

**STRUCTURE OF UNILAMELLAR
DIMYRISTOYLPHOSPHATIDYLCHOLINE VESICLE.**

SMALL-ANGLE NEUTRON SCATTERING STUDY.

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Abstract

On the basis of the separated form-factor model, a code for fitting the small-angle neutron scattering spectra of the polydispersed vesicle population has been developed. Vesicle and membrane bilayer parameters are analyzed for various hierarchical models of the neutron scattering length density across the membrane. It is shown that hydration of vesicle can be described by a linear distribution function of water molecules. For the first time, the average radius and polydispersity of the vesicle population, thickness of the membrane bilayer, thickness of hydrophobic and hydrophilic parts of bilayer, and water distribution function have been calculated from the SANS experiment, without additional methods such as dynamic light scattering or freeze-fracture electron microscopy. The results, obtained at two different spectrometers, are discussed. The appropriate conditions of the SANS experiment on vesicles are formulated as a necessity to collect the SANS curve in the region of scattering vectors from $q_{\min}=0.0033\text{\AA}^{-1}$ to $q_{\max}=0.56\text{\AA}^{-1}$.

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1. INTRODUCTION

Research to the structure of phospholipids, the main component of biological membranes, is very important from the viewpoint of structural biology and chemistry. Unilamellar vesicles are especially interesting because most biological membranes are unilamellar. On the other hand, unilamellar vesicles can be used as delivery agents; thus, the knowledge of their structure and properties are important for pharmacology [1,2].

A standard method to investigate the form and size of vesicles is the dynamic and static light scattering. However, in this way it is impossible to obtain information about the thickness and internal structure of the membrane bilayer [3,4].

A more informative method is the small angle neutron scattering (SANS). Membrane thickness can be calculated from the experimentally measured gyration radius of membrane using the Guinier approximation and the Kratky-Porod plot [5-7]. This approach was applied for the calculation of phospholipid membrane thickness [8-12] and thickness of thylakoid membranes [13] from the measured value of the gyration radius. The accuracy of the membrane thickness calculation from the experimentally measured gyration radius increases at increasing contrast [9].

The pair distance distribution function can be calculated via indirect Fourier transformation of SANS curve [14]. The indirect Fourier transformation was applied for the calculation of the scattering length density across the bilayer for the case of unilamellar vesicles from the oppositely charged surfactant [15].

The important advantage of neutron scattering relative in relation the X-ray scattering is the possibility to use deuterated solutions and samples. The contrast variation method was applied to characterize molecular volume of phospholipid molecules and membrane thickness [8,16]. The method for the evaluation of the deuterated molecule group position derived from the SANS curve was proposed in [9].

Calculation of membrane parameters from the experimental membrane radius of gyration used only a part of scattering curve in the interval of scattering vector q from 0.04 \AA^{-1} to 0.1

\AA^{-1} . Nevertheless, this approach was used for the calculation of internal membrane structure and hydration on the basis of the strip function model of neutron scattering length density [17,18]. The model of randomly oriented planar bilayer was applied for the characterization of membrane thickness [19] and internal membrane structure [18].

The possibility to determine the internal structure of membrane depends on the maximum value of scattering angle detected at experimental station [19,20] and possibility to subtract properly the incoherent background [20]. On the other hand, the possibility to evaluate the average vesicle radius increases at the detection of the scattering curve at low value of scattering vector and decreasing of system polydispersity [19,21,22].

The hollow sphere (HS) model was applied for the calculation of membrane thickness [17,22], vesicle radius [21,22] and internal membrane structure [22] from SANS experiment. The approach developed in [20] can be applied for the investigation of oligolamellar vesicles.

The application of the HS model gives opportunity to describe the internal membrane structure as two-three regions with a constant scattering length density via the strip function model of scattering length density across the membrane. The HS model has two imperfections: (1) one cannot say anything about water distribution in the hydrophilic part of the bilayer; (2) for the case of nondeuterated lipids it is impossible to define the place of the molecules inside the bilayer, i.e. this approach makes it impossible to study the multi-component systems (vesicular based delivery agents of drugs, for example).

The separated form-factors model (SFF) looks more perspective from this viewpoint: it allows one to simulate the scattering length density by any integrable function [23]. In this paper, the SFF model is used to study the structure of the polydispersed population of vesicles from the SANS data. We analyze the parameters of vesicles and the membrane bilayer for various hierarchic models of the scattering length density of neutron across the membrane. We show that the water distribution in the hydrophilic part of membrane can be described by a linear function. The parameters of the vesicle population (membrane thicknesses, average radius, polydispersity, number of linearly distributed water molecules in the membrane

bilayer) are calculated only from the SANS spectra, without additional methods usually used to characterize the vesicle size and polydispersity (dynamic light scattering, freeze-fracture electron microscopy).

