Study of the influence quinone molecules on lipids

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Introduction.

Lipids have attracted researchers' attention for a very long time. They are the most common biological organic molecules, keep a lot of secrets. One interesting application is drug delivery system in pharmaceutics. The aim of this study is the investigation of structure and phase transition for mixture of lipids and guest molecules which might be used as transporters in the future. To understand such complicated systems we should know the behavior of the easiest one. Thus, the model of one-layer lipid membranes was created. The structural aspects upon interaction with guest molecules of this model were studied at *HASYLAB* in *DESY*, Germany.

Biological background.

Lipid molecules appear in many kinds and shapes; nevertheless, they all share an important property, amphiphilicity. Every lipid is composed of a hydrophilic (polar, linking water) head and a hydrophobic (apolar, not liking water) chain. This composition governs the interaction of lipids with water and proteins and promotes the formation of lipid membranes of various shapes. In this work only one type of phospholipid, *palmitoyl-oleoyl phosphatidylcholine* (*POPE*) was used. It has two hydrocarbon chains, one 16 carbons long saturated and one 18 carbons long unsaturated between the carbon 9 and 10 and one headgroup joined by a glycerol backbone. The headgroup of *POPE* consist of tree hydrogens (fig. 1). For our experiment the *POPE* was purchased from *Avanti Polar Lipids* (Alabaster, USA).

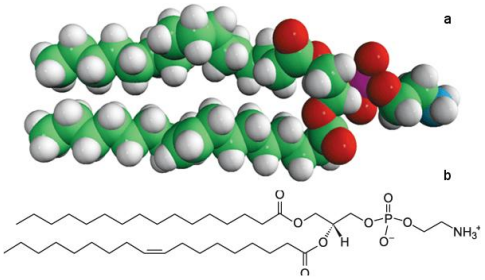

fig. 1: a) 3D image of *POPE*; b) chemical formula of *POPE*.

Experimental setup.

To investigate such complex systems, it is necessary to use models at the first step to make this process controlled and predictable. That is why for our experiments, we used only a one-layer membrane of *POPE* with additional quinone or hydroquinone molecules.

All experiments were performed at beamline *A2*, which is used for investigations small angle X-ray scattering (SAXS) at a fixed energy of *8 keV* (fig. 2).

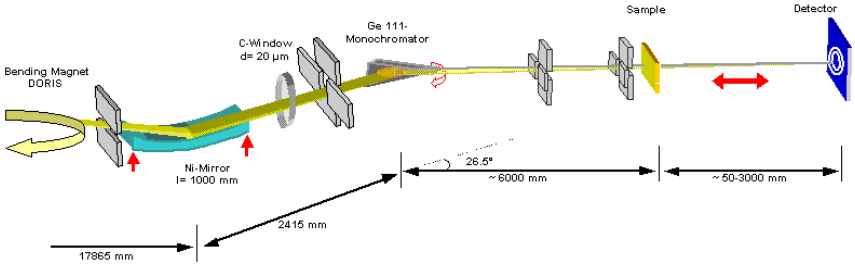

fig. 2: General scheme of beamline *A2*.

The synchrotron radiation from the storage ring *DORIS* was focused on the detector. The liquid samples were measured in air in a closed capillary, which was inserted in the capillary oven (fig. 3).

a)  b) 

fig. 3: a) capillary oven holder b) interface of *JUMO* device.

The sample holder could be moved in the horizontal and vertical direction in order to place the samples in the beam. For temperature control of the sample a *JUMO IMAGO 500* multi-channel processer and program controller connected to a computer was used.
At the *1200 mm* distance from the sample the *2D MarCCD* detector was placed, which was used for registration of the scattering pattern.

Sample preparation.

All samples were mixtures of lipid *POPE* (*6.5 -8.5 mg*) and guest molecules at ratio 100:1, correspondingly. There were two different types of guest molecules: the quinone *2,6BATQ* and the corresponding hydroquinone *2,6BATH* (fig. 3). Quinones are common constituents of biologically relevant molecules (e.g. Vitamin K1 is phylloquinone). But for our experiments it is important that they have similar carbon chains as the lipid and should integrate in lipid's structure.

