_l$  is calculated through the formula  $d_l = R_g \sqrt{12}$ .

Differential thermal analysis measurements were performed by using the Perkin Elmer DSC 4 calorimeter. For the investigation of the main phase transition the temperature interval from 0°C to 85°C and the scanning rate of 2.0 degrees per minute was used. The phase transition of Lauric acid ( $T_{ph} = 43.7^\circ\text{C}$ ,  $\Delta H = 42.58 \text{ cal/g}$ ) was used for the calibration. For the study of phase transitions associated with freezing of water and melting of ice the temperature interval from 20°C to -130°C and the scanning rate of 10 degrees per minute was used. In this case we used the melting temperature of pure ice for calibration.

## 5. RESULTS AND DISCUSSION

### 5.1. Influence of DMSO on the Structure and Main Phase Transitions

Fig.2 presents the consequence of diffraction patterns recorded with acquisition time 2 min during the heating of sample from  $T = 25^\circ\text{C}$  to  $T = 80^\circ\text{C}$ . Heating rate  $1^\circ\text{C}/\text{min}$  was used in the temperature ranges  $25^\circ\text{C} - 40^\circ\text{C}$  and  $50^\circ\text{C} - 80^\circ\text{C}$ , in the temperature range from  $40^\circ\text{C}$  to  $50^\circ\text{C}$  heating rate was decreased to  $0.5^\circ\text{C}/\text{min}$  which gave us  $1^\circ\text{C}$  step between the diffraction patterns. In the temperature interval  $25^\circ\text{C} - 33^\circ\text{C}$  the diffraction patterns (Fig. 2, patterns 1-5 from the bottom) correspond to the gel  $L\beta'$  phase. From  $T = 35^\circ\text{C}$  to  $T = 41^\circ\text{C}$  (Fig. 2, patterns 6-9 from the bottom) the membrane exhibits the ripple phase  $P\beta'$  which is characterized by the shift of the diffraction peak to the region of smaller angles and by decreasing of diffraction peak intensity. At  $T = 41^\circ\text{C}$  a membrane undergoes the phase transition from  $P\beta'$  phase to the liquid crystalline  $L\alpha$  phase, so-called main phase transition. For the sake of more precise determination of the phase transition temperature the heating rate  $0.2^\circ\text{C}/\text{min}$  was used in the region near the main phase transition, which gave us the accuracy of  $\pm 0.4^\circ\text{C}$  for determination of phase transition temperature by X-ray diffraction. With the increasing of DMSO mole fraction the region of  $P\beta'$  phase decreases and membrane undulations disappear at  $X_{DMSO} = 0.10$ , at the same time the temperature of the main phase transition increases with the increasing of DMSO mole fraction. The same result was obtained by differential thermal analysis. Fig. 3 presents the DSC curves for 6 samples with different DMSO mole fraction from  $X_{DMSO} = 0.00$  to  $X_{DMSO} = 0.10$ . For example for curve recorded from DPPC/water system the first endothermic peak (so-called pre-transition) at temperature about  $37^\circ\text{C}$  corresponds to the transition from  $L\beta'$  phase to  $P\beta'$  phase. The second endothermic peak corresponds to the main phase transition from  $P\beta'$  phase to  $L\alpha$  phase. The difference between the pre-transition and the main phase transition temperatures diminishes while  $X_{DMSO}$  increases so that at  $X_{DMSO} = 0.10$  two endothermic peaks are merged together and pre-transition disappears. The same phenomenon was observed recently [6], but with a different concentration ( $X_{DMSO} = 0.04$ ) of the pre-transition disappearance.

Fig. 4 presents the dependence of the main phase transition temperature  $T_{ch}$  and of the repeat distance  $d$  of the multilamellar structure at  $T = 20^\circ\text{C}$  on the mole DMSO fraction, which was measured by X-ray diffraction and calorimetry. The values of the main phase transition temperature obtained by X-ray diffraction and by calorimetry are in good agreement. We can divide a range of DMSO fraction used into three regions. The first one is that of small DMSO mole fractions ( $0.0 \leq X_{\text{DMSO}} \leq 0.3$ ) there important changes in the repeat distance and the phase transition temperature are observed. Second one is the region of intermediate DMSO mole fraction ( $0.3 \leq X_{\text{DMSO}} \leq 0.9$ ) there the repeat distance and the phase transition temperature are constant. In the third region, near pure DMSO solvent, the DPPC membrane undergoes a transition towards a phase characterized by a small repeat distance ( $52 \text{ \AA}$ ) and a high transition temperature ( $74.2^\circ\text{C}$ ).

