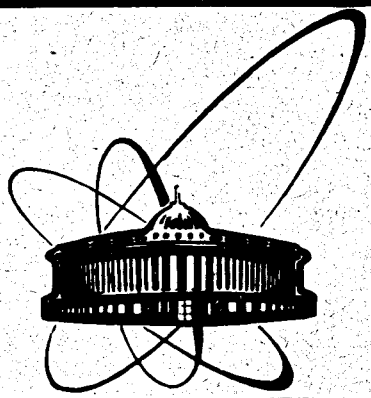

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STUDIES OF A LIPID MEMBRANE STRUCTURE WITH THE NEUTRON DIFFRACTOMETER OF JINR

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At the high flux pulsed reactor IBR-2, Laboratory of Neutron Physics, Joint Institute for Nuclear Research[1] which has been operated for over five years series of experiments with model and biological membranes have been done.

Most of these experiments have been performed at the TOF-diffractometer DN-2 with the position sensitive detector[2] and some experiments — at the small angle neutron scattering instrument (SANS)[3].

It is known that the neutron diffraction method is the most corresponding one for studies of model and biological membranes structures[4]. Besides that, the use of the pulse neutron sources gives some other advantages — the main of them is the scope for studying the unsteady-state processes in membranes[5].

1. STUDIES OF MEMBRANES WITH THE DN-2 DIFFRACTOMETER

In 1982 a neutron diffractometer for crystal structure investigations has been installed at the IBR-2 pulsed reactor. Its advantage is the application of a mirror neutron guide to shaping of an incident beam and the use of the one- or two-dimensional position sensitive detectors to count scattered neutrons. The diffractometer can be used to measure the generalized structure factor in many points of reciprocal space for small and middle transfer. The thermal neutron flux on a sample is 1.3×10^7 n sm^{-1} s^{-1}, the range of the used neutron wavelengths is $\lambda \sim 1.2 \div 20$Å. The range of transferred wave-vectors is $Q \sim 10^{-2} \div 10$ $Å^{-1}$. As was shown in the experiments, the maximum of data acquisition which is needed for construction of the bilayer Fourier-profile (the neutron scattering amplitude density projection on a normal to a bilayer plane) with ~10Å resolution is about one hour for the sample mass of about 10 mG. This value is ~10 times less than in the same experiments at the stationary reactors[2]. The diffraction spectrum from oriented multilamellar liquid-crystal membranes from egg lecithin (EYL) with the sample mass of about 5 mG (the time of data acquisition is about 80 s and diffraction angle $2\theta = 10^0$) is shown in Fig. 1. For smaller diffraction angles the time of a data acquisition is much less and equals few seconds for non-oriented phosphotidylcholine membranes. The unsteady state proces-

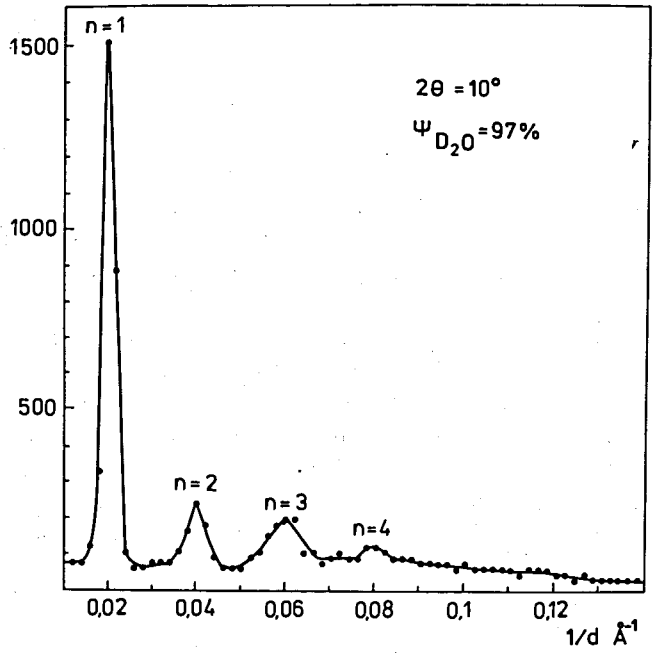

Fig. 1. Diffraction spectrum from the egg lecithin in 1/d scale, d – repeat distance, n – order number of a reflection, the time of data acquisition in 80 s.

ses in membranes can be studied because the fact that the time of data acquisition is so small.

