Experimental report

07/09/2022

Proposal: 9-12-660 **Council:** 4/2021

Title: Structural evolution under stretching of Polyacrylamide-based hydrogels cross-linked by liquid crystalline phases stabilized by Laponite

Research area: Soft condensed matter

This proposal is a new proposal

Main proposer: Cecile RERZKI-VERITE

Experimental team: Sylvain PREVOST
Alesia MIKHAILOVSKAIA
Clemence LE COEUR
Cecile RERZKI-VERITE
Fabrice COUSIN

Local contacts: Sylvain PREVOST

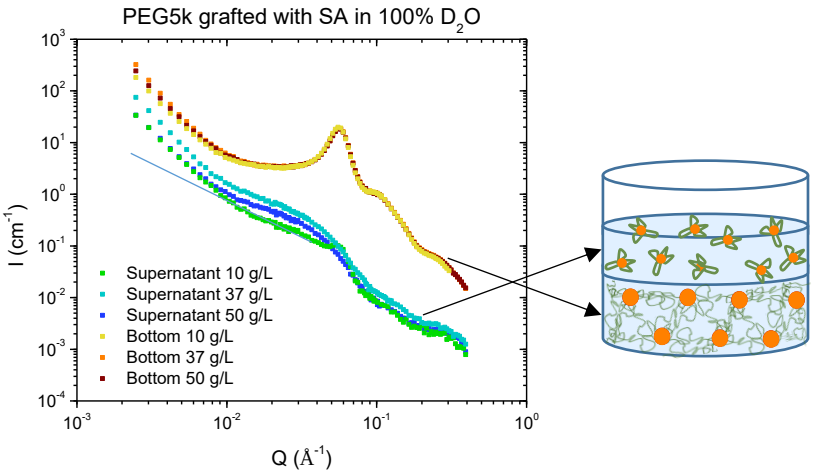
Samples: D2O
Water
acrylamide
Laponite XLG
Phytantriol
KPS
TEMED
Deuterated phytantriol

Instrument	Requested days	Allocated days	From	To
D33	3	0		
D22	3	0		
D11	3	2	07/09/2021	09/09/2021

Abstract:

We aim at determining the structure of Polyacrylamide-based hydrogels cross-linked by nodes made of lipid liquid crystalline phases, here a phytantriol cubic phase, stabilized by clay nanoplatelets of laponite. Such systems have great potential for drug delivery-based applications, since an active substance trapped in the LC phase can be released upon the hydrogel stretching. Hydrogels will be studied both at rest and under uniaxial stretching, using a tensile setup allowing their in situ deformation within the neutron beam. Contrast variation will be used to determine respectively the structure of the clay nanoplatelets of laponite and of the phytantriol cubic phase. This project is the core of the PhD program of Cécile Rerzki-Vérité.

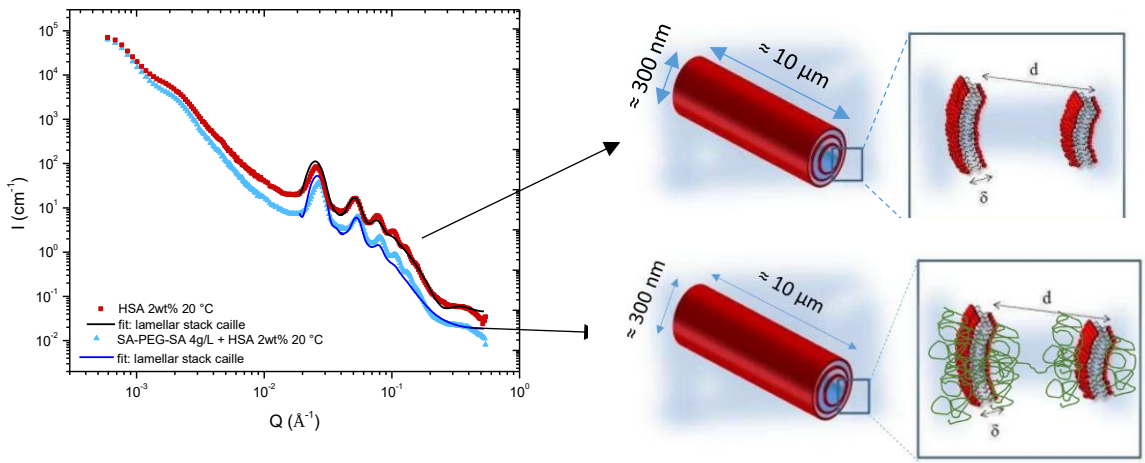
Figure 1 - Left: Intensity of PEG grafted with SA in D_2O as function of Q. Right: schematic structure of the system for the two phases. SA molecules are shown in orange and PEG chains in green.



