Experimental report

19/07/2024

Proposal: **INTER-588** **Council:** 4/2023

Title: Systematic investigation of the diffusive properties of macromolecules in bovine milk

Research area:

This proposal is a new proposal

Main proposer: **Christian BECK**

Experimental team: Ilaria MOSCA
Roody NASRO

Local contacts: Tilo SEYDEL

Samples: Bovine milk powder (nutritional grade)
Beta-casein from bovine-milk
D2O with traces of CaCl2 and Na3PO4 salts for buffering (<20mM)

Instrument	Requested days	Allocated days	From	To
IN16B	3	3	25/08/2023	28/08/2023

Abstract:

Experimental Report Inter-588

Experimental Team: Christian Beck, Ilaria Mosca, Tilo Seydel

Motivation: In biological systems, polydisperse environments influence the diffusive and static properties of their ingredients. In the past, we have investigated different proteins in monodisperse solutions [1, 2] being part of bovine milk. In addition, we have sucessfully established frameworks to analyze QENS spectra from polydisperse suspensions [3, 4]. In the present experiment we investigate the concentration dependence of commercial milk powder dissolved in heavy water. Compared to natural milk, this has the advantage that the concentration can be unambiguously determined given the preparation procedure; moreover D_2O can be used as solvent to minimize the incoherent contribution of the medium.

Performed Measurements: A systematic concentration series of milk-powder dissolved in D_2O has been performed. Samples were prepared with nominal concentrations of $c = 50, 75, 100, 130, 150, 200\,\mathrm{mg/ml}$. All samples have been measured at 280 K. To investigate the temperature dependence, the samples at $c = 50\,\mathrm{mg/ml}$ and $c = 130\,\mathrm{mg/ml}$ have been additionally investigated at 310 K and 330 K. All QENS spectra had an acquisition time of four hours.
As calibration data, an empty can, Vanadium, the empty cryofurnace and plain D_2O are measured at 280 K. All these samples were measured for 4 hours.
Since the aluminum sample holders are known to influence sometimes the containing samples, a sample holder from D33, allowing to mount a quartz cuvette in an aluminum holder to the sample stick into the cryofurnace. The sample holder with the cuvette has been used to investigate in situ the time dependence of a sample (BLG 90mg/ml; YCl_3 5.5mM in D_2O) which has shown crystallization in the lab around 30°C.

Preliminary results: The experimental data have been reduced using the framework available under `https://github.com/chribe/IN16bMantidreduction`. Already the sum over all q show significant changes as a function of concentration (Figure 1). An increase of the overall intensity is expected as a function of the concentration. Also, an overall decrease of the FWHM is observed. The temperature dependence of the sample $c = 130\,\mathrm{mg/ml}$ is shown in Figure 2.
Figure 3 displays the measurements of the empty cryofurnace, empty aluminum sample holder, empty cuvette in aluminium support as well as the measurement of the cuvette in the aluminium support filled with a D_2O solution the crystallizing sample.

References

[1] M. K. Braun, M. Grimaldo, F. Roosen-Runge, I. Hoffmann, O. Czakkel, M. Sztucki, F. Zhang, F. Schreiber, and T. Seydel, "Crowding-controlled cluster size in concentrated aqueous protein solutions: Structure, self- and collective diffusion," *The Journal of Physical Chemistry Letters*, vol. 8, pp. 2590–2596, May 2017.

[2] T. Seydel, C. Beck, S. Chakraborty, A. Grundel, L. Mateo-Minarro, T. Morozova, I. Mosca, R. Nasro, F. Roosen-Runge, and F. Schreiber, "Casein as a model intrinsically disordered protein: Impact of salt on the internal diffusive dynamics in solution," 2023. Institut Laue-Langevin (ILL) doi:10.5291/ILL-DATA.9-13-1046.

[3] M. Grimaldo, H. Lopez, C. Beck, F. Roosen-Runge, M. Moulin, J. M. Devos, V. Laux, M. Härtlein, S. Da Vela, R. Schweins, A. Mariani, F. Zhang, J.-L. Barrat, M. Oettel, V. T. Forsyth, T. Seydel, and F. Schreiber, "Protein short-time diffusion in a naturally crowded environment," *J. Phys. Chem. Lett.*, vol. 10, no. 8, pp. 1709–1715, 2019.

