# Experimental report

01/11/2016

**Proposal:** 9-13-676 **Council:** 4/2016

**Title:** Interaction of a protein with cationic surfactants in the tubular pores of SBA-15 silica

**Research area:** Soft condensed matter

**This proposal is a continuation of 9-10-1329**


**Main proposer:** Gerhard FINDENEGG

**Experimental team:** Gerhard FINDENEGG
Albert PRAUSE
Anja VON LOSPICHL

**Local contacts:** Viviana CRISTIGLIO


**Samples:** SiO2
dodecylpyridinium chloride / C17H39ClN
hexadecylpyridinium chloride / C21H47ClN
lysozyme / protein

| Instrument | Requested days | Allocated days | From | To |
|---|---|---|---|---|
| D16 | 6 | 5 | 08/09/2016 | 13/09/2016 |


**Abstract:**

We propose to study the co-adsorption and interaction of a globular protein (lysozyme) with cationic surfactants in tubular silica nanopores (diameter 8 nm) using small-angle neutron scattering. At pH values well below the isoelectric point of lysozyme (pI = 11), when the protein and surfactant are both positively charged while the silica surface is negatively charged, we expect competitive adsorption of the two components and possibly displacement of preadsorbed protein by the surfactant. Closer to pI, when the net charge of the protein is low, complexation of the protein with surfactant may also occur. Preliminary results obtained in a preceding experiment at instrument D16 indicate that pre-adsorbed lysozyme can indeed be displaced from the pores of SBA-15 silica by the cationic surfactant DPC. In the proposed experiment we will investigate if and how the assembly structures in the pores are affected (1) by pH and (2) by sample preparation (pre-adsorption of protein or surfactant, etc.). The higher neutron flux of the new D16 will enable us to study relatively dilute samples in contrast-match with acceptable sensitivity.

**Experimental report for experiment 9-13-676 at D16 (Sept. 8-13, 2016)**

A. Prause, A. F. von Lospichl and G. H. Findenegg
Berlin, October 27, 2016

## Samples and Experiment

We studied the assembly of the cationic surfactant cetylpyridinium chloride (CPC) in the cylindrical pores of SBA-15 in the presence of the globular protein lysozyme (Lyz). The pH of all samples was set to 9, where the surface of the silica pores is negatively charged. Small-angle neutron diffraction (SAND) measurements were performed at the recently upgraded D16 instrument with a neutron wavelength of 4.55 Å. We used a single detector configuration (detector angle 0°) to cover a $q$ range of 0.035 $\text{Å}^{-1} \leq q \leq 0.32$ $\text{Å}^{-1}$ with optimized collimation to resolve higher-order Bragg peaks of the pore lattice. As the solvent we chose a $H_2O/D_2O$ mixture matching the neutron scattering length density (SLD) of our SBA-15 silica. Due to the fact that the SLD of lysozyme is quite similar to the SLD of silica, the effect of protein adsorption on the structure is only visible from the change in the structure of adsorbed surfactant. Nonetheless, these contrast conditions are still the most favourable as they suppress the contribution from the silica matrix to the scattering intensity. Thanks to the recent and significant improvement of both the instrument in general and especially the increased neutron flux on the sample, we were able to measure four concentrations of CPC and three of Lyz and combinations thereof, each for both the protein and the surfactant pre-adsorbed.

Our sample preparation procedure was as follows: First, the species to be pre-adsorbed was added to a dispersion of SBA-15 and allowed to equilibrate for half a day. Then, the second species to be adsorbed was added to the dispersion and allowed to equilibrate for a few hours after re-adjusting the pH to 9. All solutions and dispersions were prepared using contrast-matching solvent for SBA-15 and adjusted to pH 9. After equilibration and sedimentation of SBA-15, the supernatant was decanted and analysed by UV-Vis spectroscopy to determine the amount of non-adsorbed CPC and Lyz. Those amounts generally confirm the adsorption isotherm previously observed, this means the remaining CPC concentration was very low except for the highest concentrations of CPC in SBA-15 (corresponding to $s > 0.6$ in figure 1, right hand side). The pasty sediment was measured by SAND in sample cells designed for pasteous samples at TU Berlin. Given the nature of the sample preparation, the volume fraction of SBA-15 in the beam (i.e. the fraction of remaining supernatant) can not be controlled exactly, thereby prohibiting data analysis in absolute scale.