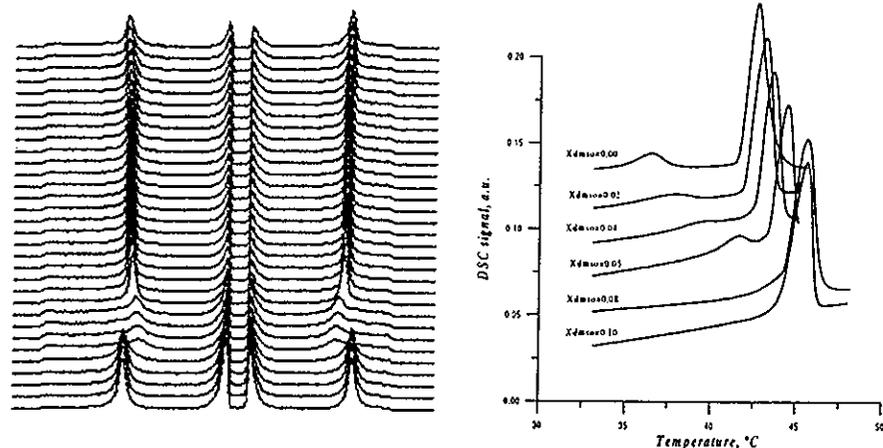


FIGURE 2. The consequence of diffraction patterns from DPPC/water system recorded with acquisition time 2min during the heating of sample from  $T = 25^\circ\text{C}$  to  $T = 80^\circ\text{C}$ . The oscillations of intensity in the center of figure correspond to the direct beam absorption by beam stop. The symmetrical to the center of beam left and right peaks correspond to the first order diffraction from multilamellar structure. The temperature region with a decreased peak intensity is  $\beta\beta'$  phase of DPPC membrane.

The repeat distance of DPPC multilayer  $d$  is connected with the membrane thickness  $d_l$  and thickness of solvent  $d_w$  in the intermembrane space via expression  $d = d_l + d_w$ . The decreasing of DPPC membrane repeat distance at  $T = 20^\circ\text{C}$  can occur as a result of  $d_l$  or  $d_w$  decreasing. In order to determine the influence of DMSO concentration on the bilayer thickness  $d_l$  SANS experiment with large unilamellar DPPC vesicles was carried out. The values of DPPC membrane thickness  $d_l$  obtained at  $T = 20^\circ\text{C}$  with the accuracy  $\pm 1 \text{ \AA}$  in Guinier approximation (1) are  $49.6 \text{ \AA}$  for  $X_{\text{DMSO}} = 0.0$ ,  $49.2 \text{ \AA}$  for  $X_{\text{DMSO}} = 0.05$  and  $50.1 \text{ \AA}$  for  $X_{\text{DMSO}} = 0.10$ . The same results concerning the absence of DMSO influence on

FIGURE 3. Calorimetric curves for main phase transition in the DPPC/DMSO/water system (long sharp peaks) at  $X_{\text{DMSO}} = 0.0, 0.02, 0.04, 0.05, 0.08, 0.1$  and pretransition (broad peaks on the left side of curves for  $X_{\text{DMSO}} = 0.0, 0.02, 0.04, 0.05$ ). Ordinate route in the relative units. The pretransition is disappeared after  $X_{\text{DMSO}} = 0.08$ .

