Experimental report

21/03/2024

Proposal: **9-13-1089** **Council:** 4/2023

Title: Simultaneous Interfacial rheology and neutron scattering studies of DPPC Langmuir films

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: d62-DPPC

Instrument	Requested days	Allocated days	From	To
FIGARO Langmuir trough	2	2	27/11/2023	30/11/2023

Abstract:

ABSTRACT (1400 characters):
Fluid interfaces are present in many living systems and technological processes. They can have a complex structural configuration and, therefore, have a non-linear response to deformations. Consequently, they have been the object of study in recent decades both from a structural and rheological point of view. However, to date, structural measurements at the molecular level have not been performed simultaneously with interfacial rheology techniques. This is of great interest since it is not easy to compare studies due to the high variations observed under different experimental conditions. Specifically, there is great interest in the study of Langmuir monolayers of phospholipids, such as DPPC, the main component of the lung surfactant, which play a role in many biophysical processes at the molecular and cellular levels. This proposal intends to develop, build, and exploit a measurement system that allows for simultaneous measurements of neutron scattering and interfacial rheology as well as to carry out studies on interfacial DPPC monolayers.

Experimental report on FIGARO #9-13-1089 (27-30 Nov 2023)

Simultaneous Interfacial rheology and neutron scattering studies of DPPC Langmuir films

Scientific Background

Langmuir monolayers are valuable model systems in biology and life sciences, particularly fatty acids and phospholipid monolayers, which mimic inner and/or outer membrane leaflets alongside membrane proteins, providing insights into biophysical processes at cellular membrane surfaces. Dipalmitoylphosphatidylcholine (DPPC), a prevalent natural phospholipid in lung surfactant and cell membranes, forms highly stable monolayers at the air-water interface, reducing surface tension effectively for improved respiration and serving as a platform for drug delivery due to its liposome properties. Techniques like Neutron and X-Ray Reflectometry [1,3], as well as Grazing-Incidence X-ray Diffraction [2], have been employed to elucidate molecular structures of Langmuir monolayers, while various instruments, including interfacial shear rheometers (ISR), have been developed to study their rheological behaviour [4]. However, challenges persist in rigorously comparing structural and rheological measurements due to the sensitivity of interfacial systems to experimental conditions.

Experimental details

The work developed during this beamtime has been focused on: (i) testing the performance of the experimental setup – developed *ad-hoc* with the help of FIGARO technician Simon Wood – that conforms the Interfacial Rheology measurement system based on rotational rheometer and the so-called Double-Wall Ring (DWR) geometry on Langmuir trough in FIGARO, and (ii) performing simultaneous Neutron Reflectometry and Interfacial Rheology measurements on DPPC, DPPC-Cholesterol (3:1) and fatty acid monolayers under different experimental conditions. Since Langmuir monolayers of DPPC is a system that has been previously studied by Neutron Reflectometry, it has been decided to perform a structural characterization (full Q) while performing interfacial shear rheology measurements in oscillatory mode at different frequency and strain conditions at different interfacial pressures. In addition, the same experimental procedure has been performed on binary mixtures of DPPC - Cholesterol since efforts are still being made to understand the effect of the presence of cholesterol in lipid systems. Fully deuterated cholesterol, provided by Krishna Batchu of the L-Lab at ILL, has been used. Therefore, different combinations of hydrogenated and deuterated versions of DPPC and Cholesterol on Air Contrasted Match Water (ACMW) and D2O has been carried out. Previous Langmuir trough, interfacial rheology, ellipsometry and Brewster Angle Microscopy studies suggest that Langmuir monolayers of fatty acids at high interfacial pressure – specifically in the LS phase – show unstable behaviour suggesting the formation of molecular domains outside the plane of the monolayer. It has therefore been decided to perform simultaneous measurements with this new experimental set-up since this is an experiment in which measuring simultaneously structural and mechanical properties is especially relevant in order to correlate both due to the unstable character of the phenomena. In this case, kinetic measurements have been performed at different incidence angles and rheology at a single frequency of 0.5 Hz at an interfacial pressure of 40 mN/m using the deuterated version of the nonadecanoic fatty acid, d37, on an ACMW subphase. No other isotopic contrasts have been used since it has been observed in previous tests that such instability does not occur in the pure D_2O subphase. This may be because the kinetic of the interfacial phenomenon is strongly affected by the bulk density. It is worth noting the complexity of the experiments, as it has been necessary to simultaneously control the FIGARO neutron reflectometer, the rotational rheometer, and the Langmuir trough, with the synchronization issues that entails. We have faced technical issues as it was the first time this sample environment was being used. Material leakage towards the outer part of the Langmuir trough barrier has been observed, likely due to minor imperfections in the machining of the components. The change in the meniscus formed at the top of the Langmuir trough at low interfacial tension has caused misalignments of the interface with respect to the beam, and even small overflows, rendering some measurements not entirely reliable (please see Figure 1). This has prompted us to seek the best trough filling to avoid both overflow on one hand and leaks beneath the barrier on the other. Work is being done to improve the experimental setup by re-machining some parts and using other hydrophilic materials.

