Experimental report

14/11/2018

Proposal: CRG-2539 **Council:** 4/2018

Title: Surfactant Modified Salivary Pellicles

Research area: Biology

This proposal is a new proposal

Main proposer: Juan francisco GONZALEZ MARTINEZ

Experimental team: Hannah BOYD
Juan francisco GONZALEZ MARTINEZ

Local contacts: Alexey KLECHIKOV
Alexei VOROBIEV
Philipp GUTFREUND

Samples: Sodium dodecyl sulfate
saliva
Lauramidopropyl betaine
pentaethylene glycol mono-n-dodecyl ether
dodecyl trimethyl ammonium bromide

Instrument	Requested days	Allocated days	From	To
SUPERADAM	5	4	23/09/2018	26/09/2018

Abstract:

Surfactants are often used in oral health products. The use these compounds has been related to several oral diseases. However, oral care products without surfactants have been unsuccessful as these components fulfil several functions. They serve as solubilizing, dispersing, emulsifying and wetting agents. They also promote foaming which provides a perception of cleanliness. Thus, a widespread use of oral care formulations without surfactants is unrealistic. It is then of interest to identify those with lower adverse effects. In this context, it is of great value to understand how surfactants interact with the first interfacial layer that they encounter i.e., salivary pellicles. These are proteinaceous nm-thick films that form on any type of surface upon exposure to saliva and that fulfil many functions e.g., they protect, lubricate and hydrate underlying oral tissues. The applicants have previously shown that Neutron Reflectometry (NR) is a powerful tool to investigate the structure of salivary pellicles. Here, we propose to use NR to study how their structure is affected by surfactants of relevance within oral care formulations.

Experimental Report

Surfactants are present in almost all health care products. They provide foaming action, which gives the perception of cleanliness and therefore making the consumer more likely to purchase. Surfactants are also the dispersion, emulsifying, solubilizing and wetting agents [1], making them important ingredients in those products. Sodium dodecyl sulfate (SDS) is the most commonly used surfactant due to its higher foaming ability. However, despite this, SDS has also been associated with many adverse effects including oral ulceration, irritation and mouth odor [2]. SDS is an ionic surfactant, it is therefore of interest to compare the effects of SDS with surfactants of different ionic character and identify those with lower adverse effects [2, 3]. The salivary pellicle is a nm-thick proteinaceous film, which forms on any surface upon exposure to bulk saliva. Within the oral cavity the pellicle is known to fulfil many functions vital to maintaining oral health, including the hydration and protection of the oral mucosa. As this is the initial layer that the surfactant will interact when using oral health products, it is applicable to consider the effect of the various surfactants on the pellicle.

NR has previously been shown to be a valuable tool to investigate the structure of the salivary pellicle [4]. Here, the change in structure upon exposure to two surfactants is observed using NR. While previous studies have been performed in surfactant-pellicle interactions [5-8], at present there are no systematic investigations on how these are affected by the properties of the surfactant. Previous results using QCM-D showed that SDS completely removes the pellicle, but upon exposure to the nonionic pentaethylene glycol monododecyl ether ($C_{12}E_5$) and amphoteric cocamidopropyl betaine**,** only a slight decrease in the overall thickness is observed. The fact that surfactants do not completely remove the pellicle and are known to be much less aggressive than SDS has been investigated in more detail using neutrons and allows an indication of how the structure of the 2-layer salivary pellicle model is changed with respect to hydration, layer thickness and penetration of the surfactant and, therefore, their overall protection of the oral tissues.

