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Ordering in Bio-Polyelectrolyte Chitosan Solutions

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Summary: The scaling of the polyelectrolyte scattering peak in chitosan solutions, as deduced from the relation $q_{max} \sim c_p^{\alpha}$ was studied by synchrotron SAXS as a function of the charge density of the polymer. We observe a variation in the α exponent corresponding to the limit of the ionic condensation, by varying the degree of acetylation of the polymer. The nature of the solution medium also affects the polyelectrolyte peak, and it is shown that in alcoholic/water mixtures, the lower dissociation of the acid induces a lower charge density, thus influencing the polyelectrolyte ordering.

Keywords: chitosan; lattice model; polyelectrolyte; SAXS

Introduction.

Chitosan is obtained by chemical modification (deacetylation) of chitin, one of the most widespread natural polymers with cellulose. Both polymers exhibit the same chemical structure, as they are linear copolymers of N-acetyl-D-glucosamine and D-glusoamine with β,(1→4) glycosidic linkages. Chitosan is obtained when the Degree of Acetylation (*DA*[1]) is below 60%. This results into a soluble polyelectrolyte polymer in diluted acidic solutions at pH<6, well below the pK_0 of the NH_3^+ moieties. Chitosan can also be obtained in other physical forms such as gels [1,2], soft and hard solids [2], bulk samples, films, fibres[3] and also more complex structures with macroscopic property gradients [4]. At last, this polymer is now well-known for its biological interest with interesting antumoral[5], antifungal[6] and antibacterial[7] activity, but mostly for bioactivity with applications for wound healing in various soft and hard tissues[8,9].

Our aim is to gain insight into the structure and microstructure of the chitosan polymers in order to better explain their macroscopic properties in their various physical states,

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[1] In the experimental conditions of our study (pH<6), the fraction of charged monomer f in chitosan polyelectrolyte polymer is related to the Degree of Acetylation *DA* by f=1-*DA*.

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together with their biological features. This work is devoted to chitosan in water and alcoholic solutions in salt-free conditions.

Experimental

Materials

Two weakly acetylated chitosans were used. They were produced from squid pens and purchased from France Chitine (batch 11.03, *DA* ~2.6%, M_w ~540000 g.mol^{-1} and batch 114, *DA* ~5.2%, M_w ~530000 g.mol^{-1}). Prior to use, the polymers were purified according to[10]. All other samples (*DA*=36.7; *DA*=46.2 and *DA*=65.5%) were prepared by homogeneous re-acetylation of the chitosan of an initial *DA*=5.2 % with acetic anhydride in a water/alcohol solution[11].

Experimental techniques

We studied the so-called 'polyelectrolyte peak' by small angle synchrotron X-ray scattering (ESRF-Grenoble France BM2-D2AM beamline). A synchrotron source was required for our study because the intensity scattered by the polyelectrolyte solution is not measurable by conventional sources.

The data were collected at an incident photon energy of 16 keV. We used a bi-dimensional detector (CCD camera from *Ropper Scientific*). All the data corrections were performed by the software *bm2img* available on D2AM beamline. The data were corrected from the dark and flat field responses of the detector. Moreover, the background (cell filled with water) was subtracted. The distortion introduced by the optical fiber bundle was also corrected. Lastly, the radial average around the image center (location of the center of the incident beam) was performed for the *q*-range calibration standard (silver behenate) and the chitosan solutions.

The viscous solutions were introduced in Low Density PolyEthylene (LDPE) cylindrical sample holders (internal diameter~5mm) with two holes perpendicular to the main axis, closed by adhesive Kapton in order to avoid the scattering contribution of LDPE.

Results

In all studied solutions, the scattering intensity could be separated into (*i*) a low angle contribution arising from long range electronic density fluctuations and associated with chain aggregation and (*ii*) the 'polyelectrolyte peak' itself. We studied the shape of the scattering curve from the phenomenological relation accounting for both scattering components:

$$I(q) = \frac{C}{q^{\gamma}} + \frac{B}{4\left[\frac{q - q_{\text{max}}}{w}\right]^{2}} \qquad (1)$$

This equation is very similar to that proposed by Wang[12], and only differs by an additionnal constant term that will be not used here. In equation 1, q_{max} is the location of the Lorentzian peak, w is the full width at half maximum, and B is the intensity of the polyelectrolyte peak. We thus consider that the value of q_{max} is close to $2\pi/d$ where d is the most probable interchain distance. An exemple of modelling is given in figure 1. Although we observed some evolutions of the parameter C and γ describing the contribution of the long range fluctuations, we will only report on the parameters associated with the polyelectrolyte peak. The dependence of q_{max} with the polymer concentration c_{p} will be described, as usual, by the scaling relation:

