

EXPERIMENTS REPORT – Proposal 02-01 852

Polyelectrolyte Complexes obtained by The Desalting of Solutions of Oppositely Charged Polysaccharides

Introduction

Hyaluronic acid (HA) is an anionic polysaccharide consisting of a repeating disaccharide unit composed of D-glucuronic acid and D-N-acetylglucosamine, linked via β -1,4 and β -1,3 glycosidic bonds. HA is naturally present in the human body, such as in the skin, cartilage and the vitreous humour. Chitosan (CS) is the only positively charged, naturally occurring polysaccharide. It is obtained from the partial deacetylation of chitin, its parent polymer extracted from the exoskeleton of crustaceans or endoskeleton of cephalopods. Hence, chitosan is a copolymer of N-acetyl glucosamine and glucosamine, known for its low toxicity, bioactivity, biodegradability and mucoadhesion.

Polyelectrolyte complexes (PECs) were prepared from CS and HA mixtures in aqueous solutions. To this end, we developed a specific processing route which can be seen as a green process since it takes place in water, without any organic solvent. This method consisted in elaborating a homogeneous mixture of the two polysaccharides at high salt concentration. Then, the mixture was dialyzed, leading to the self-assembly of the two polymers, as already described for CS and Dextran Sulfate by Costalat et al.¹

A systematic study was undertaken by varying the acetylation degree and the molar mass of chitosan. By using the dialysis method against water, we determined some critical parameters accounting for the transition from homogeneous aqueous solutions of CS and HA to coacervates or dispersed precipitates.

SAXS analysis is a convenient method to analyze the structure of polyelectrolytes offering a characterization of solution ordering by the features of the so-called polyelectrolyte peak. Polyelectrolyte complexes can also be studied to evidence their nanostructure i.e. ladder-like or scrambled egg-like associations.

Materials and methods

In this work, we used a CS from shrimp shell provided by Mahtani Pvt. Ltd. (India). Chitosan was reacylated in homogeneous media to study the impact of the DA and depolymerized by nitrous deamination to study the impact of the molecular weight.

HA was used as received from HTL (France) with a molecular weight $M_w \approx 1000$ kg/mol.

SAXS experiments were performed in D2AM Beamline from 22nd to 24th of October and from 1st to 3rd of December 2014. The sample-to-detector distance was about 1.15 meters, the incident energy of X-rays was set at 17 keV.

Results and discussion

Structure of HA in solution

First of all, we studied the conformation of the chains of HA in solution. Two important parameters were the polymer concentration and the salt concentration in the solution.

Influence of the concentration

We prepared HA solutions with varying concentrations. The corresponding SAXS profiles $I(q)$ are represented on Fig. 1 for a q range from 0.01 to 0.32 \AA^{-1} .

For all the concentrations, we observed a broad peak in the analyzed q range. This peak is characteristic of the conformation of polyelectrolyte chains in solution. Due to repulsive electrostatic interactions between the polyelectrolyte chains, the polymer chains adopt a conformation such as typical repeated distances appear, characterized by the so-called "polyelectrolyte peak". The evolution of the location of this peak with polymer concentration is related to the hydrophobicity /hydrophilicity of the polyelectrolyte in aqueous solutions.

As HA concentration increases, the SAXS diagrams revealed that the polyelectrolyte peak was shifted to higher q values, corresponding to smaller inter-chains distances (or correlation distances).

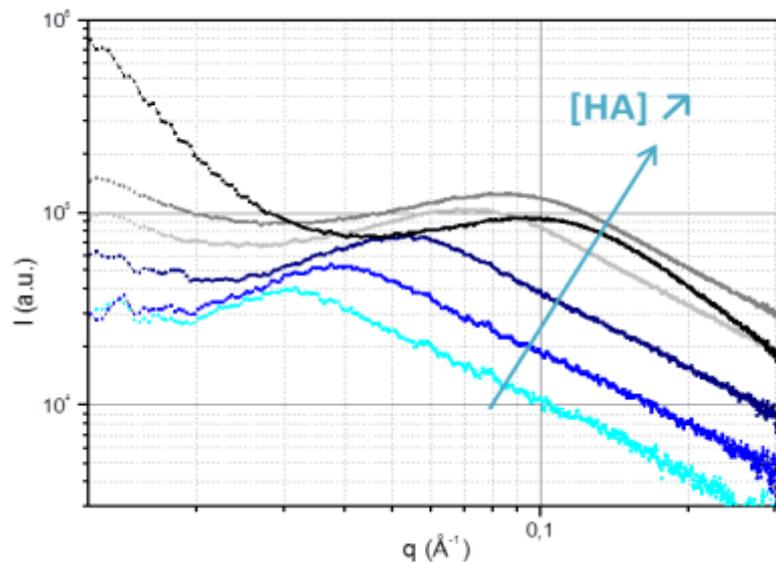


Fig. 1 : SAXS analysis of HA in solution at different concentrations.