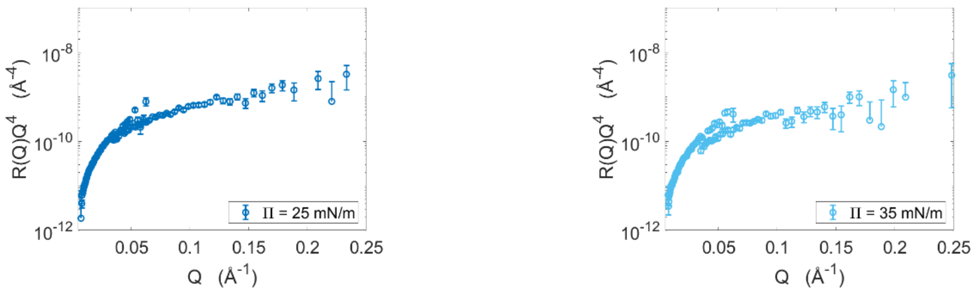

Figure 1. Examples of misalignment observed on DPPC–dChol on ACMW monolayers. This contrast is the most sensitive to misalignments of the interface throughout the full Q measurement and this causes a clear discontinuity between the parts of the reflectivity curve corresponding to the two angles of incidence used.

Results

Fittings of the experimentally obtained reflectivity curves have been made to two-layer models, separating the tails and the head of the molecules into two distinct layers due to the significant difference between their SLD (see left panel Figure 2). For monolayers containing cholesterol, their localization in the layer belonging to the tails has been considered (see right panel of Figure 2).

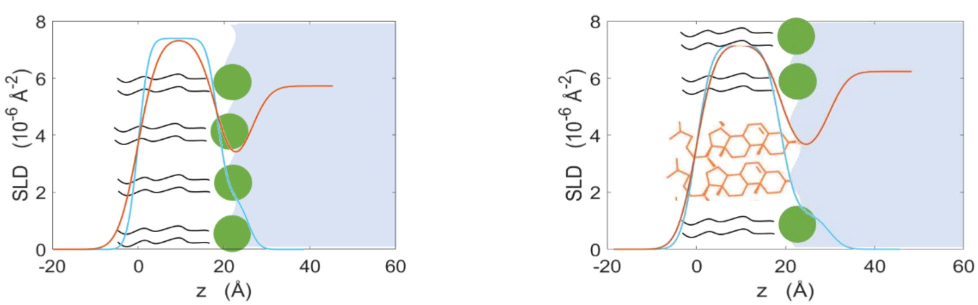

Figure 2. Models used for fitting of DPPC and DPPC-Cholesterol monolayers.

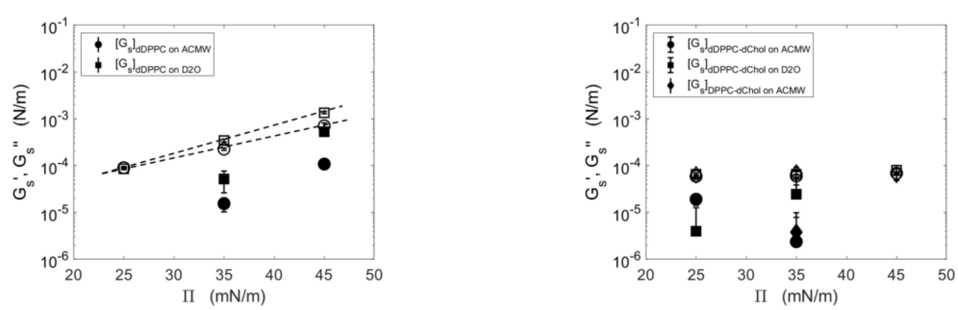

Figure 3. Dynamic shear moduli of DPPC and DPPC-Cholesterol monolayers at different interfacial pressures. The imposed strain is 3 % and the oscillatory frequency 0.5 Hz.

The preliminary results obtained are summarized in Table 1. With respect to the DPPC systems, a nice agreement with other experiments performed in FIGARO [5] is observed. The area per molecule measured in DPPC-Cholesterol binary systems is lower than in pure DPPC systems (see Table 1) and the dynamic shear moduli are also dramatically reduced (see Figure 3) as observed in previous studies [6].

Monolayer	**Π (mN/m)**	**Chains thickness (Å)**	**Headgroup thickness (Å)**	**Area per molecule (Å^2)**
DPPC	25	18.10	8	48.95
DPPC	35	18.47	7.16	44.91
DPPC	45	18.77	7.28	44.21

DPPC – Cholesterol	25	17.68	9.11	45.58
DPPC – Cholesterol	35	18.84	10.98	43.59
DPPC – Cholesterol	45	18.77	9.01	41.39

Table 1. Summary of fitted parameters of DPPC and DPPC - Cholesterol monolayers.

Small 1-min slices have been acquired during the evolution of the unstable phenomenon observed in nonadecanoic fatty acid monolayers in the LS phase. For the small incidence angle measurements, an analysis has been performed using the low Q approach which allows to obtain with high accuracy the surface excess. Different trending is observed for the dynamic shear moduli and the surface excess especially at short times (first 20 min). Measurements at intermediate incidence width reveal very relevant structural information that evidences that the interface becomes thicker with time.