## Contrast match point of SBA-15

The location of the contrast match point for our batch of SBA-15 was checked using four different $H_2O/D_2O$ mixtures. As it can easily be observed from figure 1 (left hand side), the presumed contrast match point from previous experiments with SBA-15, corresponding to $63\%_{\text{wt}}$ $D_2O$, could be confirmed.

## Adsorption of CPC to SBA-15

For increasing amount of surfactant, we see an increase in the scattering intensity for the (10) Bragg peak of the SBA-15 pore lattice (at $q \approx 0.066$ Å) until $s = 0.44$ (figure 1, right hand side). Then, the peak height decreases until it almost vanishes around $s = 0.70$ and returns when the adsorbed amount reaches its maximum ($s = 1.00$). For the diffuse

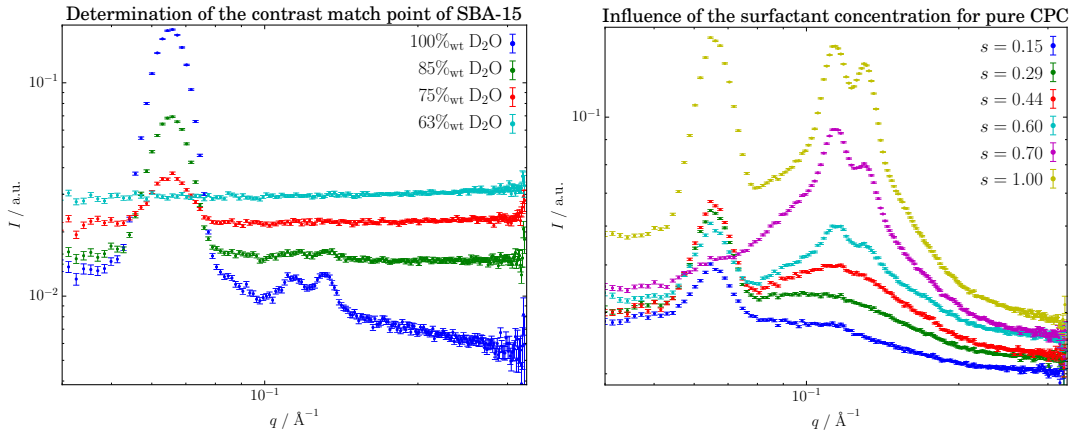


Figure 1: left: Experimental determination of the contrast match point for SBA-15. right: SAND data for different concentrations of CPC adsorbed to SBA-15. The parameter $s$ gives the fraction of surfactant adsorbed relative to the maximal adsorption possible.

scattering contribution at higher $q$ (around the (11) and (20) peaks at around 0.114 $\text{Å}^{-1}$ and 0.132 $\text{Å}^{-1}$, respectively) we see a steady increase with increasing $s$ superimposed to the appearance of the two Bragg peaks starting around $s = 0.44$.

These changes may potentially be explained by a model employed by us before [1] which involves a diffuse Teubner-Strey like contribution and a 2D-hexagonal structure factor modulated with the form factor of a cylindrical shell. Work on such a quantitative analysis is currently in progress.

