Experimental report

14/08/2017

Proposal: 9-10-1458 **Council:** 4/2016

Title: Interaction of perfluoroalkyl substances with model worm and plant membranes

Research area: Materials

This proposal is a new proposal

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Samples: perfluoroalkyl substances
lipid bilayer components

Instrument	Requested days	Allocated days	From	To
D17	5	2	22/09/2016	24/09/2016

Abstract:

Perfluoroalkyl substances (PFASs) are fully fluorinated, stabile, and surface-active compounds. They contain both a hydrophobic fluorinated carbon chain and an ionizable hydrophilic functional group, and can interact with biological membranes through different pathways, such as adsorption, penetration and displacement. PFASs have been found to accumulate in organisms in the environment, but there is a lack of knowledge on the mechanisms behind the accumulation. This proposal aims to use neutron reflection to investigate the interactions of perfluorooctane sulfonate, perfluorononanoate and perfluorohexanoate with standard eukaryotic mimic biomembrane and compare with model plant membrane to elucidate the influence of different functional groups and chain lengths on the sorption/accumulation behavior at the two different types of membranes.

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Introduction

Perfluorinated amphiphiles are widely used in industrial products such food packing, water repellent clothing, firefighting foams, carpet industry, etc [1]. Recently these contaminants of these compounds have attracted global concern since they have been shown to be persistent in the environment and toxic for wildlife and humans [2, 3]. There is a particular concern about the long chain substances hence, short chain compounds have been introduced as a safer replacement to the long chain compounds. However, the fundamentals of their interactions with membranes in a molecular level are not well understood. Phospholipids are the building block of the cell membranes and are commonly used for fundamental studies to understand the behaviour of membranes. In this study, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) was chosen as model bilayer to explore the fundamental of the interaction between perfluorinated substances with biological interfaces. The present experiment aimed to explore the interactions of short chain perfluoroalkyl sulfonate substances with phospholipid bilayers.

Method

Two cells were mounted with identical silicon crystal and similar bilayer was formed on both surfaces. Surfaces were characterised in three contrasts: H_2O, D_2O and contrast matched silicon in order to characterise the thickness of oxide layer as well as the roughness of silicon and silica layer. The bilayer was deposited by vesicle diffusion at 55 degrees on silicon crystal (111 face with an oxide layer on top). After characterizing the bilayer in D_2O, H_2O and contrast matched silicon, the PFAS solutions were injected into the reflection cell at different concentrations. Perfluorobutane sulfonate (PFBS) and perfluorohexane sulfonate (PFHxS) were chosen with 4 and 6 carbons in the chain groups, respectively, were chosen as the target PFASs. The stock solutions were prepared close to the solubility limit estimated by Want et al. 2011 in both D_2O and H_2O and were diluted to the desired concentration using HPLC pump. The reflectivity at each concentration, for each substance was measured both in H_2O and D_2O contrast and was modelled simultaneously using a modified version of reflection programs available on http://www.reflectometry.net/refprog.htm.

Results and discussion

Figure 1. shows the reflectivity measured and the model fits to the data, from the bilayer at different PFBS concentrations in D_2O contrast. Both PFHxS and PFBS showed penetration into the bilayer which increases with the concentration. As PFBS and PFHxS penetrated into the bilayer, the amount of lipid in the layer decreased. This behaviour was similar but stronger than our previous observation from PFASs. PFASs interact with the bilayer by partitioning into the layer and displacing the lipid in order to accommodate themselves. The penetrations of these compound for the same concentration was observed to be less that measured for the long chain substance (PFOS) previously. When the highest concentration of PFBS was injected into the cell, the reflection curve changed significantly and the layer could only be fitted with a rough layer of mixed PFAS and lipid. Two-dimensional scattering map for 88 $mmolL^{-1}$ showed a diffused scattering (so-called Yoneda scattering) which can appear due to the interfacial roughness. The aggregations of PFASs has been reported previously upon mixing of fluorocarbons with hydrocarbon surfactants at air/water interfaces. Similar behaviour can cause aggregation in the bilayer as mixed with hydrophanous lipids. This aggregation can make the bilayer appear rough and create possible defects in the layer.

