# Ripple Phase Behavior in Mixtures of DPPC/POPC lipids: SAXS and SANS Studies

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# Ripple Phase Behavior in Mixtures of DPPC/POPC lipids: SAXS and SANS Studies

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**Abstract**. Multilayer membranes of DPPC (1,2–dipalmitoyl–sn–glycero–3–phosphatidylcholine) and mixture DPPC/POPC (1–palmitoyl–2–oleoyl–sn–glycero–3–phosphatidylcholine) in excess of water were investigated by small angle X–ray scattering and neutron small angle scattering. Structural parameters of lipid bilayers were determined in a wide temperature range. It was shown that addition of the POPC to the DPPC/$H_2O$ does not change the temperature of the DPPC main phase transition $T_m$. The observed phase separation occurs for the system DPPC/POPC/$H_2O$ up to the pre–transition temperature $T_r$ (gel phase – ripple phase) of the DPPC multilayers.

## 1. Introduction

Studies of the physical and physics–chemical aspects of phase transitions in membranes (real biological and model lipid) have been going on for many years [1-3]. This interest is due both to the biological importance of the membranes and their promising applications (such as sensors, transducers, filters), as well as the fundamental physical issues related to phase transitions, fluctuations, and dynamics of complex systems.

Despite of the large number of papers about phase transitions, including those in biological systems, the biological role of phase transitions is still unclear. For example, some researchers believe that having a phase transition in membranes determines how temperature of warm–blooded organisms stabilize and cooperative processes of cell [4].

Intermembrane interactions are determined by the balance of forces between uncharged membranes. The main contribution is made by a strong short–range repulsion (often called the "hydration" force), long–range van der Waals attraction, and long–range Helfrich's undulation repulsion.

In the work [5] new approaches of intermembrane interactions studies are developed and the universal constant, based on a study of the temperature dependence of intermembrane interactions with the complementary use of small–angle scattering of thermal neutrons and high resolution diffraction at the synchrotron source, is determined. In particular, a value of the coupling constant, is coincides with the theoretically predicted. In addition, in [5] it was first shown what the true value of undulation forces are and they really make a significant contribution to the balance of the intermembrane interactions and, moreover, these forces become dominant at distances greater than 20Å.

Back in 1975 a theoretical model of the lipid layer [5] was proposed. The extensive surface area and the conjugate surface pressure were introduced into a cooperatively interacting hydrocarbon chain model. The exact statistical mechanical method of solution is extended to accommodate the new variables. In [6], these authors also offer a refined model of the lipid bilayer, which takes into account the weak interactions between the heads of the lipids. It is shown that even weak interactions between the lipid heads are similar to the bilayer surface pressure which influences the temperature and pressure at which the phase transition occurs. The phase diagram of lipid was calculated and the value of surface pressure was determined.

Using wide–angle diffraction, the authors [7] found two orientation distribution of lipid molecules at low temperature for mixtures of two lipids with cholesterol. This orientation ordering is observed in a wide temperature range. Is this a true microphase separation of the lipids or just the effect of "forced" changes in the concentration, still remains a question.

Numerous publications are devoted to studying the effect of various additives on the points of lipid membranes phase transitions. For example, in [8] the authors proposed a model to explain the phase transition of cylindrical micelles – bilayer vesicles in solutions of phospholipids and surfactants. The model predicts that the first order transition and transition temperature depends of the relative concentrations of two components. The phase transition boundary is calculated as a function of the area of phospholipids and surfactants. The proposed model, the authors believe, is in qualitative agreement with experimental data. In [9] the effect of electrolytes on solutions of phospholipids at a temperature below the main phase transition was studied by small angle neutron and X–ray scattering and by differential scanning calorimetry. Effect of cholesterol, anesthetics, as well as polypeptides on the main phase transition temperature of phospholipids was investigated by nuclear magnetic resonance [10] and by double electron–electron resonance. In the case of the double electron–electron resonance to the ends of the hydrocarbon chains electronic tags were attached. Using the distance between these tags the thickness of the phospholipid bilayer was determined [11].

Using X–ray diffraction and electron microscopy the effect of dichlorphenol on the main lipid phase transition in DPPC was shown [12]. It was established that the structural changes depend on the concentration of dichlorphenol.

During phase transition investigations it is interesting to change some external parameters, like temperature, pressure, pH level etc. For lipid membranes pressure is a particularly interesting option, as the natural feature of the life of some living organisms, as well as an external parameter. In the professor Winter's papers devoted to high pressure studies [10, 13-15] different research methods have been used: small–angle neutron and X–ray scattering, X–ray diffraction, nuclear magnetic resonance method, and PVT studies. The article presents the phase diagrams for the various lipids, the influence of proteins, polypeptides and some surfactants on the phase transition point. The kinetics of phase transitions and influence of phospholipid hydrocarbon tails type on the bilayer packing in the different phases was investigated [16].

