Experimental report

02/10/2016

Proposal: DIR-141 **Council:** 4/2015

Title: Adsorption of a single protein from seeds of Moringa oleifera to SiO2and Al2O3

Research area: Soft condensed matter

This proposal is a new proposal

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Samples:

Instrument	Requested days	Allocated days	From	To
D17	1	1	12/11/2015	13/11/2015

Abstract:

Adsorption to silica

The results of the neutron reflection experiments show very interesting behaviour that distinguishes the purified protein from that of the crude extract. We will first discuss what is seen clearly in the results by direct observation. As previously reported, the adsorption of the crude protein extract from solutions in D_2O to the silica layer on a silicon crystal makes a marked change to the reflectivity measured (Kwaambwa *et al.*, 2010). The data for the purified protein are shown in Figure 1 where there is clear adsorption for a solution with a concentration as low as 0.0025 wt%.

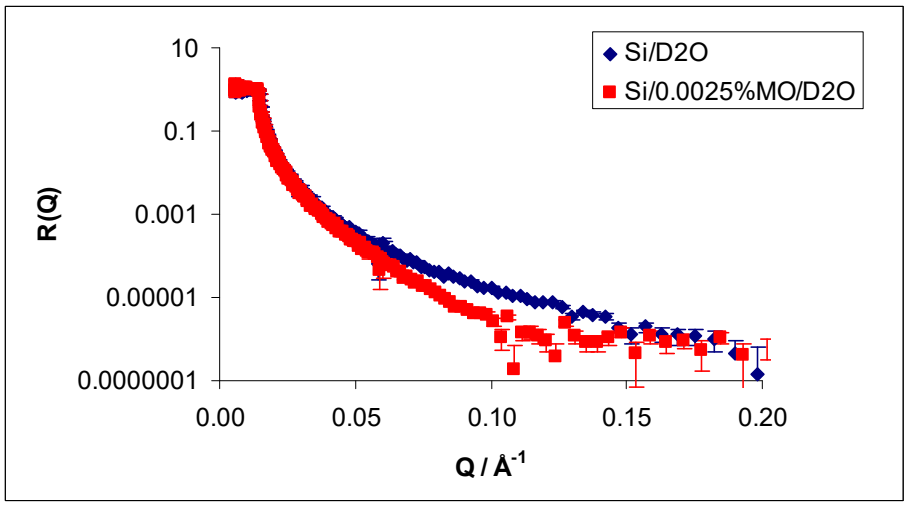

Figure 1. Neutron reflectivity data measured at silicon/silica/D_2O interface (♦) and the comparison with 0.0025 wt% solution of protein (■).

In contrast to previous results on crude extract, there was no change in the reflectivity indicative of increasing the adsorbed amount for higher concentrations (see Figure 2). The crude extract showed an increasing adsorption until a plateau amount was reached at a concentration of 0.05 wt%.

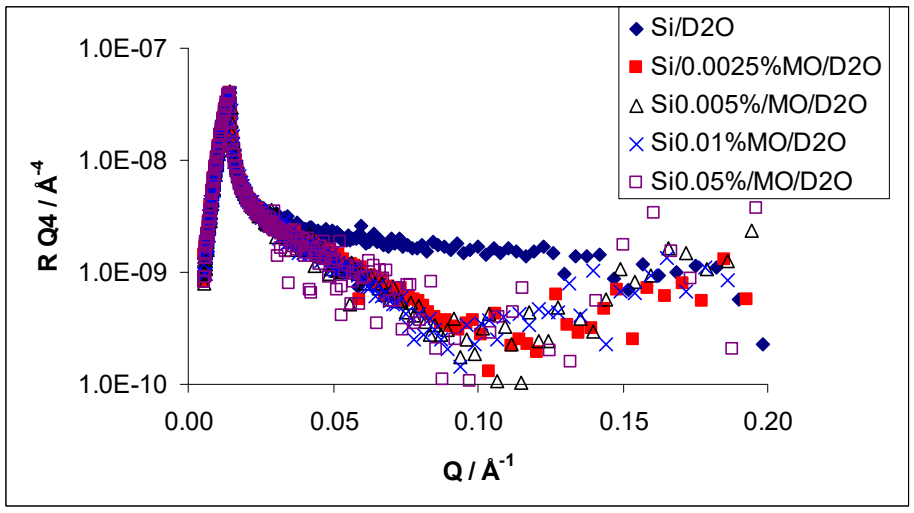

Figure 2. Reflectivity shown as RQ^4 against Q for different concentrations of protein (■ 0.025 wt%, Δ 0.005 wt%, × 0.01 wt%, □ 0.05 wt%) at the oxide layer on a silicon substrate. For comparison, the data for the clean silicon substrate are also shown (♦).

