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ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office via the User Portal:

https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Reports supporting requests for additional beam time

Reports can be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

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All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** Rhesus of double thermoresponsive composite hydrogels	**Experiment number**: 26-02-824
Beamline: BM26B	**Date of experiment**: from: 26 Jun 2017 to: 30 Jun 2017	**Date of report**:
Shifts: 12	**Local contact(s)**: Daniel Hermida Merino	*Received at ESRF:*

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Report:

Goals

The goal of the proposal was to investigate the structure of hydrogels as function of applied strain, temperature and composition. We used a strain-controlled rheometer coupled to a couvette geometry to analyse simultaneously the mechanical and structural properties of the hydrogels. The effect of temperature in the structure of hybrid hydrogels and hydrogels composed of polymers with different side groups were performed in a temperature-controlled capillary setup.

Motivation

Cells interact with their surrondings both chemically and mechanically. Over the last years increasing evidence indicates that the mechanical communication takes place in the nonlinear regime. Nearly all biological materials are designed such that they access this nonlinear regime at relatively low strains. When higher strains are applied to such systems, they stiffen up, protecting the cells inside.[1] Recently, we published a new synthetic polymer that shows exactly the same strain-stiffening behaviour as many biopolymers[2,3]; The cytoskeleton as well as the extracellular matrix are, however, not composed of single component hydrogels only. They are made of a mixture of polymers and networks and show a complex mechanical properties. Mixture of different polymers or the addition of a functional crosslinker with our synthetic polymer increases the variables of the system and can be able to mimic the complexity of cells. Further, the addition of a second network, forming a hybrid hydrogel, can present unique mechanical properties. Our group recently published a combined rheoSAXS study[3], where we proved how the structure of the hydrogel changes at the thermally induced gelation point. In the performed experiments, we aimed to understand the

molecular strucuture of our synthetic hydrogel when combined with 1) functional crosslinkers, 2) polyisocyanice-tetraethylene glycol and 3) poly(n-isopropylacrylamide) (PNIPAM).

Setup

A standard SAXS set-up with a sample-to-detector distance of 3.5 meter was used. The 2D Pilatus1M detector collected low contrast images and detected fibril alignment as a function of strain. A DHR2 rheometer (TA Instruments) was used to deform the hydrogels in the (non)linear regime. The gel was formed in a polycarbonate (PC) Couette cell with a peltier temperature controller. In the second part, a capillary setup with temperature controller was used to analyse 1) the effect of the side group in the material composition and 2) hybrid hydrogels and the structural changes with temperature.

Results and Discussion

RheoSAXS experiments:

1) Functional crosslinkers:

In the RheoSAXS setup, we analysed the hydrogel composed of polyisocyanide (0.4 wt%) chemically crosslinked with CCMV capsid (cowpea chlorotic mottle virus). Previous study in our group had demostrated that the capsid crosslinkers rupture after certain stresses are applied and therefore a conventional rheometer could follow the global mechanical changes with the rupture. With the scattering profile of the capside, we could prove that indeed is the rupture what causes the observed changes in the mechanical properties. Unfortunately, due to the differences in the geometries – we previosly used parallel plates for our tests, and the RheoSAXS setup has a Couette – the capsides did not fracture with the applied stress. Further research has to be done to determine the conditions in which the capsides can be fractured with the Couette geometry.

Capillary setup:

2) Composition

Hydrogels composed of polyisocyanide-triethylene glycol (PIC-3EG) and polyisocyanide-tetraethylene glycol (PIC-4EG) were analysed at the temperatures T=5 °C, 35 °C and 50 °C. Such small difference in the side chains, either composed of 3 or 4 ethylene glycol groups, causes the hydrogels to gelate at 18 °C and 40 °C, respectively. We investigated the scattering pattern for a mixture of the polymers and concluded that PIC-4EG bundles beyond its gelation temperature and a hybrid is formed. The results were published and can be found in: *P.H.J. Kouwer et al. / Chinese Chemical Letters 29 (2018) 281–284.*

