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Application of small-angle X-ray scattering to the characterization and quantification of the drug transport nanosystem based on the soybean phosphatidylcholine

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ABSTRACT

Phospholipid transport nanosystem (PTNS) for drug delivery is based on soybean phosphatidylcholine. The morphology of PTNS is investigated by means of small-angle X-ray scattering. The obtained results allow one to answer the key question from the viewpoint of organization of drug incorporation whether the PTNS nanoparticles have a structure of micelles or vesicles. It is demonstrated that PTNS is a vesicular system with an average vesicle radius of 160 ± 2 Å.

1. Introduction

The development of drug delivery systems allows one to essentially improve the efficiency of drug application. For today, a number of ready drug forms based on different delivery systems have already been introduced in medicine. These drugs are highly efficient and much in demand on the drug market. In this respect, phospholipid-based carriers are of sufficient interest as biodegradable, biologically inactive systems, without allergic, antigenic and pyrogenous reactions [1]. Phospholipids are highly specialized lipids which are the main component of cellular membranes of all variety of living organisms [2]. Their main function consists in the formation of a double lipid layer (bilayer) in the membranes [3]. It is known that the phospholipids contained in a large number in some food (eggs, liver, meat, sunflower seeds, corn, soybeans, etc.) cannot be considered as medical food sources of phospholipids as they contain other components (cholesterol, oils, etc.). Specially developed "essential" phospholipids (EPL) cleared of oils and undesirable impurities are applied to ensure the therapeutic effect in medicine [4]. For the first time, EPL (as a medicine to treat toxic liver damages) was extracted from soybeans by developing and using high purification technologies [5]. During 50 years which passed from the moment of receiving a dosage form of EPL, they were carefully investigated; their pharmacological properties and therapeutic effects were studied in the experiments, numerous clinical trials, and in broad medical practice. Soybean phospholipids are coproducts of soybean oil processing, the production of soybean phospholipids rises with the continuous increase of soybean oil yield. New technologies for isolation and purification of certain phospholids can enormously improve the development of medicine (biomembrane bionics, liposomes, intracellular drug carriers, etc.) and chemical industry (aggregation and dispersion of nanomaterials, etc.) [17].

The most popular phospholipid delivery nanosystems are unilamellar vesicles with a diameter in the range of 300–2000 Å [6]. In recent years, the technology of obtaining phospholipid delivery nanosystems (PTNS) with an extremely small diameter from soybean phosphatidylcholine has been developed in the V.N. Orekhovich Research Institute of Biomedical Chemistry. PTNS is

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produced as a lyophilized powder, which is stable in time [7]. The incorporation of some drugs into PTNS sufficiently increases their bioavailability and therapeutic effectiveness [8].

The carrier morphology is the key characteristic from the viewpoint of the drug incorporation technology. However, nothing is known about the PTNS morphology so far. Dynamic light scattering makes it possible to measure the average radius of PTNS as 136 ± 18 Å, but this technique cannot give a direct answer to the key question whether the PTNS nanoparticles have a structure of micelles or vesicles [7].

To clarify this point, it is necessary to apply more complex methods like small-angle X-ray scattering (SAXS). The SAXS technique is an effective method to characterize both micelles and vesicles [9]. The main difficulty in the SAXS application to characterize small unilamellar vesicles (ULV) is a weak contrast between ULVs and surrounding water. The density of electrons and hence contrast (difference between the electron density in the lipid bilayer and the solvent) can be increased by using solutions of disaccharides [10–13]. Say, the scattering intensity from dimyristoylphosphatidylcholine (DMPC) ULVs in the 40% (w/w) sucrose solution in water exceeds the intensity from the same ULVs in pure water about 100 times [10].

PTNS contains soybean phosphatidylcholine (95–97% purity) from Lipoid® and maltose, the weight ratio of phosphatidylcholine to maltose is 1:4. PTNS nanoparticles are formed in the maltose solution after dilution of lyophilized powder in water. The concentration of maltose in the sample depends on the degree of the PTNS dilution in water. This makes it possible to vary contrast for X-rays. The methodology of contrast variation by disaccharides in the X-ray scattering experiment on ULVs opens up an opportunity to investigate the PTNS structure using the SAXS technique at synchrotron facilities [10–13].

