Experimental report

04/02/2021

Proposal: 9-13-843 **Council:** 4/2019

Title: IN-SITU STUDY OF THE EFFECT OF GRAPHENE OXIDE NANOPARTICLES ON THE SURFACE PROPERTIES OF DIPALMITOYLPHOSPHATIDYLCHOLINE

Research area: Chemistry

This proposal is a new proposal

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Samples: Graphene Oxide
1,2 dipalmitoylphosphatidylcholine DPPC
Chain-deuterated DPPC

Instrument	Requested days	Allocated days	From	To
FIGARO Langmuir trough	2	2	11/09/2019	13/09/2019

Abstract:

The increasing exposure to graphene oxide nanoparticles, GO, used in a great number of technological applications, makes necessary a deeper comprehension of the potential risk and hazards related to this material for both the environment and health. Since the lungs are an important entry point of GO, one of the most important aspects is the effect that these particles produce on the respiratory function. 1,2 dipalmitoylphosphatidylcholine, DPPC is the major component of the lung surfactant and is responsible for the decrease of the surface tension and the mechanical stability of the lungs during respiratory cycles. Therefore, the study of the interaction between DPPC and GO becomes necessary to understand the potential adverse effects on respiratory functionality. Because the lung surfactant is confined between air and the alveolar cavity, Langmuir monolayers of DPPC are a good model to mimic the effect of GO nanoparticles on the interface air-liquid at the lungs. Accordingly, the main objective of this Neutron Reflectometry experiment is to develop microscopic understanding of the effect of GO on the thickness and coverage of DPPC films on the air-water interfaces.

In-situ study of the effect of graphene oxide nanoparticles on the surface properties of dipalmitoylphosphatidylcholine #9-13-843

Introduction

Dipalmitoylphosphatidylcholine (DPPC) is one of the main components of the lung surfactant. The lung surfactant constitutes a surface lining inside the alveoli, formed by 90% of lipids and 10% of proteins, which is the responsible of decreasing the surface tension in the lungs to near-zero. This helps the process of compression and the expansion of the alveoli during breathing and therefore plays an important role in the proper functioning of the lungs. Thus, understanding how the lung surfactant works and its responses to changes is fundamental to deal with diseases associated with the lungs[1].

In this project, the effect of graphene oxide could produce in the lung surfactant is investigated. Graphene oxide (GO) is formed by a single atom layer of sp^2 carbon atoms with carboxyl and hydroxyl groups in the surface, which appear during the process of exfoliation of graphite with oxidizing agents. Graphene and GO have recently enjoyed significant attention. Due to their unique properties (amphiphilicity, surface functionalizability, thermal and electrical conductivities and strong mechanical strength, among others), their use as a nanomaterial with applications in therapy, diagnosis or with electrochemical applications is emerging. Thus, the risk of inhalation of this nanoparticle is increasing, which would produce changes in the conformation of the lung surfactant.

Sample preparation

Solutions of 1 mg/mL of DPPC and d62-DPPC (Avanti Polar Lipids, Inc.) were prepared in chloroform stabilized with ethanol (Acros Organics) as a solvent. The contrast matched (CM) mixture, composed by 95% DPPC and 5% d62-DPPC, was characterized by a null SLD of the tails of the surfactants.

For the Langmuir monolayers, ultrapure water obtained by a system Millipore MiliQ was used as a subphase. For the NR monolayers, air contrast matched water (ACMW) was used instead. ACMW, a mixture of 8.1% v/v D2O in H2O, is characterized by having the scattering length density of zero. For both kinds of monolayers, a solution of 0.033 mg/mL of graphene oxide in ultrapure water was used.

DPPC monolayers were prepared using a Langmuir trough (20 800 mm^2 maximum area, microtrough G1, Kibron). A fresh Wilhelmy plate made by filter paper was used as the pressure sensor. After filling and cleaning the subphase, the lipid solution was spread using a Hamilton microsyringe until the surface pressure achieved ~1 mN/m (12 µL of DPPC solution, 19 µL of d62-DPPC and 14 µL of CM solution). The system was left for 20 min for chloroform evaporation. The area hysteresis was carried out between a maximum area of 200 cm^2 and a minimum area of 107 cm^2 (for water as a subphase) or 95 cm^2 (for GO solution as a subphase). The barriers speed during the compression was 5 mm/min. All experiments were carried out at 22C, fixed by a water bath.

Neutron reflectometry measurements were obtained in the time-of-flight neutron reflectometer FIGARO at the Institut Laue-Langevin. Measurements were recorded in six isotopic contrast: DPPC, d62-DPPC and CM as lipid monolayers and ACMW and GO/ACMW 0.033 mg/mL as sub-phases. The monolayer was compressed until a pressure of 15 mN/m, previously to initiate the NR measurements. Then, neutron beams with wavelengths lower than 20 Å incise in the monolayer with angles of $\theta = 0.62°$ and 3.8°. Measurements times between 20 and 60 min were set to optimize the data quality.

