Experimental report

15/09/2022

Proposal: 9-11-2041 **Council:** 10/2020

Title: Thermo-stimulable hydrogels based on polymer cross-linked by fatty acids self-assemblies

Research area: Soft condensed matter

This proposal is a new proposal

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Local contacts: Sylvain PREVOST

Samples: Poly (ethilene glycol) : (CH2-CH2-O)n
deuterated poly ethylene glycol
hydroxystearic acid C18H26O3

Instrument	Requested days	Allocated days	From	To
D22	2	0		
D11	2	2	22/06/2021	24/06/2021

Abstract:

We aim at determining the structure of thermo-responsive hydrogel obtained by the mixing of PEG polymers chains that are terminally grafted at both ends by small surfactants with the pure same surfactant. The chosen surfactant is 12-hydroxystearic acid (HSA), a fatty acid that forms multi-lamellar tubes at low temperature and nanometric spherical micelles above a threshold temperature. We expect that the terminal hydrophobic chain of PEG will insert in fatty acid aggregates that will play the role of thermoresponsive nodes for the resulting hydrogels. Promising first rheological measurements demonstrate that the shear modulus of hydrogels increases by a factor 1000 by playing on temperature.
We ask 2 days of beamtime of SANS in to elucidate the structure of: (i) solutions of HSA-grafted PEG without addition of free HSA molecules; (ii) hydrogels. For the last part, we will use the contrast variation technique to solve the respective structure of HSA aggregates and polymer chains in the mixtures.

Proposal number: 9-11-2041 on **D11** (22-24/06/2021) Local Contact: PREVOST Sylvain
Participants : ALMEIDA Maëva, LE CŒUR Clémence, COUSIN Fabrice

Context and objectives : 2 days were granted on D11 for the **determination of the structure of thermo-stimulable hydrogels obtained by the mixing of PEG polymers chains that are terminally grafted at both ends by 12-Hydroxystearic acid (HSA)**, a small fatty acid with pure 12-HSA molecules. Such surfactant forms multi-lamellar tubes at low temperature and nanometric spherical micelles above a given threshold temperature. We expected that the side hydrophobic chain of PEG will insert in fatty acid aggregates that will play the role of thermos-stimulable nodes for the resulting hydrogels.

The initial objective of the experiment was to determine the structure of the HSA-grafted PEG chains / HSA mixture for various concentrations and PEG mass chains (5k, 20k, 35k, 100k), for both PEG deuterated chains and hydrogenated chains in order to perform contrast variations. *Unfortunately, a few days before coming to ILL, we realized that there were some issues in the synthesis we designed for the chemical grafting of the extremities of PEG chains by HSA molecules*. All chains with masses larger than 5k were not fully soluble in water, as an unexpected reaction with the hydroxyl function in C12 leads to their aggregation. **We decided thus to measure only systems for HSA-grafted PEG chains of 5k, both for deuterated and hydrogenated PEG chains.** In order to fully optimize the beamtime, we also decided to graft such 5kPEG chains by stearic acids (SA), for which the grafting synthesis step was much simpler, and to compare mixture of HSA with either SA-PEG grafted chains or HSA-PEG grafted chains. SA differs from HSA only by the OH groups in C_{12}, but this has a huge impact on the structure of self-assemblies in aqueous solution, as pure SA systems make planar lamellar phases, and not multilamellar tubes.

Materials and Methods:

Samples. The pure fatty acids HSA and SA molecules were coupled via ion pairing with the counterion ethanolamine in order to make them soluble in water. Samples Mixtures were obtained by mixing of mother stock solutions of pure HSA molecules with solutions of pure SA-5kPEG grafted chains, respectively with HSA-5kPEG grafted chains, to target different ratio of grafted to non-grafted molecules, from low doping of tubes by pegylated fatty acids up to stoichiometric mixtures. **The concentration of pure HSA molecules was fixed to 20g/L for every sample**.

Measurements and data reduction. We used four configurations (6 Å at 1.7 m, 6 Å at 5.5 m, 6 Å at 20.5 m, and 13Å at 38 m) to reach a very large q-range spanning from 3.5 10^{-4} $Å^{-1}$ to 0.55 $Å^{-1}$. All samples were measured at 20 °C, 30 °C, 37°C, 45 °C. Transmissions, scattering of empty cell, cadmium (neutron absorber to value ambient background of experiment), scattering of hydrogenated water and differential scattering cross section of water were measured independently. Subtraction of parasitic contributions and normalization by water to take into account detectors heterogeneities were applied to raw data by the GRASP software to obtain corrected data in absolute units (cm^{-1}). Contributions from solvent and incoherent scattering were then subtracted.

