Investigation of the Lipid Vesicle Structure via Small-Angle Neutron Scattering Experiment

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Малоугловое рассеяние нейтронов (МУРН) использовалось для исследования полидисперсных популяций однослойных везикул с диаметром 500 и 1000 ангстрем из димиристоилфосфатидилхолина (ДМФХ) в трех фазах (гель, рипл и жидкой). Параметры везикулярных популяций и внутренняя структура бислоя ДМФХ были определены численно в рамках модели разделенных формфакторов с использованием гидрофобно-гидрофильного приближения для плотности длины рассеяния нейтрона поперек бислоя, а также приближения ступенчатой функцией. Показано, что форма везикул, приготовленных экструзией через поры с диаметром 500 ангстрем, изменяется от приблизительно сферической в жидкой фазе ($T = 30^oC$) до эллиптической в рипл-фазе ($T = 20^oC$) и гель-фазе ($T = 10^oC$). При этом в жидкой фазе толщина мембраны зависит от её кривизны. Определена зависимость толщины мембраны от температуры. Так, везикулы, приготовленные экструзией через поры диаметром 1000 ангстрем, имеют в жидкой, риппл- и гель-фазе толщину мембраны соответственно 45.6±0.2, 48.3±0.6 и 49.6±0.5 ангстрем.

Phospholipids are the main components of cell membranes. Research into the structure of phospholipids is important from a viewpoint of structural biology and biochemistry. Unilamellar vesicles (ULVs) are especially interesting because most biological membranes are unilamellar and the function and properties of integral membrane proteins depend on the lipid bilayer structure. Unilamellar vesicles are also used as delivery agents for drugs. Knowledge of their structure at nanoscale is important for pharmacology.

SANS technique is known as effective method to study the internal bilayer structure and hydration of vesiculae systems.

In [1], SANS on the unilamellar vesicle (ULV) populations (diameter 500 and 1000 armstrong) in D_2O was used to characterize lipid vesicles from dimyristoylphosphatidylcholine (DMPC) at three phases: gel ($T = 10^oC$), ripple ($T = 20^oC$), and liquid ($T = 30^oC$). SANS spectra were collected at SANS-1 spectrometer of the Swiss Spallation Neutron Source at the Paul Scherrer Institute (PSI), Switzerland, and at the YuMO small angle time of flight spectrometer of Frank Laboratory of Neutron Physics, JINR, Dubna, Russia.

Figures 1 and 2 demonstrate the experimentally measured (dots) and fitted (solid lines) SANS curves for the DMPC ULVs at $T = 30^oC$ prepared by extrusion through pores with the diameter 500 and 1000 armstrong, respectively.

Parameters of vesicle populations and internal structure of the DMPC bilayer were calculated on the basis of the Separated Form Factor (SFF) model [2]. This approach

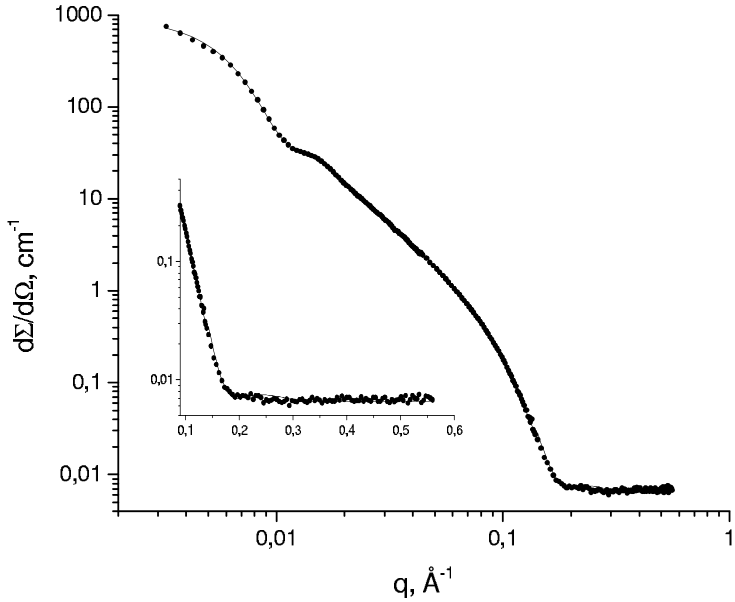

Fig. 1. Experimental cross-section of the UVLs population at T = 30^{o}C (dots) for vesicles extruded through pores of 500 armstrong diameter and fitting curve (solid line). The inset shows the magnified curve for large q