[4] C. Beck, M. Grimaldo, H. Lopez, S. Da Vela, B. Sohmen, F. Zhang, M. Oettel, J.-L. Barrat, F. Roosen-Runge, F. Schreiber, and T. Seydel, "Short-time transport properties of bidisperse suspensions of immunoglobulins and serum albumins consistent with a colloid physics picture," *J. Phys. Chem. B*, vol. 126, no. 38, pp. 7400–7408, 2022.

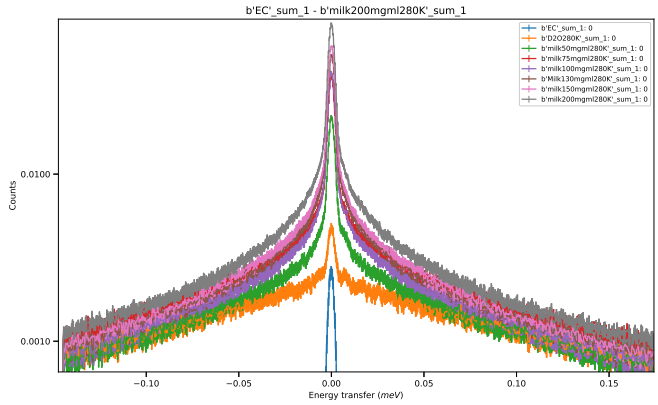

Figure 1: Influence of the concentration on the QENS spectra. A clear increase in the maximum intensity as well as a thinnening of the QENS spectra can be seen with increasing concentration.

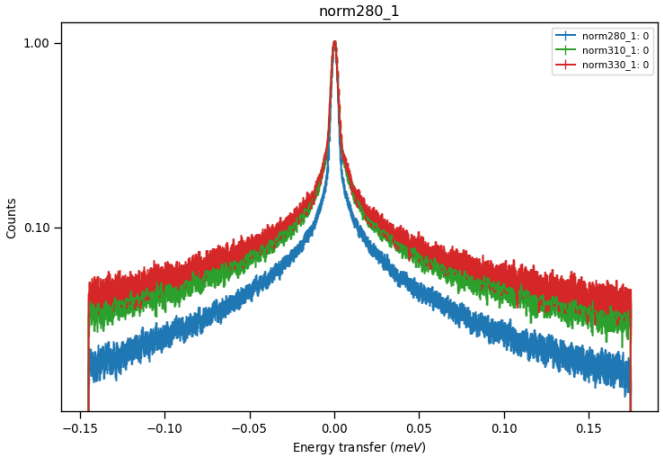

Figure 2: Normalized temperature dependence of the milk sample with $c = 130$ mg/ml. A clear broadening can be observed with increasing temperature.

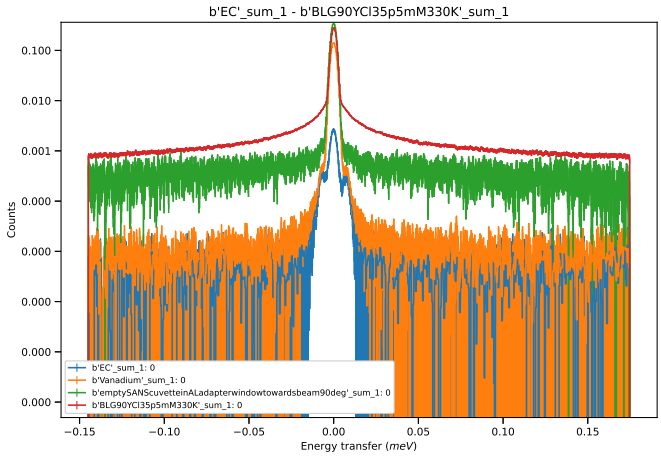

Figure 3: Comparison of the empty cell (blue), vanadium (orange), empty cuvette in aluminum holder (green) and filled cuvette. Even though the empty cuvette is characterized by a signal two orders of magnitudes higher than the empty aluminum can, a clear QENS signal can be observed in the QENS signal of the filled cuvette.