The kinetics of sorption and desorption of water and that of the exchange of H_2O and D_2O in lipid multilayers was observed with the neutron diffractometer. In the experiment one had to measure the dependence on the sample conditions of diffraction peak intensity and structural period. Minimal time of data acquisition was 80 s. It was found out that all the processes had a fast phase with the characteristic time $\tau_c <$ < 1 min and one or two slow phases with τ_c of several minutes or several dozens of minutes[6].

In series of the experiments it was shown that the bilayer could be formed in the systems of egg lecithin-ethylenglycol (EYL + EG) and egg lecithin-dimethylsulphoxid (EYL + DMSO)[7]. The repeat distance for these structures is significantly smaller than in the egg lecithin-water system: 51.0 Å for EYL + H_2O, 41,8 Å for EYL + EG, 44.0 Å for EYL + DMSO at relative humidity $\psi = 20\%$. The "compression" of the bilayer seems to be due to the phase transition from L_α to L_I (interdigitated) phase. The structure analysis was carried out for the system EYL + DMSO + water. From the Fourier-profile of the structure projection on the normal to the bilayer it is seen that the DMSO molecules are incorporated in the region of polar head groups and betweem them (Fig. 2).

Some investigation of the influence of hexane phosphonic acid diethylester (ester PAE_6) on the model lipid membrane structure from egg yolk lecithin (EYL) has been performed with a neutron diffractometer. The distribution of scattering amplitude densities in the perpendicular direction to the lamellar plane has been obtained. The distribution of water was determined

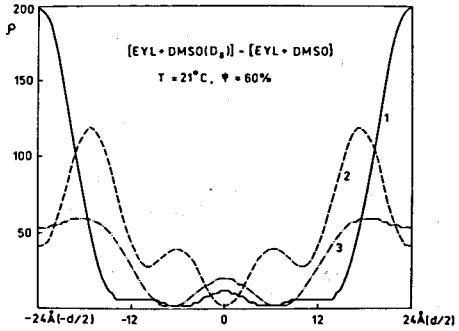

Fig. 2. Fourier-profile of the density amplitude; 1 – $\rho_W(x)$ – distribtion of the water density; 2 – $\rho_L(x)$ – density of the lipid bilayer; 3 – $\rho_{DMSO}(x)$ – density of the DMSO distribution (multiple on 5).

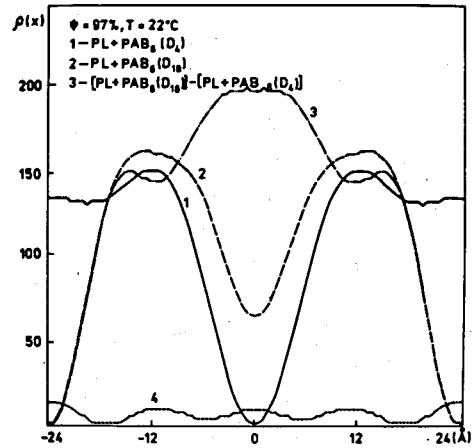

Fig. 3. Fourier-profile of the scattering density under $\psi = 97\%$ H_2O, $T = 22°$ C for a sample from: 1 – $PL + PAB_6 - D_4$; 2 – $PL + PAB_6 - D_{18}$. Curve 3 – the difference Fourier-profile of esters distribution in a membrane. Curve 4 – the level of the experimental accuracy.

for the system EYL + PAE_6 (molar relation 2:1) at relative humidities $\psi =$ $= 69\%$ and $\psi = 97\%$ (T = 20° C). With the help of the deuterium labelled esters and the Fourier difference synthesis method the ester groups of phosphonic ester were show to be placed near the carbonyl groups of lipids.

