Experimental report

14/02/2017

Proposal: TEST-2589 **Council:** 4/2016

Title: Propofol interaction and copolymerbrush conformations

Research area:

This proposal is a new proposal

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Samples: Lipids
D2O
propofol
salts
copolymers
Chlorosilane

Instrument	Requested days	Allocated days	From	To
FIGARO	2	3	06/06/2016	08/06/2016
			12/02/2017	13/02/2017

Abstract:

FIGARO TEST-2589: FINAL EXPERIMENTAL REPORT

Introduction

Many low molecular weight drugs, like classical surfactants, contain both hydrophobic and hydrophilic groups. However, due to their lower molecular weight and less distinct separation into hydrophobic and hydrophilic regions, the self-assembly of such drugs in aqueous solutions is less easy to describe in general terms. It is commonly observed that this type of drug interacts with phospholipid membranes. This is most often not the cause of the wanted effect of the drug, but it has rather been suggested to cause some of the unwanted toxic effects. To gain more understanding of the interaction between such amphiphilic drugs with phospholipid membranes, we focused our study on one particular drug molecule, propofol, which is predominantly hydrophobic but contains a hydrophilic OH-group located in the middle of the molecule.

Propofol is probably most well-known for inducing general anaesthesia, which is a physiological state of unconsciousness when the patient becomes amnesic and loses the response to pain. Propofol is also widely used as an intravenous agent, it is of importance to have a thorough knowledge about how propofol interacts with cell membranes, and it has been suggested that it penetrates the lipid bilayer forming the plasma membrane. However, information about the location of propofol in lipid membranes and the ability for membranes to squeeze out the drug with increased surface pressure remains unknown.

We have performed studies of interactions between propofol and phospholipid monolayers at various surface pressures in a Langmuir trough. The technique used is Vibrational Sum Frequency Spectroscopy (VSFS), which has enabled us to examine changes in the order, orientation, and hydration of the phospholipids induced by propofol. The phospholipids studied were DSPC, DPPC, and DMPC. An important result was the fact that at low surface pressures (lipids in fluid phase), propofol penetrated the lipid layers more than at high surface pressures (lipids in condensed phase), where propofol was squeezed out. Propofol was also shown to induce disorder in the hydrocarbon chains at low surface pressures, whereas at 25 mN/m propofol did not affect the phospholipid packing.

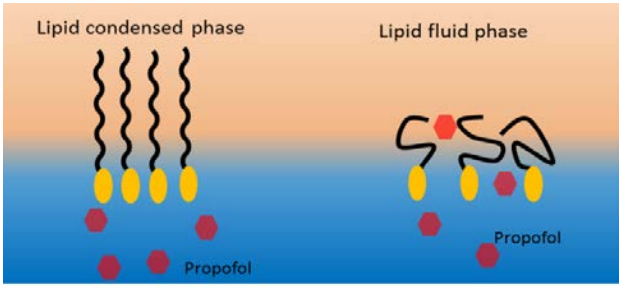


Figure 1. Propofol penetration into lipid layers.

Experiment

Petru Niga, Magnus Johnson, Erik Bergendal and Richard Campbell carried out an experiment on the FIGARO reflectometer on 6th and 7th June 2016 concerning interactions of the drug propofol with lipid monolayers on a Langmuir trough. The FIGARO experiment can be divided into four parts as follows.

PART 1 – Propofol Adsorption Isotherm

The surface excess of propofol was measured directly at 6 different bulk concentrations at low Q_z values in air contrast matched water to provide a direct measure of the adsorption isotherm.

