Experimental Report

Proposal: **9-13-564** **Council:** 4/2014

Title: Structural investigation of Hyaluronan / Pluronic aggregates: a possible system for drug delivery with specific targeting

This proposal is a new proposal

Researh Area: Soft condensed matter

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Local Contact: GRILLO Isabelle

Samples: Pluronic P123 and F127 and hyaluronic acid

Instrument	Req. Days	All. Days	From	To
D33	2	1	13/10/2014	14/10/2014

Abstract:

In the medical field and especially for cancer treatment, intensive researches are performed to deliver the drug at the right position allowing reducing dose and side effects. The poor solubility in biological fluids of drugs obliges to encapsulate them into carriers showing an amphiphilic behavior. We work here with Pluronics that form micelles above the CMT and Hyaluronan(HA), a linear semirigid polyelectrolyte, widely found in the human body. It is currently investigated as a target-specific material because many malignant cancer cells over express HA receptors. DLS measurements have been performed as a preliminary structural characterization. A systematic increase of the hydrodynamic radius with the amount of HA from 120 Å for the pure P123 micelles to 180 Å with 1% of HA is observed. Only SANS and contrast variation will give a detailed characterization of the mixed HA /Pluronic aggregates and the effect of model drug molecules. In a fundamental point of view, such system can help to understand the forces governing the formation of complexes between polyelectrolyte and nonionic micelles.

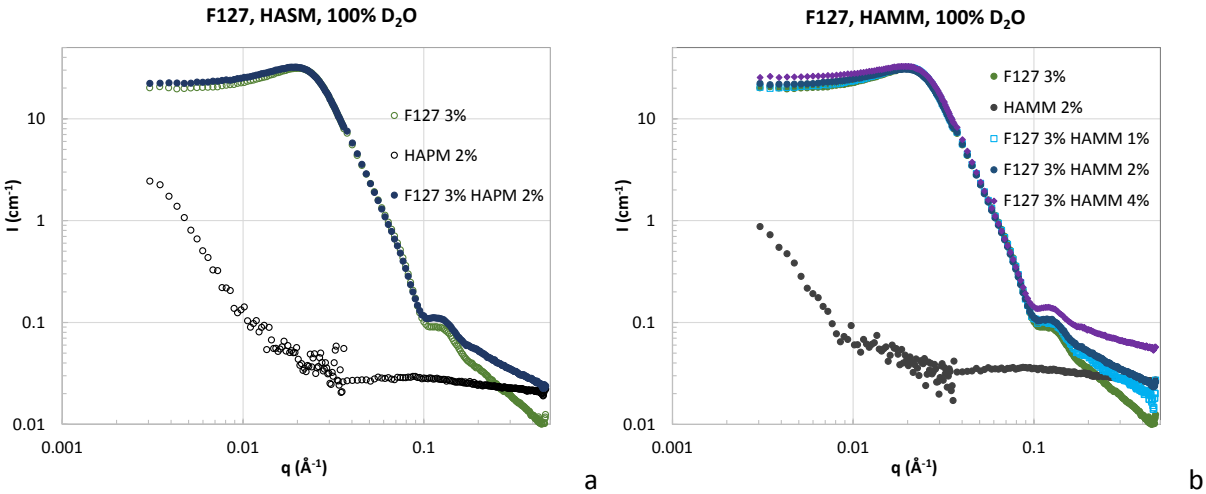
The aim of the study is to characterize the structure of assemblies formed by the F127 pluronic micelles and the hyaluronic acid (HA). These two molecules are perfectly bio-compatible. Pluronics are already used for drug encapsulation [1,2] because they are stealth carriers providing a prolonged circulation into the blood system. HA is largely found in the body but play an ambiguous role. Whereas high molecular weight HA (> 500 kDa) is anti-angiogenic, anti-inflammatory and immunosuppressive, low molecular weight HA (10 - 500 kDa) is highly angiogenic and pro-inflammatory. HA is currently investigated as a target-specific material because many malignant cancer cells overexpress HA receptors and thus incorporation of HA can be used as targeting ligand.

F127 (PEO_{100} – PPO_{65} – PEO_{100}) is purchased from Sigma-Aldrich. The molar mass is 12 570 g/mol with an average density of 1.12. For concentrations lower that 10 vol%, F127 forms spherical micelles above the CMT, which is found at 22°C for Φ =3% (DSC measurements). The scattering length densities of dehydrated EO and PO are respectively 0.67 10^{10} and 0.34 10^{10} cm^{-2}.