The clear change in reflectivity on adsorption of protein that is apparent in the data shown in Figures 1 and 2 can be interpreted quantitatively using optical matrix calculations of the reflectivity as implemented in the *cprof* program (Rennie, 2008). The plot of RQ^4 *vs* Q allows some features of the data to be identified as much the effect of the large contrast difference between silicon and D_2O is diminished. The sharp fringe in the data at about Q = 0.1 $Å^{-1}$ indicates a well defined layer. Rinsing the surface with D_2O after adsorption did not change the reflectivity and this indicates that the adsorption is irreversible. It also allows measurements to be made with a second solvent contrast that can provide more details about the interfacial layer. The fit made to the combined data for the bound layer after rinsing first with D_2O and then H_2O is shown in Figure 3. It corresponds to a layer of 15.3 ± 1 Å that consists of 53 ± 3 % protein and 47 ± 3 % water (allowance was made for exchange of protons in the protein as well as the different contrast of water). The data is modelled with a uniform protein layer and just 2 to 3 Å roughness at each of the interfaces.

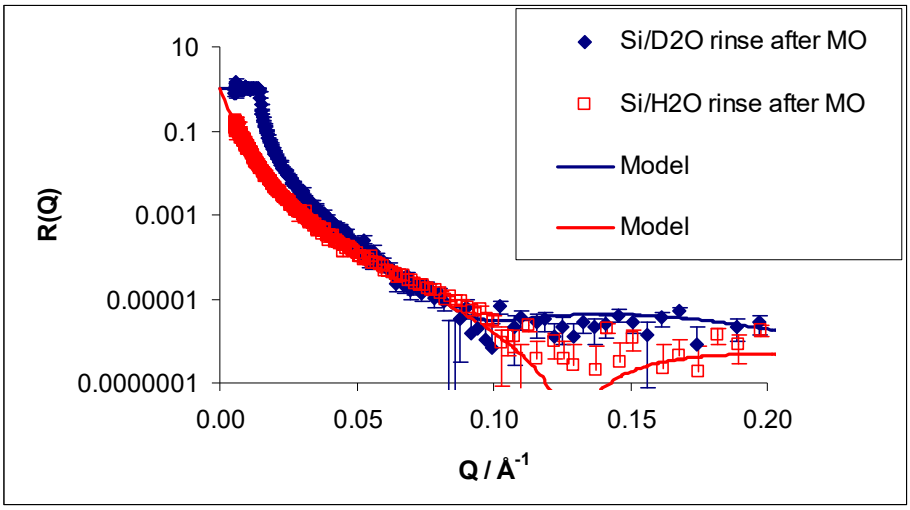

Figure 3. Reflection data for adsorbed protein layer after rinsing measured in D_2O (♦) and H_2O (□) with the curves for the model described in the text (1.3 mg m^{-2}, 47% water, thickness 15 Å).

The surface coverage of protein can be calculated from the fit parameters as the product of the volume fraction and the thickness of the layer. It corresponds to 1.3 ± 0.2 mg m^{-2}. The thin uniform layer that is hydrated could correspond to a single molecular layer of protein and contrasts with the multilayer adsorption that was found for the crude extract.

Adsorption to alumina

The design of the sample holder (Rennie *et al.*, 2015) allowed measurements to be made with the same protein solution at the alumina/solution interface. The data indicate that there is no significant change in the reflectivity and this shows that adsorption was not occurring at this surface. This contrasts with the results found previously for the crude extract of *Moringa* seed protein (Kwaambwa *et al.*, 2015) that had shown adsorption to alumina, albeit with a lower plateau coverage than that observed at the silica interface.

Discussion

The observations of a lower adsorbed amount of the purified protein to the silica interface than was seen with the crude extract and the lack of adsorption to alumina are interesting as it suggests another protein component from the seed is likely to be responsible for the previously observed multilayer adsorption (Kwaambwa *et al.*, 2010). The component that binds to alumina which is not strongly negatively charged is also not the material that has been separated and crystallised. The valuable interactions that causes the *Moringa* seed proteins to flocculate a wide range of materials (Hellsing *et al*., 2014) may therefore depend on protein other components. This data is being prepared for publication in connection with determination of the molecular structure of the protein.

References

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