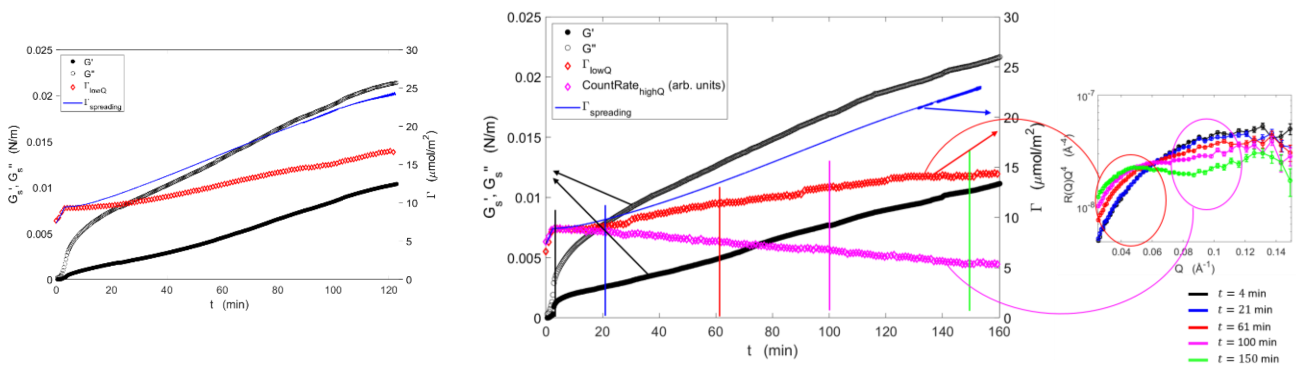

Figure 4. Summary of the results obtained on nonadecanoic fatty acid measurements at 40 mN/m. Left panel: measurements at small angle of incidence. Right panel: measurements at intermediate angle of incidence. Oscillatory rheological measurements are done at 3 % strain and 0.5 Hz.

To obtain more information about the structural evolution, fits of the reflectometry curves have been made using a model that considers two slabs, with the SLD of the complete molecule, below the monolayer, leaving the thickness and solvent fraction as free parameters. Furthermore, the surface excess of the entire modeled structure has been calculated and agrees nicely with that measured using low Q approach [7].

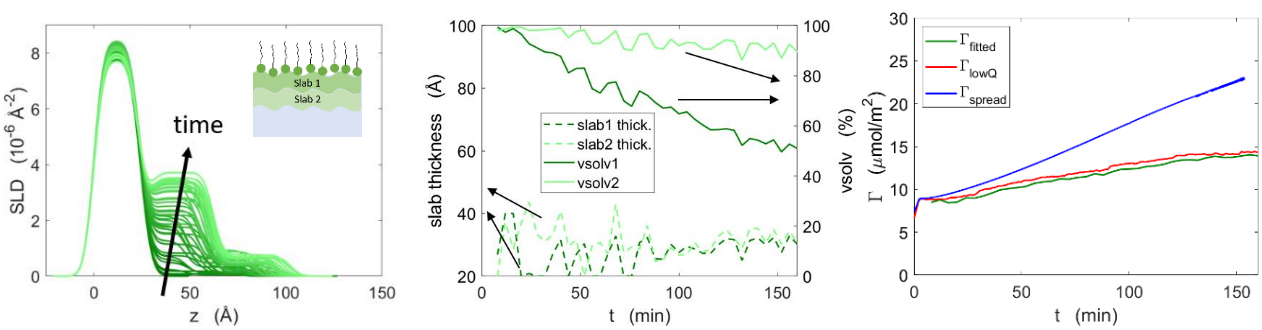

Figure 4. Summary of the fits performed on nonadecanoic fatty acid NR measurements at 40 mN/m. Left panel: measurements at small angle of incidence. Right panel: measurements at intermediate angle of incidence. Oscillatory rheological measurements are done at 3 % strain and 0.5 Hz.

Interestingly, the surface excess measured by the low Q approach and that calculated by the evolution of the area occupied by the monolayer diverge significantly. This leads us to believe that part of the material is being organized in adjacent domains that are not directly observed by reflectometry. Off-specular signal has been detected and its analysis may be of great importance to answer this question.

References

[1] A. Maestro, P. Gutfreund, Adv. Coll. Interf. Sci., 2021, 293, 102434. // [2] J. Pignat et al. J. Phys. Chem. B, 2006, 110, 22178. // [3] J. Daillant, A. Gibaud. X-ray and neutron reflectivity: principles and applications. Lecture notes in physics 770, 2008. // [4] C. F. Brooks et al. Langmuir, 1999, 15, 7. // [5] R.A. Campbell at al. *J. Colloid Interface Sci.*, 2018, 531, 98-108 // [6] K.H. Kim et al. *Proc Natl Acad Sci USA*, 2013, 110, 13 // [7] R. A. Campbell, et al. Soft Matter 2016, 12, 5304.