# Experimental report

26/04/2022

**Proposal:** 9-13-929 **Council:** 4/2020

**Title:** NR study of interactions between biocidal surfactants and a bacterialmembrane-mimic monolayer

**Research area:** Physics

**This proposal is a new proposal**


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**Experimental team:** Laura FOX

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**Samples:** DTAB (C12TAB)
CTAB (16TAB)
DPPG/DPPE

| Instrument | Requested days | Allocated days | From | To |
|---|---|---|---|---|
| FIGARO Langmuir trough | 3 | 3 | 31/05/2021 | 03/06/2021 |


## Abstract:

Quaternary ammonium compounds (QACs) are a group of cationic surfactants used as biocides in disinfectants and sanitizers for hard surface cleansing in hospitals, public hygiene and industrial food and water treatments. Although QACs are cationic surfactants with well-defined structures (charge groups, chain length and branching) and physical properties (critical micelle concentration (CMC), surface tension at CMC and solubility boundaries), there has been little effort to try to link such data to their efficacy against bacteria. The missing link lies in understanding how QACs interact with lipid membranes in the context relevant to bactericidal actions.
The broad aim of this project is to establish a knowledge base at the molecular level and use such information to understand antimicrobial actions of QACs at the cellular level.
With help of contrast variations NR can determine the composition of lipids and surfactants and their extent of mixing from which the structural damage to the lipid film caused by different biocide surfactants can be assessed. Such information can't be obtained by other means.

# NR study of interactions between biocidal surfactants and a bacterial membrane-mimic monolayer

## Introduction

Quaternary ammonium compounds (QACs) are a group of cationic surfactants used as biocides in disinfectants and sanitizers for hard surface cleansing in hospitals, public hygiene and industrial food and water treatments. QACs have high inhibitory and bactericidal activity against a wide spectrum of bacteria, but little is currently known about how their molecular structures affect their efficacy and toxicity in spite of their use as important biocides since 1930s.

Although QACs are cationic surfactants with well-defined structures (charge groups, chain length and branching) and physical properties (critical micelle concentration (CMC), surface tension at CMC and solubility boundaries), there has been little effort to try to link such data to their efficacy against bacteria. In spite of some reported studies of surfactant binding to model lipid membranes, the missing link lies in understanding how QACs interact with lipid membranes in the context relevant to bactericidal actions. This work was set to bridge this gap and neutron reflection a key method for studying such mixed molecular films with help of deuterium labelling.

## Materials and Method

Due to issues with the arrival of samples to the ILL site (held at customs), the proposed experiment was adapted to include materials available to us which complimented the systems stated in the proposal. DPPS lipids were used to replace DPPG lipids as the charged component of the monolayer mimicking Gram Positive bacteria membranes. The QAC studied interacting with this membrane was DDAB at varied concentrations. The most important contrasts for analysis of the lipid monolayer structure in the absence and presence of the QAC were also available to us (d-QAC/h-lipid and h-QAC/d-lipid in NRW and D2O).

DPPS was dissolved in chloroform/methanol (6:1, v/v) at a concentration of 0.5 mg/mL. DDAB was prepared in 140mM NaCl buffer at a concentration of 2 μM and 20 μM corresponding to 1 x CMC and 10 x CMC of DDAB.

DPPS was dropcast onto a trough surface containing 80 mL of 140 mM NaCl buffer. The structure of the DPPS monolayer, mimicking G+ bacteria lipid membranes, was first examined at a constant surface pressure of 30 mN/m. Then, DDAB was injected from underneath the monolayer using a long needle, producing final concentrations in the subphase of 0.02 μM and 0.2 μM. Dynamic scans of the monolayer structure were then measured for a minimum of 90 minutes and surface pressure equilibrium was reached. A final structural scan was then measured with better statistics for improved data fitting. There were a total of 4 isotropic contrasts carried out for each concentration, increasing the credibility of data analysis.