2) Hybrid hydrogels

Double-network hydrogels composed with PIC and PNIPAM were analysed in the temperature range from 30 °C to 40 °C. The network of PNIPAM collapses with temperature above 32 °C and strains the PIC network. The goal of the experiments was to determine the structural change in the PIC network with the PNIPAM collapse. We measured two compositions of PIC/PNIPAM, with concentrations of 4/6 and 4/17 mg mL^{-1} in order to assess the impact of concentration into the network. As a control, samples of the single-networks of PNIPAM and PIC were analysed. The scattered data were fitted with a combination of Kholodenko worm-like chain term and correlation length model to describe the polydisperse PIC network: $I_{network}(q) \propto I_0[1 + (q\xi)^p]^{-1}$, where $I(q)$ is the experimental scattering intensity. The result (Figure 1) showed that as the PNIPAM collapses, the pore size of PIC network halves and the network becomes denser. The results brought essential insights to the understanding of the interaction between both components at molecular level. The SAXS results are included to a manuscript recently submitted: *Cytoskeletal Stiffening in Synthetic Hydrogels*, *submitted*, P. de Almeida, M. Jaspers, S. Vaessen, O, Tagit, G. Portale, A. E. Rowan, P. H. J. Kouwer.

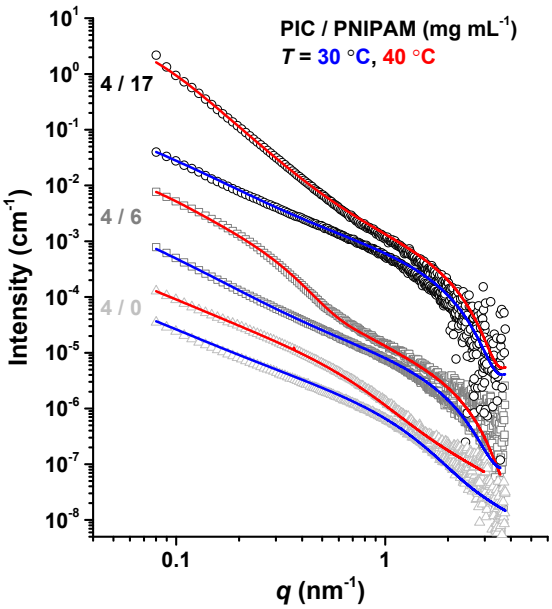

Figure 1. SAXS scattering profiles of the PIC/PNIPAM (4/17) double-network at temperatures 30 (blue fit) and 40 °C (red fit). The PNIPAM contribution was subtracted. The high angle concave curvature (in all samples) originates from the scattering contribution of the fraction of dissolved PIC chains not incorporated in the gel that is well described by a Kholodenko worm-like chain term with contour length L = 72 nm, persistence length l_p = 8 nm and radius R = 1.0 nm. The fits (solid lines) follow the correlation length model, with the exception of 4/6 at 40 °C, which also contains a Kholodenko bundle term with L_B > 200 nm, $l_{p,B}$ > 200 nm (both outside the experimental window) and bundle radius R_B = 6.7 nm. The inset tabulates the key fitting parameters, correlation length ξ (in nm) and Porod exponent p. Data is vertically offset for better visualisation.

Conclusions

SAXS is a reliable technique to study the structural changes of our polymeric system. With that, we could understand the stiffening effect of our PNIPAM/PIC system and to evaluate the bundle process of a mixture of PIC with different gelation temperatures. For the rheo-SAXS system, more investigation is required with the couvette apparatus.

References and notes

1. Wen, Q. & Janmey, P. A. Polymer physics of the cytoskeleton. *Curr. Opin. Solid State Mater. Sci.* **15,** 177–182 (2011).
2. Kouwer, P. H. J. *et al.* Responsive biomimetic networks from polyisocyanopeptide hydrogels. *Nature* **493,** 651–655 (2013).
3. Jaspers, M. *et al.* Bundle formation in biomimetic hydrogels. *Biomacromolecules* **17,** 2642–2649 (2016).