Hyaluronic acid (HA) is a gift from Soliance (Pomacle, France). HA is a linear semirigid polyelectrolyte with the repeating disaccharide structure poly((1→3)-β-D-GlcNAc-(1→4)-β-D-GlcA), with global formula $C_{14}H_{20}NO_{11}Na$. The molar mass of the repeat unit is 401.3 g/mol, the density is 1.65 and the segment length 10 Å; the calculated length scattering density is 2.3 10^{10} cm^{-2}. Two different molecular weights are used; HASM Mw = 16500 g/mol and HAMM Mw = 300 000 g/mol. The contour lengths are respectively 400 and 7500 Å.

The composition of the system is given by Φ_F, the volume fraction of Pluronic and Φ_{HA}, the volume fraction of HA. The Pluronic concentration is fixed at 3vol%. Three HA volume fractions 1, 2 and 4 vol% are investigated. The samples are prepared in pure water or in 0.1M NaCl salt solution to be close to the biological conditions. Three contrasts are used: in 100% D_2O, the total signal is seen; in 40% D_2O only the F127 is visible and in 85% D_2O, the polyelectrolyte is visible. The temperature is set at 37°C. All the samples are perfectly transparent. The experiment is performed on D33 with three instrument settings; λ = 6 Å and two detector distances 2 and 12.5 m and λ = 13 Å with D= 12.5 m to cover a q-range from 0.0015 to 0.45 $Å^{-1}$. The data reduction is performed using the Lamp software.

The scattering curves obtained at full contrast in D_2O, at the 2 ionic strengths and for the two HA molecular weights are shown in figures 1, a to d.



a b

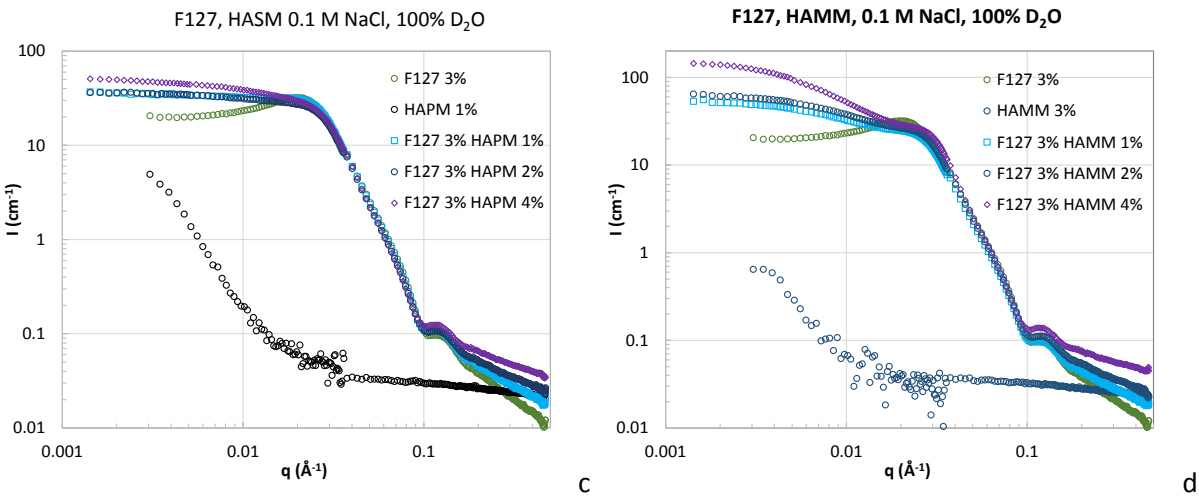


Figure 1: Scattering intensity at full contrast: a and b in pureD_2O for HASM and HAMM; c and d in 0.1M NaCl for HASM and HAMM.

The HA signal is very weak compared to the F127 signal. In pure D_2O, a weak so-called polyelectrolyte peak is observed around 0.09 $Å^{-1}$. In brine, due to the screening of the electrostatic charges, the HA chain adopts a globular conformation.

Both in D_2O and in 0.1M NaCl, F127 forms monodisperse micelles. The oscillation at 0.1 $Å^{-1}$, is characteristic from the micellar core with a radius of 40 Å. The PEO shell is hydrated at 90% and is almost invisible by neutrons. At 2 10^{-3} $Å^{-1}$, a structure factor is clearly visible corresponding to an average distance between two adjacent micelles of 317 Å.

HA does not modify the position of the oscillation, an evidence that the micellar core size remains unchanged. In D_2O, HA as only a very small effect on the intensity a low q. On the opposite, in brine, we observe the disappearance of the interaction peak and an increase of the scattering intensity at low angles depending on the HA concentration and its mass. The curves still reach a plateau at the lowest q values, the signature of the presence of larger objects with nevertheless a finite size.

