Casein micelles as soft, deformable spheres verified by combined GISAXS and filtration experiments

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Casein micelles (CM) are highly aggregated protein agglomerates, which solubilize calcium phosphate in milk. In an aqueous environment, CM form spherical structures with a mean diameter of 150 nm. An inhomogeneous surface as well as water-filled channels and cavities in their internal structure were resolved by electron microscopy [1, 2]. Recent small angle X-ray experiments showed that CM behave as soft, deformable objects when subjected to osmotic stress or forces acting during drying processes [3, 4]. Nanocluster [5] and sponge-like models [3, 6] account for the main structural features and the reaction of the micelles during processing operations. Asymmetric scattering patterns recorded in an in-situ frontal filtration cell were first indications for a micelle shape change induced by a fluid flow field [7]. Because of the large beam size, the study was performed 200 µm above the membrane and thus insensitive for surface-near structures mainly contributing to the filtration resistance.

We applied surface-sensitive GISAXS in combination with a µm-sized X-ray beam on deposit structures to overcome previous limitation. We prepared deposits of CM under fluid-flow conditions on smooth silicon micro-sieves, which allow for surface-sensitive X-ray experiments. To determine the influence of packing effects and filtration forces on the structure of CM we varied thickness and trans-membrane pressure during the deposit formation. We performed GISAXS scans on the solid-air interface of the so generated deposits taking the porous microstructure of the micro-sieve into account. We found that spherical CM undergo a structural transition and assume a non-spherical shape after their deposition [8]. We analysed structural changes by simulating two-dimensional GISAXS patterns by an elastic scattering approach [9]. We found that an ellipsoidal shape matches the structure of CM in deposits best. Above the porous areas, the ellipsoidal micelles are aligned perpendicularly to the surface of the micro-sieve. The degree of the ellipsoidal shape change depends on the deposit height, the pressure difference across the micro-sieve as well as on the location on the micro-sieve (above a porous or a non-porous area). For the deposit height we found that the ellipsoidal shape changes were all the greater the higher the deposit layer. The results can be explained by a mechanism which takes both packing effects and fluid forces into account [9].

Investigating the deposit formation on the solid/liquid interface requires the use of time-resolved in-situ experiments. Supported by a DFG-grand and a long-term project from DESY/HASYLAB [10], we developed a cross-flow filtration cell (Figure 1) for in-situ GISAXS experiments. The cell consists of two parts made of polycarbonate. The upper part containing the cross-flow channel (width: 1mm) is pressed on the porous area of the micro-sieve embedded in the bottom part. Integrated supply lines for the retentate (inflow: F1 and outflow: F2) and permeate (F3) are indicated. Recently, we have investigated deposit formation of CM under flowing liquid conditions. The recorded GISAXS patterns contain well resolved Yoneda and specular peaks and change with increasing filtration time. In future, we will investigate the initial phase of deposit formation under various milieu and fluid flow conditions. The proposed experiments demand a 3rd generation synchrotron source and a beamline which provides focused micro-sized X-ray beams.