Earlier we investigated the aqueous solution of DMPC with PVT method and showed the critical behavior of the isothermal compressibility at the main phase transition [17, 18].

Despite on the considerable list of publications about the effect of high hydrostatic pressure on the phase transitions, the key experiments have not yet been delivered. In our view, this is due to two factors: the technical implementation of the experiment and the difficulty in preparing the samples.

In this work multilayer membrane DPPC and DPPC/POPC (3:1) in excess water are investigated by small–angle X–ray and neutron scattering. These two lipids have similar length of hydrocarbon chains but different temperatures of the main phase transition.

The aim of our work is evaluation the impact on the structure of the lipid close to the temperature shift of the phase transition point at atmospheric pressure. Though, the effect of certain additives (such as alcohols, hydrocarbon chains, cholesterol) on the phase transition is well–studied and there are several models of this semiphenomenological influence, however, there is still no clarity in the main phase transition. Additional difficulties in the study of phase transitions of mixtures are due to the fact that the components of the system at a sufficient degree of homogenization may be in a nonequilibrium state. Arising in this state are connected primarily with the effects of hydration–dehydration, which essentially determines the morphology of the membranes.

Lipid mixtures investigations have great interest for understanding of the structure and the important functions of membranes. Biological membranes are multicomponent lipid systems. Many biomembranes have about 100 different lipids. It is also of considerable interest from the standpoint of understanding the influence of the lipid bilayer on the function of membrane proteins. In addition, these studies are important for understanding the crystallization of membrane proteins in lipid membranes.

Our task is investigation of the structural changes with temperature in DPPC and POPC multilayer membranes in the case of two–component mixture.

## 2. Material and methods

Synthetic 1,2–dipalmitoyl–sn–glycero–3–phosphatidylcholine (DPPC) and 1–palmitoyl–2–oleoyl–sn–glycero–3– phosphatidylcholine (POPC) was purchased from Avanti (Birmingham, Al) and was used without further purification. Water (18 MV/cm) was obtained with the help of Millipore (USA).

Multilamellar vesicles (MLVs) were prepared in the following way: DPPC or DPPC/POPC (3:1) was added to the water. For mixing the system a shaker was used. Homogeneous dispersion was obtained by freezing – heating method about ±20°C below and above the main phase transition temperature. For SAXS experiments the mixture was placed into borosilicate capillary with 1.5 mm diameter and 0.01 mm wall thickness (W. Muller, Berlin, Germany). The capillary was hermetically sealed, and the specimens were held at the temperature higher than that of phase transition for several hours. For SANS experiments the mixture was placed in quartz cell.

The final concentration of the lipid/water was 10 mg/ml. We have used the same way for preparation of all samples. The samples prepared for neutron measurements had final concentration 2% (lipids/heavy water wt/wt).

The investigations by small angle X–ray (SAXS) method were performed on a pinhole camera Molecular Metrology SAXS System at the Institute of Macromolecular Chemistry CAS (Prague, Czech Republic).

A pinhole camera Molecular Metrology SAXS System was attached to a microfocused X–ray beam generator (Osmic MicroMax 002) operating at 45 kV and 0.66 mA (30 W). The camera was equipped with a multiwire, gas–filled area detector with an active area diameter of 20 cm (Gabriel design). Two experimental setups were used to cover the q range of 0.0045 – 1.1 Å ($q$=(4 $\pi/\lambda$)sin$\Theta$, where $\lambda$ is the wavelength and $2\Theta$ is the scattering angle).

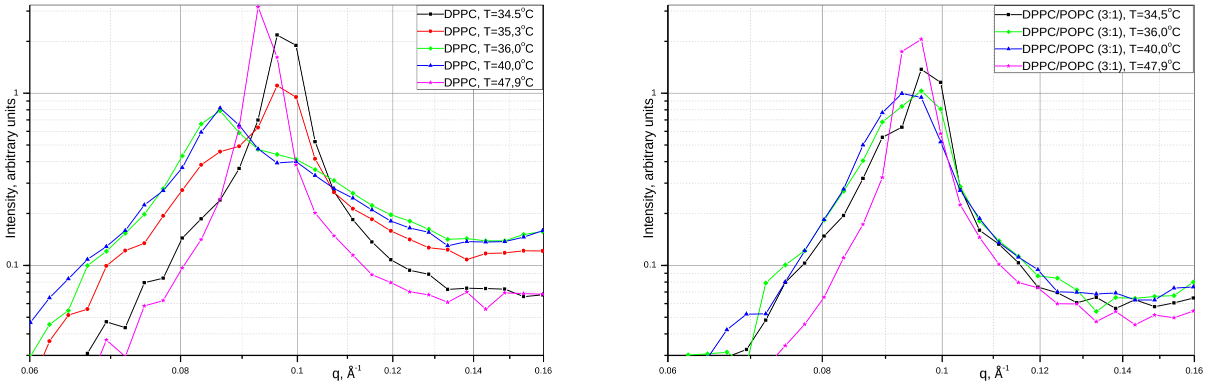
The neutron experiments were performed at YuMO spectrometer using two–detector set up with ring wire detectors [19, 20]. The beam was collimated to a diameter of 14 mm on the sample. The data treatment was performed by SAS program with smoothing mode [21, 22].