After injecting the highest concentration of PFASs the reflection cell was rinsed with water (with approximately 10 times higher volume of water that the volume of the cell) and the

reflection was measured in both contrasts. Rinsing the layer showed to remove PFASs but leave a less dense and rougher bilayer behind.

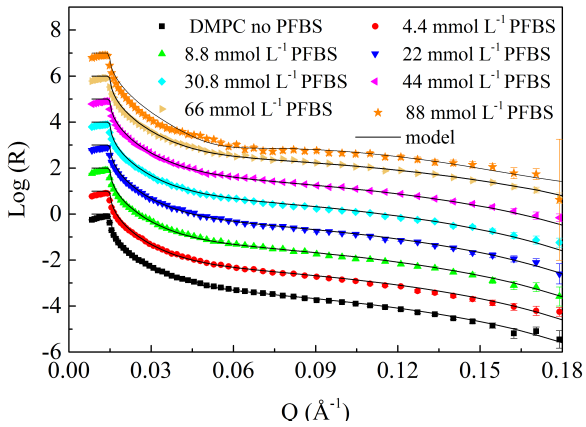

Figure 1. Reflectivity from the bilayer exposed to PFBS in different concentrations in D_2O contrast [3].

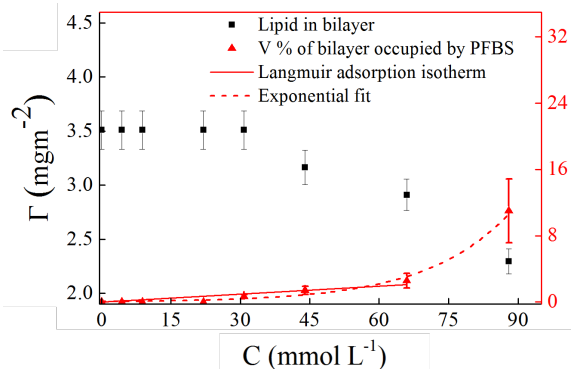

Figure 2. The amount of lipid in the bilayer (right axis) versus the penetration of PFBS into the bilayer (right axis) in different concentrations. The penetration of PFBS into the bilayer in low concentrations could be fitted using the Langmuir adsorption isotherm [3].

Conclusions

- Short chain substances interact with the bilayer in a similar way as long chian substances but at higher concentrations.
- Both PFBS and PFHxS penetrate into the bilayer and their interactions with bilayer increase with concentration.
- As PFBS and PFHxS penetrate into the bilayer lipid is detached from the layer.
- For the same concentration PFHxS has stronger interaction compared to PFBS, suggesting that the effect increases with the chain length
- As the concentrations of both groups of PFASs increases, the layer becomes rougher, less dense and more disordered.
- Rinsing removed most of most of the PFASs, but the bilayer after rinsing was different than the original one.

Outlook

This experiment has been a part of a larger study comparing the interactions of PFASs with different chain length and functional group with phospholipid bilayers. The result is compared to that obtained for various other PFASs and the manuscript has been submitted to the journal of colloid and interface science. The present work can pave the way for understanding the details of PFASs interactions with more complex membrane systems. The future work is planned to investigate how PFASs partition into the plant like or worn like membranes.

References

1. R. C. Buck et al. (2011), *Integrated Environmental Assesment and Management*, 7, 513-541.
2. L. Ahrens & M. Bundschuh (2014), *Environmental Toxicology and Chemistry*, 33, 1921–1929.
3. J. W. Martin et al. (2003), *Environmental Toxicology and Chemistry*, 22, 196–204.
4. S. Nouhi et al. (2017), *submitted manuscript*.