Experimental report

02/03/2020

Proposal: **9-13-797** **Council:** 10/2018

Title: Mucin Films under Mechanical Confinement

Research area: Soft condensed matter

This proposal is a resubmission of 8-05-437

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Samples: mucin solution

Instrument	Requested days	Allocated days	From	To
D17	3	3	21/06/2019	24/06/2019
FIGARO	3	0		

Abstract:

Nowadays, a significant research effort is devoted to understand and mimic biological lubricants which, in contrast to most man-made lubricants, are based on water. It has been extensively shown that nature overcomes the poor lubricity of water with the addition of biological molecules, mostly proteins. Among these proteins, mucins are recognized as instrumental for biological lubrication. However, the molecular details of their lubricating properties are yet poorly understood. While structural studies of mucins at surfaces have given some insight into this aspect, the fact is that very little is known on the structure of confined mucin films, i.e. the really relevant system in the study of mucin lubrication. We propose to study this system by means of neutron reflectivity and grazing-incidence small-angle scattering employing a recently developed surface force type apparatus that allows the investigation of confined thin films.

Mucin Films under Mechanical Confinement (Proposal 9-13-797)

Nowadays, a significant research effort is devoted to aqueous lubrication [1]. Nature overcomes the poor lubricity of water by modifying the sheared surfaces with biological molecules. In the search for key components of biological lubricants, long glycoproteins like mucins seem to be a cornerstone [2]. While it has been proposed that this type of molecules provide efficient boundary lubrication due to a combination of entropic and hydration lubrication effects [2], the underlying molecular mechanisms are far from being understood. Specifically, little is known on the structure of confined mucin films. In this regard, Neutron Reflectometry (NR) is a promising tool for extracting thin film structural information. Recently, a team partly formed by the applicants developed a sample environment for NR studies of mechanically confined thin soft films [3]. In this setup (Fig. 1), a flexible membrane (Melinex 401) 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 for several NR studies at ILL [3-6].

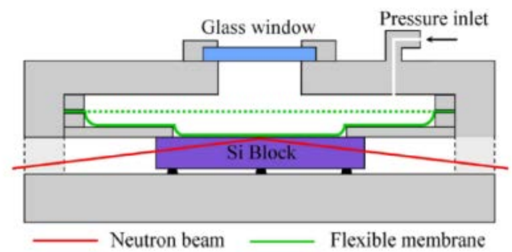


Figure 1. Currently available NR confinement cell [3].

In the scope of the beamtime 9-13-797, this setup was used to investigate mucin (oral MUC5B) films under mechanical confinement.

First, non-confined mucins were investigated at solid-liquid interfaces. NR data analysis revealed differences between measurements in deuterated and hydrogenated PBS buffer solution as shown in Fig. 2 and Table 1. The main differences obtained from the fit are the SLD value (higher in D_2O) and the solvent content (higher in H_2O). The fits are represented as RQ^4 vs. Q, to graphically emphasize the difference between the curves.

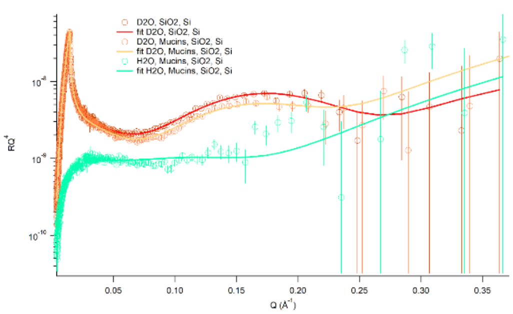
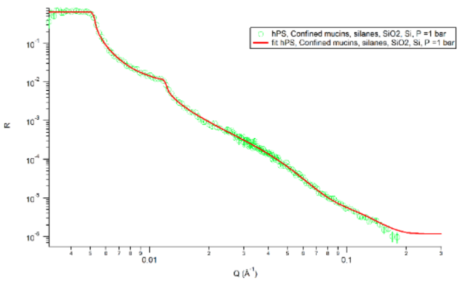