Results

Please note that **this report does not present exhaustively all results gathered during the experimental campaign** (38 samples were measured at 4 or 5 different temperatures, giving around ~ 160 spectra). Only the more representative results, obtained at 20°C, are presented and discussed here. **Results obtained above 37°C**, that is the transition threshold for tubes to micelles for the pure HSA system, **show that hairy micelles made of a fatty acid core with a PEG shell are obtained in every case.**

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

The scattering of the reference sample of a pure sample of HSA (20g/L) in aqueous solution is shown in Figure 1. It displays the characteristics features of lamellar phases at intermediate and large Q, i.e. a strong correlation peak at $Q_0 = 0.025$ $Å^{-1}$ followed by its harmonics (at $2Q_0$, $3Q_0$, $4Q_0$ *etc* ... visible here up to the 6^{th} 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. This would give an order of magnitude of ~ 300 nm, which is consistent with observations from microscopies. 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.

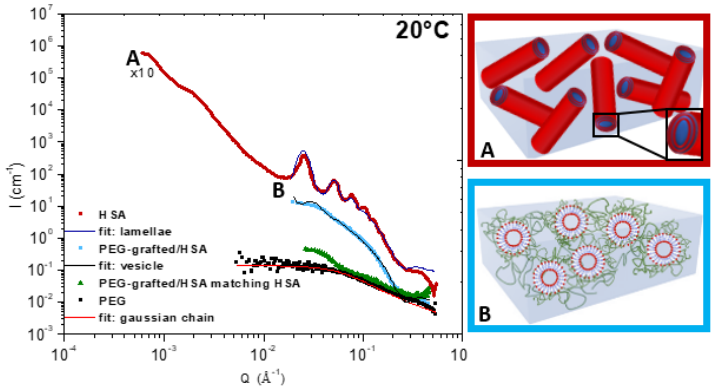

Figure 1 – Left: SANS intensity for pure HSA solution and HSA-PEG-HSA / HSA mixtures for 2 contrasts (D_2O in light blue and 87%H_2O/13%D_2O in green) and PEG5k in water. Right : Schematic structure of the system. HSA molecules are shown in red and PEG chains in green

Figure 1 also shows the scattering of the structures formed by mixtures of HSA-grafted PEG chains with HSA molecules with various contrast conditions. 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. Macroscopically, the sample is a turbid liquid solution. In full contrast, *i.e.* with both hydrogenated components in D_2O, it immediately appears that the system does not any longer form multilamellar tubes since there are no more Bragg peaks at intermediate Q. The scattering pattern displays the characteristic shape of a small unilamellar vesicle with : (*i*) a Guinier plateau at low Q; (*ii*) a Q^{-2} decay at intermediate Q with an oscillation associated with the radius; (*iii*) a crossover between a Q^{-4} and Q^{-2} at large Q and an oscillation at ~ 0.25 $Å^{-1}$ linked to the lamella thickness. It was successfully fitted by the form factor of the vesicle with an internal radius of 110 Å and a shell thickness of 23 Å. It is worth noting that the overall diameter of the vesicle (~ 246 Å) does almost exactly corresponds to the interlamellar distance within the tubes of pure HSA fatty acids at such concentration. In order to refine the structure of such a vesicle, we made an additional experiment with deuterated PEG chains in the 13%H_2O/87%D_2O solvent that matches HSA molecules, making only the deuterated PEG chains visible in the system. The exact SLD of HSA molecules was determined at beginning of experiment by measuring a series of different H_2O/D_2O mixtures solvents ranging from 40%/60% to 0%/100%. At large Q, where the local conformation of the chains is probed, the scattering decays like $Q^{-1/\nu}$, where ν is the Flory exponent. Experimentally, the scattering decays like Q^{-2} ($\nu = 2$), evidencing that the HSA-PEG grafted have locally a Gaussian behavior. It also overlaps at large Q with the scattering of a sample of pure non-grafted 5k chains measured independently and fitted by a Debye function, giving a gyration radius of 23 Å. This unambiguously demonstrates that the small vesicles are decorated by the 5k PEG chains. At intermediate and low Q, the scattering does not overlap anymore to those of the pure chains but its shape follows those of the scattering of the vesicle in full contrast condition to a constant factor. This originates from the fact that the scattering of the ghost contrast-matched vesicle is revealed by the decorating non-matched PEG chains.

This experiment with short grafted 5k PEG chains demonstrate that the doping of lamella of HSA by PEG-grafted HSA molecules, that have a different packing parameter since the volume of polar head is strongly increased owing to the PEG chains, is sufficient to induce a transition from lamellar tubes to vesicles.

