Experimental report

15/02/2023

Proposal: 9-10-1661 **Council:** 4/2020

Title: Protein adsorption and foul resistance of PDMS and amphiphile doped PDMS coatings

Research area: Physics

This proposal is a continuation of 9-11-1927

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Samples: PDMS CAS 9016-00-6
Bovine Serum Albumin cas CAS 9048-46-8
PFPE doped PDMS thin film PFPE-PEO fluorolink e10 CAS number:1260733-08-1 not classed as hazardous
Mytilus edulis foot protein-1
Dextran Polysaccharide Cas: 9004-54-0

Instrument	Requested days	Allocated days	From	To
D17	3	1	10/06/2021	11/06/2021

Abstract:

As the continuation of a recent, successful study of the surface reconstruction of polydimethlysiloxane (PDMS) coatings in water by the spreading of a layer of amphiphilic perfluoropolyethers (PFPE) we seek further beam time to study the adhesive behaviour and subsequent removal of Proteins on PDMS and PFPE-PEO doped PDMS surfaces in a stop flow experiment.
This would be both a novel study of the mechanism of Protein fouling on PDMS via neutron reflectivity and a comparative study assessing how wetting layers of PFPE can improve the foul resistance of PDMS coatings. Through this we would be able to better understand some of the fundamental mechanisms of early biofouling and why some molecules are so effective at resisting this fouling.

ILL Experimental report - 9-10-1661 on D17

The objective of this experiment was to study the adsorption of proteins on model foul release surfaces, that have been developed as industry. Oligomers of amphiphilic Perfluoropolyether-polyethylene oxide (PFPE-PEO) have been shown to be effective as additives for foul release materials.
In a pervious neutron reflectivity experiment(9-11-27) we studied the solid liquid interface of a model foul release coating comprising a thin PDMS film upon which a thin layer of PFPE-PEO had been top coated. We found that in air this top coated layer of PFPE-PEO dewetted completely into surface micro droplets and which were undetectable with neutron reflectivity but when in water, a partial wetting monolayer of PFPE-PEO covered the solid liquid interface of the PDMS film. Further experiments using QCM to examine bioadhesion on these model coatings have demonstrated that these PDMS/PFPE-PEO topcoat films have far greater resistance to protein adsorption than PDMS films, with negligible adsorption of BSA protein being observed on PDMS/PFPE-PEO surfaces. This experiment studied the adsorption of Bovine Serum Albumin (BSA) protein on both PDMS and PDMS/PFPE-PEO surfaces using neutron reflectivity to corroborate these findings and study the structure (thickness/density/degree of hydration) of any adsorbed protein layer on these surfaces.

Materials and coating design: Vinyl, terminated PDMS Mw 25,000 was purchased from Sigma Aldrich along- side 950 Mw (50%) methylhydro- co- (50%) dimethylsiloxane and Karstedt's catalyst platinum complex 1% in Xylene. 50x50x10mm Silicon reflectivity blocks were purchased from Sil'tronix for use as substrates.
BSA sourced from Sigma Aldrich was prepared in a buffer 200mM CaCl2 dissolved in deionised H2O. D2O was sourced from the ILL and 200mM CaCl2 was added to D2O to produce a deuterated salt buffer.
Thin films of PDMS were prepared by spin coating from solution: crosslinker HMDS-co-PDMS was mixed with vinyl terminated PDMS at a ratio of 10% the mass of PDMS and then dissolved in Hexane to a concentration of 1% by wt. Karstedt's catayst was then added to the PDMS solution at a concentration of 13ppm of the overall solution.
PDMS films were formed by spin coating this solution on Silicon blocks at 3000rpm and then curing the films at 65C^{o} for 4 hours. Before film deposition, the oxide layer on both Silicon blocks was measured with ellipsometry, this technique was also used to measure the thickness of each PDMS film once crosslinked.
The PDMS/PFPE-PEO surface was prepared by selecting one of the 2 samples and depositing a dewetting layer of PFPE-PEO Fluorolink E10/6 (Solvay) on top from a 2% dispersion in ethanol at 3000 rpm.

Neutron Reflectivity Experiment:
2 Samples, one a thin PDMS film the other a thin PDMS film with a PFPE-PEO topcoat, were loaded into ILL standard solid/liquid cells and filled with water buffer solution. Samples were measured in both D2O and H2O contrasts exchanged using a HPLC pump prior to the injection of a 50 μg/ml BSA solution in H2O contrast buffer. Kinetic 1 minute scans were taken for 50 minutes during the initial protein exposure phase before a final full measurement after 2.5 hours. To examine protein desorption, salt buffered solution was then flowed through solid liquid cells and two final neutron measurements were taken in H2O and then D2O water contrasts.