4 Self-assemblies of HSA molecules in aqueous solution at 20°C

The scattering of the reference sample of a pure sample of HSA (20g/L) in aqueous solution is shown in Figure 3. It displays the characteristics features of lamellar phases at intermediate and large Q, i.e. a strong correlation peak at $Q_0 = 0.025$ Å^{-} followed by its harmonics (at $2Q_0$, $3Q_0$, $4Q_0$ *etc* ... visible here up to the 6th order) that accounts for the Bragg peak associated with the interlamellar distance and an oscillation at around 0.3 Å^{-1} associated with the thickness of the lamellae. The scattering was fitted on this Q-range by the Nallet model allowing to determine the structural parameters of lamella (thickness of lamella of 24 Å, *d*-spacing of the lamella of 243 Å) and the Caillé parameter that is linked to the fluctuations of the membrane. We obtained a value of 0.075 that demonstrates that lamella are rigid. Remarkably, the extended low Q range of D11 enabled us to reveal an oscillation at ~ 2 10^{-3} Å^{-1}, that must arise from the form factor of tube diameter, and was not observed on scattering curves of the same system reported in literature as they were all limited to a reduced Q-range. This would give an order of magnitude of ~ 300 nm, which is consistent with observations from microscopies (TEM, cryo-TEM, phase-contrast, *data not shown*). The overall decay is however like Q-3 in the very low Q region, and not like Q^{-1}, as expected for the form factor of a tube, which remains to be understood.

Figure 2 - Left: Intensity of HSA and a 4 g/L of polymer hydrogel as function of Q. Right: schematic structure of the system for the two phases. SA molecules are shown in orange, HSA molecules in red and PEG chains in green.



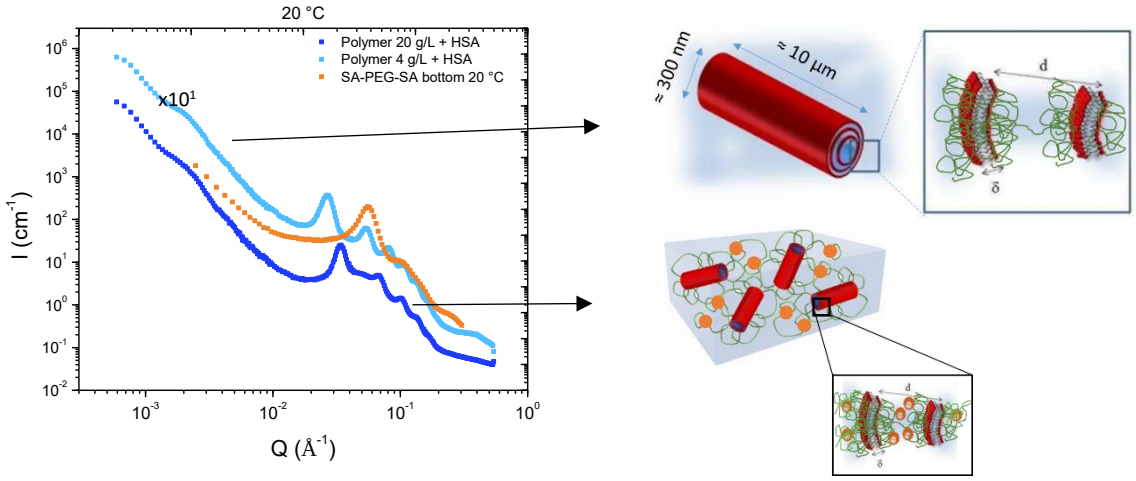
5 Self-assemblies of SA-grafted PEG chains/HSA molecules mixtures

Then we measured different mixture made of SA-grafted PEG chains with various concentration and HSA molecules at the same concentration of 20g/L as for the reference sample.

For a low content of SA-grafted PEG chains at a concentration of 4 g/L, the solution is macroscopically jellified but the scattering pattern is very similar to those of the pure HSA reference sample (oscillation of form factor at low Q, Bragg peaks at intermediate Q, and oscillation at large Q) demonstrating that the mixture also form multi-lamellar tubes. However, the overall intensity of the doped sample is lower, which show that there are less fatty acids within the sample. This can come either from a partial sedimentation of molecules within the hellma cell measured and/or from the fact that the concentration was not uniform within the jellified sample, and the part of the sample collected from the vial by pipetting to fill the cell would have a lower concentration. Although the overall structure remains similar as for the pure HSA system, the structural parameters of the lamella differ from it as there is slight shift of the Bragg peaks towards large Q, evidencing a decrease of the interlamellar distance. For its part, the tube diameter almost does not vary. The large Q and large Q part of the scattering curves were also fitted by the lamellar stack Caillé model. The obtained value of the lamella thickness is probably not meaningful given that the PEG chains must contribute to the scattering at large Q, even if their scattering if negligible compared to those of the self-assembled fatty acids at intermediate Q and low Q. The other obtained parameters are by cons very interesting as they reveal the influence of the grafted PEG chains on the structure of the tubes. The Caillé parameter value is 0.113, which is larger than for the pure HSA system (0.075) that demonstrates that the charged lamella repel themselves less. This arises from the replacement of HSA fatty acids whom head is negatively charged head owing to the presence of COO- group by neutral PEGylated heads. This also influence the lamellar spacing which has a value of 234 Å, reduced of around 10 Å from the reference sample. This gives an overall picture of the system that forms multilamellar tubes with membranes decorated by small PEG chains (Figure 3).

