SFF analysis of the small angle scattering data for investigation of a vesicle systems structure

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Abstract. Experimental data on the small angle synchrotron X-ray scattering (SAXS) are analyzed on a basis of Separated form factors method (SFF) for a study of the drug delivery Phospholipid Transport Nano System (PTNS). Basic parameters of polydispersed population of PTNS nanoparticles (average radius of PTNS-particles, polydispersity of radius, thickness of membrane) have been determined. The results are discussed in comparison with the SFF results for the “classical” vesicular system of dimyristoylphosphocholine (DMPC).

1. Introduction

Small angle scattering of neutrons (SANS) and of X-rays (SAXS) is well known as a powerful tool for investigation of structure of nanosystems including unilamellar vesicles (ULVs) of phospholipids in water solutions of disaccharides [1, 2, 3, 4]. Phospholipid vesicles (liposomes, nanospheres) have a a vide range of applications in modern biochemistry and pharmacology. One of important directions is a development of the drug delivery systems on the basis of phospholipids [5, 6]. In recent years, the Phospholipid Transport Nano System (PTNS) with extremely small diameter (200–300˚A) has been obtained from the soybean phosphatidylcholine in the V.N. Orekhovich Research Institute of Biomedical Chemistry (Moscow, Russia) [7]. Incorporation of drugs into PTNS is shown to sufficiently increase the therapeutic effectiveness [8]. From the point of practical applications, the information on the structure and properties of PTNS nanoparticles in different media is needed. In the recent work [9], on the basis of SAXS data collected at the Kurchatov Synchrotron Radiation Source of the National Research Center “Kurchatov Institute” (Moscow, Russia), the conclusion about vesicular structure of PTNS nanoparticles has been made and an estimation of average size of PTNS particles has been obtained.

In this contribution, we present the results of analysis of the PTNS system by means the Separated Form Factors method (SFF) . SFF is an effective approach to obtain information about a structure of vesicular systems from the small angle scattering data [4, 10, 11]. The SFF results are presented in comparison with the results of analysis on the basis of the Hollow sphere model (HS) and with the results of SFF analysis of “classical” dimyristoylphosphocholine (DMPC) vesicles in water solution of sucrose with different concentration.
2. Experiment
The measurements of the PTNS particles have been performed at room temperature at the DICSI
station of the Kurchatov Synchrotron Radiation Source in Moscow, Russia. The procedure
of SAXS measurement and the sample preparation is the same as in [9]. The measurements
were carried out at two sample-to-detector distances of \( L_{sd} = 30 \) and 243.5 cm with a photon
wavelength of \( \lambda = 1.62 \text{ Å} \). The scattering intensity \( I \) of photons is measured in the SAXS
experiment as a function of the scattering vector \( q = 4\pi \sin(\theta)/\lambda \), where \( 2\theta \) is the photons
scattering angle. For clarity, the layout of the SAXS experiment is reproduced from [9] in Fig. 1.

![Figure 1. The layout of the small-angle scattering experiment.](image)

The PTNS sample for the SAXS measurement is prepared via dilution of lyophilized
PTNS (25% w/w) in water. The sample composition after dilution is as follows (w/w): 5%
phospholipid, 20% maltose, 75% water. More details of the sample preparation are given in [9].

3. Model
In the SAXS data analysis, we employ two approaches based on the Separated Form Factor
method (SFF) [11] and the Hollow Spheres model (HS) [12]. Both approaches suggest the
vesicular structure of the PTNS particles as shown in Fig. 2.