The data acquisition was performed using the SONIX Software Package [23].

## 3. Results and discussions

### 3.1. SAXS results

In Figure 1 the small angle X–ray scattering curves of pure DPPC (a) and mixture DPPC/POPC (3:1 wt/wt) (b) near phase transition temperature points are shown. Above 34.5°C we observe the shift of the diffraction peak, which corresponds to the change repeat distances of lipid bilayers, and thus manifests lipid phase transition.



**a** **b**

**Figure 1.** SAXS patterns comparison for DPPC (**a**) and DPPC/POPC (3/1) lipid mixture (**b**) in water excess.

As expected, from 20°C to 34.5°C DPPC membranes are in the gel phase and at the temperature range from 35,3°C to 36°C they are in the ripple phase, and at temperature range from 40°C to 47,9°C they are in the liquid phase. This is the test of the experimental accuracy.

The repeat distance of multilamellar bilayers was calculated by the equation (1):

$$D = \frac{2\pi}{q_{peak}}, \tag{1}$$

where $D$ is repeat distances of the multilammelar bilayer, $q_{peak}$ corresponds to the maximum of the diffraction peak.

In Figure 2 we present the results of the calculation of the periodicity of multilayers of the DPPC and DPPC/POPC (3:1) membranes vs temperature. Due to sufficiently high resolution we observed second peak on scattering curves for two lipids DPPC/POPC mixture.

As follow from the data, presented in Figure 2, the adding of POPC to DPPC (1:3) reduce the lattice constant of the ripple phase in comparison with pure DPPC solution.

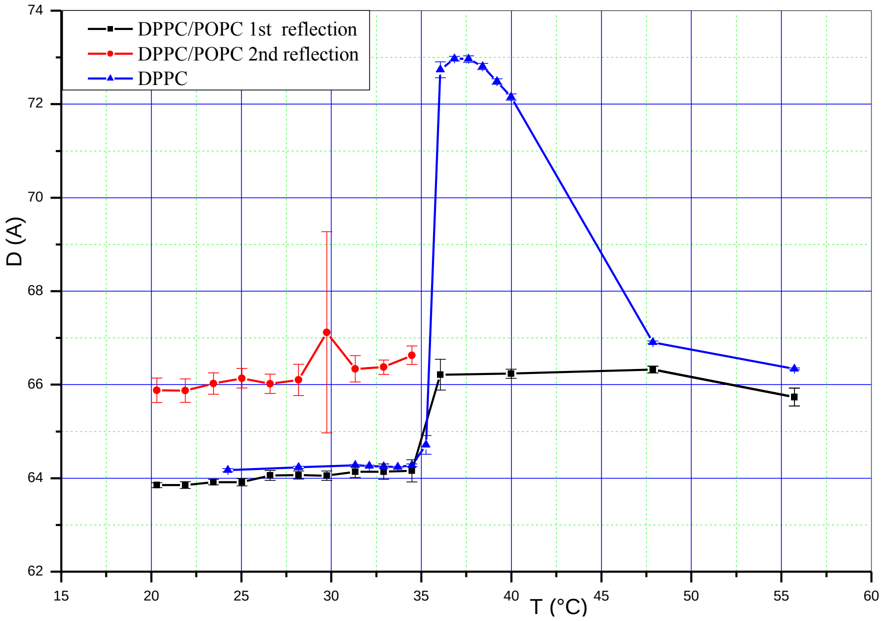
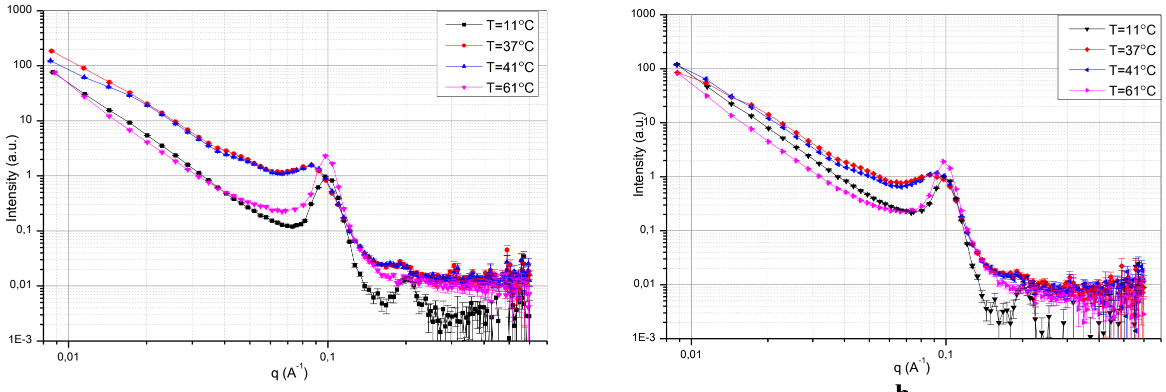