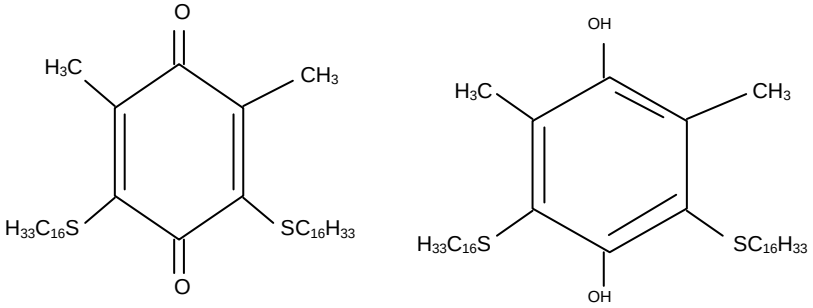

fig. 3: a) *2,6BATQ* b) *2,6BATH*

For observation of the behaviour of this mixture at different pH value a buffer was added to keep the pH constant. The value of pH varied in the range from 3.2 to 7.5 pH with buffer concentration *1 mol*.

At the last stage of sample preparation two different fractions were observed. The sample in the capillary was separated into transparent volume and conglomerate of molecules which was deposited on the bottom or arranged in the layer/ring on the top of the sample, which was called "orange ring". That is why the measurements were performed at several points of the capillary.

Summary table of samples:

Number of sample	components	pH	Mass POPE, *m* (*mg*)	Mole of POPE, *n* (10^{-5}*mol*)	Ratio 100:1, mol of molecule, 10^{-7}	Volume of guest molecule solution, *ml*
1	POPE+buffer+2,6BATH	3.2	7.54	1.05	1.05	0.049
2	POPE+buffer+2,6BATH	6.0	8.79	1.224	1.224	0.057
3	POPE+buffer+2,6BATH	7.5	6.94	0.9666	0.9666	0.045
4	POPE+buffer+2,6BATQ	3.2	7.57	1.054	1.054	0.056
5	POPE+buffer+2,6BATQ	6.0	8.09	1.127	1.127	0.06

6	POPE+buffer+2,6BATQ	7.5	7.66	1.067	1.067	0.056
8	POPE+buffer	3.2	2.23	0.3106	0.3106	Pure lipids
9	POPE+buffer	6.0	3.45	0.4805	0.4805	Pure lipids
11	POPE+buffer	7.5	4.16	0.579	0.579	Pure lipids

Data acquisition and analysis.

During temperature scans, the samples were heated from 25 to 83°*C* in 60 *min* and the diffractograms were recorded for 24 *s* every two minutes. After the experiments all diffraction patterns were saved as function intensity versus pixels. For transforming this function to a more suitable form the *A2tool* program was applied. This program makes the transformation by using known information about the strongest peaks of calibration sample *rat-tail tendon* (RTT). At the next step all data was treated in the *Origin 7.5* program to plot the function intensity versus peak position. As a result the set of 1D diffraction patterns, each taken at a different temperature, was plotted for each sample (fig. 4).

