Experimental report

29/11/2023

Proposal:	**9-11-2103**	**Council:**	10/2022
Title:	Investigation of the Mesoscopic Structure of Three Mucin-Mimicking Hydrogels		
Research area:	Materials		

This proposal is a new proposal

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Samples: PEO/PMAA
Polyglycerol
Peptide Hydrogels

Instrument	Requested days	Allocated days	From	To
D22	2	1	10/04/2023	11/04/2023
D33	2	0		

Abstract:

Biological hydrogels such as mucus, found at surfaces in the human body, act as a protective barrier against pathogens. In this experiment, we aim to develop a thorough understanding of how the rheological properties of mucin-mimicking hydrogels are related to their mesoscopic structure. For three different model hydrogel systems, we will systematically vary polymer chain length, concentration, and pH, which yields a broad range of viscoelastic behaviour. Using SANS, we want to establish a connection to the associated structural changes on the mesoscopic scale. From the resulting spectra, we will be able to extract important hydrogel parameters such as mesh size and the fractal dimension of the polymer network. These parameters are required as input for a detailed theoretical description of the hydrogels based on bottom-up modelling techniques.

Experimental report for Proposal 9-11-2023

Performed: April 10-11, 2023

Version: November 29, 2023

Investigation of the Mesoscopic Structure of Three Mucin-Mimicking Hydrogels

In this experiment we studied the mesoscopic structure of different model hydrogel systems that mimic the properties of mucus - a biological hydrogel found at surfaces in the human body that acts as a protective barrier against pathogens. The systems studied are

a) Aqueous solutions of poly(ethylene oxide) (PEO); this very simple system is used as a reference since it is very easy to prepare and has reproducible viscoelastic properties.
b) Mixed solutions of PEO and poly(methacrylic acid) (PMAA); the acid moieties of PMAA form hydrogen bonds with PEO and thereby introduce pH-dependent crosslinks into the system.
c) Chemically crosslinked polyglycerol hydrogels; synthetic polyglycerols are dynamically crosslinked via thiol end groups which yields a hydrogel with very similar rheological properties to mucus.
d) Coiled-coil peptide hydrogels; these coiled-coil peptides self-assemble under the formation of a network structure, which introduces gel-character. They can be functionalized with glycans to study the effect glycans have on the rheological and structural properties in mucus, which is also highly glycosylated.

For this experiment, we focused mainly on the PEO/PMAA hydrogels, as well as the effect of additives, such as CaCl2, NaCl, Glucose and Lysozyme on the mesoscopic structure.

Experimental procedure

All samples were prepared in filtered D_2O containing the respective additives in the appropriate concentrations. Small-angle neutron scattering (SANS) experiments were performed on the D22 instrument. We used a neutron wavelength of 6 Å and a collimation length of 17.6 m to yield a total *q*-space of $0.00256 < q < 0.64061$ Å^{-1}. The samples were measured in 1 mm and 2 mm cylindrical quartz cuvettes and a round aperture of 12 mm was used.

Results

PEO/PMAA hydrogels For the PEO/PMAA hydrogels, we chose a ratio of PMAA-acid to PEO-ether moieties of x = 0.5. Then, the pH was varied between 4.75 and 5.75. At low pH, the acid moieties are protonated, meaning that H-bonds can be formed between PMAA and PEO. At high pH, almost all acid moieties are deprotonated, so we expect almost no interaction between the two polymers. The scattering spectra are shown in Figure 1 A). A pure PEO sample is shown by light blue squares for reference. The pure PEO sample is homogeneous that can be described by a two-correlation length model, as shown by Hammouda et al., a longer time ago [1,2]. At low pH, we can see the emergence of a scattering peak at intermediate *q*. This scattering peak indicates that isolated scattering centers are formed which have more or less regular spacing. If the pH is increased, the scattering peak is lost again until the curve at pH 6.25 looks essentially the same as the pure PEO sample. To accentuate the changes between the curves, we can calculate an effective structure factor by dividing the scattering intensity of the PEO/PMAA samples by the pure PEO sample, $S_{\mathrm{eff}}(q) = I_{\mathrm{mixed}}(q)/I_{\mathrm{PEO}}(q)$. The effective structure factor is shown in Figure 1 B). We clearly see a structure factor peak at low pH, which reduces in strength with increasing pH. This indicates that the homogeneous gel structure is lost in exchange for more pronounced scattering centers. Taking the known number concentration of PMAA molecules ${}^{1}N$, the average spacing of PMAA molecules in the solution, assuming a cubic arrangement, is given by $d = ({}^{1}N)^{1/3}$. The expected scattering peak position for that spacing is given by $q_{\mathrm{max}} = 2\pi/d$, which is shown as a vertical black line in Figure 1 B). Although the position of the true scattering peak and the prediction do not perfectly match, they are very close, which warrants the assumption that the scattering centers are formed around each PMAA molecule. The change in structure is accompanied by a 1000-fold increase of the storage modulus *G*' at low pH, indicating a vastly increased hydrogel character. Combining the results from SANS and rheology, a comprehensive picture for this system emerges: at low pH, the acid moieties of PMAA form hydrogen bonds with the much longer PEO chains, which disrupts the otherwise homogeneous PEO solution and forms defined aggregates, which act as scattering centers. Almost every PMAA molecule forms its own scattering center. The aggregates are interconnected by the long PEO chains, which constitutes a crosslinked network structure, explaining the dramatic increase of the elastic modulus. At high pH, the hydrogen bonds are lost and the PEO solution is unaffected by the addition of PMAA.