**Figure 2.** Repeat distance of multilayers of DPPC and DPPC/POPC membranes vs temperature (SAXS data). Triangles corresponds to the repeat distance calculated from scattering curves of DPPC sample, rectangle – the repeat distance calculated from scattering curves used first peak and circles corresponds to the data calculated from scattering curves used second peak DPPC/POPC (3:1) sample.

### 3.2. SANS results

The measurements at YuMO small angle neutron scattering spectrometer were done under the following conditions: the size of the neutron beam is 14 mm instead of 0.3 mm for the X–ray beam, the concentration of lipid/heavy water is 2 % (wt/wt) instead of 10% lipid/water (wt/wt), and volume of sample is 1cm$^3$ instead of 0.5 mm$^3$.

In Figure 3 the typical small angle neutron scattering curves of DPPC (a) and DPPC/POPC (b) in heavy water solution (2% wt/wt) for 11°C, 37°C, 41°C, 61°C and pressure 1 atm are shown.



**a** **b**

**Figure 3.** Small–angle neutron scattering curves of DPPC (**a**) and DPPC/POPC membranes (**b**) in heavy water (2% wt/wt) for 11°C, 37°C, 41°C, 61°C and pressure 1 atm.

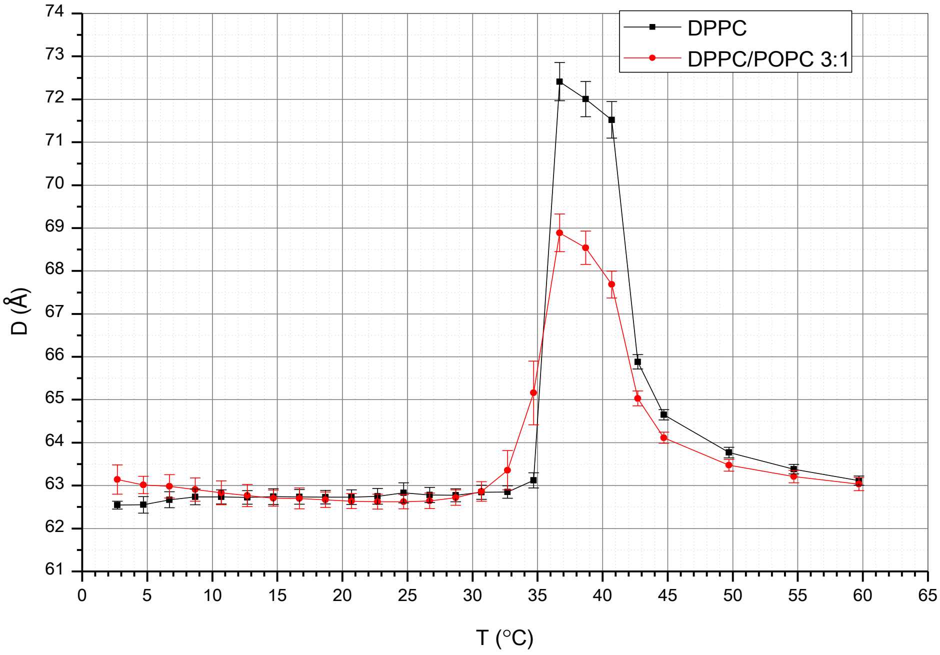


**Figure 4.** The repeat distance of the multilayers DPPC and DPPC/POPC vs temperature obtaining at the YuMO spectrometer. Rectangle corresponds to DPPC, circle to DPPC/POPC (3:1) membranes.

In Figure 4 the repeat distance of multilamellar structures of DPPC and DPPC/POPC (3:1) mixture in heavy water are presented. Behavior of the scattering curves is different in ripple phase, namely in the former case the periodicity is 72 Å and for the mixture with POPC it is equal to 69 Å.

Using two different methods (SAXS and SANS) for two concentration and different volumes of water (heavy water) for the mixture of POPC/DPPC we found that by adding of POPC (1:3) the periodicity of the ripple phase of DPPC is suppressed in comparison with pure DPPC solution.

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