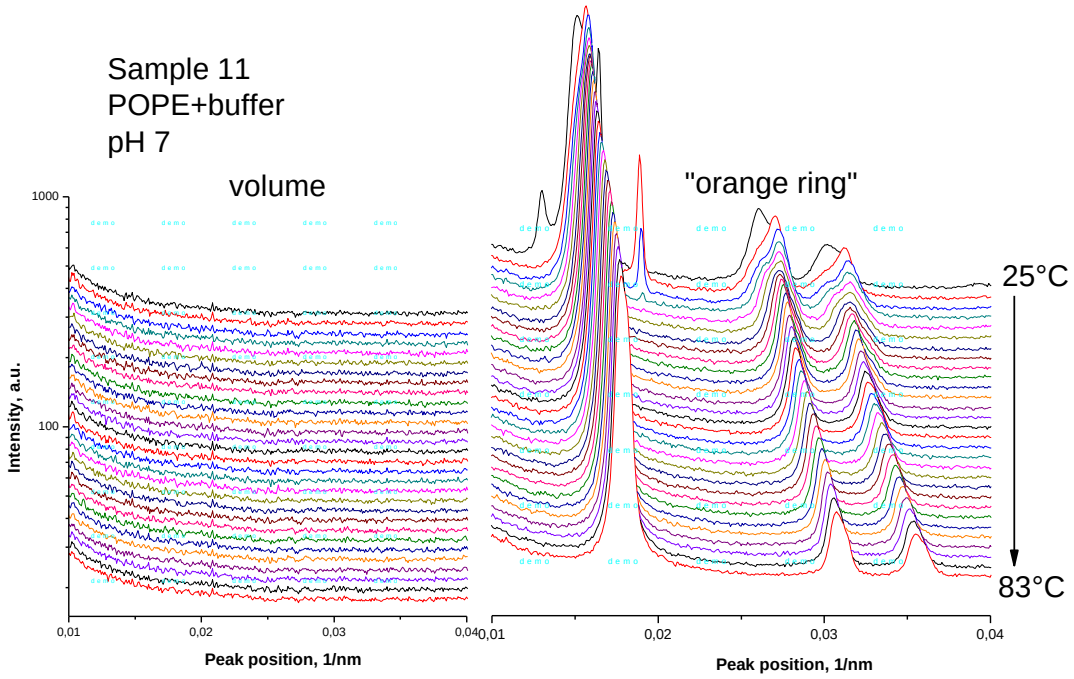

fig. 4: The set of 1D diffraction patterns in volume and “orange ring” points.

To determine the structure of the sample all peak positions were found and their ratios calculated.

Results and discussion.

Almost all measurements in the volume point did not show any structure except the sample 4. For this sample a phase transition from a lamellar to a hexagonal phase was observed at 28°*C*. The same behavior was registered for sample 9, but at the “orange ring” measuring point and at 38°*C*.

A more complicated mixture of structures was observed for samples 2, 4, 8 and 11 at “orange ring point”. In these cases a combination of a cubic and a lamellar or a hexagonal structure was found before phase transition and a cubic and a hexagonal or only a hexagonal after (fig. 5). The composition, pH and transition temperature are different in all those cases.

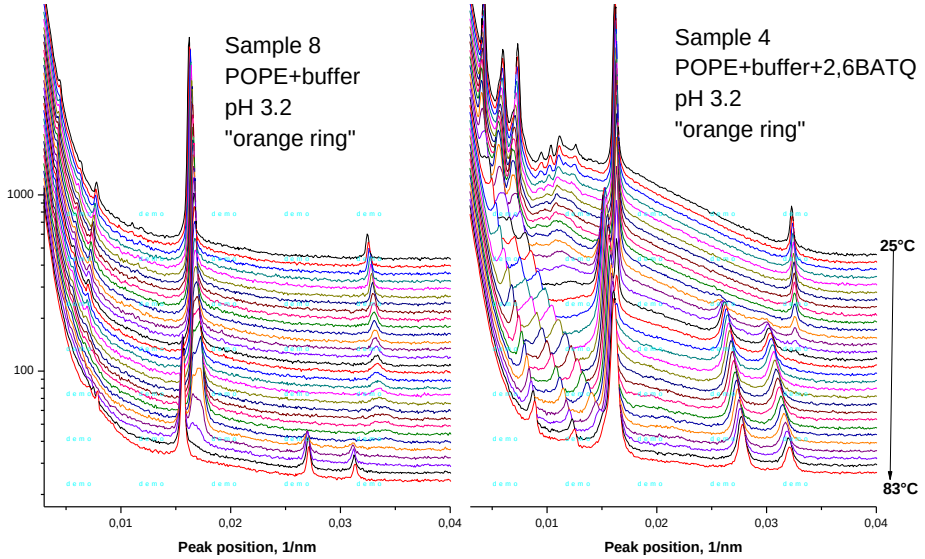

fig 4: The set of 1D diffraction patterns in “orange ring” points for samples 8 and 4.

There was no structure during both scans for samples 1, 5 and 6 and for sample 3 only one peak at “orange ring” measurement, which can come into any structure family.

Conclusions.

The goal of this work was to investigate the effects of the guest molecules (quinone and hydroquinone) on the lipid model membrane. The model membrane in this study was prepared from zwitterionic lipid POPE abundant in bacterial cell membranes. The structure of mixed lipid-quinones membranes was determined and it changed during heating from 25 to 83°C. Also the phase transition parameters were found and discussed. It is known from the literature that POPE forms the lamellar structure at low and hexagonal phase at high temperature [?]. In our case the thermotropic phase behavior was changed even by adding buffer. The more destructible structure effect was observed in mixtures with quinones at different pH. This work will be expanded on the usage of buffer at different concentration and should help to understand the effect of guest molecules on the structure of the lipid membranes

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