Journal of Physics: Conference Series

PAPER • **OPEN ACCESS**

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To cite this article: Yu Volkov *et al* 2019 *J. Phys.: Conf. Ser.* **1186** 012010

20th International School on Condensed Matter Physics IOP Publishing
IOP Conf. Series: Journal of Physics: Conf. Series **1186** (2019) 012010 doi:10.1088/1742-6596/1186/1/012010

Model-independent X-ray reflectometry analysis of phospholipid thin films on liquid substrates

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Abstract. We present a brief review of application of a model-independent analysis of X-ray reflectometry data to our recent investigations of thin films of phospholipids DSPC and DMPS on a surfaces of water and colloidal silica hydrosol. Comparison of the results against analytical models of phospholipid membranes known in literature allowed us to extract more extensive information on the samples in question. The effect of spontaneous ordering of lipid films on a surface of colloidal silica is discussed. We also demonstrate the possibilities to influence the structure of prepared membranes by enriching the substrate with alkali ions.

1. Introduction

Planar phospholipid systems are of interest both for condensed matter physics and biophysics, as they are considered as the simplest models of a cell membranes [1, 2]. Preparation of macroscopic samples of phospholipid films in practice is limited by the effect of spontaneous curvature of lipid bilayer in an aqueous medium; for this reason, most structural studies of lipid bilayers and multilayers were performed for vesicles and lamellar aggregates [3, 4]. However, the method of obtaining a macroscopically flat lipid multilayers on a substrate of silica hydrosol was proposed in [5].

The colloidal solution of SiO_2 nanoparticles in water stabilized by a small amount of NaOH (also known as silica hydrosol) forms a strongly polarized air/liquid boundary [6]. The gradient of electric potential at the surface exists due to the difference between the potentials of the electric image forces for strongly negative charged nanoparticles and Na^+ cations. In this work, we present a review of our investigations on the effect of spontaneous ordering within a membranes of standard phospholipids 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and 1,2-dimyristoyl-sn-glycero-3-phospho-L-serine (DMPS) deposited on various aqueous substrates.

The structure of lipid films and near-surface layers has been obtained by the X-ray reflectometry method, with the use of a model-independent approach to the reconstruction of distribution of electron concentration [7, 8]. Its significant advantage over the more widely used model-based approaches (see, for example, [9]) is that it does not require any *a priori* assumptions on a shape of the structure under study. The reconstruction method consists in an extrapolation of asymptotic behavior of the angular dependence of reflectivity factor $R(q_z)$ over the region of high q_z, which allows one to significantly reduce the number of possible solutions. In general case, only two unique distributions of dielectric permittivity $\varepsilon(z)$ (and, respectively, electron concentration $\rho(z) = \pi(1-\varepsilon(z))/(r_o\lambda^2)$ [10]) exist that satisfy both the experimental reflectivity $R_{exp}(q_z)$ measured in a limited range of q_z and the estimated asymptotic $R(q_z \to \infty)$ at the same time. The problem of uniqueness for the obtained solution is discussed in-depth in [7].

2. Preparation of samples and experimental setups

Samples were prepared and studied at room temperature $T = 295$K in a hermetically sealed cell with transparent windows according to the method described in [5]. Phospholipid solutions in chloroform (~ 50 mmol/L) were deposited by the use of a calibrated syringe on a surface of liquid substrate placed in a polytetrafluoroethylene dish with diameter of 100 mm. In each case the amount of lipid in a droplet has been estimated for the formation of about ten lipid monolayers after its spreading among the surface; any excess of the surfactant is accumulated in macroscopic three-dimensional aggregates being in equilibrium with the adsorbed film.

Most of the experimental measurements of X-ray specular reflectivity R were performed at synchrotron station ID31 at ESRF [11]. Monochromatic beam with photons wavelength $\lambda = 0.1747 \pm 0.0003\,\text{Å}$ (energy $E \approx 71\,\text{keV}$) has been focused to size $10 \times 250\,\mu$m with integral intensity $\sim 10^{10}$ photons/s. Reflected intensity signal has been collected by 2-D CCD detector MaxiPix [12], matrix size 256×256 pixels with resolution $55\,\mu$m/px, then integrated over the exposure area. However, part of the measurements has been performed at FSRC "Crystallography and Photonics" RAS using a multifunctional butterfly-type diffractometer with laboratory X-ray tube [13]. Monochromatic beam with photons $\lambda = 1.5405 \pm 0.0001\,\text{Å}$ ($E \approx 8\,\text{keV}$), focused to $0.1 \times 10\,$mm, provided integral intensity 3×10^5 photons/s. Signal has been collected by 0-D scintillation detector Radicon SCSD-4.

Comparison between the data sets obtained with different sources is shown by figure 1 (circles, squares and triangles were measured at ID31, diamonds were measured at laboratory setup). It demonstrates that at least for the case of samples under discussion the laboratory diffractometer has been able to provide experimental results comparable to the synchrotron beam at the cost of substantial increase in measurement times, while on the other hand considerably lowering radiation damage to the samples.