Two small-angle neutron scattering spectrometers with different range of accessible scattering vector were used: the YuMO instrument in JINR, Dubna, with range of q from 0.0083\AA^{-1} to 0.2\AA^{-1} and the SANS-1 instrument at PSI with range of q from 0.0033\AA^{-1} to 0.56\AA^{-1} .

2. THE FITTING PROBLEM IN THE FRAMEWORK OF THE SFF MODEL

The macroscopic coherent scattering of monodispersed population of vesicles is defined by the formula [24]:

$$\frac{d\Sigma}{d\Omega_{mon}}(q) = n \cdot A^2(q) \cdot S(q) \quad (1)$$

where n is the number of vesicles per unit volume, $A(q)$ is the scattering amplitude of vesicle, $S(q)$ is the vesicle structure factor, q is the length of scattering vector ($q = 4\pi \sin(\theta/2)/\lambda$, θ - the scattering angle, λ - the neutron wavelength).

The scattering amplitude $A(q)$ in the spherically symmetric case is equal [24] to

$$A(q) = 4\pi \cdot \int \rho_c(r) \cdot \frac{\sin(qr)}{qr} \cdot r^2 \cdot dr. \quad (2)$$

Here $\rho_c(r) = \rho(r) - \rho(\text{D}_2\text{O})$ is the neutron contrast between the scattering length density of the lipid bilayer $\rho(r)$ and D_2O ($\rho(\text{D}_2\text{O}) = 6.4 \cdot 10^{10} \text{ cm}^{-2}$), R is the vesicle radius that corresponds to the radius of bilayer center. Substituting $r = R + x$ and integrating over the space where $\rho_c(x) \neq 0$, gives the following expression:

$$A(q) = 4\pi \cdot \int_{-d/2}^{d/2} \rho_c(x) \cdot \frac{\sin[q \cdot (R + x)]}{q} \cdot (R + x) \cdot dx. \quad (3)$$

Here, d is the membrane thickness. Assuming $R \gg d/2$ and approximating $R + x \approx R$ one can rewrite Eq.(3) in the form

$$A(q) = 4\pi \cdot \frac{R}{q} \int_{-d/2}^{d/2} \rho_C(x) \cdot \sin[q \cdot (R + x)] \cdot dx \quad (4)$$

We assume in our model that $\rho_C(x)$ is a symmetric function relative to the bilayer center ($x=0$). (In actuality, $\rho_C(x)$ is not symmetric due to membrane curvature and, for many component system, due to a possible asymmetry of different components.) Thus, we obtain from Eq.(4):

$$A_{\text{SFF}}(q) = 4\pi \cdot \frac{R}{q} \cdot \sin(qR) \cdot \int_{-d/2}^{d/2} \rho_C(x) \cdot \cos(qx) \cdot dx. \quad (5)$$

(We used the relation $\sin[q \cdot (R + x)] = \sin(qR) \cdot \cos(qx) + \cos(qR) \cdot \sin(qx)$.) Finally, Eq. (6) can be rewritten in a more suitable form

$$A_{\text{SFF}}(q) = 4\pi \cdot \frac{R^2}{qR} \cdot \sin(qR) \cdot \int_{-d/2}^{d/2} \rho_C(x) \cdot \cos(qx) \cdot dx. \quad (6)$$

We have obtained the equation for scattering amplitude where internal structure of bilayer (integral over $-d/2$ to $d/2$) is separated from the vesicle radius R . Hence, the macroscopic cross-section of the monodispersed population of vesicles can be written as

$$\frac{d\Sigma}{d\Omega_{\text{mon}}} (q, R, d) = n \cdot F_s(q, R) \cdot F_b(q, d) \cdot S(q) \quad (7)$$

where $F_s(q, R)$ is a form-factor of the infinitely thin sphere with radius R [21,25]

$$F_s(q, R) = \left(4\pi \cdot \frac{R^2}{qR} \cdot \sin(qR) \right)^2 \quad (8)$$

and $F_b(q, d)$ is a form-factor of the symmetric lipid bilayer.

$$F_b(q, d) = \left(\int_{-d/2}^{d/2} \rho_C(x) \cdot \cos(qx) \cdot dx \right)^2. \quad (9)$$

Eqs.(8)-(10) present the separated form-factor model (SFF) for large unilamellar vesicles [23].

The structure factor $S(q)$ was included into the model as in Ref. [26]. For the case of the 1% concentration of DMPC one can put $S(q) \approx 1$.