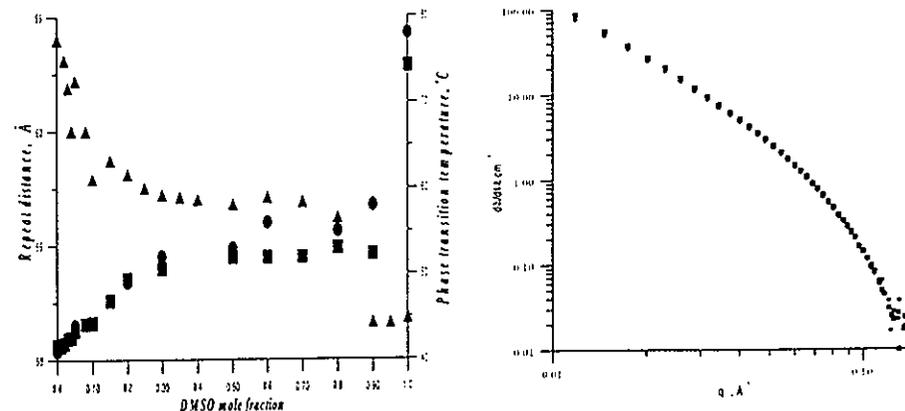


FIGURE 4. The dependence of the main transition temperature and the repeat distance of DPPC membrane on the mole fraction of DMSO in excess solvent.  $\bullet$  - main phase transition temperature obtained from diffraction measurements with heating rate  $0.2^\circ\text{C}/\text{min}$  and acquisition time 2min per spectrum,  $\blacksquare$  - main phase transition temperature obtained from calorimetric measurements with heating rate  $2.0^\circ\text{C}/\text{min}$ ,  $\blacktriangle$  - repeat distance of multilamellar liposomes at  $T = 20^\circ\text{C}$ .

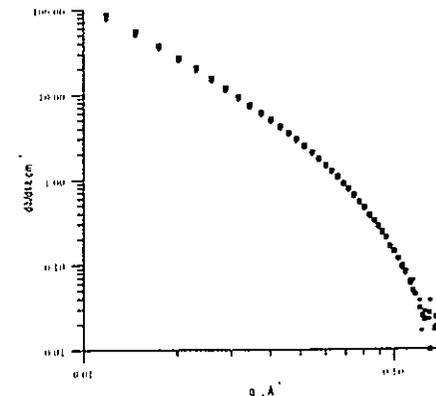


FIGURE 5. SANS curves for extruded DPPC vesicles at  $T = 20^\circ\text{C}$ :  $\bullet$  - in  $\text{D}_2\text{O}$  solvent.  $R_1 = 14.3 \pm 0.3 \text{ \AA}$ ;  $\blacksquare$  - in  $\text{DMSO}/\text{D}_2\text{O}$  solvent with  $X_{\text{DMSO}} = 0.1$ .  $R_1 = 14.5 \pm 0.3 \text{ \AA}$ . The macroscopic cross section  $d\Sigma/d\Omega$  proportional to the  $q^{-2}$  at small values of scattering vector  $q$ , which correspond to the scattering from large vesicles.

Fig. 5 demonstrates SANS curves for DPPC vesicles in  $\text{DMSO}/\text{D}_2\text{O}$  solvent. Scattering curves from vesicles in pure  $\text{D}_2\text{O}$  and from vesicles in  $\text{DMSO}/\text{D}_2\text{O}$  solvent with  $X_{\text{DMSO}} = 0.1$  practically coincide. As it is seen from Fig. 4 the region  $0.0 \leq X_{\text{DMSO}} \leq 0.10$  is the region where the main changes in the DPPC membrane repeat distance occur. The repeat distance  $d$  decreases from  $64.0 \pm 0.8 \text{ \AA}$  at  $X_{\text{DMSO}} = 0.0$  to  $57.9 \pm 0.8 \text{ \AA}$  at  $X_{\text{DMSO}} = 0.1$  and with further DMSO fraction increasing the repeat distance diminishes slowly to the constant value of about  $57 \text{ \AA}$ . These changes in the membrane structure correspond to the decreasing of intermembrane solvent thickness  $d_w$  from  $14.4 \pm 1.8 \text{ \AA}$  at  $X_{\text{DMSO}} = 0.0$  to  $7.8 \pm 1.8 \text{ \AA}$  at  $X_{\text{DMSO}} = 0.1$ , which can be explained as a reduction of the solvent amount in the intermembrane space.