For each surfactant the following procedure was used. First the silicon surface was characterized in a standard solid liquid cell set up. Then, bulk saliva was allowed to adsorb onto the surface for 1 hour. PBS was then used to rinse the surface, before characterizing with 3 contrasts. The surfactant at a concentration of 2.5 CMC is then injected and allowed to interact for 1 hour. PBS was again used to rinse, and the surfactant treated saliva was characterized using three contrasts. Figure 1 shows the D2O contrasts for the silica, rinsed saliva and rinsed surfactant treated saliva. For both data sets the salivary pellicle is present and visible to neutrons. For the $C_{12}E_5$ treated pellicle, the surfactant does have an effect on the structure of the pellicle but does not completely remove everything on the surface. However, the cocamidopropyl betaine does not seem to change the pellicle. It will be of interest to understand the change in structure of the pellicle after treatment by the different surfactants through further analysis. This analysis will allow a better understanding on how surfactants of different ionic characteristics interact with the salivary pellicle with respect to hydration and individual later thickness and will further explain the results seen from QCM-D and ellipsometry, which both show a decrease in thickness but cannot differentiate between the layers and components of the pellicle. It is particularly interesting to see if the pellicle remains intact, or if the surfactant is penetrating the film or replacing the salivary proteins.

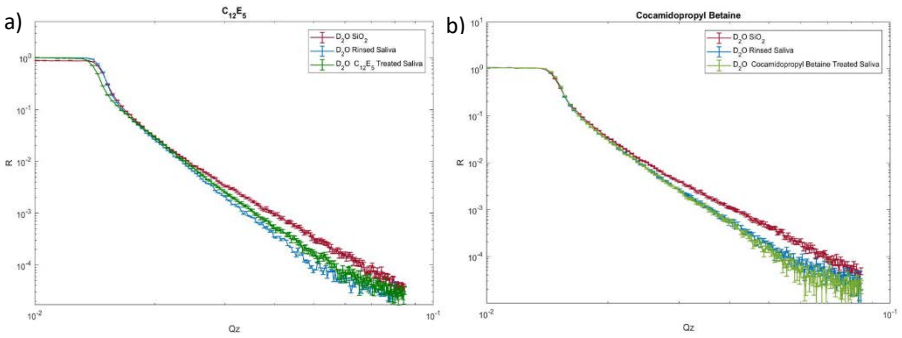

Figure 1. NR curve obtained with SuperADAM for Saliva adsorbed onto silica, before and after exposure to a) $C_{12}E_5$ and b) cocamidopropyl betaine, compared to clean silica.

References

[1] Pader, M., Surfactants in Oral Hygiene Products, in Surfactants in Cosmetics. Marcel Dekker Inc. New York, 1997. [2] Moore, C., et al. Int. J. Dent. Hyg., 2008. 6, 193-198. [3] Herlofson, B.B.; Barkvoll, P. Acta Odontol. Scand., 1996. 54, 150-153. [4] Cárdenas, M., et al. Biomacromolecules, 2007. 8, 65-69. [5] Arnebrant, T.; Simonsson, T. Acta Odontol. Scand., 1991. 49, 281-288. [6] Berg, I.C.H., et al. Biofouling, 2001. 17, 173-187. [7] Vassilakos, N., et al. Biofouling, 1992. 5, 277-286. [8] Vassilakos, N., et al. Acta Odontol. Scand., 1992. 50, 179-188.

Confinement Cell Experiment

Grazing Incidence Small-Angle Neutron Scattering (GISANS) is a promising tool for extracting structural information of boundary lubricant films under confinement and shear. Recently, a team partly formed by the applicants developed a sample environment for NR studies of mechanically confined thin soft films [1]. In this setup (Fig. 1), a flexible membrane (Melinex) that can conform to long range waviness or bend around any entrained dust is inflated against a solid hard surface. This setup has been successfully used at several studies at ILL [3-6]. Within the framework of a collaborative project between Malmö University, Kent University, ISIS, ESS and ILL recently funded by the Swedish Research Council and the Nordforsk Foundation, the applicants would like to test for the first time if GISANS could be used in combination with the developed confinement cell and if this confinement cell could be used for future confinement experiments in SuperADAM.