We performed a Lorentz fitting on the curves, which led to the position of the maximum intensity of the peak q_{\max} . The values are reported on Table 1. Then, the position q_{\max} is plotted as a function of the HA concentration (Fig. 2).

For polyelectrolytes in solution, a scaling model proposed by Dobrynin et al.² consists in considering the polyelectrolyte chains conformation as beads necklaces. Depending on the polymer concentration, it predicts a transition from a string-controlled conformation (where the correlation length ξ is larger than the string length; $q_{\max} \propto C_p^{-1/2}$) to a pearl necklace model where the chain

conformation is governed by the beads (when ξ is of the order of the distance between the beads; $q_{max} \propto C_p^{1/3}$).

HA Concentration	Position q_{max} (\AA^{-1})
C_1	0.031
C_2	0.040
C_3	0.055
C_4	0.073
C_5	0.085
C_6	0.092

Table 1: Results of the Lorentz fitting on SAXS curves.

In the following complexation study, the HA concentration will be about 3% so the conformation of HA chains will be in the beads-controlled domain. The correlation length, i.e the distance between the beads can be determined with equation (1). The calculated correlation length ξ was found about 74 \AA .

$$\xi = 2\pi/q_{max} \quad (1)$$

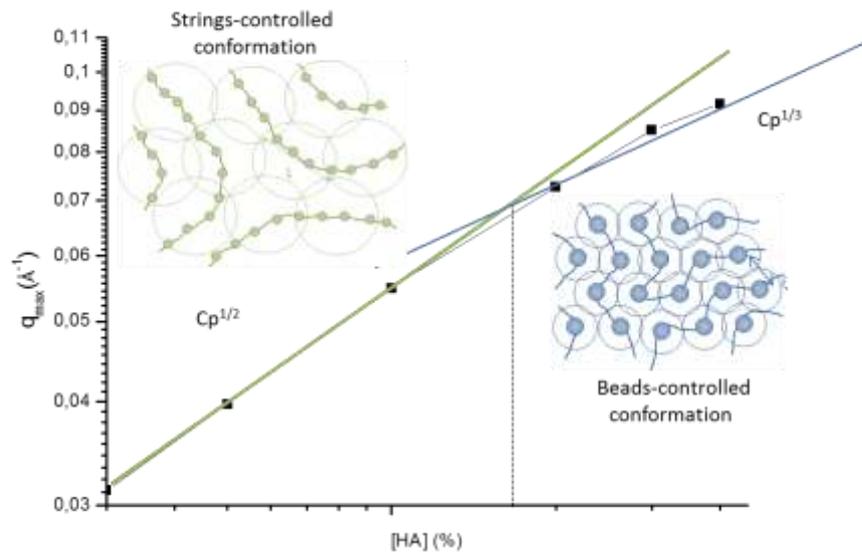


Fig. 2 : Evolution of the position q_{max} as a function of HA concentration.

Influence of the salt concentration

As we prepare the HA and CS mixture in presence of salts, we studied the evolution of the chain conformation in the presence of salts.

We prepared HA solutions at fixed concentrations and with various amounts of added sodium chloride. The SAXS diagrams (Fig. 3) revealed an outstanding reproducibility. In the salt-free solution, we found again the well-defined polyelectrolyte peak. As the salt concentration increases, the scattered intensity increases and the polyelectrolyte peak disappears for sodium chloride concentration higher than 0.1 mol.L^{-1} . These diagrams also revealed the presence of a pseudo-isobestic point, reflecting the existence of only two possible structural states. The first state corresponds to the classical polyelectrolyte conformation of a salt-free polyelectrolyte solution, and the other state corresponds to a more hydrophobic context with aggregated chains.