The values of  $d = 64.0 \pm 0.8 \text{ \AA}$  and  $d_l = 49.6 \pm 1.0 \text{ \AA}$  obtained in our work for DPPC/water system at  $T = 20^\circ\text{C}$  are in good agreement with the values from electron density modeling of X-ray small-angle diffraction (SAXS):  $d = 63.7 \pm 0.3 \text{ \AA}$  and  $d_l = 49.8 \pm 2.3 \text{ \AA}$  [11].

### 5.2. Crystallization of water and melting of ice

Fig. 6a presents the calorimetric curves ( $X_{\text{DMSO}} = 0.0, 0.01, 0.03, 0.05, 0.08, 0.1, 0.15$ ) for the binary  $\text{DMSO}/\text{water}$  system under cooling from  $20^\circ\text{C}$  down to  $-130^\circ\text{C}$  with the rate  $-10^\circ\text{C}/\text{min}$ . For the binary  $\text{DMSO}/\text{water}$  system only one transition is observed in this temperature range. The shape of the transition remains narrow up to  $X_{\text{DMSO}} = 0.1$ , but for

larger cryoprotector concentration  $X_{\text{DMSO}} = 0.15$  the transition becomes broader, as can be noted from Fig. 6a. A multiplication factor  $k$  was introduced to scale the curves so that the amplitude of the transition remains approximately constant. This factor was found to be necessary because of the fast decrease of the transition enthalpy when DMSO is added. The transitions for solutions with  $X_{\text{DMSO}} \leq 0.10$  correspond to creation of binary system which consists of ice and liquid DMSO/water mixture. The eutectic temperature for DMSO/water system is  $-63^\circ \pm 1^\circ \text{C}$  [4]. Consequently the transition in the solution with  $X_{\text{DMSO}} = 0.10$  corresponds to the creation of ice and solid DMSO  $\cdot 3 \text{H}_2\text{O}$  hydrate.

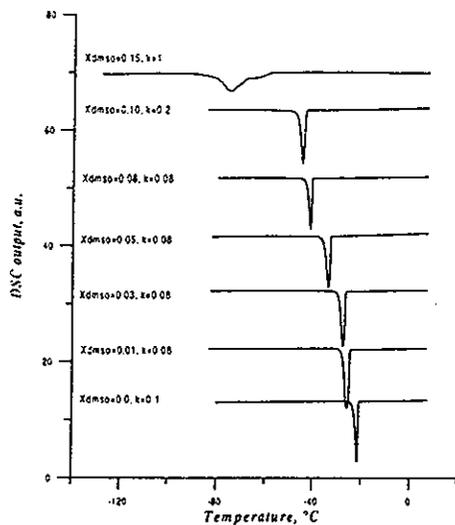


FIGURE 6a. DSC recordings of the curves for the cooling of DMSO/water mixtures from  $20^\circ\text{C}$  to  $-120^\circ\text{C}$  with the rate  $-10^\circ\text{C}/\text{min}$ . All peaks are normalized on the peak height (normalized coefficients  $k$  are presented). Ordinate root in the relative unites. The cooling event at  $X_{\text{DMSO}} = 0.15$  corresponds to the water crystallization and eutectic trihydrate DMSO, output DSC signal become smaller and peak broader.

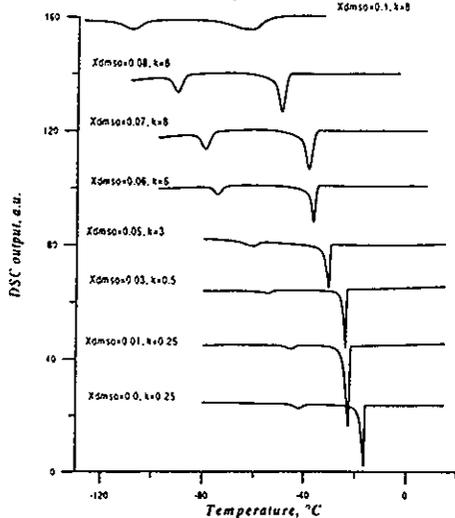


FIGURE 6b. DSC recordings of the curves for the cooling DPPC/DMSO/water mixtures at the same conditions as Fig. 6a. All peaks are normalised on the peak height (normalised coefficients  $k$  are presented). Ordinate root in the relative unites. Two phase transitions have been measured for all DMSO concentrations. At  $X_{\text{DMSO}} = 0.1$  the both phase transitions became similar and difficult to detectable due to weak output DSC signal. First transition corresponds to the ice formation in the bulk excess solvent, second one to the ice homogeneous nucleation in to intermembrane space.