The purpose of communication is to present the first result on the characterization of the PTNS structure at the Kurchatov Synchrotron Radiation Source of the National Research Center «Kurchatov Institute» in Moscow. The analysis of the PTNS SAXS spectrum in comparison with the SAXS data from the DMPC unilamellar vesicles allows one to make conclusion about the vesicular structure of PTNS nanoparticles.

2. Materials and methods

2.1. Materials

Lipoid S100 (purchased from Lipoid GmbH, Germany) was used for obtaining nanoparticles with a content of phosphatidylcholine of at least 95%. Maltose monohydrate (purchased from company MERCK, Germany) was used for the lyophilization of nanoparticles.

2.2. Obtaining of phospholipid nanoparticle emulsion

The phospholipid nanoparticle emulsion was obtained in two stages: (1) obtaining of a crude emulsion using a mechanical stirrer (RW 20.N, IKA, Germany) and (2) obtaining of a thin (nano) emulsion using microfluidizer M110EH30K, Microfluidics, USA.

Obtaining of crude emulsion: 5 g of S100 Lipoid was dispersed at room temperature (T = 25 °C) in 200 ml of deionized water obtained in Milli-Q (Millipore, USA) using a mechanical stirrer at a speed of 500–700 rpm/min for 5 min until a homogeneous emulsion with no visible agglomerates was formed.

Obtaining of thin emulsion: The previously obtained crude emulsion was poured into the homogenizer or microfluidizer receiving tanks. The emulsion was subjected to homogenization at a pressure of 1000 atm at a temperature of 45 °C and the number of cycles from 1–8. The temperature of the resulting emulsion was maintained using the homogenizer or microfluidizer built-in cooling system. Sampling for control measurements was carried out after each cycle of homogenization.

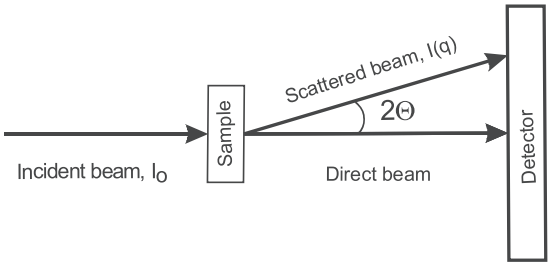

Fig. 1. The layout of the small-angle scattering experiment. The incident photon beam with the intensity I_o is scattered from the sample at an angle 2θ. The intensity of the scattered beam $I(q)$ is measured by a position-sensitive detector.

2.3. Method of data analysis

The scattering intensity of photons $I(q)$ is measured in the SAXS experiment as a function of the scattering vector $q = \frac{4\pi \times \sin(\theta)}{\lambda}$, where 2θ is the scattering angle of photons, and λ is the photon wavelength. The layout of the SAXS experiment is presented in Fig. 1. The scattering intensity $I(q)$ is measured by a position-sensitive detector. For the case of monodisperse vesicle population within the framework of the separated form factor model [15,16], $I(q)$ is given by the following expression

$$I(q) = I_0 \times n \times F_s(q, R) \times F_b(q, d_m), \quad (1)$$

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

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

$$F_b(q, d_m) = \left(\int_{-d_{m/2}}^{d_{m/2}} \rho_c(x) \times \cos(qx) \times dx\right)^2. \quad (3)$$

Here $\rho_c(x)$ is the contrast (difference) between the scattering length density of the lipid bilayer and the solvent [16]. For the case of X-rays, 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 the electron density of the lipid bilayer and the surrounding solvent.

Eq. (3) can be simplified in the case of a homogeneous lipid bilayer $\rho_c(x) \equiv \Delta\rho$ = const:

$$F_b(q, d_m) = \left(\frac{2\Delta\rho}{q} \times \sin\left(\frac{qd_m}{2}\right)\right)^2. \quad (4)$$

The homogeneous approximation $\rho_C(x) \equiv \Delta\rho$ = const is widely used due to the simplicity of calculations.

It follows from the Eq. (2) that the first minimum in the form factor F_s of the spherical surface with radius R is calculated as

$$q_R = \frac{\pi}{R}. \quad (5)$$

The SAXS curves from PTNS and DMPC describe the size of vesicles in the range of small values of q, see the left parts of the curves in Fig. 2. The oscillations in the experimental SAXS spectra are smeared by vesicle polydispersity and spectrometer resolution.