## Results and Discussion

Figure 1a shows the NR profiles and the inset shows fitted SLD profiles for a DPPS monolayer in 4 contrasts. From these fitting results the area per molecule of DPPS was 45.8 $Å^2$/molecule. The thickness of the tail and head groups fitted with a slab model were 19.6 Å and 12.0 Å respectively with a solvent volume fraction of 54% in the headgroup slab. The surface concentration was calculated to be 3.63 $\mu mol/m^2$.

After pressure equilibration, following injection of 2 μM DDAB (final 0.02 μM), the total area per molecule was found to decrease to 43.49 $Å^2$/molecule with a surface concentration of surfactants of 0.93 $\mu mol/m^2$ within the lipid tails slab. For d-DDAB injection the thickness of the tail region was found to decrease to 16.4 Å whilst the headgroup thickness increased slightly to 12.2 Å. Since DDAB has

shorter alkyl chains (C12) compared to DPPS (C16), the reduction in thickness, alongside the reduction in area per molecule is indicative of DDAB insertion into the monolayer. Fittings to the dynamic measurements of d-DDAB insertion in h-DPPS monolayers with restraints on total thickness (31.6 Å) show an increase in SLD of the monolayer over time, again indicating insertion of DDAB into the DPPS monolayer.

Similar results are observed for the injection of 20 μM DDAB (final 0.2 μM), again showing a decrease in tail thickness 17.4 Å and increase in head thickness 13.4 Å after pressure equilibration. Further to this the fittings of dynamic scans of d-DDAB insertion into h-DPPS monolayers, Figure 1b, reveal a greater change in SLD, setting monolayer thickness to constant as before, as compared to injection at a lower concentration. This shows a greater insertion of DDAB into the DPPS monolayer at higher concentration.

Due to the sample transport issues described, this experiment did not result in a complete data set for this system. It is hoped with further measurements this data will be fit for future publication, further exploring the effect of biocide structure (single chain C12TAB) and monolayer composition by varying ratio of anionic and zwitterionic lipids.

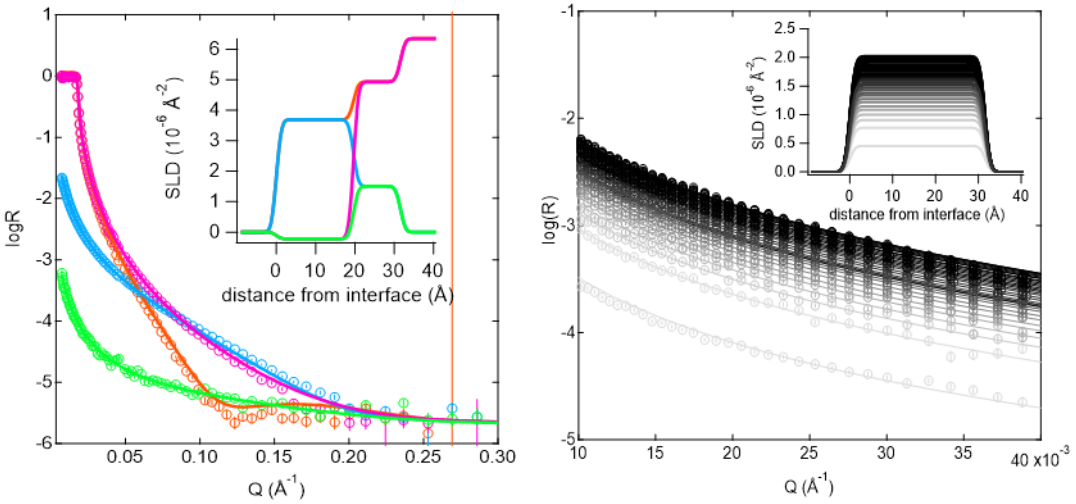


Figure 1 (a) Reflectivity profiles of DPPS in four contrasts and (inset) SLD profiles of fits to reflectivity data (b) Reflectivity profiles of dynamic scans during d-DDAB interaction with DPPS monolayer with (inset) SLD profiles of fits to reflectivity data with constant thickness showing insertion of DDAB over time.