Fig. 6b presents the calorimetric curves ( $X_{\text{DMSO}} = 0.0, 0.01, 0.03, 0.05, 0.06, 0.07, 0.08, 0.1$ ) for the ternary DPPC/DMSO/water systems, obtained under the same conditions as described above for the binary system. Two transitions are observed on cooling from  $T = 20^\circ\text{C}$  down to  $T = -130^\circ\text{C}$ . The first one (its temperature is close to the transition temperature of the solvent) can be attributed to the ice formation in the DMSO/water solvent (ice formation out of intermembrane space in the bulk solvent). The second transition (its temperature is from 20 to  $45^\circ\text{C}$  lower than that of the first) can be attributed to the freezing of the solvent trapped into bilayers [5]. A multiplication factor was also introduced here to scale the data. It can be noticed that these two transitions have distinct amplitude variations with DMSO concentration. While the amplitude of the first transition is kept roughly constant the relative importance of the second one increases while DMSO is added. According to our results and the phase diagram of DMSO/water system [4] the ratio between the first and the second exothermic transitions in the ternary system DPPC/DMSO/water corresponds to the decrease of the amount of ice created out of intermembrane space compare to the amount of ice created into intermembrane space. For  $X_{\text{DMSO}} \geq 0.05$  the second phase transition corresponds to eutectic transition.

Characteristics of studied systems important for the future discussion were obtained from the DSC curves recorded under the heating. The melting temperature of ice was determined as the offset temperature (the intersection point of the baseline with the tangent to the back side of the endothermic peak). The offset temperature was chosen because it corresponds to the end of the melting process (when the last ice crystallites is melted) so that the composition of the liquid (mole DMSO fraction) in the ternary system was equal to the system composition without ice.

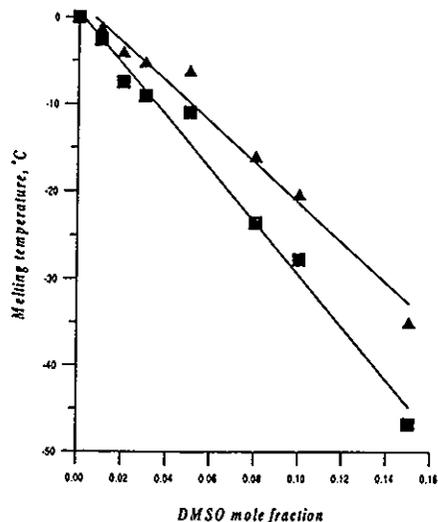


FIGURE 7. The dependence of melting temperature ( $^\circ\text{C}$ ) of ice on DMSO concentration for the DPPC/DMSO/water system,  $\blacksquare$  pixel and for DMSO/water solvent,  $\blacktriangle$  pixel. The melting point was determined as offset temperature on DSC curves.

Fig. 7 presents the differences in the dependence of melting temperature upon DMSO fraction for the binary and the ternary system. For each DMSO mole fraction the melting temperature of DPPC/DMSO/water system is lower compare with the melting temperature of the DMSO/water solvent. Taking into account that the melting temperature depends on the melting of ice in the extraliposomal space, these differences can be attributed to different

DMSO mole fraction in the binary system and in the bulk solvent around of multimembrane structure in the ternary system. The explanation of this phenomenon is that some fraction of water molecules are connected with the polar head groups of membranes and are not mixed with the DMSO molecules in the ternary DPPC/DMSO/water system. As a result the real DMSO concentration  $X'_{DMSO}$  in the bulk solvent of the ternary system will be higher compare with DMSO/water solvent used for the ternary system preparation. It is possible to estimate the number of water molecules that are not mixed with the DMSO molecules from the results presented at Fig. 7. The line fitting for binary and ternary systems of the melting temperature dependence on the DMSO fraction gives the values:  $T_{bin}(X_{DMSO}) = -230.3 \cdot X_{DMSO} + 1.7$  and  $T_{ter}(X_{DMSO}) = -302.4 \cdot X_{DMSO} + 0.58$  respectively. From the equation  $T_{bin}(X_{DMSO}) = T_{ter}(X'_{DMSO})$  and condition  $(N_{DMSO} + N_w) / N_{lip} = 40.8$  it is possible to calculate that  $10 \pm 2.6$  H<sub>2</sub>O molecules per one DPPC molecule are never frozen and  $X'_{DMSO} = 1.32 X_{DMSO}$ . The obtained value of unfrozen water molecules is in agreement with a well-known number of water molecules connected with polar head group of one DPPC molecule, which was determined from DSC measurement and is equal to  $10 \div 11$  molecules [5].

