Experimental Report

Proposal: **9-13-518** **Council:** 10/2012

Title: Detailed hydration and temperaturestudy of phosphocholine lipids.

This proposal is a new proposal

Researh Area: Soft condensed matter

Main proposer: **PRESCOTT Stuart**

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Samples: 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC)
1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC)

Instrument	Req. Days	All. Days	From	To
D16	7	7	27/05/2013	03/06/2013

Abstract:

The behaviour of lipid bilayers under various types of pressure is a well established research area, as bilayer stacks provide a good model for biological systems. We have recently developed a unique surface force style apparatus which combines a confining pressure with neutron reflection measurements to determine the structure of thin films. This has been applied to a stack of lipid bilayers in a recent experiment on D17 with very interesting results. Below and above the gel to liquid phase transition in the zwitterionic phosphocholine lipid, DPPC, different behaviour was observed. Despite a loss of the hydration layer in both, in the gel phase the inter layer spacing decreased as one would expect but in the liquid phase it increased. This implies a phase change; however the mechanical pressure applied (5 bar) is significantly less than the hydrostatic pressure required for this change. The influence of hydration therefore needs to be thoroughly determined across a range of both temperatures and hydration. The relative humidity cell on D16 will allows us to correlate changes in internal structure with relative humidity at temperatures corresponding directly to our previous data.

Title: Detailed hydration and temperature study of phosphocholine lipids.

Experiment: 9-13-518 Dates: 27/05/2013 - 03/06/2013 Instrument: D16
Team: L.L.E. Mears, R. Barker, S.B. Abbott, W.M. de Vos, S.W. Prescott, R.M. Richardson.
Local contact: R. Barker

Abstract

A temperature and humidity controlled sample environment was used on D16 to investigate the response of a stack of DMPC lipid bilayers to small changes in relative humidity, close to full hydration, in both the fluid and gel phases. This experiment explains whether the effects of confinement, applied to lipid bilayer stacks in other neutron reflection experiments, should solely be attributed to dehydration or if confinement has a unique effect, caused by the combination of dehydration and mechanical pressure.

Introduction

The phase behaviour of lipid bilayers is influenced by many conditions, most notably temperature and humidity. In some recent experiments using the D17 reflectometer our novel surface force type apparatus [1] was used to probe the effect confinement has on spin coated lipid bilayer stacks [2-4]. In those experiments hydrogenous lipids were hydrated with D_2O vapour prior to confinement. The data suggested that under confinement some of this D_2O is removed from the stack, a consistent feature for lipids of varying tail length and in the fluid and gel phases. For lipids in the gel phase the shift in Bragg peak position indicated an associated thinning of the interlayer spacing. However for lipids with a longer tail length in the fluid phase there was a surprising increase in the repeat distance.

Comparison with the existing literature on the effect of hydrostatic pressure confirmed that the confinement applied in our apparatus has a different effect from an equivalent pressure. This led to the question whether the structural changes are purely caused by the dehydration induced osmotic pressure or; the unique combination of dehydration and mechanical pressure, created during confinement, has its own effect which cannot be contributed to either component individually.

Experimental results

Samples were prepared by evaporation from chloroform onto 2 ½" Si wafers, to form ~1000 or more layers of hydrogenous 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) or deuterated tail d54-DMPC and annealed at 50°C in an oven before use. These were clamped into the humidity cell in its standard vertical geometry and hydrated with D_2O and H_2O respectively. They were taken through at least one heating and cooling cycle to anneal the sample in situ with the correct hydration contrast. The sample was rotated with respect to the incident beam, wavelength 4.767 Å, with the detector at a fixed angle of 12.5° which allowed Bragg peaks of multiple orders to be detected. Two temperatures were used for the water bath controlling the sample temperature, 13 and 40°C, i.e. below and above the main transition temperature at 23°C. The water bath controlling the temperature of the reservoir of water below the sample, hence dictating the humidity within the chamber, was decreased in small increments. The relative humidity was then calculated from the temperatures recorded by probes next to the sample and the water reservoir.

In the case of the sample temperature set at 40°C the Bragg peak position appeared uniform over the whole height of the detector indicating homogeneity in the interlayer spacing across the sample for all temperature differences (humidities). Although for the majority of the 13°C sample

temperature measurements the sample remained homogenous, at a very small number of high humidities there was a difference in the Bragg peak position between the top and bottom of the data on the detector. This was observed after lengthy equilibration and for both contrasts. We are therefore investigating this effect further.