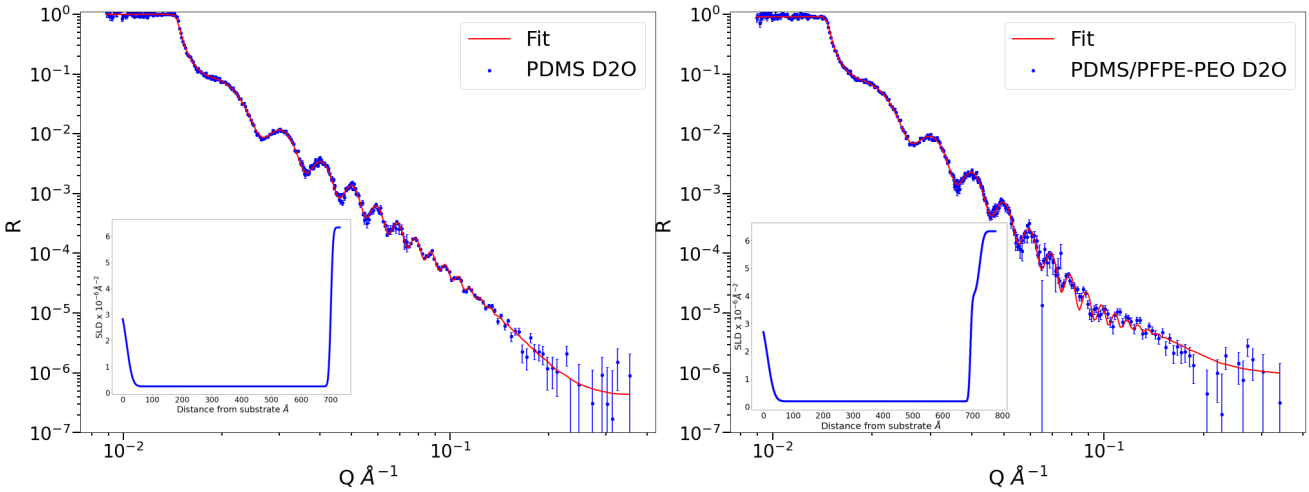
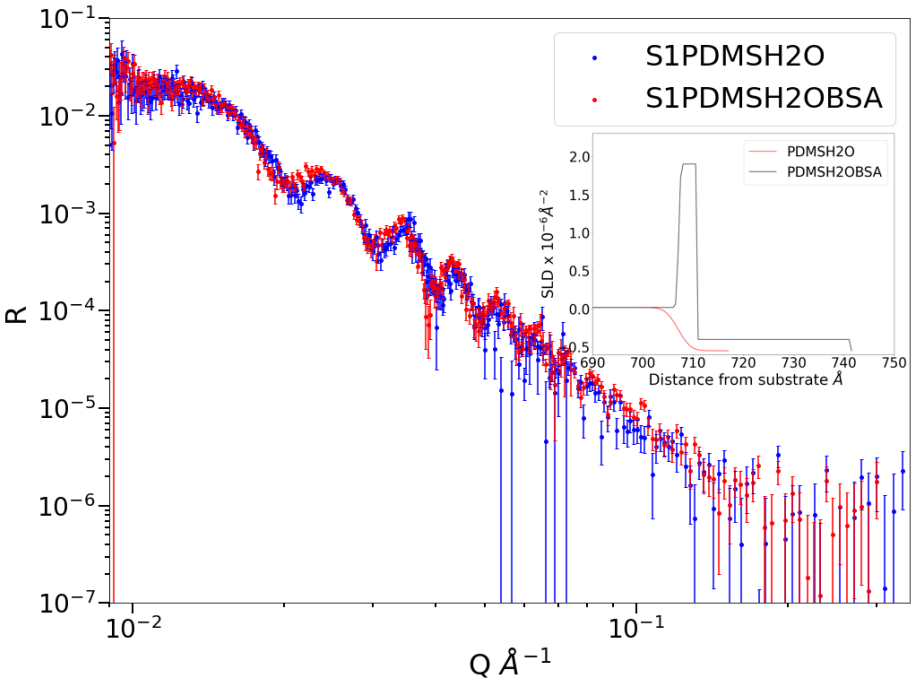


Figure 1a and 1b: Neutron reflectivity for PDMS and PDMS/PFPE-PEO film in D2O with SLD models from fits, note shelf in SLD profile at 700Å in 1b.

Results:

Initially, samples were analysed using neutron reflectivity in D2O and H2O ensuring the samples were well characterised prior to exposure to BSA protein.

Results from protein adsorption:



When considering the PDMS sample in H2O contrast before BSA and 2.5 hours after BSA exposure it is clear that there is a change in fringe spacing in the neutron reflectivity data and some adsorption of protein can be observed

Figure 3: Neutron Reflectivity from a PDMS film in H2O before and after exposure to BSA solution. With SLD model fit.