Besides that, the studies of the influence of the hexame phosphonic acid diester (PAB_6) on the model lipid membrane structure from a total lipid fraction of egg (PL) (molar relation of PL + PAB_6 is 2:1) have been performed with a neutron diffractometer. The two-phase coexistence with a significant difference in the lattice constants at relative humidities $\psi = 15\%$ and 60% (T = 22° C). Only one L_α-phase with d = 48.1 Å is observed at $\psi =$ $= 97\%$. Using the deuterium labelled esters and Fourier difference synthesis method, the distribution of PAB_6 and water, other structure parameters of membranes were determined. It is shown that the molecules of PAB_6 are mainly distributed in the lipid region with the maximum value of density discribution in the central carbon (hydrophobic) part of membrane (Fig.3).

2. INVESTIGATIONS OF A MEMBRANE WITH THE SMALL-ANGLE NEUTRON SCATTERING INSTRUMENT (SANS)

A small-angle neutron scattering installation is oriented on solving the structural problems of solid state physics, biology, colloid chemistry, physics of polymers. Its specific properties are as follows: the use of the time-of-flight method for the determination of the neutron wavelength and the use of the ring-like multiwire ^{3}He neutron detector for counting scattered neutrons. The thermal neutron flux on a sample is 6.5×10^7 n cm^{-2} s^{-1}. The range of the used neutron wavelength is $\lambda \sim 0.7 \div 4$ Å. The range of transferred wave-vector is $Q \div 8 \times 10^{-3} \div 2.0$ Å^{-1}.

The small-angle neutron scattering (SANS) on highly oriented dipalmithoylphosphatidilchline (DPPC) multilayers in liquid (L_α) phase (lateral diffraction) clearly shows the existence of extra-inhomogeneities of scattering density (water-filled defects) appearing when a sample is wetted by a saturated heavy water vapour. The existence of defects, their size and concentration found from SANS data are in excellent agreement with predictions of a dynamically clustered bilayer model (Ivkov, 1984) (Fig. 4). In a solid ($L_{\beta'}$) phase of DPPC no extradefects are observed after the sample wetting (the diffusion scattering intensity was at least several times less). It follows from the experiments described: the mean cluster radius $R = (29 \pm 2)$ Å, for the area occupied by the defects on the membrane surface $\Sigma \sim 16\%$, increases in the bilayer carbon part volume due to implanted defects $\Delta v/v \sim 2\%$. These and other experiments on studies of a membrane structure by the neutron diffraction method have demonstrated high efficiency in the field of a membrane structure investigations with the DN-2 diffractometer and small-angle neutron scattering installations.

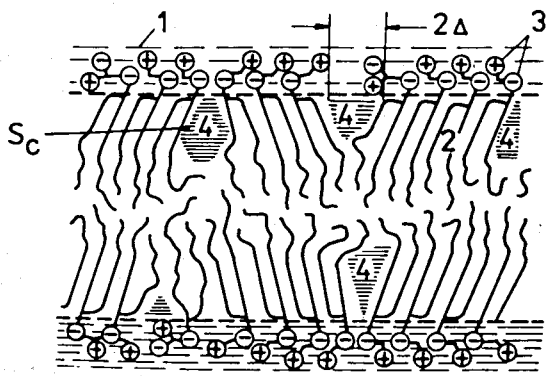

Fig. 4. The cross-section of the membrane with defects in a cluster model. 1 - water; 2 – hydrocarbon of lipid chains; 3 – polar heads of lipids; 4 – defects with water; S_Δ – cross-section of a defect; 2Δ – the size of a defect which is near the polar head.

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