## Co-adsorption of CPC and Lysozyme

In figure 2 we show some examples of co-adsorption of CPC and lysozyme. On the left hand side, we can see the influence of the amount of pre-adsorbed lysozyme on the scattering spectra of the sample. We can observe a decrease in intensity of the (10) peak with increasing lysozyme concentration and an increase of the diffuse scattering intensity and the (11) peak at higher $q$. Notable here is the discrepance between nominal fraction of protein adsorbed $p_0$ and the experimentally observed value $p$ (from UV-Vis spectroscopy). While there is no trend visible for the nominal loading, the intensity of the (10) peak decreases with increasing experimental protein loading $p$, whereas the diffuse scattering contribution and the intensity of the (10) peak increase.

On the right hand side, we observe that the scattering intensity is largely unaffected by the order of adsorption at high $q$ while it differs significantly for the (10) peak. Qualitatively, there seems to be a significant influence of the pre-adsorbed species on the adsorption of the second species.

For the diffuse part of the scattering intensity, an increase with increasing protein loading seems plausible as the increasing presence of a second species with different contrast is expected to increase the Teubner-Strey like scattering if both protein and surfactant form small domains on the pore wall.

As for the intensity of the (10) peak almost no change is visible from a pure CPC sample to a sample with CPC pre-adsorbed. Nonetheless, as the diffuse Teubner-Strey

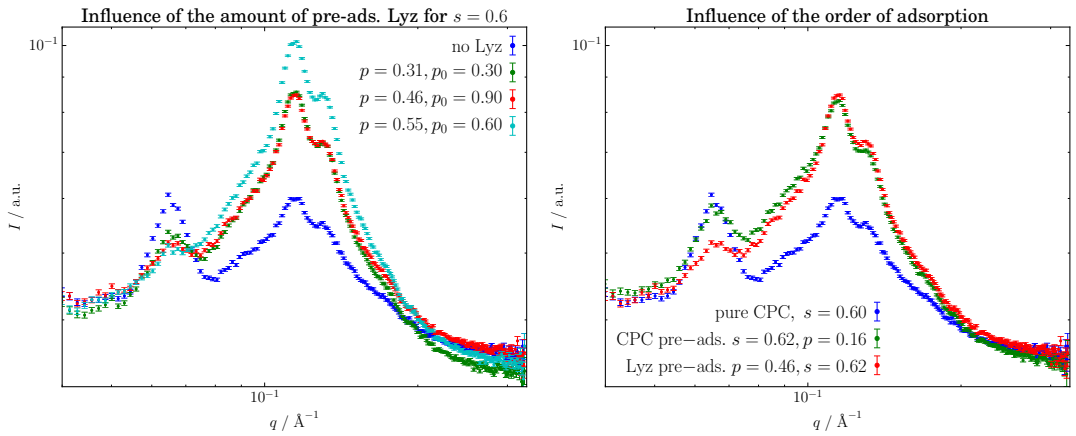


Figure 2: Left: SAND intensity for different amounts of pre-ads. lysozyme while keeping the CPC concentration constant. $p$ and $p_0$ give the amount of actually and nominally adsorbed protein relative to the maximum amount possible.
Right: Influence of the pre-adsorbed species on the spectra. Note that the amount of adsorbed protein $p$ determined by UV-Vis spectroscopy is different even though the nominal composition of the sample is identical.

background is much larger for the samples with lysozyme, the actual peak heights are likely to differ and will have to be determined by quantitative analysis. In the other case with lysozyme pre-adsorbed, we see an influence of lysozyme as compared to the pure surfactant sample. This finding does also match the experimental protein loading fraction $p$ from UV-Vis spectroscopy, which is significantly lower for the case of surfactant pre-adsorbed.

In conclusion, the experimental scattering profiles of the surfactant + protein mixtures in pores exhibit systematic trends hinting at some preferential adsorption of the surfactant. It appears that the scattering profiles can be analysed in terms of the model previously adopted for pure surfactants [1]. We are currently working on the quantitative analysis of the data along this line.

## References

[1] D. Müter, T. Shin, B. Demé, P. Fratzl, O. Paris, and G. H. Findenegg. Surfactant self-assembly in cylindrical silica nanopores. *J. Phys. Chem. Lett.*, 1:1442–1446, 2010.