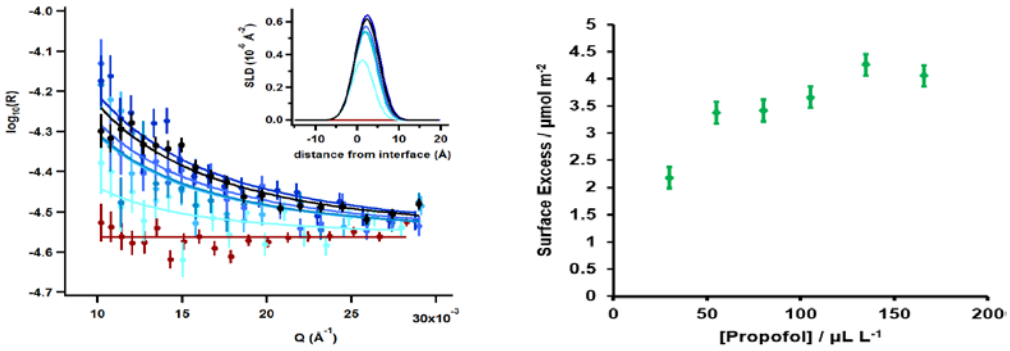
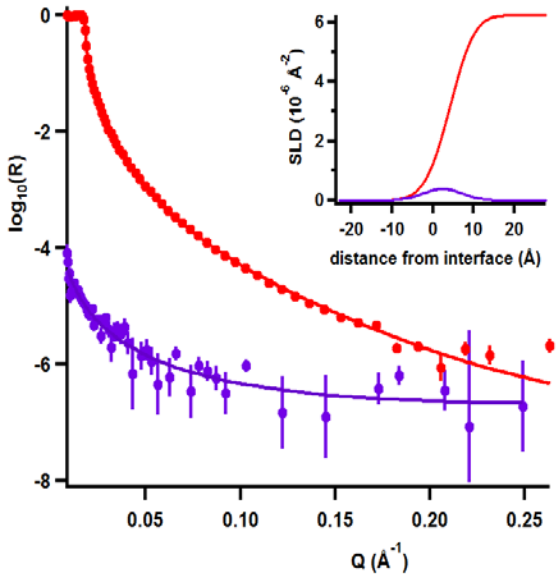


Figure 2. (A) Neutron reflectivity data and model fits for propofol solutions at the air/water interface where the six bulk concentrations in panel B are progressively darker in color; the pure air contrast matched water background data and fit shown in red. The inset shows the scattering length density profiles. (B) The resulting adsorption isotherm.

PART 2 – Propofol Interfacial Structure

The structure of propofol at the air/water interface was measured by exploiting isotopic substitution of the subphase and performing measurements over the whole accessible Q_z range.



The inter-layer roughnesses were constrained to 4.5 Å, the residual background was fitted to 2 x 10^{-7}, and the thickness of a propofol monolayer and its volume fraction were fitted. The fit result using a genetic algorithm converged to a propofol layer of 4.8 Å with a volume fraction of 1. The high volume fraction demonstrates that the layer is uniform and propofol does not form islands at the interface. We know that the density of propofol cannot exceed that of the pure compound, so in order to accommodate all the measured molecules at the interface the monolayer thickness cannot be less than 4.8 Å. Nevertheless, due to the coupling of the thickness information with the inter-layer roughness values, which have not been measured independently for this system, an upper limit on the monolayer thickness of an additional Ångström seems appropriate. The results from parts 1 and 2 will shortly be submitted.

Figure 3. Neutron reflectivity data and model fits for propofol solutions at the air/water interface recorded in D_2O (red) and air contrast matched water (purple) revealing a dense monolayer.

PART 3 – Dynamic Interfacial Composition of Propofol with DPPC, DSPC, and DMPC

Traditionally it takes about 2 hours to resolve the interfacial composition of a binary mixture at the air/water interface using neutron reflectometry. Measurements are typically made in three isotopic contrasts over the full Q_z-range. Recently we pioneered a new method to resolve the interfacial composition by measuring two scattering excesses of different isotopic contrasts both in air contrast matched water only at low Q_z values.[#C] The method is much faster and is more accurate that the traditional approach and has allowed us to access the interfacial composition of a soft matter system under dynamic conditions of compression/expansion isotherms for the first time.[#D] However, the method has never been applied to biologically-relevant systems. Determination of the background value is essential and in this case it was fitted from a measurement of pure air contrast matched water as 2.73 x 10^{-5}. The squeezing out of propofol from the monolayer upon compression, and its re-adsorption during expansion is shown clearly for all three lipid systems. The data for the lipid has lower scatter because of the use of a deuterated sample; the scatter in the propofol data, however, is not too bad and rough fits to the data can allow us to resolve the interfacial stoichiometry.