Data analysis of the monolayer in the absence of GO was developed by a global fit (performed using the Aurore software). The monolayer was modelled as two stratified layers separated by air and liquid. The air layer corresponds to the hydrophobic tails of the lipids, while the liquid layer to their hydrophilic heads. The variables for both layers were the thickness (t) and the fraction of water in the second layer (fw), while the scattering length density (ρ) and the roughness (σ) were given a constant value. The value of ρ was defined by the scattering length (b) and the volume (V) of each individual layer and lipid (**Table 1**). The roughness was fixed as 3 Å. The characterization of the monolayer in the presence of GO was developed with a global fit. In this case, the structure was modelled based on the assumption that the two layers from the previous model (tails and heads) were defined by the same values of thickness and

roughness but with different ones for the scattering length density. The final model was obtained by adding additional layers to those two mentioned before. The fitting was minimized by a least-squares function, χ^2, in order to obtain the best set of parameters. The parameters obtained were finally refined by a Bootstrap analysis.

		DPPC	d_{62}-DPPC	CM
AIR	b (fm)	-32.1	612	0.185
	V (Å^3)	822	892	826
LIQUID	b (fm)	59.9	59.9	59.9
	V (Å^3)	322	322	322

Table 1. Scattering length (*b*) and molecular volume (V) of each layer and lipid. As shown in the right figure, air layer corresponds to the tails of the lipids and the liquid layer to their heads.

Results. By fitting the experimental RQ^4 data for both DPPC and DPPC-GO monolayer (**Fig. 1**), the system was characterized by a model of three layers. In this approach, molecules of GO would interact with the hydrophilic heads of the lipids and, moreover, GO sheets would form an additional film under the heads layer (**Fig. 2**). The thickness of both first two stratums was kept constant (15.1 Å and 8.5 Å). The result of this system of equations conclude in that the second layer is composed by 20% of water, 67% of lipids heads and 13% of graphene oxide. Finally, a third layer, 32.1 Å thick, was found to be composed by 99% water and 1% GO.

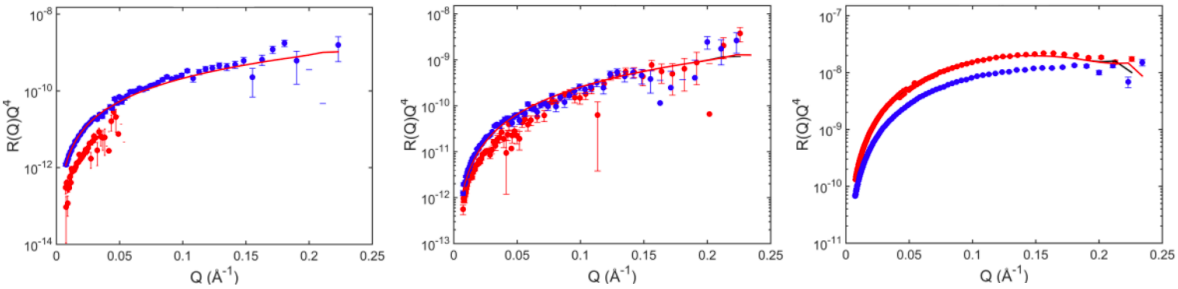

Fig. 1 Experimental reflectivity data (dots) and fit curves (lines) for DPPC (left), CM (middle) and d_{62}-DPPC (right) monolayers in 0.033 mg/mL of GO modelled by three layers.

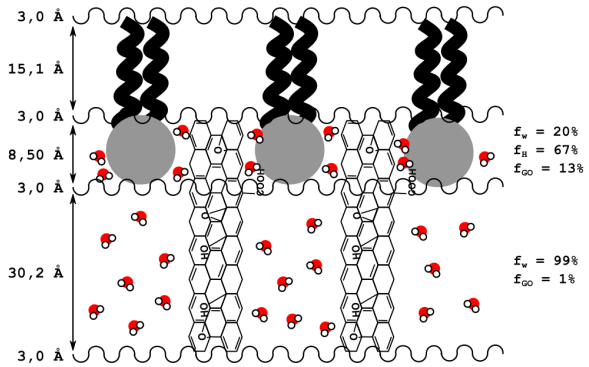

Fig. 2 Schematic representation of the model of three layers resulting from the interaction between DPPC monolayer and GO. The first layer, 15.1 Å thick, is constituted by the tails of the lipid. The second layer, 8.5 Å thick, is constituted by 67% heads, 15% GO and 13% water. The third layer, 30.2 Å thick, is constituted by 99% water and 1% GO. Roughness was, in all cases, fixed as 3.0 Å.