The number of vesicles per unit volume $n=n(R,d)$ can be obtained in the following way. It is known that the molecular volume of DMPC in the liquid phase is equal to 1101\AA^3 [27]. The volume of the lipid bilayer in one vesicle can be calculated by formula

$$V = 4\pi/3 [(R+d/2)^3 - (R-d/2)^3]. \quad (10)$$

So, $M=V/1101$ is the number of DMPC molecules in a single vesicle. The concentration of DMPC in our experiment was 15mM. The number of DMPC molecules in cm^3 is calculated as $C = 15 \cdot 10^{-3} \cdot N_A \cdot 10^{-3} = 90.4 \cdot 10^{17}$ where N_A is the Avogadro number. Hence, $n(R,d)=C/M$.

A coherent macroscopic cross section of polydispersed vesicle population $I_{theor}(q, \bar{R}, d)$ is determined by the formula:

$$I_{theor}(q, \bar{R}, d) = \frac{\int_{R_{min}}^{R_{max}} \frac{d\Sigma}{d\Omega_{mon}}(q, R, d) \cdot G(R, \bar{R}) \cdot dR}{\int_{R_{min}}^{R_{max}} G(R, \bar{R}) \cdot dR}, \quad (11)$$

where $R_{min}=100 \text{\AA}$, $R_{max}=1000 \text{\AA}$, \bar{R} is an average vesicle radius, and G is the vesicle polydispersity that is described by the Schulz distribution [20,28] with the polydispersity coefficient m :

$$G(R) = \frac{R^m}{m!} \cdot \left(\frac{m+1}{\bar{R}} \right)^{m+1} \cdot \exp\left[-\frac{(m+1) \cdot R}{\bar{R}} \right], \quad (12)$$

Relative standard deviation of vesicle radius is $\sigma = \sqrt{\frac{1}{(m+1)}}$.

The experimentally measured macroscopic cross-section is not equal to the theoretically calculated value of coherent macroscopic cross-section $I_{theor}(q, \bar{R}, d)$ due to the incoherent scattering background I_{IB} from sample [20] and spectrometer resolution distortions. The method developed in [29], was used to make a correction to the resolution function (note that it is approximately equal $\Delta q/q=20\%$ at small q and $\Delta q/q=10\%$ at large q). Final expression for the macroscopic cross-section $I_{model}(q)$ has the following form

$$I_{model}(q) = I_{theor}(q, \bar{R}, d) + \frac{1}{2} \cdot \Delta^2 \cdot \frac{d^2 I_{theor}(q, \bar{R}, d)}{dq^2} + I_{IB}. \quad (13)$$

where Δ^2 is a second moment of the resolution function [29,30].

To fit the SANS data in the framework of SFF model, the Fortran code was developed using the minimization code DFUMIL from the JINRLIB library (JINR, Dubna). In order to estimate the fit quality, we used the following formula:

$$R_I = \frac{1}{N} \cdot \sum_{i=1}^N \left(\frac{\log[I_{model}(q_i)] - \log[I(q_i)]}{\log[I(q_i)]} \right)^2 \quad (14)$$

where N is a number of experiment points, q_i is experimentally measured value of scattering vector, $I(q_i)$ – experimentally measured macroscopic cross sections.

The fitting parameters are the average vesicle radius \bar{R} , coefficient of polydispersity m , thickness of the lipid bilayer d , and parameters of function $\rho(x)$ modeling the neutron scattering length density of bilayer. We considered three types of $\rho(x)$ function presented at Fig. 1a,b,c. Additional fit parameters are: (a) average contrast $\Delta\rho$ between D₂O and average scattering length density of bilayer ρ_{av} ; (b) average scattering length density of hydrophilic part ρ_{PH} and thickness of hydrophobic membrane part D ; (c) ρ_{PH1} scattering length density at the hydrophilic-hydrophobic boundary $x=\pm D/2$ and value of D . In the cases (b) and (c) we put $\rho_{CH} = -0.36 \cdot 10^{10} \text{ cm}^{-2}$ [20,31]. The scattering length density ρ_{PH2} at the boundary between bulk D₂O and membrane $x=\pm d/2$ was a fixed parameter. We considered two cases (c1) and (c2): (c1) corresponds to $\rho_{PH2} = \rho_{D2O} = 6.4 \cdot 10^{10} \text{ cm}^{-2}$; (c2) corresponds to $\rho_{PH2} = 5.4 \cdot 10^{10} \text{ cm}^{-2}$ (this value was used in [32]).

Besides, one can consider the incoherent background I_{IB} as another unknown parameter of the model. The value of I_{IB} for the case of 15mM DMPC concentration is theoretically estimated as 0.00546 cm^{-1} . In the fitting of the YuMO spectrometer data, this value of the incoherent background was used. For the case of PSI SANS spectrometer, I_{IB} was a fitted parameter.