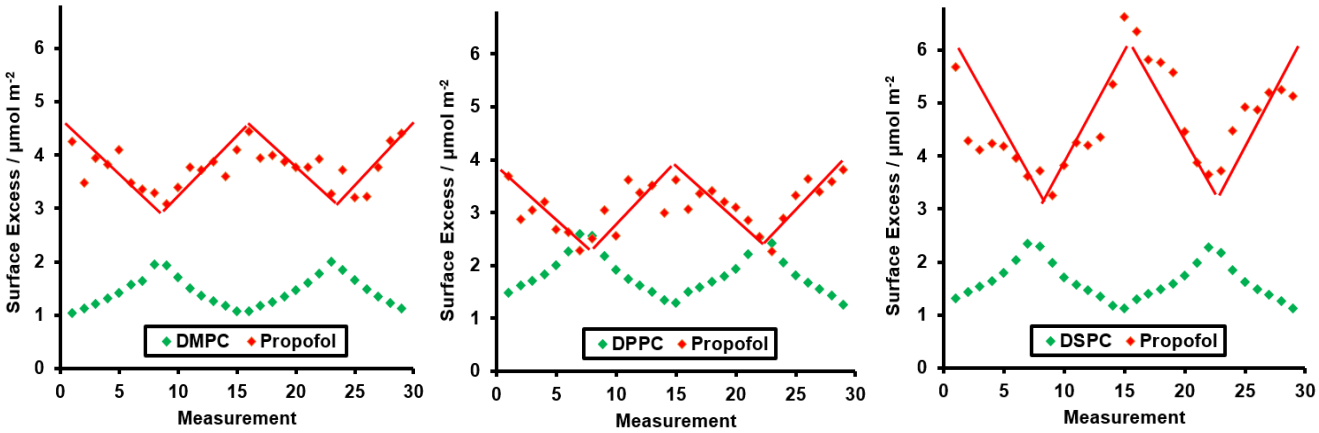
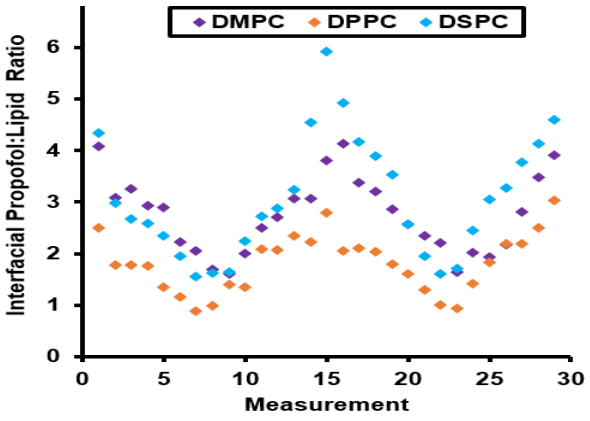


