Thickness of lipid bilayer and lipid surface area in unilamellar DMPC and DPPC liposomes evaluated from small-angle neutron scattering curves measured at different contrasts

KUČERKA, N. [1], UHRÍKOVÁ, D. [1], ISLAMOV, A. [2], BALGAVÝ, P. [1]

[1] Faculty of Pharmacy, J. A. Comenius University, 832 32 Bratislava, Slovakia

[2] Frank's Laboratory of Neutron Physics, JINR, 141980 Dubna, Russia

Introduction

The study of the biological membranes is one of the most popular parts of biophysics. Its questions consist of the determination of membrane components, their physical properties and interactions, determination of solvent effects and changes of their physical properties due to some admixtures, e.g. drug molecules. Because the biological membranes are very complex objects, it is convenient to study their physical properties by using model systems, such as phospholipid bilayers. The basic parts of phospholipid molecule are the polar head group region and the nonpolar hydrocarbon region consisting of the chains of methylene groups (CH_2) and of the region of methyl groups (CH_3). In the bilayer, some limited number of water molecules can penetrate into the head group region. Then the basic physical parameters of these systems are the thickness of the phospholipid bilayer, d_L, the surface area per lipid on the bilayer-aqueous phase interface, A_L, and the number of water molecules per lipid penetrated into the polar region of the bilayer, N_L. In the present report we demonstrate that the bilayer thickness d_L, the surface area A_L, and the number of water molecules N_L can be obtained simultaneously from the small-angle neutron scattering (SANS) curves measured at different contrasts.

Material and Methods

The unilamellar 1,2-dimyristoylphosphatidylcholine (DMPC) and 1,2-dipalmitoylphosphatidylcholine (DPPC) liposomes were prepared by extrusion through two polycarbonate filters with pores of diameter 500 Å. The samples were subjected to 51 passes through the filters at temperature about 60 °C. The SANS measurements were performed at 65 °C and contrasts $N_{D_2O}/(N_{D_2O} + N_{H_2O})$ =1.0, 0.6 and 0.4 at the small-angle time-of-flight axially symetric neutron scattering spectrometer YuMO at the IBR-2 fast pulsed reactor of the Frank's laboratory of Neutron Physics, JINR in Dubna.

Results and discussion

SANS curves of extruded unilamellar liposomes from DMPC and DPPC in the aqueous phase are evaluated by using a multishell model, which divides the lipid bilayer of liposomes into the polar head group region, and the nonpolar hydrocarbon

region consisting of the chains of methylene groups and of the region of methyl groups. In the each of these regions, the coherent neutron scattering length density is supposed to be homogeneous. The evaluation is based on obtaining of gyration radius (R_g) from the Kratky-Porod plot of SANS data in the Guinier region of small scattering vector values 0.001 Å^{-2}>Q^2≥0.006 Å^{-2} [1]. From gyration radii obtained at several different molar fractions $N_{D_2O}/(N_{D_2O}+N_{H_2O})$ in the aqueous phase (contrasts) and independent volumetric data [2], the lipid surface area A_L (or the bilayer thickness d_L) and the number of water molecules N_L penetrated into the bilayer polar region can be evaluated in the following steps: The value of $R_g(exp)$ is obtained from the scattering curve by fitting the data in the region of 0.001 Å^{-2}≤Q^2≤0.006 Å^{-2}. Then, the A_L value is calculated for a given N_L value from the interval 0≤N_L≤20. This is done by fixing the N_L value, calculating the scattering curves for different A_L values using the multishell model of the liposome, and fitting them by linear functions in the region of 0.001 Å^{-2}≤Q^2≤0.006 Å^{-2} till their R_g value fulfils the condition |R_g-$R_g(exp)$|≤0.001 Å. The set of paired A_L and N_L values is obtained at given contrast and this is plotted as a continuous curve by fitting the paired A_L and N_L points by a smooth polynomial function (Fig.1). The whole procedure is repeated for another contrast. It is seen, that the continuous A_L vs. N_L curves obtained at two different contrasts intersect in one point. From this point, the values of A_L and N_L are obtained. The same procedure gives also the d_L value. Using this method the values d_L=41.2 Å, A_L=59.0 Å^2 and N_L=3.2 for DMPC and d_L=45.1 Å, A_L=62.7 Å^2 and N_L=5.9 for DPPC were obtained. The method is very sensitive to the precision of SANS data.

Fig. 1: Dependence of the surface area A_L on the number of water molecule N_L calculated from the SANS data of **A-** DMPC; **B-** DPPC
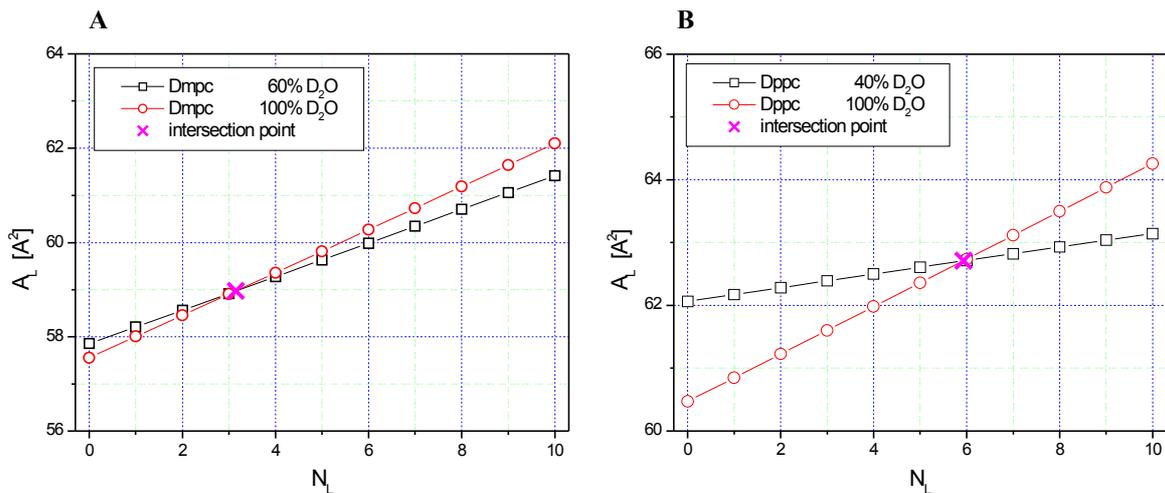
[1]   Knoll, W., Haas, J., Stuhrmann, H. B., Fuldner, H. H., Vogel, H., and Sackman, E.: *J. Appl. Cryst.* **14** (1981) 191

[2]   Nagle, J. F. and Wilkinson, D. A.: *Biophys. J.* **23** (1978) 159