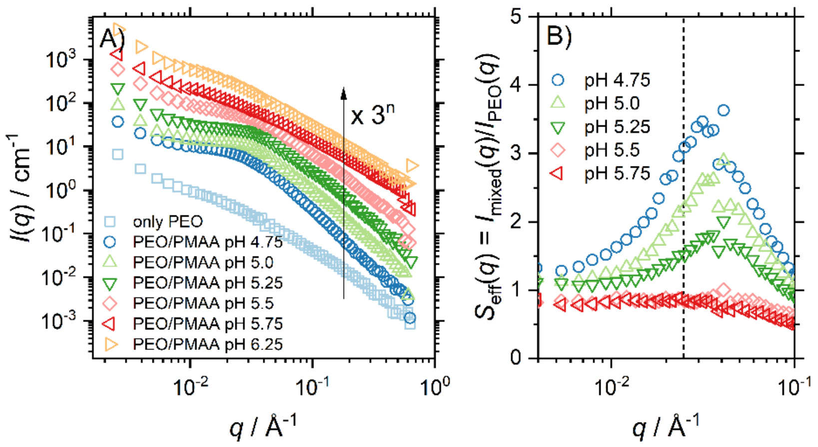

Figure 1: A) SANS scattering spectra for mixed PEO/PMAA solutions at a -COOH to -O ratio of 0.5 and a total polymer concentration of 2 wt%. A comparable pure PEO solution is shown for reference. The spectra were multiplied by factors of 3^n for better lucidity. The pure PEO sample is in cm^{-1}. B) Dividing the scattering intensities of the PEO/PMAA samples by that of the pure PEO sample, we obtain an effective structure factor, which illustrates the emergence of scattering peak at low pH indicating the formation of defined scattering centers. The vertical black line denotes the average spacing of PMAA molecules in solution.

Influence of Additives on Mucin-Mimicking Hydrogels The second part of the experiment focused on the influence certain additives have on the mesoscopic structure of three different mucin-mimicking hydrogels. As additives we used CaCl2, NaCl, Glucose and Lysozyme. They were tested on the pure PEO solutions, on the polyglycerol hydrogels and on the peptide hydrogels. To accentuate the change induced by the additives, the scattering spectra are divided by the spectrum of a comparable sample in pure D_2O. The results are shown in Figure 2.

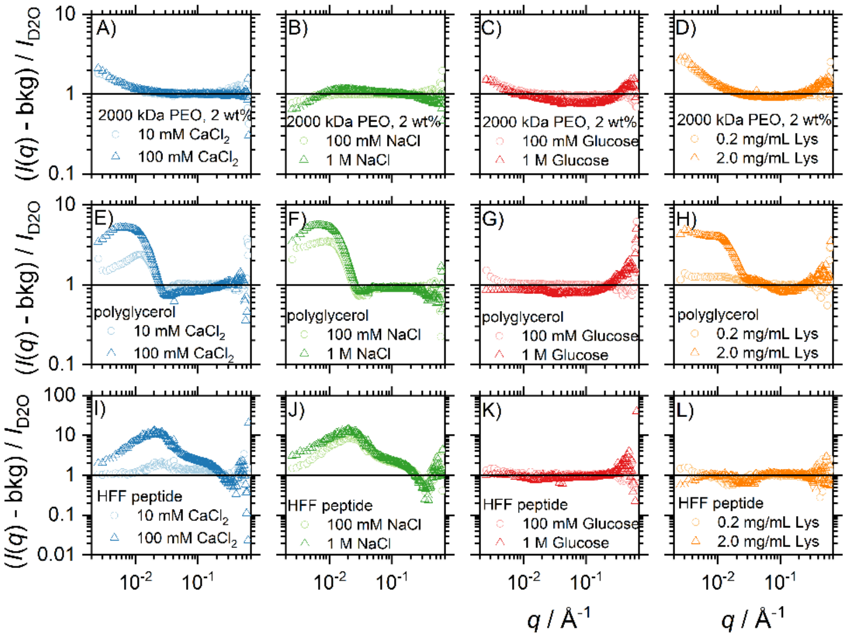

Figure 2: Influence of additives on the scattering spectra of A)-D) PEO solutions, E)-H) polyglycerol hydrogels, I)-L) peptide hydrogels. The PEO solutions are hardly affected and the effect is strongest for the peptide hydrogels.