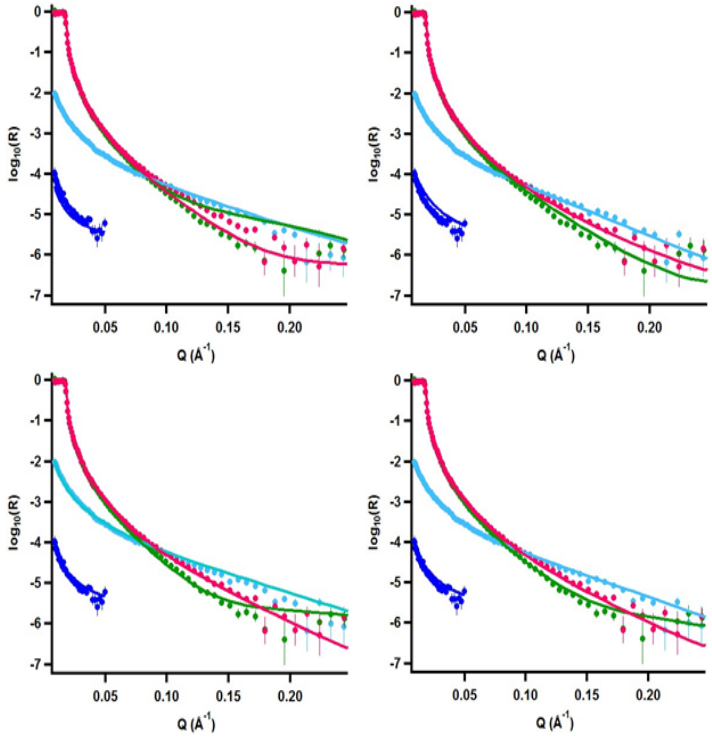
Figure 4. Surface compression/expansion isotherms on a Langmuir trough for the interactions of propofol with monolayers of DMPC, DPPC and DSPC. The lines are shown just to guide the eye.



The amount of propofol incorporated in the fully expanded monolayers takes the order DSPC > DMPC > DPPC, which is not monotonic with the chain lengths. Interestingly, 60% of the propofol is squeezed out of the monolayer upon compression for each system, and re-adsorption is faster than the time scale of the measurements because there is no observable hysteresis exhibited in the data. Such quantitative data was not previously accessible for this system.

Figure 5. Dynamic interfacial stoichiometries of the data in figure 4 – a first for a bio-system.

PART 4 – Structural analysis of DPPC and propofol



Structural NR data mostly over the full dynamic Q-range were collected for DPPC monolayers compressed to 5 mN/m in four isotopic contrasts: (1) d75-DPPC/D_2O, (2) d75-DPPC/ACMW, (3) DPPC/D_2O and (4) DPPC/ACMW. Further, data were recorded in the same 4 isotopic contrasts but in this case the DPPC was spread on propofol solution before its compression to 5 mN/m. The approach here was to compare different models of the location of propofol at the interface, and judge the quality of the model fits to the experimental data to make some statements about where the drug is sitting in the interfacial layer. Four different models are shown where the last one with propofol located both between the chains and in the head groups best fits the experimental results. Further, we can say categorically that it does not sit underneath the lipid head groups. Such information about the location of propofol in lipid monolayers had not been previously determined.

Figure 6. Experimental data and model fits in 4 contrasts of DPPC monolayers at 5 mN/m on propofol solution: (top-left) 3 layers: chains / head groups / propofol, (top-right) 2 layers: chains / head groups + propofol, (bottom-left) 2 layers: chains + propofol / head groups, and (bottom-right) 2 layers: chains + propofol / head groups + propofol.

FUTURE WORK

Following this extremely successful test experiment in June 2016, with all the aims achieved and results fully analysed (with some included in a manuscript that is almost ready for submission), the next part of this project is to extend the type of lipid from phosphatidylcholines to ones with different head groups. A provision of some more days of beam time on FIGARO will allow us to succeed in several further aims, e.g., to extend the structural characterization of this system to lipids with different acyl chain lengths, and to extend the dynamic interfacial compositional characterization to lipids with differently charged head groups. These results will enable us to publish a second full paper in this project.

REFERENCES

#A. ACS Chem. Neurosci., 2015, 6, 927−935. // #B. Biophysical Journal, 1998, 75, 734–744. // #C. Langmuir, 2013, .29, 11554–11559. // #D. Soft Matter, 2016, 12, 1504–1512.