3. EXPERIMENT

Unilamellar dimyristoylphosphatidylcholine vesicles (DMPC) were prepared by handle extrusion of 15mM (about 1% w/w) suspension of DMPC in D₂O through filters with a pore diameter of 500Å [33]. The SANS spectra from unilamellar vesicles at T=30°C were collected at two different spectrometers.

1. The YuMO time-of-flight spectrometer of the IBR-2 pulsed reactor at the Joint Institute for Nuclear Research (JINR), Dubna, Russia [29]. Two sample-to-detector distances were used: 4.38m and 13.70m. The spectra were normalized on the macroscopic cross-section of vanadium [34].

2. The SANS spectrometer of the Swiss Spallation Neutron Source at the Paul Scherrer Institute (PSI), Switzerland. Three sample-to-detector distances were used: 2m, 6m, and 20m. Neutron wavelength was $4.7 \pm 0.47 \text{Å}$. The spectra were normalized on the macroscopic cross-section of H₂O.

4. RESULTS AND DISCUSSION.

Results of fitting the DMPC vesicle spectrum of the YuMO spectrometer are given on Fig.2 and Table 1. Parameters $\bar{R}=277$ and $m=10$ were calculated only for the uniform density (case (a)). They were fixed for the cases (b) and (c). This approach reduces the number of unknown parameters for a more complex function $\rho(x)$. We put $\rho_{\text{CH}} = -0.36 \cdot 10^{10} \text{cm}^{-2}$, $S(q)=1$, $I_B = 0.00546 \text{cm}^{-1}$.

The introduction of the internal structure of the membrane containing hydrophobic and hydrophilic parts, leads to the increase of the thickness of membrane on 5.4 Å (see variants (a) and (b) of the Table 1 and Fig.1). The calculated thickness of the hydrophobic part of the DMPC membrane $13.2 \pm 0.7 \text{Å}$ is in agreement with the result for a hydrophobic part of the POPC membrane in [20] $13 \pm 1 \text{Å}$. The calculated membrane thickness $42.1 \pm 0.4 \text{Å}$ is a little smaller than the value 44.2Å obtained in [27] from the X-ray diffraction experiment at multilamellar DMPC vesicles. Our results show that the phospholipid hydrophilic part 14.5Å

is significantly higher than the size of its polar head 9 Å [27], i.e. water molecules penetrate into the region of hydrocarbon tails on approximately 5 Å (it corresponds to the length of two methylene groups).

Two variants of the linear water distribution are presented in Table 1: c1 and c2. Variant c2 corresponds to the case of density plotted on Fig. 1c by the dashed line. Variant c1 (solid line on Fig. 1c) shows a situation where $\rho_{PH2} = \rho_{D2O} = 6.4 \cdot 10^{10} \text{ cm}^{-2}$. Both variants give similar results; however, the c1 case provides a little smaller value of residual R_I .

From our calculation, one can make a conclusion about water distribution inside hydrophilic part of the bilayer. Let us apply the results of variant c1 for estimation of the number of water molecules N_W per one DMPC molecule penetrating into the bilayer. Assuming that all water molecules are distributed linearly across the bilayer, value N_W can be calculated as follows:

$$\frac{(\rho_{PH2} - \rho_{PH1})}{2} \cdot \frac{(d - D)}{2} \cdot A = N_W \cdot l_{D2O} \quad (15)$$

where $A = 59.6 \text{ Å}^2$ – the membrane surface area per one DMPC molecule [27], $l_{D2O} = 1.914 \cdot 10^{-12} \text{ cm}$ – scattering length of D_2O molecule. One can obtain from eq.(15) $N_W = 5.7 \pm 0.3$. This value is smaller than value 7.2 obtained from the X-ray diffraction on multilamellar vesicles [27] and value 6.8 ± 0.2 obtained in [18] from analysis of the same SANS curve in the Guinier region of membrane and it is in agreement with the value 7 ± 2 calculated from SANS in [17].

Fitting results for the spectra of the PSI SANS experiment are presented on Fig.3 and Table 2. The fit parameters for three different models of $\rho(x)$ (Fig.1) were: \bar{R} , m , d , D , $\rho(x)$, I_{IB} . It is seen that the values of \bar{R} given in Tables 1 and 2, are in good agreement.

The calculation of water quantity with linear distribution across the hydrophilic part of membrane (Eq.(15)) gives $N_W = 3.9 \pm 0.03$. It is smaller than the water molecules number in the polar head groups region obtained in [17,18,27]. It is a reasonable result: from molecular

dynamic simulation, the water distribution across the bilayer is more similar to sigmoidal function [35]. Only middle part of the sigmoidal function has a linear form. Thus, modeling of water distribution across the bilayer gives underestimated value of water molecules.