The membrane repeat distance  $d$  is connected with membrane thickness  $d_l$  and the number of solvent molecules per one DPPC molecule as:

$$d = d_l + \frac{2V_w}{A} n_w + \frac{2V_{DMSO}}{A} n_{DMSO} \quad (2)$$

$n_w = n_{wb2} + n_{wf}$  is the number of bound water molecules  $n_{wb2}$  and free water molecules  $n_{wf}$ , the both mentioned type of the molecules exist in the intermembrane space.  $n_{wb2} = n_{wb} - n'_w$ ,  $n'_w = 2.2$  [7] is the number of water molecules embedded in to region of the polar head groups.  $n_{DMSO}$  is the number of DMSO molecules per one DPPC molecule in the intermembrane space.  $V_w = 29.9 \text{ \AA}^3$  and  $V_{DMSO} = 118 \text{ \AA}^3$  are the volumes of water and DMSO molecules at  $T = 20^\circ\text{C}$  respectively,  $A = 47.9 \text{ \AA}^2$  is the area of one DPPC molecule at the membrane surface in the gel  $L\beta'$  phase [7].  $d_l = 49.6 \pm 1 \text{ \AA}$  is value which not dependent on DMSO fraction. The number of DMSO molecules can be easy calculated from DMSO mole fraction in the assumption that DMSO mole fraction in extramembrane space is equal to DMSO mole fraction in the "free" intermembrane solvent

$$n_{DMSO} = \frac{X'_{DMSO}}{1 - X'_{DMSO}} n_{wf} \quad (3)$$

From equations (2), (3) and the experimental DSC result which concerns the constant value of the amount of bound water  $n_{wb} = 10 \pm 2.6$  the decrease of repeat distance  $d$  in the region  $0.0 \leq X_{DMSO} \leq 0.3$  can be explained as a decreasing of the quantity of free water molecules in the intermembrane space.

The line interpolation of the  $d(X_{DMSO})$  function (see Fig. 3) in the region  $0.0 \leq X_{DMSO} \leq 0.1$  gives:

$$d = 64 - 48.66 X_{DMSO} \quad (4)$$

In the region  $0.3 \leq X_{DMSO} \leq 0.9$  the repeat distance can be estimated as a constant value of  $57 \text{ \AA}$ . From (4) the value of DMSO mole fraction at which can be reached equilibrium value of  $d = 57 \text{ \AA}$  is 0.14. In this case the decreasing of repeat distance from  $64 \text{ \AA}$  to  $57 \text{ \AA}$  corresponds to the decreasing of the number of free water molecules on the value of  $2.9 \pm 0.4$ . It is only part of free water molecules which exist in the fully hydrated DPPC membranes. The number of free water molecules per one DPPC molecule was determined as a result of DSC

study of the ice formation in the DPPC/water system [5], this value is 4 water molecules per one DPPC molecule.

As a result the region of small DMSO mole fraction from pure water to  $X_{DMSO} = 0.3$  corresponds to the decreasing of the number of free water molecules inside of intermembrane space from 4 to  $1.1 \pm 0.4$  at  $X_{DMSO} = 0.14$ . The DMSO-induced dehydration of intermembrane space increases the main phase transition temperature from  $T_{ch} = 41^\circ\text{C}$  at  $X_{DMSO} = 0$  to  $T_{ch} \approx 52^\circ\text{C}$  at  $X_{DMSO} = 0.3$ . It is important to note that DMSO and water molecules form hydrogen bonds in the relation  $1/2$  ( $X_{DMSO} = 0.33$ ) or  $1/3$  ( $X_{DMSO} = 0.25$ ) [3]. From this approach the dehydration of intermembrane space can be tentatively explained as a result of the strong connection of free water molecules and DMSO molecules in the bulk solvent. At  $0.3 \leq X_{DMSO} \leq 0.9$  only the bound water exists in the DPPC/DMSO/water system, in this region repeat distance  $d = 57 \text{ \AA}$  and the main phase transition temperature  $T_{ch} \approx 52^\circ\text{C}$  are constant values.