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

Prior to their mixing with pure HSA molecules, we remarked that a macroscopic phase separation occurred within the mother stock solutions of SA-grafted PEG chains, with the bottom phase that formed a strong gel while the supernatant was liquid, which prompted us to measure the scattering of both phases (Figure 2.a). In the bottom phase, all curves almost overlap showing that they have the same structure and end up with almost the same concentration, although the samples having different initial concentrations prior to the phase separation. On the reverse, the overall scattering increases with concentration in the supernatant phase. This

suggests that **the phase separation is a first-order transition**. For the supernatant phase, all scattering share the same features : (*i*) in the low Q region, an increase of intensity with a scattering decay like Q^{-3}, that suggests the presence of large inhomogeneities within the sample; (*ii*) in the intermediate Q region, a Q^{-4} scattering decay preceded by a plateau, that would consistent with the form factor of 3-D objects of rather low size; (*iii*) at large Q, a Q^{-2} decay that is consistent with the local Gaussian behavior of the PEG chains in solution. Although these scattering curves are not yet fitted with satisfactory models, this gives the overall picture of the **formation of hairy micelles**. The scattering of the bottom phase is markedly different. It also shows a Q^{-3} decay at low Q , but a strong correlation peak at $Q_0 \sim 5\ 10^{-2}$ Å^{-1} followed by other correlation peaks of lower intensity and oscillations at larger Q, and an overall decay like Q^{-4} at large Q. The Q-positions of the correlation peaks of higher order are not the harmonics of Q_0, which plaids for the formation of **a network of hairy micelles organized with a crystalline structure**, as often observed in telechelic systems. Such a cross-linked structure is consistent with the formation of the macroscopic gel. Some schematic sketches are proposed to depict the obtained structures in Figure 2.

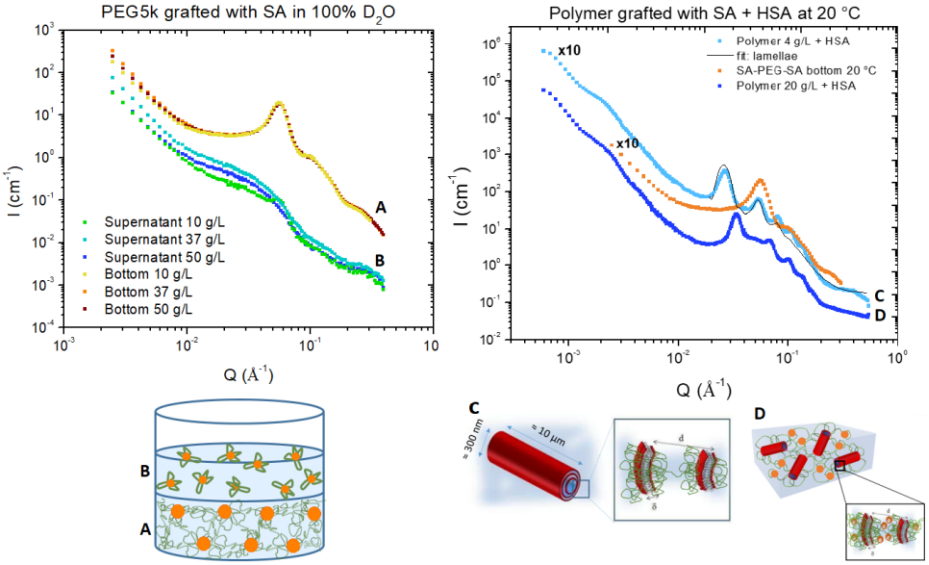

Figure 1 – Top panel. Left: SANS Intensity of pure solutions SA-PEG grafted chains. Right: SANS Intensity of mixtures of SA-PEG grafted chains with HSA molecules. Lower panel : Schematic structure of the systems. HSA molecules are shown in red, SA molecules in orange and PEG chains in green.

Then we measured different mixture made of such SA-grafted PEG chains at various concentrations with HSA molecules at 20g/L. For a low content of SA-grafted PEG chains (4 g/L), the solution is macroscopically jellified even if the scattering pattern is very similar to those of the pure HSA reference sample demonstrating that the mixture also form multi-lamellar tubes. However, the structural parameters of the lamella differ from there reference sample as there is slight shift of the Bragg peaks towards large Q, evidencing a decrease of the interlamellar distance, while 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 Caillé parameter value is 0.113, which is larger than for the pure HSA system 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 2.b).

If the concentration of SA-grafted PEG chains is largely increased up to 20 g/L, the structure of the resulting hydrogel is no longer made of multilamellar tubes only. Indeed, although that there oscillations at large Q (form factor of the tube) and large Q (form factor of lamella), 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. It happens that the dark blue curve of the concentrated mixture may be obtained from a linear combination of the concentrated phase of the demixed SA-grafted PEG chains pure solution 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.