As we have already mentioned, at the YuMO experiment data fitting, \bar{R} and m were fitted only for $\rho(x) \equiv \text{const}$. On the contrary, in case of the PSI SANS data fitting, the values of \bar{R} and m were fit parameters for all the model calculations. It was possible because the experimental conditions of the PSI small-angle spectrometer are better for the determination of vesicle radius: at YuMO spectrometer $q_{\min}=0.0083 \text{ \AA}^{-1}$ while at PSI SANS spectrometer $q_{\min}=0.0033 \text{ \AA}^{-1}$. According to the SFF model, the possibility to measure the scattering curve in a small value of q is important for the vesicle radius evaluation (see Eq.(8)). That is why \bar{R} can be directly fitted for all scattering length density models in the case $q_{\min}=0.0033 \text{ \AA}^{-1}$.

The relative standard deviation of vesicle radius $\sigma=0.30$ from experiment at YuMO spectrometer and $\sigma=0.27$ from experiment at the PSI SANS spectrometer. This small difference can arise due to the differences in the value of q_{\min} , accuracy of resolution function calculation and differences in the samples. (It is important to note, that two different handle extrusions at the same conditions cannot produce exactly the same vesicle population.)

Information on the internal membrane structure, obtained at two different spectrometers, confirms that the calculated membrane parameters strongly depend on the used range of scattering vector as was shown in [36,37]. At the PSI SANS spectrometer, the maximum value of q corresponds to 0.56 \AA^{-1} (while the value 0.2 \AA^{-1} at the YuMO spectrometer). The statistical errors at the end portion of the scattering curve of the YuMO spectrometer are large; in fact, this curve was measured with good statistics only to $q_{\max}=0.15 \text{ \AA}^{-1}$. According to the SFF model, the end of scattering curve corresponds to the form-factor of bilayer (see Eq.(5)). The accuracy of restoring the DMPC membrane structure depends on the possibility to collect the SANS curve in the region of large values of q , as it was done with PSI SANS spectrometer [36,37].

The important parameter is incoherent background I_{IB} . Due to the large value of q_{max} at the PSI SANS spectrometer, this value can be used as a fit parameter. (For the case of the YuMO we should estimate this value from the quantity of lipids in the sample.) The calculated values are in the range of $0.0050 - 0.0059 \text{ cm}^{-1}$ and correspond to the theoretical value for 15mM DMPC concentration, 0.0055 cm^{-1} .

The value of membrane thickness 47.4\AA and thickness of hydrophobic membrane part 17.3\AA from the PSI experiment exceed the corresponding values 42.5\AA and 11\AA obtained from the YuMO experiment. The values of the hydrophilic part of DMPC membrane calculated from the PSI experiment, 15.1\AA , and from the YuMO experiment, 15.8\AA , are in reasonable agreement. The calculated value of DMPC membrane thickness exceeds the value 44.2\AA obtained in [27] from the X-ray diffraction experiment and value of 44.5 obtained from SANS in [18].

5. CONCLUSION

On the basis of the SFF model, the scheme and code of fitting the SANS spectra of polydispersed vesicle population have been developed taking into account a structural factor, a spectrometer resolution function, and internal structure of vesicles.

The accuracy of the vesicle structure fitting depends on the experimentally measured range of the scattering vector. This means that the restored parameters of the internal membrane structure depend on the value of q measured experimentally. For the systems under study, the best experimental conditions were realized for the PSI SANS spectrometer with possibility to collect a scattering curve in the q range from $q_{min}=0.0033\text{\AA}^{-1}$ to $q_{max}=0.56\text{\AA}^{-1}$.

The SFF model for the SANS data of the PSI spectrometer allowed one to calculate parameters of the polydispersed DMPC vesicle population: average radius $275\pm 0.4 \text{ \AA}$, polydispersity 27%, lipid bilayer thickness $47.4\pm 0.04\text{\AA}$; thickness of its hydrophobic and hydrophilic parts $17.3\pm 0.05 \text{ \AA}$ and $15.05\pm 0.09 \text{ \AA}$, respectively. The number of water

molecules per one DMPC molecule, which are linearly distributed across the hydrophilic part, is calculated as 3.9 ± 0.03 .