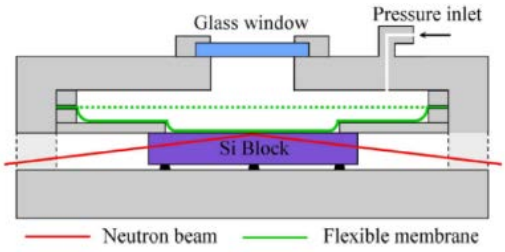

Figure 1. Currently available NR confinement cell [3].

In order to study the viability of a GISANS experiment under confinement, an ideal sample was needed. To simplify the analysis of GISANS data, a sample with a known structure should be tested using NR. SuperADAM has been used to test the ideal sample that will be described in the following paragraphs. A silicon block (3 inches of diameter, 1 cm of thickness, Si 111) has been treated using a method proposed by Andrea Tummino and Philipp Gutfreund. In this method, silica nanoparticles are spin coated on the active silica surface (cleaned with RCA and treated with UV). In the ideal case, the particles should form a well distributed monolayer covering an area large enough for the neutron footprint.

Although this was not the case for our test (Fig. 2), the covered surface was enough to probe that a relatively well-ordered surface could be used for a GISANS experiment. In particular, the test was remarkably useful because the GISANS test performed in D22 (beamtime 8-05-437) was successful (Fig. 4).

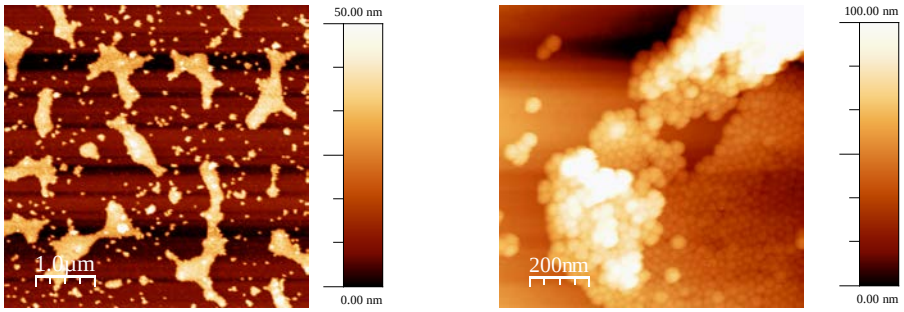

Figure 2. Topography images from the spin coated silica surface.

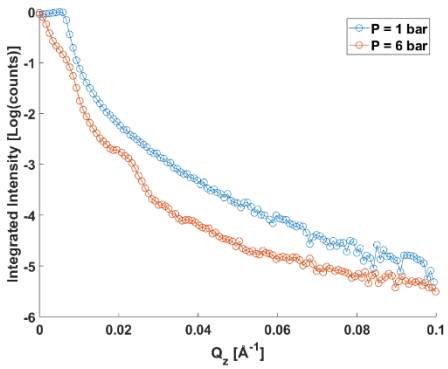

Figure 3. NR curve obtained with SuperADAM using the confinement cell at 1 and 6 bars, showing the presence of a silica

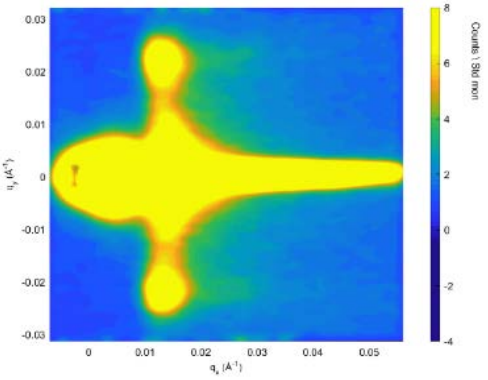

Figure 4. GISANS image from D22.

References:

[1] Review of Scientific Instruments, 83(11), 113903. [2] Macromolecules, 2016, 49: 4349. [3] Macromolecules, 2014. 47: 3263. [4] Macromolecules, 2013. 46: 1027. [5] Macromolecules 2015, 48 2224-2234.