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Experiment Report Form

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The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

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All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** Structure of protein deposits plugging pores of micro-sieves	**Experiment number**: **SC-3315**
Beamline: ID-13	**Date of experiment**: from: 16 Nov 2011 to: 18 Nov 2011	**Date of report**: 19.04.2012
Shifts: 6	**Local contact(s)**: Manfred Burghammer	*Received at ESRF:*

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Tim Steinhauer*

Patricia Meyer*

Ulrich Kulozik

Preliminary Report:

Introduction

We investigate casein micelles in deposits using small angle X-ray scattering. Casein deposits deposits lead to a number of undesired effects in technological applications such as in the widely used microfiltration. In this process, a trans-membrane pressure acts as a driving force for a volume flow of casein solution through a membrane. The casein micelles it contains can either form a deposit layer on top of the membrane known as external fouling or block the pores of the membrane causing the so-called internal fouling. The internal fouling is believed to be the initial step of the entire fouling process whose study is thus of the utmost importance. Micro-sieves as membranes provide cylindrical pores which allow structural investigation of the deposits in transmission experiments. To resolve structural changes of casein micelles in dependence of their spatial location within the pores and an acting trans-membrane pressure, nano-beam small angle X-ray scattering in combination with nanometer resolution scans is the method of choice. Results contribute to the general understanding of the casein micelle structure in membrane deposits, which has been so far investigated in external fouling layers by SAXS[1] and GISAXS[2].

Materials and Methods

We used a size-fractionated casein micelle sample prepared using ultra-centrifugation[3]. The used micro-sieves (Nano-Filtertechnik-GmbH, München, Germany) had a defined pore-size of 800 nm and an active area of 0.5%[4]. We prepared two casein fouling layers by solution casting and by using a trans-membrane pressure, generated by a diaphragm pump (MZ2C, Vacuubrand). Pores on the micro-sieves were roughly aligned using a vertical beamline microscope and a hexapod scanner in the EH3-hutch of the ID13-beamline. We recorded X-ray scattering patterns during fine scans using a piezo-scanner and FReLoN camera after selecting one single pore on the micro-sieve.

Results

Fig. 1A shows an X-ray mesh scan of 12 symmetrically arranged pores. Patterns from the pore border contain

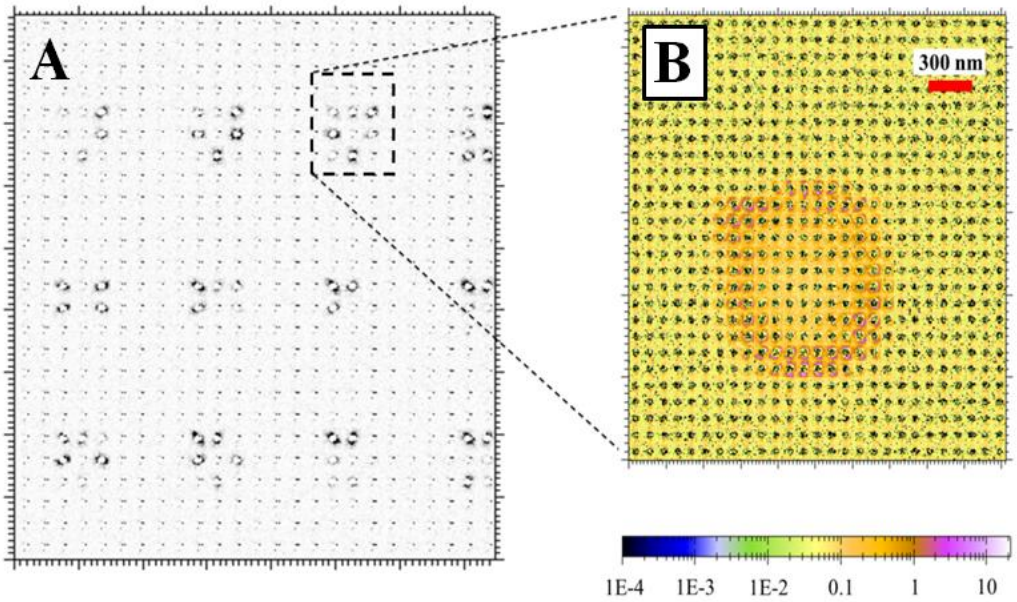

Fig. 1: Rough X-ray scan of the membrane area of the micro-sieve (A) and fine-scan across a single selected pore together with a color-intensity scale (B)

the highest intensity due to interface reflections. Compared to the non-porous parts, the scattering signal inside the pores is stronger. This can be seen more precisely in Fig. 1B. We performed a fine-scan with a step-size of 100 nm across a single pore. Scattering inside the pore (orange color) is more than one-order of magnitude larger than from outside (yellow), but most of the scattering arises from the pore border (red), which is 200 nm in size. The scattering from casein micelles inside the pores has to be corrected regarding the background by a comparable measurement of an empty pore. We obtained scattering functions for casein deposits after 1). Grouping and averaging of the scattering patterns inside the pores, 2). Radial averaging and 3). Transformation to the q-scale. From the scattered intensity of the provided q-range we extracted information at distances between 10-20 nm, where the casein micelle has a characteristic sub-structure.

Conclusions

The experimental results provided us information on how the inner structure of casein micelles is affected by filtration forces. From the experiments we can further asses how the overall structure of the casein micelle is influenced taking the different substructure models into account[5]. Together with recent results from surface

scattering experiments on external fouling layers[2], the nano-SAXS experiments could contribute to a total understanding of the deposit formation of soft colloidal particles on membranes during the microfiltration process. We conclude that nano-beam SAXS is a suitable method for probing the nano-structure of deposit materials inside individual micro-sieve pores.

References:

(1) David C, Pignon F, Narayanan T, Sztucki M, Gesan-Guiziou G & Magnin A (2008) Spatial and Temporal in Situ Evolution of the Concentration Profile during Casein Micelle Ultrafiltration Probed by Small-Angle X-ray Scattering: Langmuir. *Langmuir* **24:** 4523–4529

(2) Gebhardt R, Steinhauer T, Meyer P, Sterr J, Perlich J & Kulozik U (2012) Structural changes of deposited casein micelles induced by membrane filtration. *Faraday Discuss* DOI: 10.1039/C2FD20022H

(3) Gebhardt R and Vendrely C and Kulozik U (2011) Structural characterization of casein micelles: shape changes during film formation. *Journal of Physics: Condensed Matter* **23:** 444201

(4) Gebhardt R, Holzmüller W, Zhong Q, Müller-Buschbaum P & Kulozik U (2011) Structural ordering of casein micelles on silicon nitride micro-sieves during filtration. *Colloids and Surfaces B: Biointerfaces* **88:** 240–245

(5) Bouchoux A, Gèsan-Guiziou G, Pèrez J & Cabane B (2010) How to Squeeze a Sponge: Casein Micelles under Osmotic Stress, a SAXS Study. *Biophys J* **99:** 3754–3762