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References

- [1] A.Nagayasu, K.Uchiyama, H.Kiwada. *Adv. Drug Delivery Rev.*, 40 (1999) 75-87.
- [2] G.Cevc, A.Schatzlein, H. Richardsen. *Biochim. Biophys. Acta*, 1564 (2002) 21-30
- [3] J.Pencer, G.F.White, F.R.Hallet. *Biophys. J.*, 81 (2001) 2716-2728.
- [4] A.J.Jin, D.Huster, K.Gawrisch, R.Nossal. *Eur. Biophys. J.*, 28 (1999) 187-199.
- [5] A.Guinier, G.Fournet. *Small-angle scattering of X-rays* (Chapman and Hall, London, 1955).
- [6] O.Kratky. *Prog. Biophys.*, 13 (1963) 105-173.
- [7] O.Glatter, O.Kratky. *Small-angle X-ray scattering* (Academic Press, New York, 1982).
- [8] W.Knoll, J.Haas, H.Stuhrmann, H.H. Fulner, H. Vogel. *J. Appl. Cryst.*, 14 (1981) 191-202.
- [9] V.I. Gordeliy, L.V. Golubchikova, A.I. Kuklin, A.G. Strykh, A. Watts. *Progr. Colloid Polym. Sci.* 93 (1993) 252-257.
- [10] M.Dubnickova, M.Kiselev, S.Kutuzov, F.Devinsky, V.Gordeliy, P.Balgavy. *Gen. Physiol. Biophys.*, 16, 175-188, 1997.
- [11] S.N.Shashkov, M.A.Kiselev, S.N.Tioutiounnikov, A.M.Kisselev, P.Lesieur. *Physica B*, 271 (1999) 184-191.
- [12] T. Gutberlet, M. Kiselev, H. Heerklotz, G. Klose. *Physica B*, 381-383, (2000), 276-278.
- [13] V.I. Gordeliy, V.G. Cherezov, A.D. Tugan-Baranovskaya, L.S. Yagujinskij. *Biochem. Mol. Biol. Int.*, 38 (1996) 485-491.
- [14] O.Glatter. *J. Appl. Cryst.*, 10 (1977) 415-421.
- [15] D.J.Iampietro, L.L.Brasher, E.W.Kaler, A.Stradner, O.Glatter. *J. Phys. Chem. B*, 102 (1998) 3105-3113.
- [16] D.M. Sadler, F.Reiss-Husson, E.Rivas. *Chem. Phys. Lipids*, 52 (1990) 41-48.

- [17] P.Balgavy, M.Dubničková, N.Kučerka, M.A.Kiselev, S.P.Yaradaikin, and D.Uhrikova. *Biochim. Biophys. Acta* 1521 (2001) 40-52.
- [18] N.Kucerka, M.A.Kiselev, P.Balgavy. *Europ. Biophys.J.*, in press (2003).
- [19] J.Pencer, R.Hallet. *Phys. Rev. E*, 61 (2000) 3003-3008.
- [20] H.Schmiedel, P.Joerchel, M.Kiselev, G.Klose. *J. Phys. Chem. B*, 105 (2001) 111-117.
- [21] P.Lesieur, M.A.Kiselev, L.I.Barsukov, D.Lombardo: *J. Appl. Cryst.* 33, 623 (2000).
- [22] M.A.Kiselev, S.Wartewig, M. Janich, P.Lesieur, A.M.Kiselev, M.Ollivon, R.Neubert. *Chem.Phys.Lipids*123 (2003) 31-44.
- [23] M.A.Kiselev, P.Lesieur, A.M.Kiselev, D.Lombardo, V.L.Aksenov. *Applied Physics A*, 74 (2002) S1654-S1656.
- [24] L.A.Feigin, D.I.Svergun: *Structure analysis by small-angle X-Ray and neutron scattering* (Plenum Publishing Corporation, New York, 1987).
- [25] M.Bergstrom, J.S.Pedersen, P.Schurtenberger, S.U.Egelhaaf. *J. Phys. Chem. B*, 103 (1999) 9888-9897.
- [26] M.A.Kiselev, D.Lombardo, A.M.Kiselev, P.Lesieur. *JINR Preprint* E19-2003-33, Dubna, 2003.
- [27] J.F.Nagle, S.Tristram-Nagle. *Biochim. Biophys. Acta*, 1469 (2000) 159-195.
- [28] F.R.Hallet, J.Watton, P.Krygsman: *Biophys. J.* 59, 357 (1991).
- [29] Y.M.Ostanevich: *Makromol. Chem., Macromol. Symp.* 15 (1988) 91-103.
- [30] I.A.Gladkih, A.B.Kunchenko, Yu.M.Ostanevich, L.Cser. *JINR Comm* P3-11487, Dubna, 1978.
- [31] E.V.Zemlyanaya, M.A.Kiselev. *JINR preprint* P3-2002-163, Dubna, 2002.
- [32] N.Kucerka, M.A.Kiselev, P.Balgavy. <http://preprint.chemweb.com/physchem/0210004>.
- [33] R.C.MacDonald, R.I.MacDonald, B.Ph.M.Menco, K.Takeshita, N.K.Subbarao, L.Hu. *Biochim. Biophys. Acta*, 1061 (1991) 297-303.
- [34] V.Yu.Bezzabotnov, Yu.M.Ostanevich. *JINR Comm.* P3-88-394, Dubna, 1988.
- [35] R.S.Armen, O.D.Uitto, S.E.Feller. *Biophys. J.*, 75 (1998) 734-744.
- [36] M.Schalke, M.Losche. *Adv. Colloid Interf. Sci.*, 88 (2000) 243-274.
- [37] M.Schalke, P.Kruger, M.Weygand, M.Losche. *Biochim. Biophys. Acta*, 1464 (2000) 113-126.