Figure 2. First experiment: *Non confinement measurements in D_2O and H_2O.*

Layer	Thickness (Å)	SLD (Å⁻²)	Solvent	Roughness (Å)
Si	-	2.07	-	-
SiO_2	19.06	3.47	0	3.13
Silanes	14.59	0.66	0	10.19
Mucins	101.52	2.97	99.99	26.41
H_2O	-	-0.56	-	29.67

Layer	Thickness (Å)	SLD (Å⁻²)	Solvent	Roughness (Å)
Si	-	2.07	-	-
SiO_2	19.06	3.47	0	3.13
Silanes	14.59	0.66	0	10.19
Mucins	104.74	5.56	25.89	5.67
D_2O	-	6.37	-	89.64

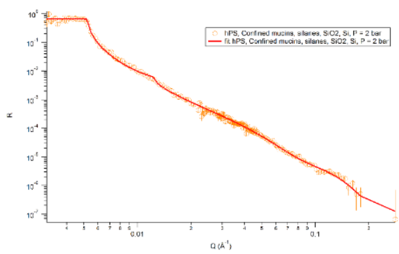
When mucins are confined, some bubbles with dust or a mix of dust, water and air could be trapped between confining membrane (Melinex) and the sample. To model these water inclusions, Philipp Gutfreund designed a model for Motofit, were reflectometry curves for the layers and for the water inclusions are combined. The model works when two critical angles are present in the data, one for the Melinex and its coating and other for the deuterated buffer pockets. Figure 3 shows the fractional fit made using the previous obtained values for the silicon block and the SLD of mucins. The table shows the fitted values to the fractional model. The novelty of this approach is that it is possible to check how much water inclusions have been trapped. In this case, a fraction of 1.2 % of the reflectivity corresponds to water inclusions. Also, confinement modified the thickness of mucins, up to 91 % of the original size. A "backing layer" has been used as a last layer, consisting of hydrogenated polysterene (hPS). No other layers were added to the fit, as it was considered that nearly all the water was already squeezed out of the space between Melinex and the sample.



Layer	Thickness	SLD	Roughness	Fraction
Si	-	2.07	-	-
SiO_2	19.06	3.47	3.13	-
Silanes	14.59	0.66	10.19	-
Mucins	8.89	5.56	12.09	-
Water inclusions	-	2.61	14.29	0.988
Backing layer	-	4.94	36.3	-

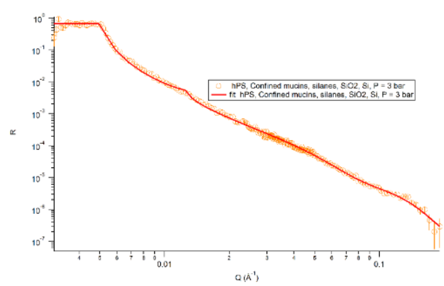
Figure 3. Experiment at 1 bar. *Left: fractional fit that includes the water inclusions between Melinex and the mucin sample. Right: table with the fitted values for the confined mucins.*

Experiments for pressures higher than 1 bar, do not show any further modification of the mucins thickness. Main difference is found in the water inclusions fraction as it decreases at higher pressures (Figs. 4 and 5).



Layer	Thickness	SLD	Roughness	Fraction
Si	-	2.07	-	-
SiO_2	19.06	3.47	3.13	-
Silanes	14.59	0.66	10.19	-
Mucins	8.89	5.56	12.25	-
Water inclusions	-	2.62	14.29	0.995
Backing layer	-	5.16	33.61	-

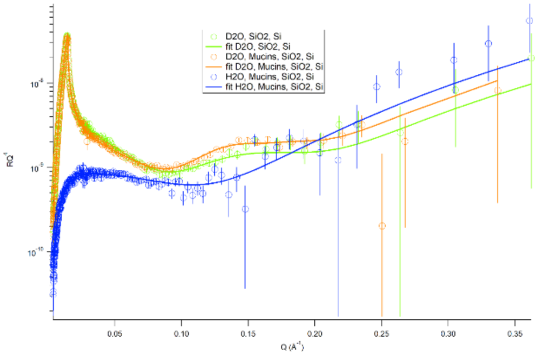
Figure 4. Experiment at 2 bars. *Left: fractional fit that includes the water inclusions between Melinex and the sample. Right: table with the fitted values.*