Within the framework of the SFF model, in the case of the monodisperse vesicle population,
the intensity \( I(q) \) is given by the following expression:

\[
I_{SFF}(q) = n I_o F_s(q, R) F_b(q, d),
\]

where \( I_o \) is the intensity of the incident beam, \( n \) — a number of vesicles in cm\(^3\), \( F_s(q, R) \) —
form factor of the spherical surface with radius \( R \), \( F_b(q, d) \) — form factor of the symmetric lipid
bilayer, \( d \) — thickness of the lipid bilayer of ULVs. \( F_s(q, R) \) and \( F_b(q, d) \) are determined as follows:

\[
F_s(q, R) = \left( 4\pi R^2 \frac{\sin(qR)}{qR} \right)^2, \quad F_b(q, \theta_b) = \left( \int_{-d/2}^{+d/2} \rho_s(x) \cos(qx)dx \right)^2.
\]

Here \( \rho_s(x) \) is a contrast between the scattering length density of the lipid bilayer and the density
of the solvent [13]. For the case of X-ray, the scattering length density is proportional to the
density of electrons in the lipids or the solvent. Respectively, the contrast is proportional to
the difference between electron density of lipid bilayer and surrounded solvent. We utilize the “step” form of the density distribution across bilayer, see Fig. 2. Here $\rho_0$ is the scattering length density of the solvent, $\rho_{CH2}$ – scattering length density of the hydrocarbon chains, $\rho_{PH}$ – scattering length density of polar head region.

In case of the HS model, the intensity $I(q)$ is determined as follows:

$$I_{HS}(q) = n I_o \left(\frac{4\pi}{q^3}\right)^2 \left[\sum_{i=1}^{n_s} \rho_i(A_{i+1} - A_i)\right]^2,$$

where $A_i = \sin(qR_i) - qR_i \cos(qR_i)$.

In our calculations, we put $n_s = 3$ and determine $R_1 = R - d/2$, $R_2 = R - D/2$, $R_3 = R + D/2$, $R_4 = R + d/2$, $\rho_1 = \rho_3 = \rho_{PH} - \rho_0$, $\rho_2 = \rho_{CH2} - \rho_0$ in order to reproduce the density profile as in Fig. 2.

Polymdispersity $\sigma$ of the vesicular radius $R$ relative to average radius $\bar{R}$ is accounted utilizing the Schulz distribution with coefficient $m$:

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

The intensity $I_m$ for the polydispersed ULV system is obtained by means of a standard convolution procedure:

$$I_m(q) = \frac{\int_{R_{\min}}^{R_{\max}} I_{SFF,HS}(q, \tilde{R}) G(\tilde{R}, R) d\tilde{R}}{\int_{R_{\min}}^{R_{\max}} G(\tilde{R}, R) d\tilde{R}}.$$

The final expression of intensity $I(q)$ has the following form (details are given in [1, 2, 4, 10, 11]):

$$I(q) = I_m(q) + \frac{1}{2} \Delta^2 \frac{d^2 I_m(q)}{dq^2} + I_B,$$

Here $I_B$ – parameter of incoherent background, $\Delta^2 = 3.6 \times 10^{-7} \text{ cm}^{-2}$ – the second momentum of a resolution spectrometer function [13].

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**Figure 2.** Left panel: structure of a phospholipid unilamellar vesicle with radius $R$. Right panel: the “step” distribution of the scattering length density across the bilayer.
4. Results and Discussion

The experimental SAXS curve $I_{\text{exper}}(q)$ from 25% of PTNS solution in water is shown in Fig. 3 by circles. Plotted is the subtraction of the PTNS intensity and the pure water intensity.

Calculation on the basis SFF model with the “step” model of density distribution across bilayer (see Fig. 2) gives the following values of the average radius and bilayer thickness: $R_{\text{PTNS}} = 134.3 \pm 1.4$ Å and $d_{\text{PTNS}} = 43.5 \pm 0.5$ Å. Respective curve $I(q)$ is plotted in Fig. 3 by solid line. It is seen that the calculated intensity is in good agreement with the experimental data excluding a region of large $q (q > 0.3$ Å$^{-1}$).

