Experimental report

29/05/2024

Proposal: 9-13-1075 **Council:** 4/2023

Title: Internal Dynamics of Biological Hydrogels

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: PEO/PMAA
Bovine Submaxillary Mucin

Instrument	Requested days	Allocated days	From	To
IN15	3	3	17/11/2023	20/11/2023
D22	1	0		
D33	1	1	23/11/2023	24/11/2023

Abstract:

Hydrogels are highly interesting with respect to their structure and properties, especially in the context of biology. Here mucus plays a central role and in our experiments we want to study mucus BSM and compare it to a much simplified synthetic hydrogel of PEO/PMAA tailored to possess similar viscoelastic properties. For understanding their transport and viscous properties knowledge of the dynamics of chains and local domains is essential. For obtaining such information NSE with the high contrast in D2O is unique and shall be employed here as a function of hydrogel concentration and also to study the effects of osmolyte and salt, that are known to play important roles in biological hydrogels. In order to cover the relevant size range of 0.5 to 50 nm and the time range of 0.1 to 500 ns IN15 is uniquely suited to give the required information once combined with the SANS experiments done in parallel. Combining the experimental data with theoretical modelling shall then yield a much enhanced insight into the workings of biological hydrogels as they are required in order to advance both fundamental physics research as well as medical application.

Experimental report for Proposal 9-13-1075

Performed on

D33 23/11/2023 – 24/11/2023

IN15 17/11/2023 – 20/11/2023

This experiment dealt with the internal dynamics of soft hydrogels. Mucus is a very important soft biological hydrogel that is difficult to study due to limited sample availability and reproducibility. The interplay between rheology and structure of mucus is not well understood. For these reasons, we study soft synthetic model hydrogels that mimic the physical properties of mucus that are available in large quantities. The model systems we studied highlight various interaction mechanisms that are of relevance for mucus:

a) Solutions of high molecular weight poly(ethylene oxide) (PEO) → physical entanglement of polymer chains
b) Solutions of PEO and hydrophobically-modified poly(methacrylic acid) (PMMA) → hydrogen bonding & formation of hydrophobic domains
c) Modified tetraPEG hydrogels with linear spacers → covalent crosslinks
d) Solutions of Bovine Submaxillary Mucin (BSM) → glycosylated proteins with many means of interaction

All systems have been studied comprehensively in terms of their rheological behavior. Where appropriate, the collective dynamics were determined using dynamic light scattering. The static structure has also been studied in previous SANS beamtimes.

Neutron Spin Echo Results

Using NSE, we now focused on the internal dynamics of these hydrogel systems. Unfortunately, the samples from c) phase separated and could therefore not be studied using NSE. The spin echo data of one sample from each group are shown in Figure 1. To get a first idea, the S(q,t) data were fitted with a simple exponential according to

$$S(q,t) = A \cdot \exp(-D_{\text{eff}} q^2 t) \qquad (1)$$

where A is the y-intercept and D_{eff} is the effective diffusion coefficient. The fit generally works quite well, although some deviations are seen. Therefore, a more detailed analysis is needed. For most samples, D_{eff} reaches a plateau at low q, indicating Fickian diffusion over large length scales. For higher q, $D_{\text{eff}} \propto q$, which indicates internal motion with hydrodynamic interactions (Zimm).

The PEO-based hydrogels all behave similarly as shown on the left in Figure 2. Apparently the choice of concentration, molecular weight or the presence of chemical crosslinks does not influence on the dynamics on a local scale. For the PEO/PMAA mixtures, shown in Figure 2 (middle), we see a clear dependence on pH. At lower pH, the carboxylic acid moieties on PMAA are protonated leading to the formation of hydrogen bonds with the ether moieties on PEO. This slows down the dynamics of the overall system. With increasing pH, increasing portions of PMAA are deprotonated, leading to again faster dynamics of the system. At pH 5.75, D_{eff} is only slightly smaller than that of a pure PEO solution. The BSM solutions are much stiffer than both the pure PEO and the mixed PEO/PMAA solutions, as shown in Figure 4. All NSE results will be studied in further detail and compared with simulation results from our cooperation partners.