Table 1. Parameters of DMPC vesicles (T=30°C) calculated in the framework of SFF model for different forms of the scattering length density of neutron across lipid bilayer (the YuMO spectrometer).

	$\bar{R}, \text{Å}$	$d, D, \text{Å}$	$\rho, 10^{10} \text{cm}^{-2}$	$R_I, \%$
(a)	277±5	36.7±0.1	$\Delta\rho=5.1\pm0.01$	1.3
(b)	277(fix)	42.1±0.4 13.2±0.7	$\rho_{\text{PH}}=2.5\pm0.1$	2.6
(c1)	277(fix)	42.5±0.3 11.0±0.9	$\rho_{\text{PH1}}=4.1\pm0.1$ $\rho_{\text{PH2}}=6.4(\text{fix})$	2.4
(c2)	277(fix)	42.7±0.4 12.7±0.9	$\rho_{\text{PH1}}=4.4\pm0.09$ $\rho_{\text{PH2}}=5.4(\text{fix})$	2.5

Table 2. Parameters of DMPC vesicles (T=30 °C) calculated in the framework of SFF model for different forms of the scattering length density of neutron across lipid bilayer (the PSI SANS spectrometer).

	$\bar{R}, \text{Å}$	m	$d, D, \text{Å}$	$\rho, 10^{10} \text{cm}^{-2}$	$I_{IB}, 10^{-3} \text{cm}^{-1}$	$R_I, \%$
(a)	272.9±0.4	12	36.7±0.021	$\Delta\rho=4.91\pm0.005$	5.01±0.01	0.55
(b)	275.3±0.4	13	46.4±0.03 18.1±0.03	$\rho_{\text{PH}}=3.4\pm0.003$	5.76±0.01	0.16
(c)	275.0±0.4	13	47.4±0.04 17.3±0.05	$\rho_{\text{PH1}}=4.9\pm0.001$ $\rho_{\text{PH2}}=6.4(\text{fix})$	5.899±0.01	0.15

Fig.1 (a) – the uniform scattering length density of neutron; (b) – the ‘step’ scattering length density; (c) – the density of the linear function type. ρ_{D_2O} , ρ_{PH} , ρ_{CH} – the scattering length density of the D_2O , hydrophilic and hydrophobic parts of lipid bilayer, respectively. D and d are thickness of hydrophobic part of membrane and thickness of lipid bilayer, respectively.