Fitting and analysis: In previous neutron experiments, a poly ethyl silicate cross linker had been used to cure Oh terminated PDMS films via a condensation cure which resulted in significant silica formation within the film as a by-product. This resulted in a more complex inhomogeneous PDMS film. By using a vinyl terminated thermally curing PDMS we have been able to produce a thin polymer film that can be modelled as a single homogenous layer, as seen in both 1a and 1b, simplifying the overall data fitting significantly. Strong fits were achieved; in 1b, the PDMS/PFPE-PEO sample in water, the layer at 700 Å at the solid liquid interface was modelled with an SLD 3.9x10^{-6} A^{-2} and thickness 3.1nm for a χ^2 1.58. This is in good accord with previous measurements and consistent with our model of a monolayer of PFPE-PEO coating the PDMS film.

For the PDMS sample, kinetic measurements show that most of the protein adsorption occurred within the first 15 minutes. Further, reflectivity measurements taken after subjecting the sample to a rinsing buffer flow showed minimal protein desorption from the surface. Both these observations are consistent with QCM protein adsorption experiments performed on similar PDMS surfaces.

The data shown in Figure 3 for the PDMS film after BSA exposure was modelled with a bilayer fit; a thin layer of 3.5Å with an SLD of 1.9 and a larger upper layer of thickness 3.09nm but with a much lower SLD of -0.4.

This structure is inconsistent with the dimensions of BSA in solution but describe a denatured layer of adsorbed protein, which has adhered to the surface via hydrophobic interactions. With a very thin wholly dehydrated protein layer directly on top of the PDMS and a much larger, much more hydrated layer exposed to the water interface. Using neutron reflectivity, similar hydrophobically absorbed layers have been observed previously of protein adsorbed on a hydrophobic polystyrene monolayer[1].

As shown in Figure 4, for the PDMS/PFPE-PEO sample there is no observable difference in the scattering spectra before and after protein exposure. Without any appreciable difference in scattering it's hard to justify attempting to fit any kind of adhered layer to the surface, as the reflectivity can be adequately modelled by the existing fit for the pristine film. This is in good agreement with the results from the QCM tests which suggest little to no protein adsorption on these PDMS surfaces treated with PFPE-PEO.

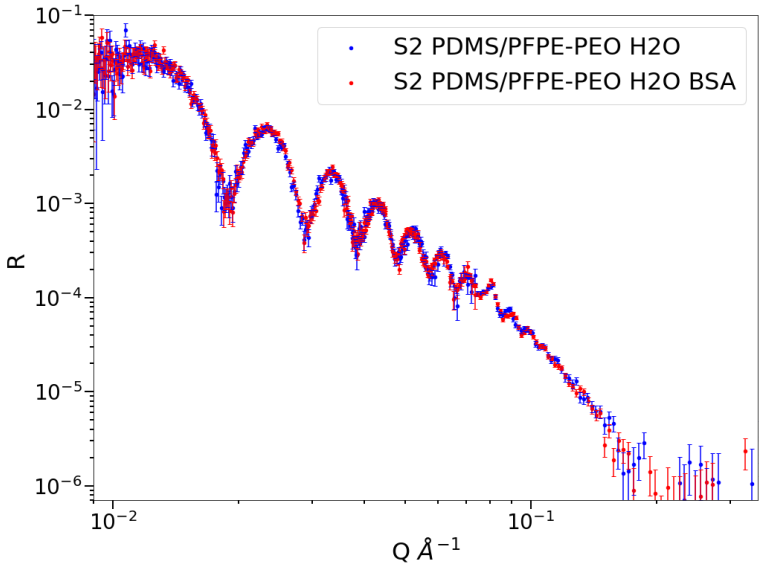


Figure 4: Neutron reflectivity for PDMS/PFPE-PEO sample in H2O before and after exposure to BSA protein.

We intend to publish the full results of this experiment alongside our other work on bioadsorption in a journal article.

Our Local Contact for this experiment was Dr Samantha Micciulla whose assistance proved indispensable for the success of the experiment. The experiment had to be conducted remotely due to the Covid pandemic and Dr Micciulla's professionalism and willingness to help prepare and run the experiment to our specifications ensured we were able to acquire all desired data in the 24 hour timeframe despite these difficult circumstances.

[1]N.Brouette, G.Fragneto, F.Cousin, M.Moulin, M.Haertlein, and M.Sferrazza.
A neutron reflection study of adsorbed deuterated myoglobin layers on hydrophobic surfaces. Journal of Colloid and Interface Science, 390(1):114–120, 2013