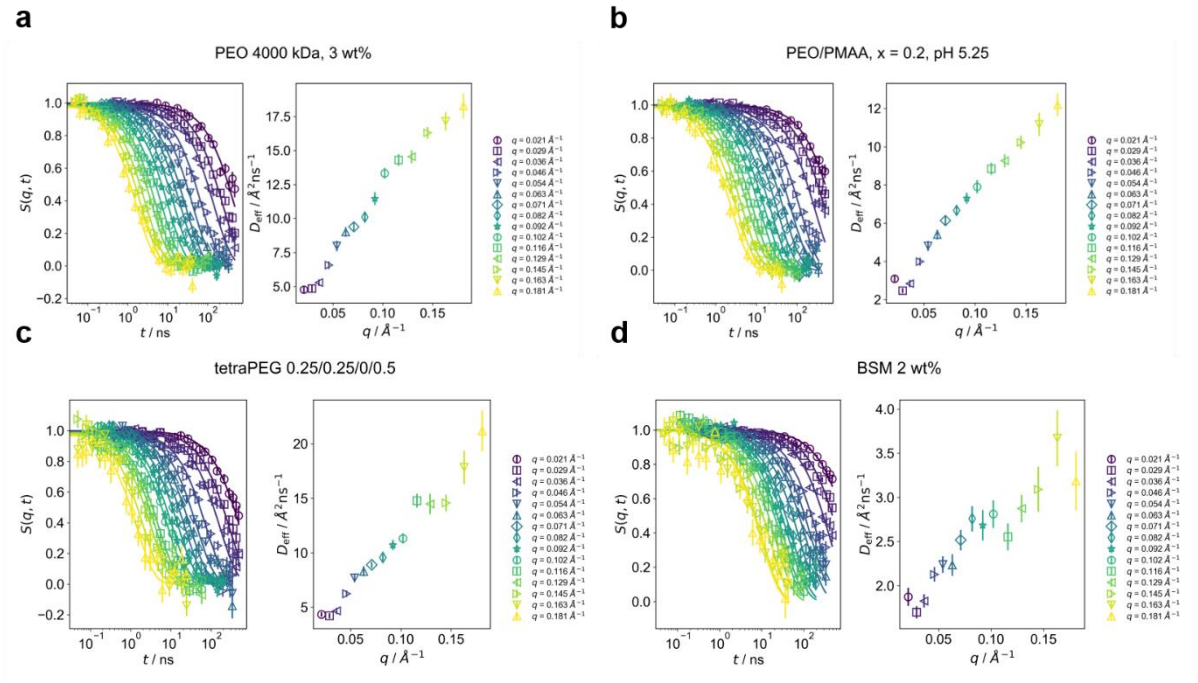

Figure 1 NSE data from different mucin-mimicking hydrogels: **a** PEO solution. **b** PEO/PMAA interpolymer complex. **c** modified tetraPEG hydrogel and **d** BSM solution. The $S(q,t)$ data were fitted with a simple exponential. The resulting effective diffusion coefficient is shown as a function of the scattering vector q on the right side of each plot.

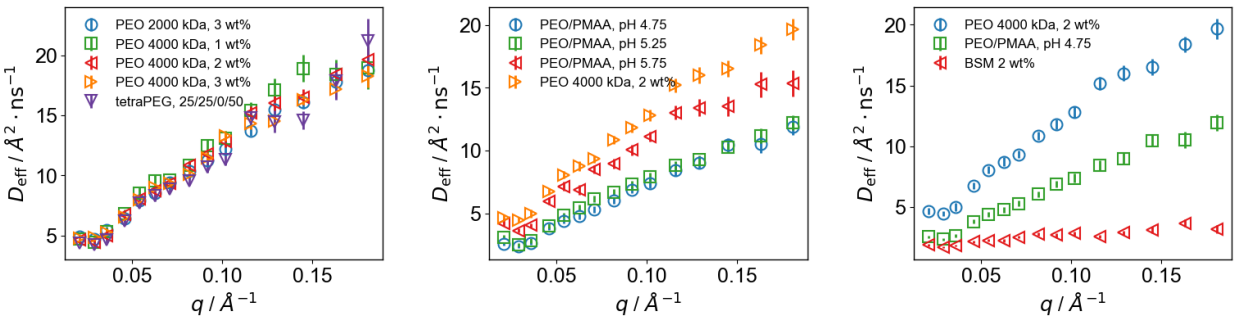

Figure 2 Effective diffusion coefficient for PEO-based hydrogels (left), PEO/PMAA mixtures (middle) and the three different systems in comparison (right).

SANS results

Regular SANS measurements were performed on D33, where all systems were studied in terms of their static mesoscopic structure. Some PEO/PMAA samples are shown in Figure 3. For low pH, the acid moieties of PMAA are protonated leading to the formation of hydrogen bonds to the PEO ether moieties. This is accompanied by an increase of intensity in mid-q. To accentuate the differences, we can determine an effective structure factor by normalizing the SANS spectra with the scattering intensity of the sample at the highest pH, 4.75, where close to no hydrogen bonds are formed. At lower pH, we clearly see the appearance of a peak in the effective structure factor, indicating the formation of distinct scattering centers.