For the PEO solutions, we see only slight changes with the additives, mostly in low-*q*. Here it is unclear, whether these small changes are really brought about by the additives or if they stem from small differences in concentration or measurement inaccuracies. The situation is quite different for the polyglycerol hydrogels. The scattering spectra are significantly increased in low-*q* for $CaCl_2$ and NaCl, as well as for Lysozyme. There is no significant effect for Glucose. Lysozyme has 8 positive charges at neutral pH. Since Glucose is the only uncharged additive, we hypothesize that the change in structure is brought about by an increased screening of electrostatic interactions. For the peptide hydrogels, we also see a strong effect of the two salts, which bring about an up to 20-fold increase in intensity in intermediate-*q*. Interestingly, Lysozyme has no effect in this case, which questions whether this is a purely electrostatic effect.

For the polyglycerol and the peptide hydrogel samples, which were both affected by the additives, rheology experiments were performed to determine the storage modulus *G*', which is indicative of the hydrogel properties. The results for *G*', as well as the full scattering curves are shown in Figure 3.

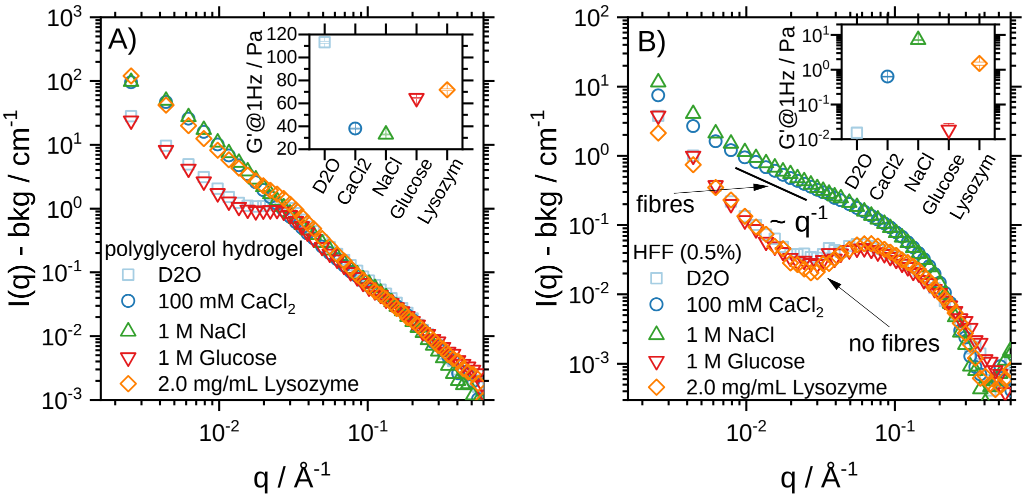

Figure 3: Scattering curves and results for G' for A) polyglycerol hydrogels. and B) peptide hydrogels. In the case of the polyglycerol hydrogels, the hydrogel character is decreased by the additives, while for the peptide hydrogel, it is increased.

For the polyglycerol hydrogels, seen in Figure 3 A), addition of salt leads to a marked decrease of *G*' and an increase of the scattering intensity, especially in low-*q*. We can therefore deduce that the formation of a stiff hydrogel is hindered by electrostatic interactions. Interestingly, *G*' also decreases when Glucose is added, which does not affect the scattering intensity. For the peptide hydrogels, seen in Figure 3 B), previous studies reported the presence of fibers, which are responsible for the hydrogel character [3]. The fibers are associated with a q^{-1} power law scaling in mid-*q*. In our case, addition of salt leads to the appearance of the q^{-1} scaling and increases *G*', indicating that the screening of charges is necessary for fiber formation. However, for Lysozyme, we see an increase in *G*' but no evidence for fibers. Potentially, Lysozyme facilitates a different gelation mechanism.

References

[1] B. Hammouda, D. Ho, S. Kline, *Macromolecules* **2002**, *35*, 8578–8585.

[2] B. Hammouda, D. L. Ho, S. Kline, *Macromolecules* **2004**, *37*, 6932–6937.

[3] K. S. Hellmund, B. Lospichl, C. Böttcher, K. Ludwig, U. Keiderling, L. Noirez, A. Weiß, D. J. Mikolajczak, M. Gradzielski, B. Koksch, *Peptide Science* **2021**, *113*, e24201.