Experimental report

08/01/2016

Proposal: 9-12-417 **Council:** 4/2015

Title: Formation of pluronic micellar clusters induced by the change of chain conformation of hyaluronic acid after addition of salt

Research area: Soft condensed matter

This proposal is a continuation of 9-13-564

Main proposer: **Isabelle GRILLO**

Experimental team: Isabelle GRILLO
Isabelle MORFIN

Local contacts: Isabelle GRILLO

Samples: D2O
Hyaluronic acid
Pluronic F127
salt : NaCl, KCl, NaBr, KBr

Instrument	Requested days	Allocated days	From	To
D22	2	0		
D11	2	0		
D33	2	2	10/12/2015	12/12/2015

Abstract:

The structure of complexes formed between nonionic surfactants and polyelectrolytes as well as the nature of the interactions are still discussed in literature. We work with the F127 Pluronic, a nonionic triblock copolymer and hyaluronan (HA), a linear semirigid polyelectrolyte. A first SANS experiment has evidenced the formation of small micelle cluster after addition of NaCl (0.1M) which induces the change of conformation of the polyelectrolyte chain. We propose to continue the SANS characterization by varying the nature of the salt, the salt concentration and the temperature in order to evidence the nature of the forces involved in the complex formation.
In addition, such mixed system has a potential application for drug encapsulation and delivery. F127 is biocompatible and form micelles of ca. 200 Å, a perfectly adequate size for pharmaceutical applications. HA is currently investigated as a target-specific material because many malignant cancer cells overexpress HA receptors such as cluster determinant 44 (CD44) and thus incorporation or conjugation of HA can be used as targeting ligand.

Pluronic micellar clusters induced by the change of chain conformation of hyaluronic acid after addition of salt: a SANS structural characterisation

The aim of the study is to characterize the structure of assemblies formed by the F127 pluronic micelles and the hyaluronic acid (HA), two perfectly bio-compatible molecules. 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 is purchase from Sigma. HA, with two molecular weights - 12.6 and 300 kDa, called HA20 and HA300 respectivel, are kindly provided by Soliance. The composition of the system is given by Φ_F, the volume fraction of Pluronic and Φ_{HA}, the volume fraction of HA.

From a previous SANS experiment (see report 9-13-564, D33, October 2014) we have learnt that HA does not modify the spherical shape of the micelle, nor the size of the inner hydrophobic core and of the shell thickness. Without added salt, the spatial micelle arrangement is not either modified by HA. With 0.1M of NaCl, one observes the disappearance of the peak of the structure factor and an increase of the scattering at low q which augments with the amount of HA and with its molar mass. Consequently, there is the formation of micellar clusters with a finite size.

In this new experiment, for Φ_F = 3% and in presence of 0.1M of NaCl, the HA volume fraction has been increased up to 10%. The scattering curves are presented in Figure 1. The increase of the HA concentration induces an increase of the scattering at low q, which at a given volume fraction is more pronounced for HA300 than for HA20. After a critical volume fraction, Φ^*_{HA}, found at 8% for HA20 and 4% for HA300, the sample become turbid and the micelles collapse into a face centred cubic (fcc) phase.

The increase of the NaCl concentration has no effect on the pure F127 micelles up to 2M. For Φ_F = 3% and Φ_{HA} = 0.2%, with HA20, the increase of the NaCl salt concentration induces up to 0.5M a strong increase of the scattering intensity at low q, followed by a phase separation at 1M, where upper phase is enriched in micelles separated by 160 Å, as calculated with the position of the correlation peak. The same effect is observed with HA300, but at a lower salt concentration where first a cubic phase appear (0.15M) and then phase separation occur (0.5M). (Figure 2)

The effect of the nature of the salt at a concentration of 0.1M has also been investigated (Figure 3). For the monovalent salts, NaCl, NaBr, KCl and KBr, only small differences are observed. NaBr and KBr induce a slightly more pronounced increase of the intensity at low angles than NaCl and KCl. In the Hofmeister series Br^- is more hydrophobic (chaotropic) than Cl^- and should have the tendency to adsorb in the pluronic shell [3]. The divalent salt $CaCl_2$, however has a strong effect on the formation of micellar clusters with HA20 and induces the collapse and the formation of a fcc phase with HA300.

Finally, the temperature increase has a similar effect as the salt concentration. For Φ_F = 3% and Φ_{HA} = 0.2%, the fcc phase is formed at 45°C and 55°C for HA300 and HA20 respectively.

The motor of the reorganisation is related to the modification of the conformation of the HA chains. Addition of salt screens the electrostatic interaction leading to a more folded and globular conformation of the polyelectrolyte chain. The formation of micellar clusters by increasing the ionic strength, the HA volume fraction or the HA molar mass are indications for a depletion mechanism. Depletion interaction may occur when a non adsorbing polymer is added to a colloidal suspension. The conformational entropy restriction of the chains at the particle surface creates a depletion zone from where the polymer is preferentially excluded.