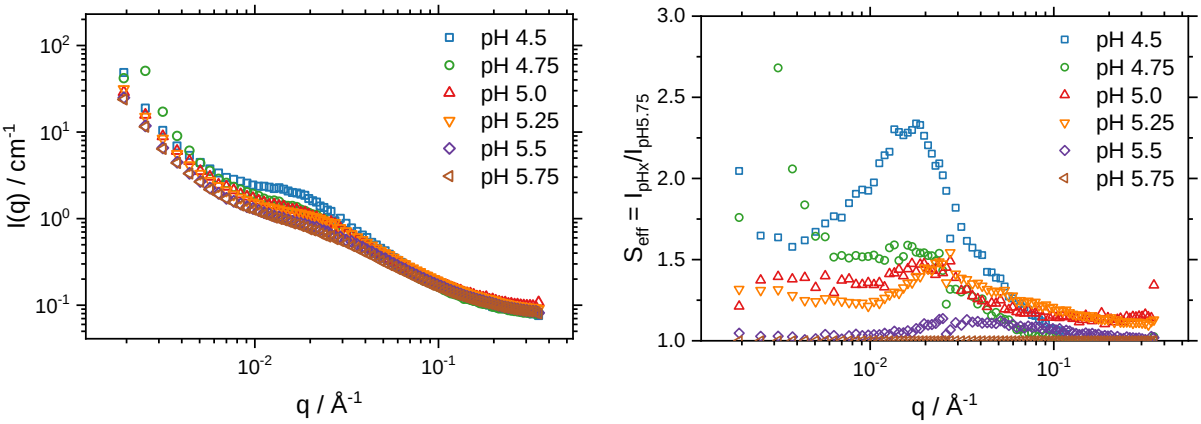

Figure 3 Left: SANS spectra of mixed PEO/PMAA samples at different pH. Right: The SANS spectra were normalized by the pH 5.75 sample, where almost no hydrogen bonds are formed resulting in an effective structure factor.

The data for the modified tetraPEG hydrogels are shown in Figure 4. In these samples, 4-arm precursor polymers are replaced by 2-arm polymers, which leads to a wider structure and weaker hydrogel character. With decreasing 4-arm concentration, the scattering intensity in mid-q decreases, although the overall polymer content is kept constant at 1%. At the same time, the low-q intensity upturn also shows significant changes, although they seem to be less predictable. We are currently thinking about how to best interpret these results. All SANS data will be analyzed in further detail by model fitting. The results will then be correlated with rheology data, as well as the dynamic data from DLS and NSE.

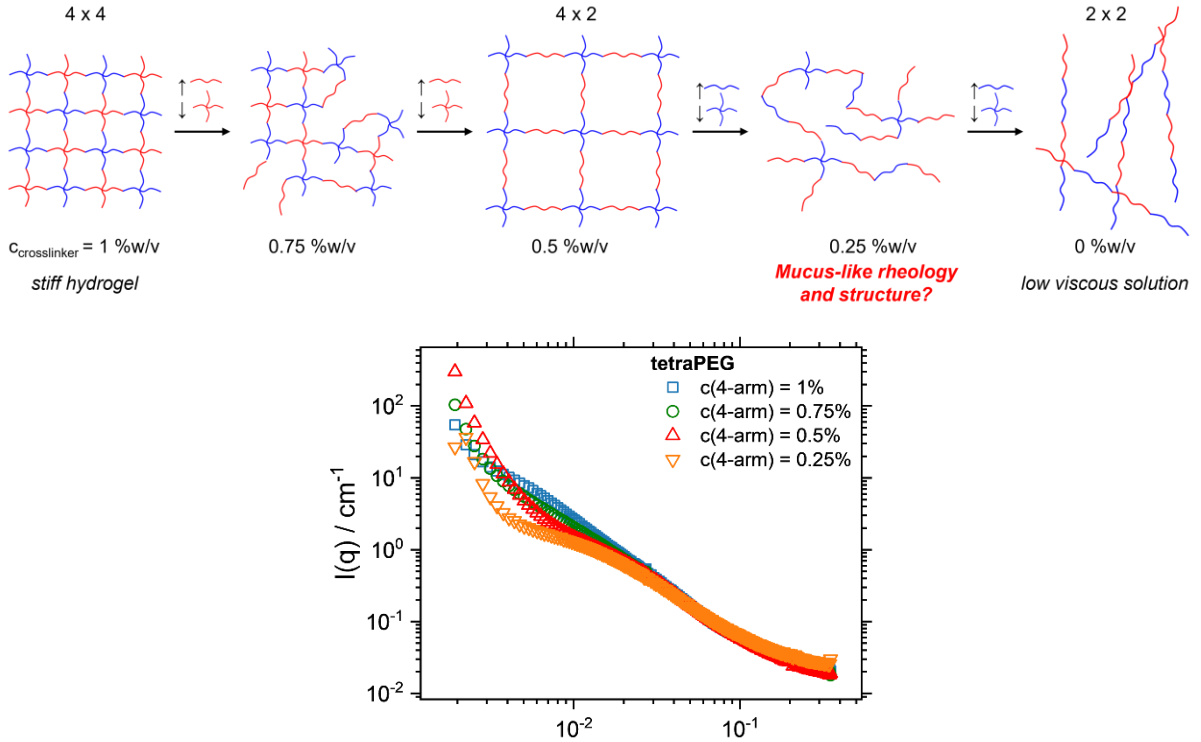

Figure 4 Top: Proposed structural change of modified tetraPEG hydrogels with decreasing 4-arm concentration. Bottom: Scattering spectra of tetraPEG hydrogels.