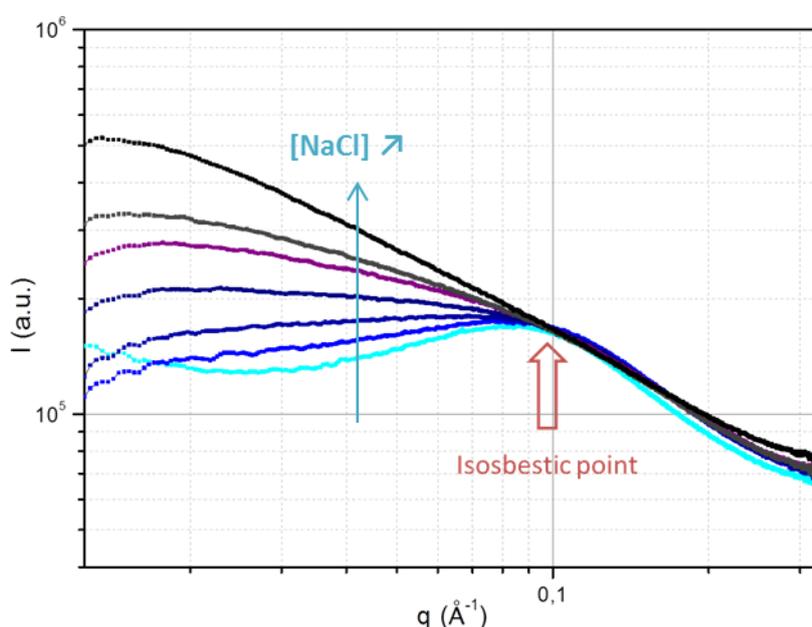


Fig. 3 : Influence of the salt concentration on the chains conformation of HA in solution.

Study of the structure of PECs

A series of PECs have been prepared varying the charge ratio of CS with respect to the charges of HA. The HA concentration was kept fixed for the whole study and the CS concentration was varied depending on the expected ratio and the DA. This part of the work focuses on the comparison of polyelectrolyte complexes obtained at two charge ratios.

Charge ratio $r = r_1$

Polyelectrolyte complexes were formed by desalting a homogeneous mixture of HA and CS with a charge ratio $r = r_1$. The starting salt concentration was the same for all the samples and desalting was achieved by dialysis against deionized water. The resulting PECs (opalescent/turbid solutions) were analyzed by SAXS. Here, we compared the structure of PECs with different DA for CS with medium molecular weight. The diagram of the free-salt HA solution at the same concentration has been added for the sake of comparison (Fig. 4).

For all the DAs, the SAXS diagrams revealed the existence of the polyelectrolyte peak. The position of this peak is almost independent of the DA of CS. As the CS is added in default, we can assume that all the CS is completely complexed with the HA and the polyelectrolyte peak can be attributed to partially-complexed chains of HA. Compared to the HA solution, the position q_{\max} is slightly different and is shifted to lower q values, i.e. to higher values of the correlation length ξ .

At low q values (inferior to 0.04 \AA^{-1}) scattering is due to aggregates that increase with DA. These observations are consistent with the macroscopic observations of turbidity.

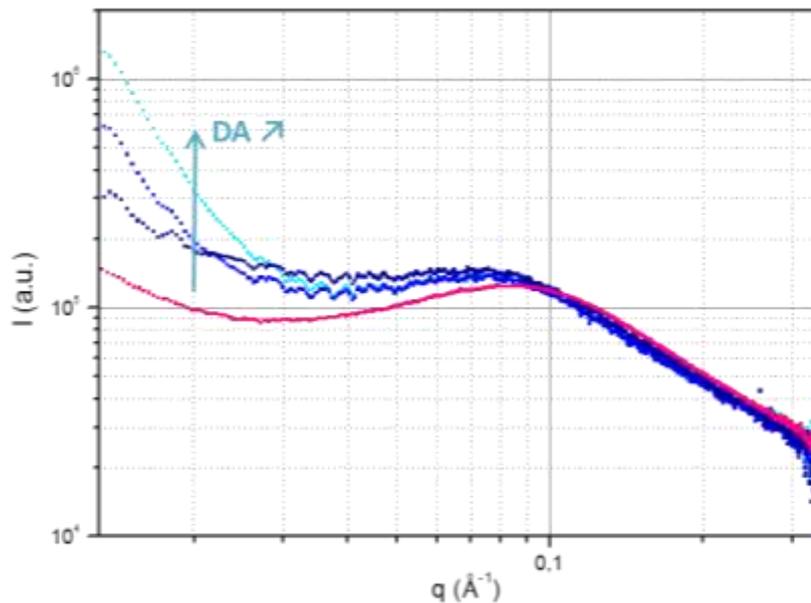


Fig. 4 : Influence of the DA for PECs containing HA at fixed concentration and CS with a charge ratio $r = r_1$ and comparison to a salt-free HA solution.