3. Results and discussion

Structure of the transient layer at the air/liquid interface of colloidal silica has been studied previously in [6, 14], however the X-ray reflectivity data in these was interpreted according to the 4-layer analytical model. Example of experimental reflectivity curve $R(q_z)$ for the hydrosol containing silica nanoparticles of diameter $D = 12$ nm (30 wt% SiO_2, 0.5 wt% NaOH, pH$\sim$10.5) is shown in figure 1 (circles). Model-independent $\rho(z)$ distribution reconstructed from that data, shown in figure 2a (black line), is in a very good agreement with analytical model taken from [6] (color lines); it is possible to discern the sub-layers of suspended alkali ions, depleted region, and loose monolayer of nanoparticles. Calculation of the surface concentration of Na^+ from the model-independent profile yields $\sim 4 \times 10^{18}$ m^{-2}, which corresponds to the values estimated independently in [14]. Behavior of SiO_2 nanoparticles in the loose monolayer, including the effect of condensation of nanoparticles at air/liquid interface in the presence of phospholipids, was discussed further in our work [15].

Kinetics of the spontaneous ordering within phospholipid film deposited on a surface of silica hydrosol was investigated in our publications [16] and [17]. Reflectivity $R(q_z)$ from the membrane of DSPC (or $C_{44}H_{88}NO_8P$) on the hydrosol with 12 nm silica nanoparticles is shown in figure 1 (squares); presence of the periodic peaks on the curve indicates a regular structure. Reconstructed model-independent distribution (figure 2b, black line) contains five-layer stack

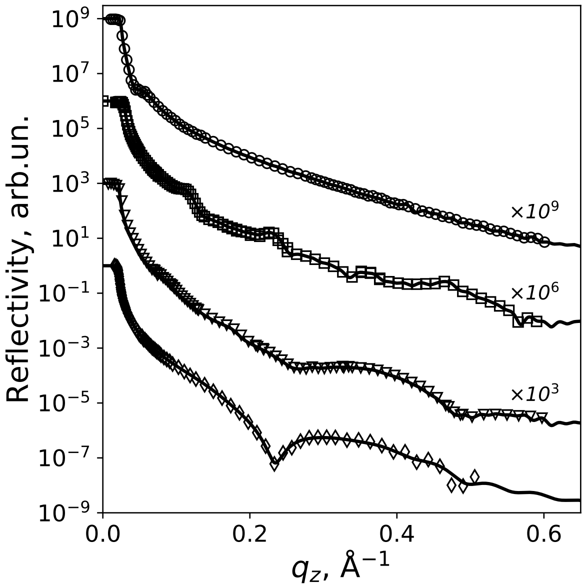

Figure 1. Reflectivity curves measured for the surface of 12-nm sol (circles), DSPC multilayer on 12-nm sol (squares), DSPC bilayer on 7-nm sol enriched with NaOH (triangles), DMPS monolayer on water (diamonds). The solid lines are simulated from the reconstruction.

with period of (68±1) Å, which corresponds to the doubled length of DSPC molecule (2×34.1 Å), however their electron concentration exceeds theoretical value for the bilayer by 15–20%.

Decomposition of the analytical model is also shown in figure 2b in colors; parameters for bilayer has been chosen according to [6], bilayers in the stack implied to be separated with a small amount of water. Assuming the lipid molecules in bilayers are in crystal phase with area per hydrocarbon chain $A = 20.8$ Å^2, the excess number of electrons per lipid molecule is 86±2, which corresponds to nine Na^+ ions. In [17] we proposed a hypothesis that the accumulation of Na^+ is caused by the electroporation effect, as the electric field intensity at the surface of hydrosol exceeds the critical electroporation value for lipid membranes by few orders of magnitude. Other aspects of the spontaneous ordering of lipid multilayer according to that hypothesis is also studied and discussed in [17].

Dependence of the structure and properties of lipid membranes on the properties of a hydrosol substrate was briefly considered in [16]. Reflectivity $R(q_z)$ from the thin membrane of DSPC on the hydrosol containing SiO_2 particles of diameter $D = 7$ nm and enriched with additional NaOH (16 wt% SiO_2, 1.3 wt% NaOH, pH∼12) is presented in figure 1 (triangles); the respective $\rho(z)$ distribution (figure 2c, black) shows the collapse of the whole volume of lipid into a bilayer of thickness ≈ 70 Å.

Comparison with a theoretical model (figure 2c, colors), giving the thicknesses, electron densities and area-per-unit for hydrocarbon chains and polar groups the same as above, yields an excess number of electrons for the polar groups is 10±1, which equals to 1 Na^+ ion per lipid molecule. However, the relative orientation of lipid molecules in the bilayer is inverted to the one in the multilayer stack. Another layer of Na^+ ions is present on lipid/substrate interface, and a layer of SiO_2 particles are found adjacent to that interface within the substrate, their concentration being considerably lower than for the case of a clean surface of hydrosol.

