ESRF	**Experiment title:** **Structure of gluten proteins and model sequences concentrated in a microfluidic device**	**Experiment number**: SC-3927
Beamline: ID02	**Date of experiment**: from: 28/11/2014 to: 01/12/2014	**Date of report**: 13 May 2015
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Introduction

Wheat gluten proteins are among the most complex protein networks in nature, due in particular to their poor solubility in water and to their viscoelastic behavior. Gluten is composed of many proteins, classified in two main groups according to their ability to form intermolecular disulfide bonds (gliadin and glutenins), and displays a broad molecular weight distribution. In order to decipher the role of each group of protein, we study the structure of gliadin and glutenin rich extracts as function of concentration in different solvents. To go further in the understanding of the primary sequence contribution, we plan to perform similar studies on model peptides whose sequence is inspired from repetitive domains of glutenins. As only very small quantities of peptides can be obtained, a microfluidic tool will be used to easily scan the concentration phase diagram of nano-quantities of synthesized peptides. The principle of microevaporator is to introduce a diluted protein solution into a PDMS end-closed microchannel equipped with a thin membrane which enables the pervaporation of the solvent. Pervaporation of the solvent through the membrane results in a concentration gradient along the channel.The aim of this experiment was to study the structure of different gluten protein extracts as function of concentration and to test the possibility to perform such study using microevaporators [2,3].

Experimental and Results

The beamtime on ID02 was allocated from the 28th November to 1st December, 2014. The measurements were carried out with a energy of 12.5 keV. Different types of sample-to-detector distances (SD) were used. For measurements of samples in capillaries three SDs, 1m, 5m and 30m were used covering a q-range of 2.35 x 10^{-3} – 9.97 nm^{-1}. For the measurement of samples in microevaporators a SD of 5 m was used, covering a q-range of 0.013 – 1.64 nm^{-1}. The beam size for all measurements was 50 x 50 µm. This beam size was chosen to focus the analysis in different regions of the micoevaporator channel as function of evaporation time. Two gluten extracts were analysed. Firstly, samples at different concentrations were measured into capillaries, then the same diluted samples were introduced into the microfluidic chips and measured as function of the channel position and evaporation time.
The microchannels were covered by 26 µm thick PDMS membranes on both sides. A long tube, which fullfils the role of the sample reservoir was attached to the microchannels. The microevaporater was mounted on the sample stage and the X-ray beam was aligned to the microchannel. The protein profile within the channel was scanned by measuring the scattering curves at several position along the channel, from the closed end up to the sample reservoir. The time between the filling and the first measurements was recorded.

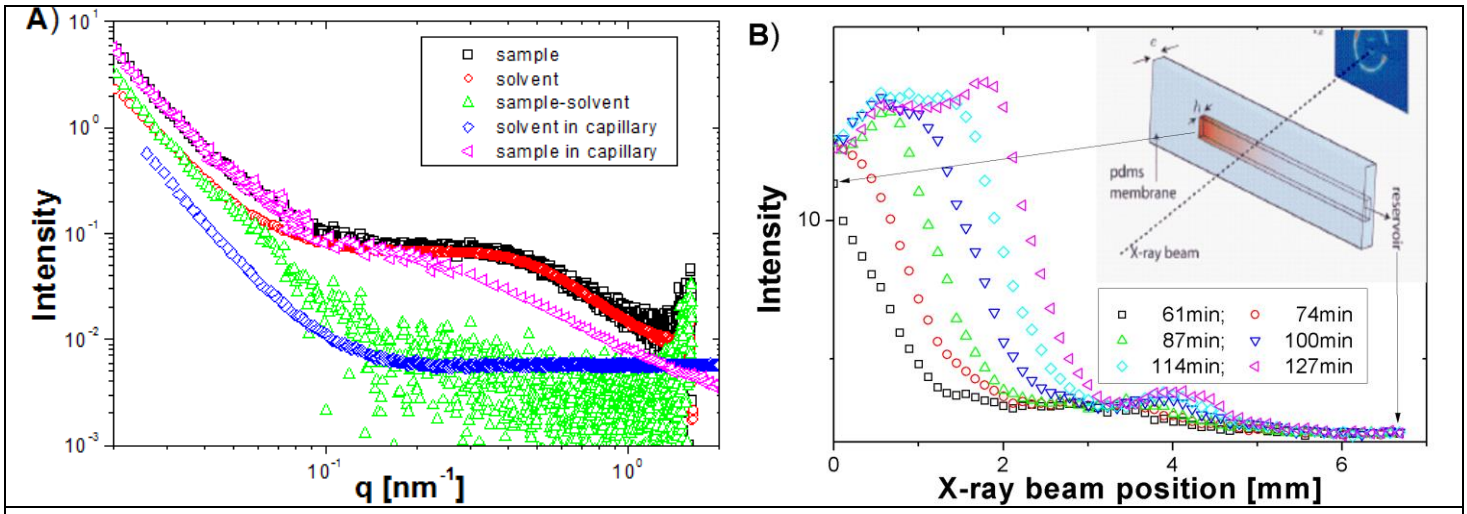

Fig. 1: A) Comparison between the signal of the protein sample in the microchannel, of the solvent in the microchannel, of the sample signal minus the solvent signal in the microchannel and the solvent and sample signal measured in a capillary. B) An example of the intensity at q=0.02nm^{-1} along the microchannel is plotted for different evaporation times. A sketch of the experimental setup is shown in the inset.

The scattering intensities at q=0.02 nm-1 were used to determine the protein concentration gradient over time inside the microchannel. The observation of the sample signal at higher q-values was not possible because of the high scattering background of the used PDMS membranes. The problem of the high scattering background of PDMS is shown in Fig. 1A), where the scattering curves of the sample (black squares), the solvent (red circles), the background subtracted sample signal (green triangles) and the solvent measured in a capillary (blue diamonds) are plotted. In the high-q and medium-q range the black and red curve are nearly identical. From the differences between the red and blue curve it is visible that these regions are dominated by the PDMS scattering signal. An example for the time

dependence of the concentration profile along the channel is shown in Fig. 1, where the intensity profiles at varying time steps are plotted. An increase of the scattering intensities close to the dead end of the channel can be observed whereas the scattering intensities close to the reservoir stay constant. With increasing time more solvent evaporates and the protein concentration close to the end of the channel increases. An increase of the maximum intensity can be observed. In addition, the position of the maximum intensity shifts with time and gets closer to the reservoir. Further analysis of the scattering curves and an improvement of the microevaporators are necessary in order to reduce the scattering background.

References

[1] Dahesh, M.; Banc, A.; Duri, A.; Morel M.-H. and Ramos, L. *J. Phys. Chem. B* **2014**, 118, 11065-11076.
[2] Merlin, A.; Angly, J.; Daubersies, L.; Madeira, C.; Leng, J.; J.-B, Salmon. *Eur. Phys. J. E* **2011,** 34, 58.
[3] Daubersies, L; Leng, J; Salmon JB - *Lab on a Chip*, **2013**, 910-919.