Charge ratio $r = r_2$

Here, polyelectrolyte complexes were formed by desalting a homogeneous mixture of HA and CS with a charge ratio $r = r_2$. Again, the started salt concentration was the same for all the samples and desalting was achieved by dialysis against deionized water. The resulting PECs (coacervates) were analyzed by SAXS. Here, we compared the structure of PECs with CS of various DA but similar molecular weight.

For all the DAs, the SAXS diagrams (Fig. 5) do not disclosed the existence of the classical polyelectrolyte peak. The CS concentration was important enough to suppress the polyelectrolyte behavior. The observed differences in the scattered intensities are probably impacted by the concentration variations as the syneresis leading to coacervates is difficult to control.

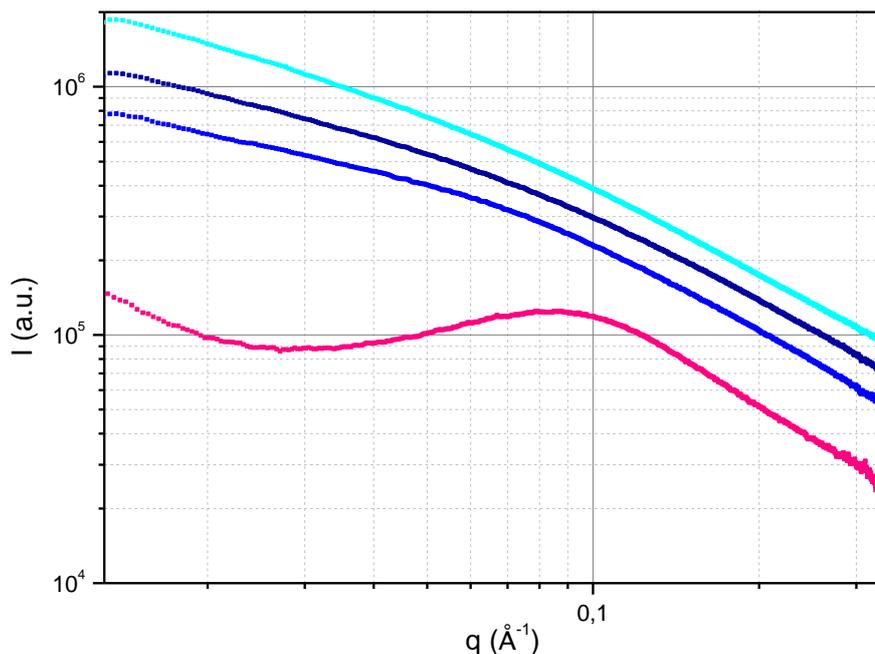


Fig. 5: Influence of the DA for PECs containing HA at 3% (w/v) and CS with a charge ratio $r=0.5$ and comparison to a salt-free HA solution.

Conclusions and perspectives

We studied the conformation of HA in salt-free solution and in presence of salts. We further characterized the structure of the polyelectrolyte complexes formed by association of HA with CS by mixed solution desalting. At low CS concentration, the SAXS diagrams revealed that the conformation of HA chains is not heavily affected and that the soluble PECs exhibit a behavior similar to that of HA alone. When the CS quantity is increased, the PECs take the form of coacervates, i.e. a dense phase containing the two complexed polymers with a completely different nanostructure.

In order to improve our understanding of the formation of the PECs, in particular for coacervate systems, it could be interesting to evaluate the impact of the concentration of CS on the chains conformation (depending on the DA and the molar mass of CS).

On the other hand, we also found out that desalting a homogeneous solution of CS and HA in an acidic media ($\text{pH} < 4$) led to highly stretchable coacervates. The SAXS characterization could give us information about the structure which could explain the interesting mechanical behavior of these materials.

References

1. Costalat, M.; David, L.; Delair, T. *Carbohydr. Polym.*, **2014**, *102*, 717–26.
2. Dobrynin, A.V.; Rubinstein, M. *Macromolecules*, **1999**, *32*(3), 915–22.