If the concentration SA-grafted PEG chains is largely increased up to concentration where grafted SA fatty acids molecules and HSA fatty acids are of the order of stoichiometric ratio, the structure of the resulting hydrogel do no longer form multilamellar tubes only. This is shown in Figure 4 that compares the scattering of a mixture containing 20g/L of SA-grafted PEG chains and 20g/L of HSA molecules (dark blue curve) with the scattering curve of the mixture containing 4g/L of SA-grafted PEG chains depicted here before (light blue curve). Indeed, although that oscillations at large Q (form factor of the tube) and large Q (form factor of lamella) remain, the intermediate Q region where Bragg peaks are located has completely changed, given that some harmonics of the correlation peak have vanished. This suggest that the tubes co-exist in solution with another type of structure, which may possibly be aggregates of pure SA-grafted PEG chains. We have then recalled in Figure 4 the typical scattering curve of the concentrated phase of the demixed SA-grafted PEG chains pure solution (in orange). It happens that the dark blue curve of the concentrated mixture may be obtained from the a linear combination of this phase and those of tubes doped with a low amount of SA-grafted PEG chains. Our hypothesis is that the SA-PEG grafted chains progressively insert within the HSA lamella of the HSA multilamellar tubes up to the point where such lamella cannot accommodate more of the SA-PEG grafted molecules. The SA-PEG grafted molecules remain thus in water and aggregate themselves, coexisting with the tubes. They can even lead to a swelling of the tubes.

Figure 3 - Left: SANS intensity profiles for polymer grafted with SA (4g/L light blue and 20 g/L dark blue) hydrogels and PEG grafted with SA bottom, as function of Q. Right: schematic structure of the system. SA molecules are shown in orange, HSA molecules in red and PEG chains in green.



Behavior when increasing temperature upon the tubes/micelles transition threshold temperature

As recalled in introduction section, our hydrogels are thermos-sensitive with a tunable transition temperature between tubes and micelles. We have set this transition temperature to 37 °C by an appropriate HSA to counter-ion ratio. Figure 5 shows the evolution of the structure upon an increase of temperatures. By increasing the temperature to 30 °C we obtained the same mixed structure of coexisting aggregates SA-grafted PEG chains and tubes as at 20°C, with a decrease of interlamellar distance revealed by the shift of the Bragg peaks towards large Q. At 37 °C, there are no longer tubes but charged micelles interacting through electrostatics repulsions, as the scattering pattern is characterstic of a system of repulsive spheres, as well as at 45 °C.

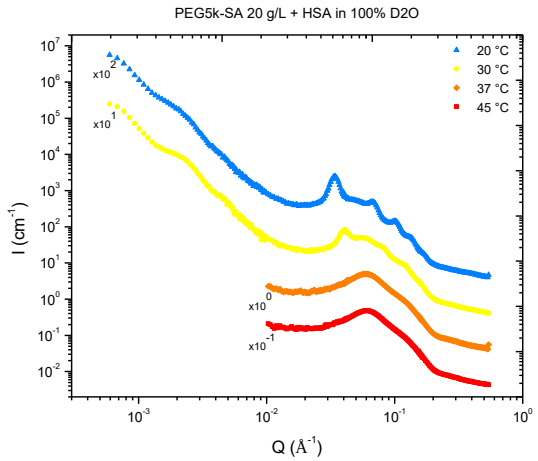


Figure 4 - SANS intensity profile for SA-PEG-SA 20 g/L + HSA 2wt% Hydrogel in function of Q at 20 °C, 30 °C, 37 °C and 45 °C.

6 Self-assemblies of HSA-grafted PEG chains/SA molecules mixtures at 20°C

We present now here the structures formed by mixtures of HSA-grafted PEG chains with HSA molecules. Due to the low amount of HSA-grafted PEG chains available, we focused on one sample containing a large amount of HSA-grafted PEG chains (20 g/L) and 20g/L of HSA. Please note that both components are at stoichiometry from mass of point of view but not from molecules point of view since the molar mass of the 5k PEG chain is much larger than that of a HSA molecule. Macroscopically,