allows simulation of the neutron scattering length density across the bilayer ρ, using any appropriated function. In [3], the fluctuation approximation of ρ was applied to numerically analize a structure of unilamellar DMPC vesicle population. In [4], the hidrophobic-hidrophylic approximation (HH) was presented to approximate ρ and interprete the SANS data for polidispersed populations of unilamellar vesicles of one-, two- and four-component lipid systems.

In [1] we applied the HH approximation of neutron scattering length density across the bilayer ρ at $T = 30^oC$, and the Step Function (SF) approximation [5] of ρ at $T = 10^oC$ and $T = 20^oC$.

It is shown in [1] that the DMPC vesicle shape changes from nearly spherical (eccentricity 1.1) in the liquid phase to elliptical in the ripple and gel phases (eccentricity 1.6). DMPC membrane thickness in the liquid phase demonstrates a dependence on the membrane curvature for extruded vesicles.

It has been found that prepared via extrusion through 500 armstrong diameter pores, vesicle population in the liquid phase has the following characteristics: average value of minor semi-axis 266±2 armstrong, ellipse eccentricity 1.11±0.02 armstrong, polydispersity 26%, thickness of the membrane 48.9±0.2 armstrong and of hydrophobic core 19.9±0.4 armstrong, surface area 60.7±0.5 armstrong^2 and number of water molecules 12.8±0.3 per DMPC molecule. Vesicles prepared via extrusion through pores with the diameter 1000A have the polydispersity of 48 %, and the membrane thickness of 45.5±0.6 armstrong in the liquid phase. SF approximation was used to describe the DMPC membrane structure in gel ($T = 10^oC$) and ripple ($T = 20^oC$) phases. Extruded DMPC vesicles in D_2O have membrane thickness of 49.6±0.5 armstrong in the gel phase and 48.3±0.6 armstrong in the ripple phase. The dependence of the DMPC membrane thickness on temperature was restored from the SANS experiment.

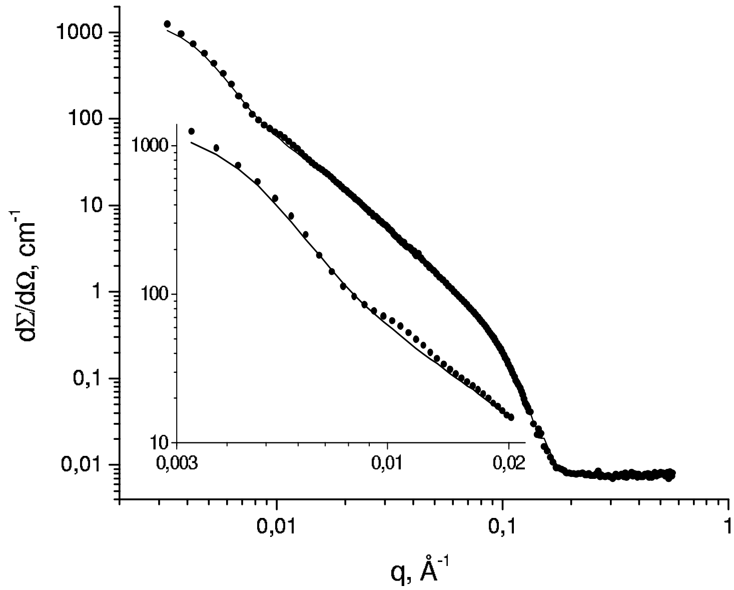

Fig. 2. Experimental cross-section of the ULVs population at T = 30^oC (dots) for vesicles extruded through pores of 1000 armstrong diameter and fitting curve (solid line). The inset shows the magnified curve for small q

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