In dilute condition when there is no overlap, the osmotic pressure on the spheres due to the polymer solution is isotropic. For overlapping depletion layers the osmotic pressure on the spheres is unbalanced and exert an attractive force. A schematic picture from the micellar organization is proposed in Figure 4.

Due to a lake of time, the second part of the experiment dedicated to the formation of the micellar clusters followed by the collapse in a fcc structure and the reversibility of the transition by using the in-situ dialysis cell has not been performed.

Experimental: the experiment was performed on D33. 3 instrument configurations, λ= 13 Å, D= 12.5m; λ= 6 Å, D= 12.5m and 2m were used to cover a q range from 1.5 10^{-3} to 0.45 $Å^{-1}$. For the cubic organization, an intermediate configuration at 5m with a long collimation of 12.6m is used to obtain a good resolution of the Bragg peaks.

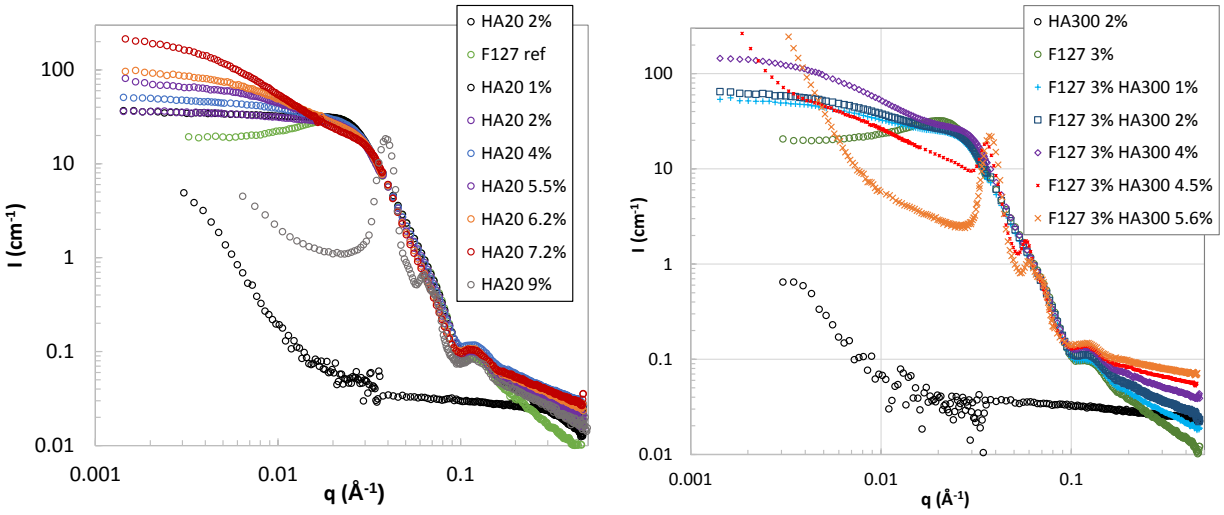

Figure 1: T=37°C. [NaCl) = 0.1M. Influence of HA volume fraction on the structure of HA/ F127 micelles. (Left) HA20; (Right) HA300.

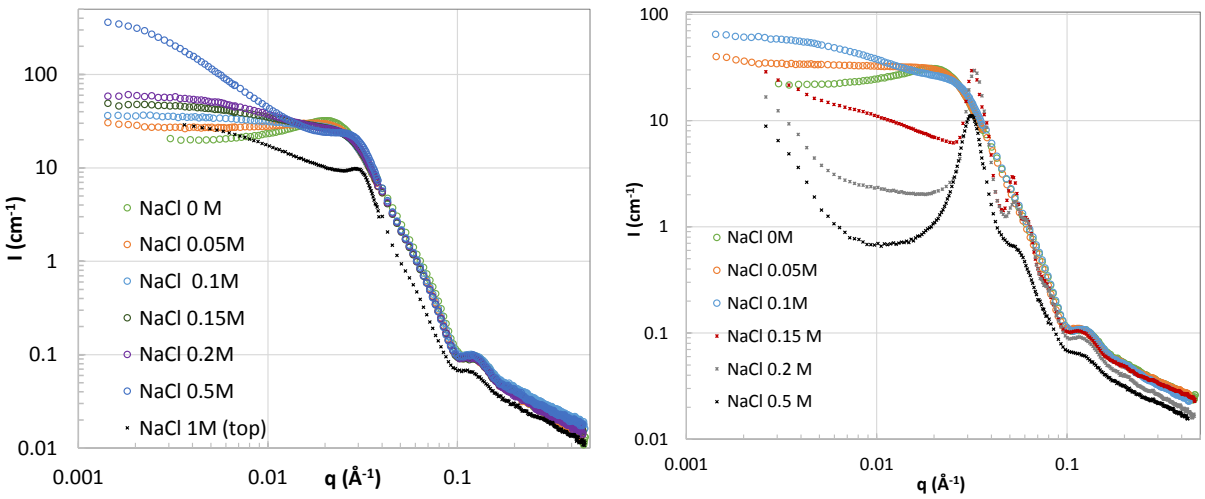

Figure 2: T=37°C. Φ_F = 3% and Φ_{HA} = 0.2%. Effect of the NaCl concentration on the structure of HA/ F127 micelles. (Left) HA20; (Right) HA300.