Layer	Thickness	SLD	Roughness	Fraction
Si	-	2.07	-	-
SiO_2	19.06	3.47	3.13	-
Silanes	14.59	0.66	10.19	-
Mucins	8.89	5.56	12.23	-
Water inclusions	-	2.62	14.29	0.996
Backing layer	-	5.37	32.24	-

Figure 5. Experiment at 3 bars. *Left: fractional fit that includes the water inclusions between Melinex and the sample. Right: table with the fitted values.*

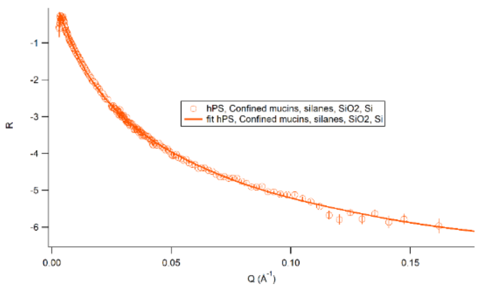
The same experiment was repeated, although with the confinement in hydrogenated buffer. The results for the characterization of the silicon block (without confinement) are shown Fig. 6 and Table 2. The silicon block used was the same as in the first experiment, so similar results were obtained for the characterization. For the confinement in hydrogenated buffer, a compression factor of 88% was found, again without changes in thickness between 1 bar and 2 bars (Fig. 7 and Fig. 8). These fits were done without the fractional fit model, as no water inclusions peaks were found in the curve.



Layer	Thickness	SLD	Solvent	Roughness
Si	-	2.07	-	-
SiO_2	19.06	3.47	0	3.13
Silanes	14.08	0.66	0	17.08
Mucins	98.76	5.56	89.43	9.23
D_2O	-	6.37	-	6.05

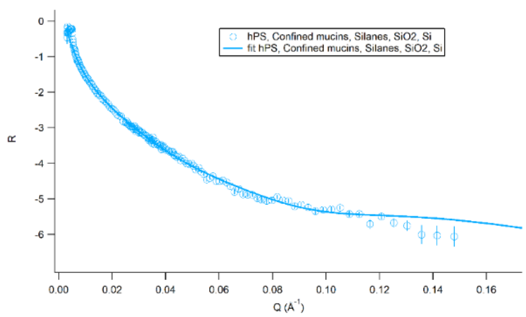
Layer	Thickness	SLD	Solvent	Roughness
Si	-	2.07	-	-
SiO_2	19.06	3.47	0	3.13
Silanes	14.08	0.66	0	17.08
Mucins	214.37	2.99	98.87	20.07
H_2O	-	-0.56	-	63.07

Figure 6. Second experiment: *Non confinement measurements in* H_2O.



Layer	Thickness	SLD	Roughness	Solvent
Si	-	2.07	-	-
SiO_2	19.06	3.47	3.13	-
Silanes	14.08	0.66	21.67	-
Mucins	26.01	2.39	370	56.78
Backing layer	-	1.41	21.31	-

Figure 7. Experiment at 1 bar. *Left: confined mucins with* H_2O *between Melinex and the sample. Right: table with the fitted values.*



Layer	Thickness	SLD	Roughness	Solvent
Si	-	2.07	-	-
SiO_2	19.06	3.47	3.13	-
Silanes	14.08	0.66	21.67	-
Mucins	26.01	2.39	370	56.78
Backing layer	-	1.41	21.31	-

Figure 8. Experiment at 2 bars. *Left: confined mucins with* H_2O *between Melinex and the sample. Right: table with the fitted values.*

In conclusion, during this experiment information regarding the SLD of mucins and their behavior under pressure were obtained. These data that will be useful for the future experiment at Figaro (beamtime 1-10-41) where shear will be included in the experiment.

References: [1] Aqueous lubrication: Natural and Biomimetic Approaches. 2014: World Scientific Publishing. [2] Curr. Opin. Colloid Interface Sci., 2010. 15: 406. [3] Rev. Sci. Instrum., 2012. 83: 113903. [4] Macromolecules, 2016, 49: 4349. [5] Macromolecules, 2014. 47: 3263. [6] Macromolecules, 2013. 46: 1027.