The overall trend for the change in interlayer spacing with humidity is as expected, showing a rapid increase at the highest humidities. Figure 1 indicates, on a separate axis, the spacing which was achieved by applying 1 bar to a stack of DMPC bilayers using our confinement apparatus. Although a similar spacing was obtained for the lipid in its gel phase, the same was not achieved for the fluid phase. Further analysis to understand more about the distribution of water in the layer, using Fourier synthesis for the diffraction data and model fitting of the reflectivity data from the other experiments, is ongoing.

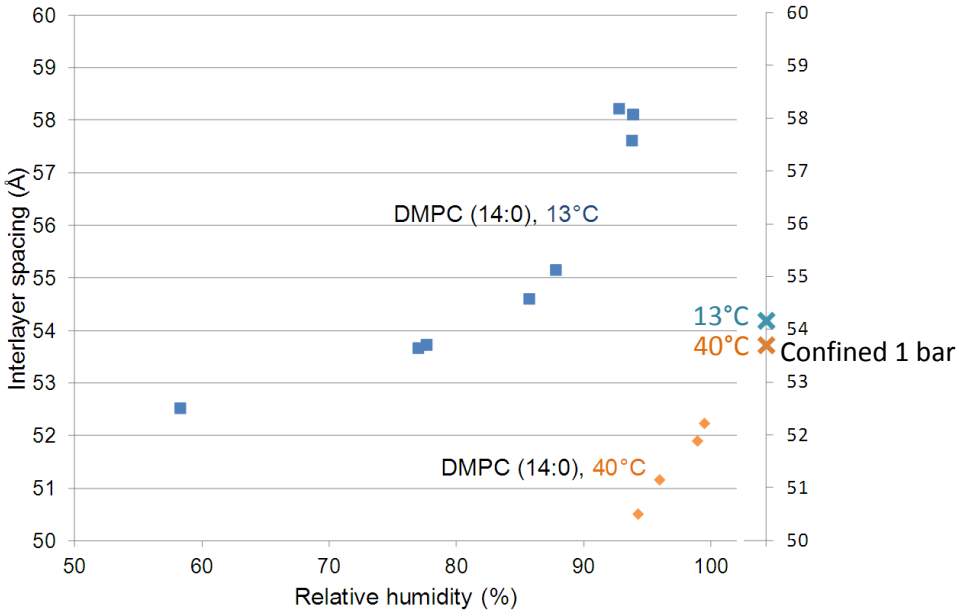

Figure 1 Interlayer spacings taken from the Bragg peak positions for DMPC stacks at different relative humidities in the gel and fluid phases. The axis on the right indicates the thickness of a set of bilayers under 1 bar of confinement taken from an associated experiment [3].

Conclusions

The experiment has given detailed information about the humidity dependence of the structure of DMPC bilayer stacks in both the gel and fluid phases. There was an interesting inhomogeneity to the sample at certain humidities in the gel phase. A similar interlayer spacing, to the one obtained under confinement was achieved for the gel phase but was not quite possible for the higher temperature, fluid phase. Further analysis will be carried out, on both the data from this experiment and from the neutron reflection experiments, in order to fully understand the contribution of dehydration to the phase behaviour of lipids under confinement.

References

1. W.M. de Vos, L.L.E. Mears, R.M. Richardson, T. Cosgrove, R.M. Dalgliesh, S.W. Prescott, *Rev. Sci. Instrum.*, 83, 113903 (2012).
2. W.M. de Vos, L. L. E. Mears, S. W. Prescott, R. M. Richardson, T. Cosgrove, and R. Barker, *Exp. report ILL, D17*, 9-13-375 (2011).
3. L. L. E. Mears, W.M. de Vos, S. B. Abbott, S. W. Prescott, T. Cosgrove, R. M. Richardson, and R. Barker, *Exp. report ILL, D17*, 9-13-454 (2012).
4. L. L. E. Mears, W.M. de Vos, S. B. Abbott, S. W. Prescott, T. Cosgrove, R. M. Richardson, and R. Barker, *Exp. report ILL, D17*, 9-13-496 (2013).