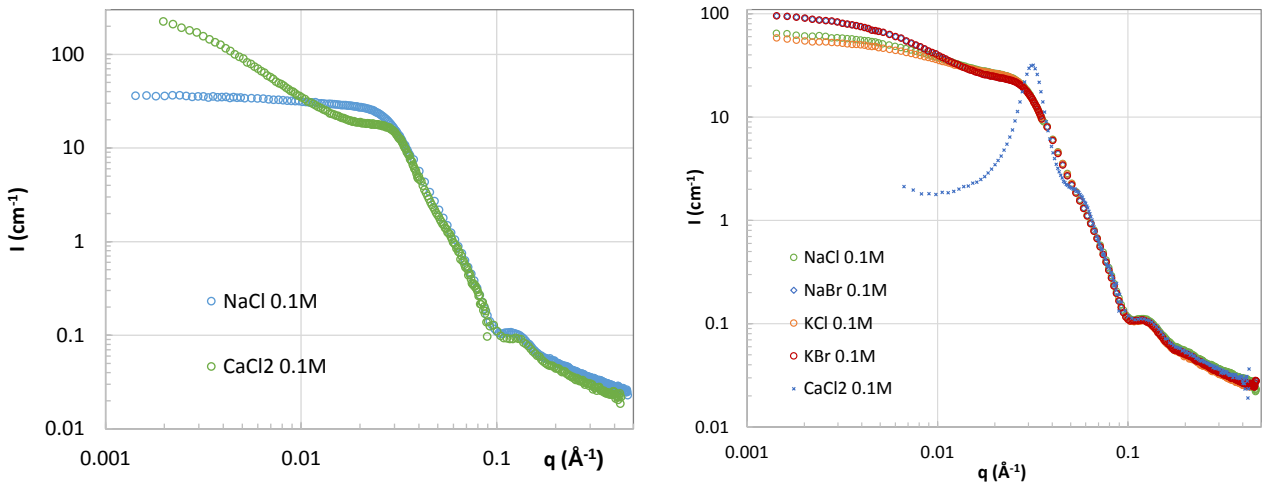

Figure 3: T=37°C. Φ_F = 3% and Φ_{HA} = 0.2%. Effect of the nature of the salt on the structure of HA/ F127 micelles. (Left) HA20; (Right) HA300.

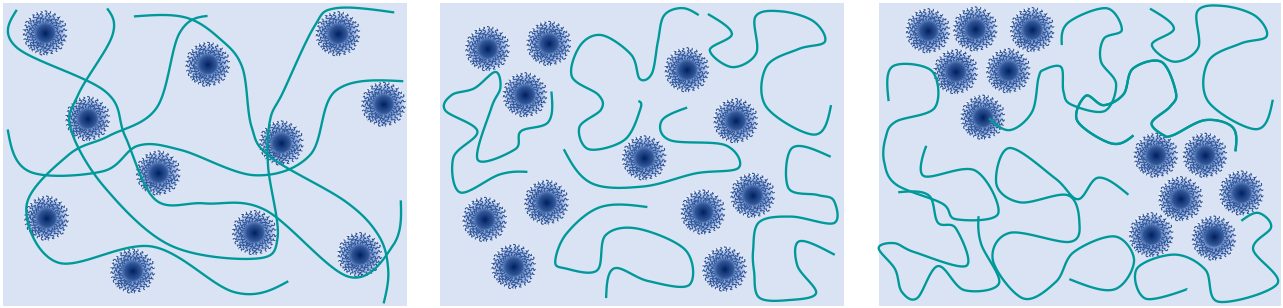

Figure 4: Schematic representation of the HA / Pluronic structure. (Left) In D_2O, the micellar organisation is not affected by the presence of HA. (Middle) In presence of salt, the HA chain adopts of more globular shape that induce the formation of small micellar clusters. (Right) The increase of the HA concentration in presence of salt induces the formation of a fcc phase through a depletion mechanism.

References:

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2 Alexander S., Cosgrove T., Castle T., Grillo I., Prescott S. J. Phys. Chem. B (2012) 116, 11545−11551
3 Manet et Al. J. Phys. Chem. B (2011) 115, 11318–11329