A discrepancy between theoretical and experimental values of intensity

$$
\chi^2 = \frac{1}{N - k} \sum_{j=1}^{N} \left( \frac{I(q_j) - I_{\text{exper}}(q_j)}{\delta_{\text{exper}}(q_j)} \right)^2
$$

(where $N$ – number of experimental points and $k$ – number of fitted parameters, $\delta$ – experimental errors) is equal to $\chi^2 = 2.6$. Similar calculations on the basis of the HS model give close results: $R_{\text{PTNS}} = 135.4 \pm 0.6$ Å, $d_{\text{PTNS}} = 36.9 \pm 0.2$ Å, $\chi^2 = 3.5$. Also, the calculated average radius is in agreement with the estimation $136 \pm 18$ Å from the dynamic light scattering experiment [7]. On the other hand, the calculated $R_{\text{PTNS}}$ is about 15% less the estimation in [9], see Table 1.

The disagreement between experimental data and numerical results at $q > 0.3$ can be explained by fluctuations of bilayer. The fluctuations effect as well as more complex models of the density distribution has to be accounted in further analysis [10].

For comparison, we give in Table 1 parameters of the DMPC ULVs system in water solutions of sucrose with different concentrations calculated within the framework of SFF analysis of SAXS and SANS experimental data. The SANS spectra for sucrose concentration 0% - 20% in D$_2$O have been measured on YuMO small-angle spectrometer of IBR-2 reactor (JINR, Dubna, Russia). The SAXS measurements in the case of 40% sucrose concentration in water were performed on the A2 system of the Doris III synchrotron source (DESY, Hamburg, Germany). Details of experiment and fitting procedure are given in [10].

It is seen that the size of PTNS particles is smaller the DMPC ULVs size. Indeed, the minimal value of average radius of DMPS ULVs $R_{\text{DMPC}} = 190.6 \pm 1.6$ Å obtained in case of 40% sucrose.
Table 1. Parameters of PTNS and DMPC vesicular systems. Parameters of PTNS vesicles have been obtained by means of SFF and HS analysis of SAXS spectrum. Parameters of DMPC ULVs for different concentrations of sucrose in water are obtained by the SFF analysis of SANS and SAXS spectra.

| System  | Experiment | Model or Ref. | R, Å | d, Å | σ, % | χ² |
|---------|------------|---------------|------|------|------|-----|
| PTNS    | SAXS       | SFF           | 134.3±1.4 | 43.5±0.5 | 27 | 2.6 |
| PTNS    | SAXS       | HS            | 135.4±1 | 36.9±0.2 | 33 | 3.5 |
| PTNS    | SAXS       | Ref.[7]       | 136 ±18 | 36.9 ±18 | 33 | 3.5 |
| PTNS    | SAXS       | Ref.[9]       | 160±2 | 27.2±0.1 | 22 |     |
| DMPC 0% | SANS       | SFF           | 307.5±5 | 49.8±1 | 30 | 1.1 |
| DMPC 5% | SANS       | SFF           | 270.6±5 | 48.1±2 | 31 | 5.9 |
| DMPC 10%| SANS       | SFF           | 243.4±3 | 47.6±1 | 27 | 6.6 |
| DMPC 20%| SANS       | SFF           | 213.4±2 | 44.5±2 | 26 | 9.1 |
| DMPC 40%| SANS       | SFF           | 190.6±1.6 | 34.8±0.6 | 26 | 1.7 |
| DMPC 40%| SAXS       | Ref.[9]       | 210±2 | 20.5±0.1 |     |     |

solution in water (SFF-analysis of SAXS data), is about 30% exceeds the numerically adjusted to SAXS data average radius of PTNS ULVs $R_{PTNS} = 134.3 ± 1.4$ Å.

The calculations show that the polydispersity $\sigma$ of radius $R$ in both DMPC and PTNS vesicle populations is about 30%, i.e. it is relatively low.

5. Summary

The SFF method is shown to be appropriate approach for analysis of SAXS and SANS data from polydispersed ULVs systems including PTNS particles. The SFF results of analysis of PTNS ULVs are shown to be in reasonable agreement with analysis on the basis of HS model. Calculated basic parameters of the polydispersed PTNS system do not contradict theoretical predictions and the SFF results of DMPC ULVs population.

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