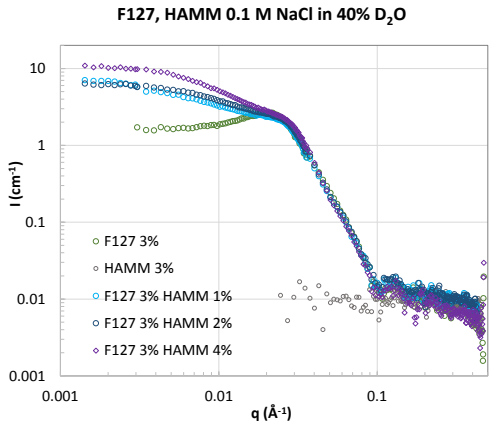


Figure 2: Scattering intensity in 40%D_2O:F127 3vol% in 0.1M NaCl with HAMM.

The scattering data in 40%D_2O are shown in Figure 2. The curves are similar to those measured in full contrast in D_2O, after normalization by the contrast factor $(\Delta\rho)^2$. In the last contrast, 15%D_2O / 85%H_2O, it was unfortunately not possible to extract the HA scattering signal from the incoherent scattering coming from the solvent.

In first conclusions:

Complementary SAXS measurements performed on D2AM (ESRF) have confirmed that the addition of HA does not modify the structure (shape and inner and outer radii) of the F127 micelles. In pure D_2O, the micelle spatial arrangement is not affected by the polyelectrolyte. In 0.1M NaCl, the increase of the scattering intensity at low angle reveals the formation of small micelle clusters with a finite size (Figure 3). The motor of the controlled aggregation is still under discussion. In such nonionic / charge system, hydrogen bounds play an important role [3]. First, the HA chain conformation change by addition of salt can drag the micelles linked to the polyelectrolyte chain. Or the second possibility is that by adopting a more globular conformation and a larger excluded volume, the HA expelled the micelles that form small clusters. The two hypothesis are in agreement with the fact that the longest chain, either by dragging more micelles or with a larger excluded volume induces the formation of larger clusters.

From this one day experiment emerges many interrogations to understand these mixed assemblies, the detailed structure and the driving forces. A major question is the role of the hydrophobic forces and solvation. The salt concentration, the nature of the salt (Hofmeister series) and the temperature are the three different parameters which allows to tune continuously the water solvation. In parallel, micro-DSC measurements (equipment available in the PSCM) are performed to investigate how the CMT is modified.

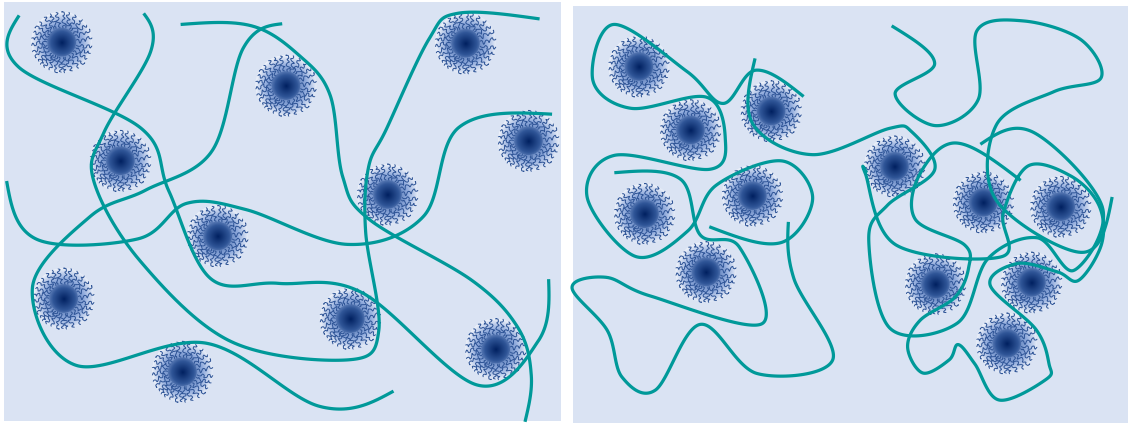


Figure 3: Schematic representation of the structure of the F127 / HA system. Left: without salt; right: with salt.

References

1 Valero M., Grillo I., Dreiss C. J. Phys. Chem. B (2012),
2 Alexander S., Cosgrove T., Castle T., Grillo I., Prescott S. J. Phys. Chem. B (2012) 116, 11545−11551
3 Cabane B., Duplessix R., J. Physique (1982), 43, 1529-1549