It should be noted that even for the simpler samples, such as classical Langmuir films, simultaneous application of model-independent approach along the model-based method can provide more extensive description of the lipid membrane. In [18] and [19] we demonstrated the

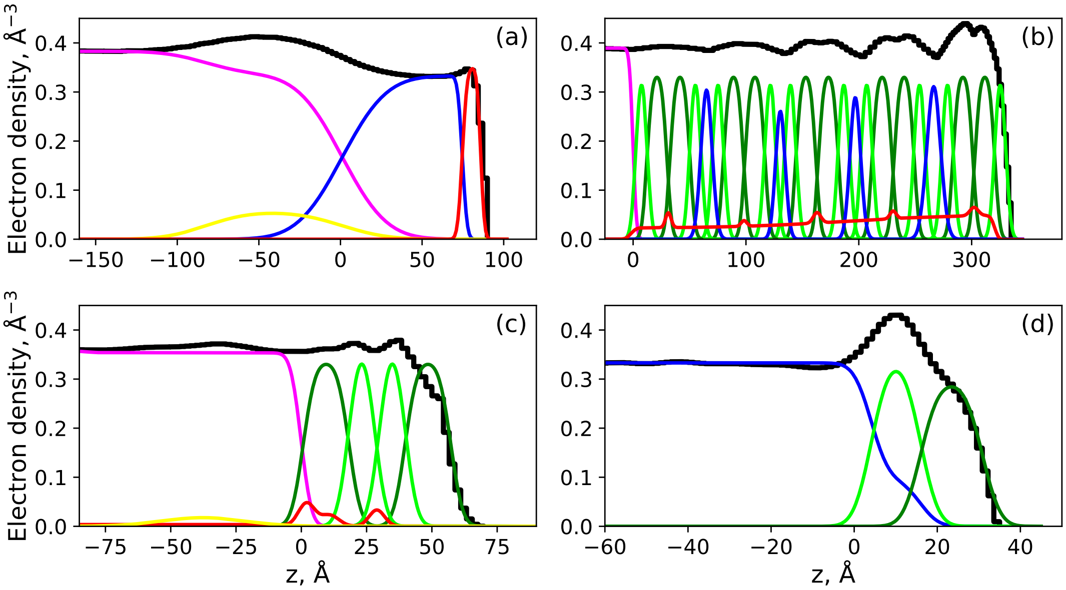

Figure 2. *(Colors online)* Electron density distributions $\rho(z)$ for (a) 12-nm silica sol, (b) DSPC multilayer on 12-nm sol, (c) DSPC bilayer on 7-nm sol and (d) DMPS monolayer. Model-independent reconstruction is denoted by black lines. Components of analytical models by-color: lipid polar groups (light green), hydrocarbon chains (dark green), Na^+ ions (red), H_2O (blue), SiO_2 particles (yellow), and bulk hydrosol (magenta).

possibility of such analysis applied to the two-dimensional liquid/gel phase transition in Langmuir monolayer. Figure 1 (diamonds) shows the reflectivity curve for the DMPS (or $C_{34}H_{65}NO_{10}PNa$) membrane prepared on a substrate of a KCl solution ($\sim$ 10 mmol/L) in deionized water (pH= 7), with the estimated area per lipid molecule $A \approx 45$ Å^2. Figure 2d compares the result of model-independent reconstruction (black) against the decomposition of two-layer theoretical model (colors). In the regions corresponding to the hydrocarbon chains both the model and "free-form" solutions are in perfect agreement, which confirms the initial estimation of A value. However, in the region of serine polar groups model-independent electron concentration exceeds the theoretical one by $\approx$27%, presumably by the effect of hydration of lipid polar groups on water. Integration of the excessive electron concentration yields the hydration level of 5 molecules of H_2O per lipid molecule. In [19] this combined approach allowed us to experimentally observe the changes in hydration level and orientation of hydrocarbon chains at the different stages of phase transition within DMPS monolayer.

4. Conclusions

To summarize, in a series of papers [15, 16, 17, 18, 19] we demonstrated the perspectives of model-independent approach to the analysis of X-ray reflectivity data in application to the studies of macroscopically flat phospholipid membranes on a liquid substrates. It is shown that lipid films on a colloidal silica sols exhibit a spontaneous ordering effect. Investigations on the kinetics of such spontaneous ordering reveal the dependence of the structure and configuration of lipid membrane on the electric properties of air/substrate interface and concentration of alkali ions within the substrate. Possibilities for the quantitative study of a more complex processes, namely two-dimensional phase transitions in lipid membranes, with combined X-ray reflectivity analysis, have been justified for the usage of laboratory sources.

Acknowledgements
Experiments at the station ID31 have been performed under the research projects SC-4246 and SC-4461 of European Synchrotron Research Facility, Grenoble, France. Theoretical part of the work is supported by the Russian Scientific Foundation (project #18-12-00108).

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