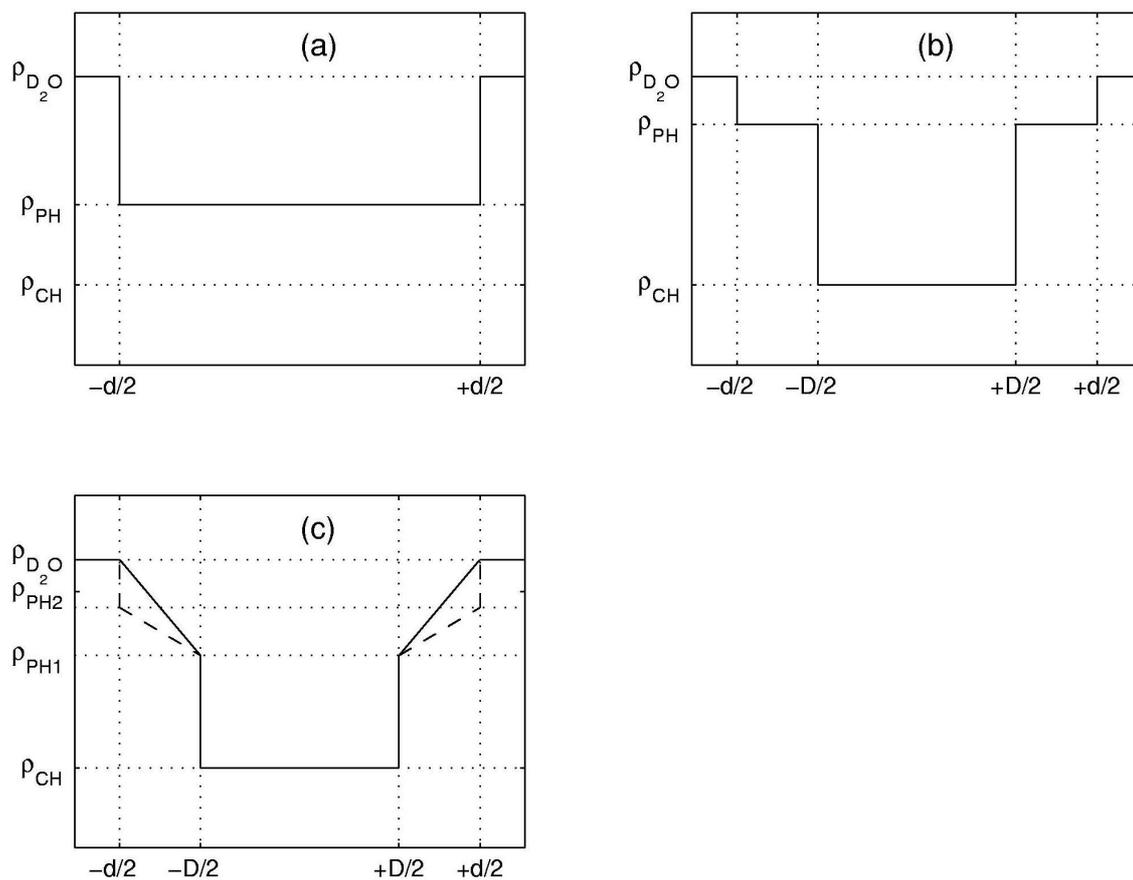


Fig.2. Results of fitting of the DMPC vesicle spectrum for three variants of the internal structure of lipid bilayer given in Fig.1 (the YuMO spectrometer)

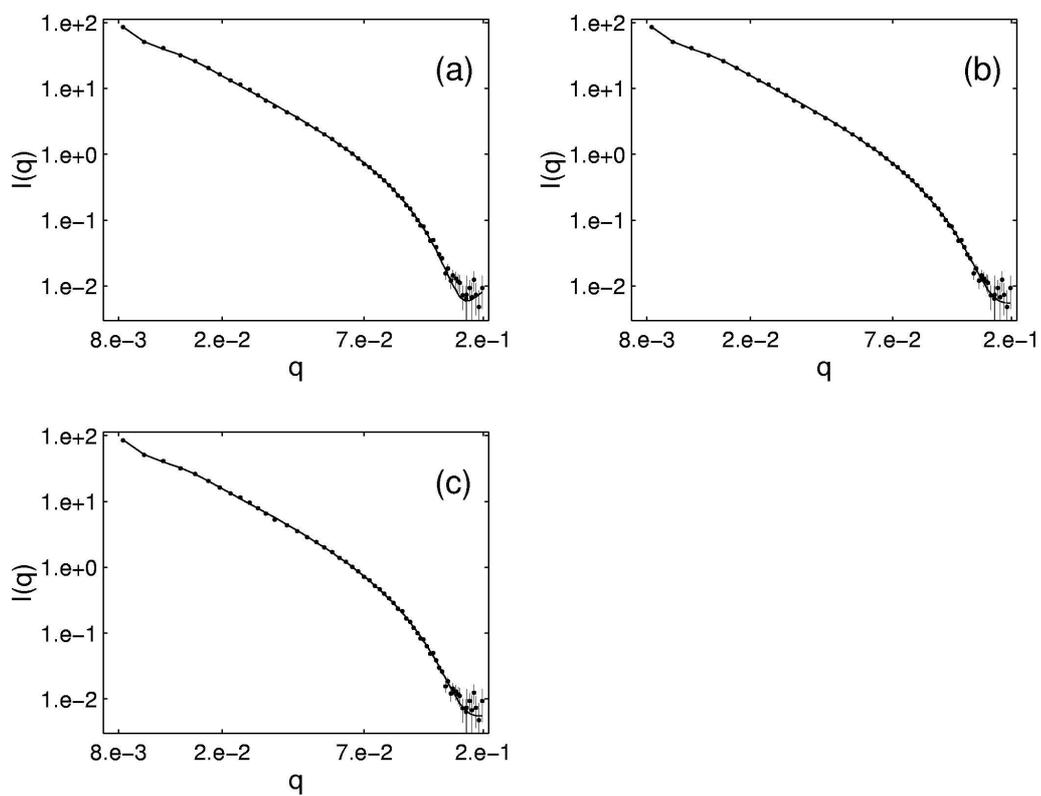


Fig.3. Results of fitting of the DMPC vesicle spectrum for three variants of the internal structure of lipid bilayer given in Fig.1 (the PSI SANS spectrometer)