## 6. CONCLUSIONS

DMSO molecules strongly interact with water molecules by creation of hydrogen bonds, this interaction is more intensive compare with the interaction between DMSO and polar head group of DPPC membranes. DMSO molecules do not penetrate into the region of polar head groups and thus do not influence on the membrane thickness, nevertheless they strongly influence on intermembrane solvent thickness and as a result create the DMSO-induced dehydration of intermembrane space. At a room temperature the quantity of free water molecules in the intermembrane solvent decreases with the increasing of the DMSO mole fraction and at  $X_{DMSO} = 0.14$  only  $1.1 \pm 0.4$  water molecules per DPPC molecules exist without connection with polar head groups.

## 7. ACKNOWLEDGMENTS

The authors are highly grateful to Dr. Sylviane Lesieur (URA 1218 of CNRS) for her help in the sample preparation. One of us M.K. acknowledge the CEA Saclay for the grant which allowed these measurements to be performed.

## 8. REFERENCES

- [1] Ashwood-Smith, M.J. 1967. Radioprotective and cryoprotective properties of dimethyl sulfoxide in cellular systems. *In Biological Action of Dimethyl Sulfoxide*. Ed. Edward M. Weyer. Ann. NY Acad. Scien., 141: 45-62.
- [2] Meryman, H.T. 1971. Osmotic stress as a mechanism of freezing injury. *Cryobiology*. 8: 489.
- [3] Szmant, H.H. 1975. Physical properties of dimethyl sulfoxide and its function in biological systems. *In Biological actions of dimethyl sulfoxide*. Ed. S.W. Jacob and R. Herschler. Ann. NY Acad. Scien., 243: 20-23.
- [4] Rasmussen, D.H., and A.P. MacKenzie. 1968. Phase diagram for the system water-dimethylsulfoxide. *Nature*. 220: 1315-1317.
- [5] Gabrielle-Madélmont C., and R. Perron. 1983. Calorimetric studies on phospholipid-water systems. *J. Colloid Interface Sci.* 95: 483-493.
- [6] Yu, Zhi-Wu, and Peter J. Quinn. 1995. Phase stability of phosphatidylcholines in dimethylsulfoxide Solutions. *Biophys. J.* 69: 1456-1463.
- [7] Nagle, J.F. et al. 1996. X-ray structure determination of fully hydrated  $L_{\alpha}$  phase dipalmitoylphosphatidylcholine bilayers. *Biophys. J.* 70: 1419-1431

- [8] MacDonald R. C., R.I. MacDonald, B.Ph.M. Menco, K. Takeshita, N.K. Subbarao, L.-R. Hu. 1991. Small-volume extrusion apparatus for preparation of large, unilamellar vesicles. *Biochim. Biophys. Acta.* 1061, 297-303.
- [9] Gordeliy, V.I., L.V. Golubchikova, A.I. Kuklin, A.G. Syrykh, A. Watts. 1993. The study of single biological and model membranes via small-angle neutron scattering. *Prog. Colloid Polym. Sci.* 93, 252-257.
- [10] Dubnickova, M.; M. Kiselev, S. Kutuzov, F. Devinsky, V. Gordeliy, P. Balgavy. 1997. Effect of N-Lauryl-N,N-Dimethylamine N-Oxide on Dimyristoyl Phosphatidylcholine Bilayer Thickness: A Small-Angle Neutron Scattering Study. *Gen. Physiol. Biophys.* 16, 175-188.
- [11] Wiener, M.C., R.M. Suter, and J.F. Nagle. 1989. Structure of the fully hydrated gel phase of dipalmitoylphosphatidylcholine. *Biophys. J.* 55: 315-325.

Received by Publishing Department  
on April 15, 1998.