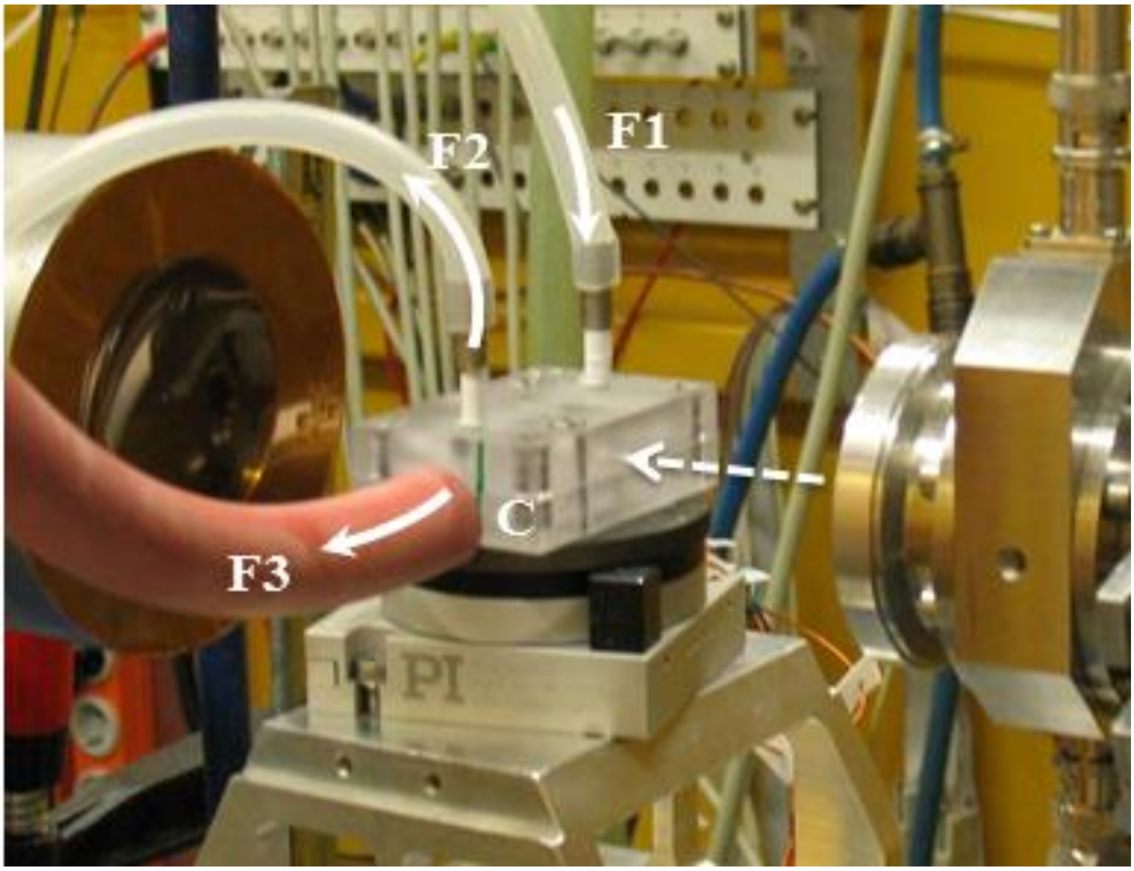

Figure 1: Cross-flow filtration cell (C) for in-situ GISAXS experiments integrated in the experimental setup of the BW4 beamline. Arrows denote retentate inflow (F1), outflow (F2) and permeate outflow (F3); the dashed arrow indicates the beam direction.

References

[1] D. G. Dalgleish, P. A. Spagnuolo and H. Douglas Goff, International Dairy Journal **14**, 1025 (2004).

[2] R. Trejo, T. Dokland, J. Jurat-Fuentes and F. Harte, Journal of Dairy Science **94**, 5770 (2011).

[3] A. Bouchoux, G. Gésan-Guiziou, J. Pérez and B. Cabane, Biophys. J., **99**, 3754 (2010).

[4] R. Gebhardt and C. Vendrely and U. Kulozik, Journal of Physics: Condensed Matter **23**, 444201 (2011).

[5] C. G. de Kruif, T. Huppertz, V. S. Urban, A. V. Petukhov, Advances in Colloid and Interface Science **171-172**, 36 (2012).

[6] D. G. Dalgleish, M. Corredig, Annu. Rev. Food Sci. Technol. **3**, 449 (2012)

[7] F. Pignon, G. Belina, T. Narayanan, X. Paubel, A. Magnin and G. Gesan-Guiziou, The Journal of Chemical Physics **121**, 8138 (2004).

[8] R. Gebhardt, T. Steinhauer, P. Meyer, J. Sterr, J. Perlich, U. Kulozik, Faraday Discuss. **158**, 77 (2012).

[9] R. Lazzari, J. Appl. Cryst. **35**, 406 (2002).

[10]DFG-grand (DFG GE 2375/1-1) and II-20100114