$$q_{\text{max}} = q_0 . c_p^{\alpha} \qquad (2)$$

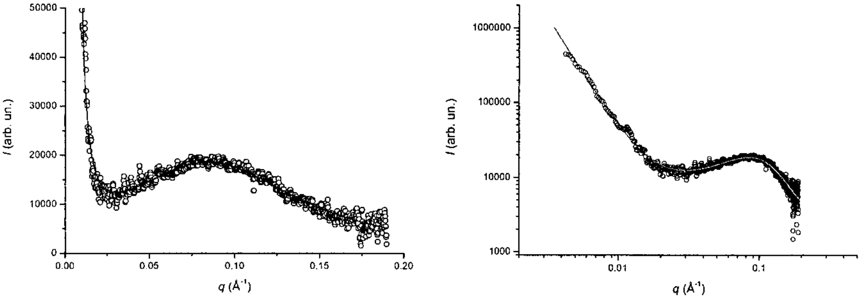

Figure 1. Example of modeling of a scattering curve according to equation 1 (solid line with C=0.045 arb.un. Å$^{\gamma}$; γ=3; B=18500 arb.un. Å^2; q_{max}=0.085 Å^{-1}; w=0.13 Å^{-1}) and experimental data (○) for chitosan acetate aqueous solution, with DA=5.2% and polymer concentration c_{p}=0.015 g/g. Left: linear representation, Right: log-log representation.

Influence of *DA* on the polyelectrolyte peak

The concentration dependence of the location of the polyelectrolyte peak, for different *DA* values was characterized in the case of chitosan acetate in water. The power law (equation 2) is well adapted to describe the results in a large concentration range. The power laws observed for low *DA* (*i.e.* at 2.6 and 5.2%) exhibit a higher exponent than for the high *DA* values (46.2% and 65.5%). The values of the scaling exponent α are given by figure 2 as a function of the degree of acetylation.

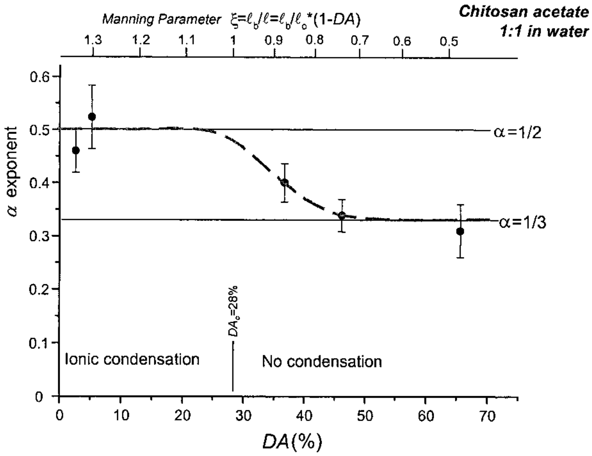

Figure 2. Change in the α exponent of the scaling relation (2), as a function of *DA* for chitosan acetate in water. The transition *DA* between α~1/2 to α~1/3 is close to the ionic condensation limit, as predicted by Manning theory.

The transition observed from low to high *DA* bears many similarities with that reported by Baigl[13] in the case of partly sulfonated polystyrene. The transition looks continuous, and the polyelectrolyte peak is broader close to the ionic condensation[14] limit, at *DA*~28%[15] as shown on figure 3. Indeed the Manning parameter is theoretically related to the *DA* value by $\xi=[\ell_B/\ell_o](1\text{-}DA)$ where ℓ_B is the Bjerrum length (7.1 Å in water) and ℓ_o is the length of the repeating unit (5.1 Å in the case of chitosan).

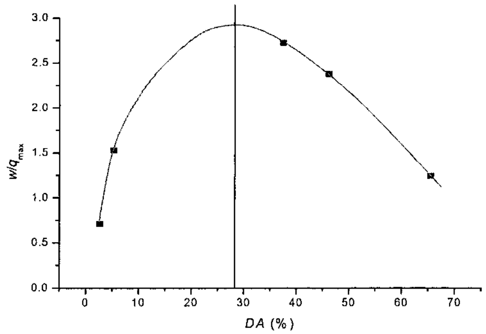

Figure 3. Evolution of the breadth parameter of the polyelectrolyte peak, w/q_{max} as a function of *DA* for chitosan acetate in water at c_p=1.5%

We can interpret these data as the transition between a structure consisting in a collection of parallel polyelectrolyte rod-like chains (with $d\sim c_{\mathrm{p}}^{-1/2}$)[16] at low *DA* when ionic condensation is present, to a solution with punctual electrostatic interaction sites (with $d\sim c_{\mathrm{p}}^{-1/3}$) when the apparent charge density of the polymer decreases, escaping from ionic condensation. In the *DA* range associated with a Manning parameter ξ close to unity, the coexistance of both structural regimes can explain the increase of the breadth of the polyelectrolyte peak.

Influence of the solution medium on the polyelectrolyte peak

For a very low *DA* chitosan (2.6%), addition of 1,2 propanediol to the solution decreased the amplitude of the polyelectrolyte peak and shifted the polyelectrolyte peak to larger distances (see figure 4). Moreover, the breadth parameter of the polyelectrolyte peak was largely increased in the high q range, so that the Lorentzian modeling is not adequate in this case. At higher alcohol concentrations, we observed a disappearance of the polyelectrolyte peak and the formation of a gel. An evolution of the dielectric constant of the solution medium alone can not be invoked to explain the results: the decrease of the relative dielectric constant form 80 in water to 27.5 in pure alcoholic medium leads to a larger Bjerrum length $\ell_{\mathrm{B}}=\frac{q^2}{\varepsilon kT}$ and thus should promote Manning condensation ordering in the polyelectrolyte solution. As a result, we interpret our results in terms of a decrease of apparent density of charge of the chains, since the dissociation of acetic acid is lower in hydro-alcoholic media.

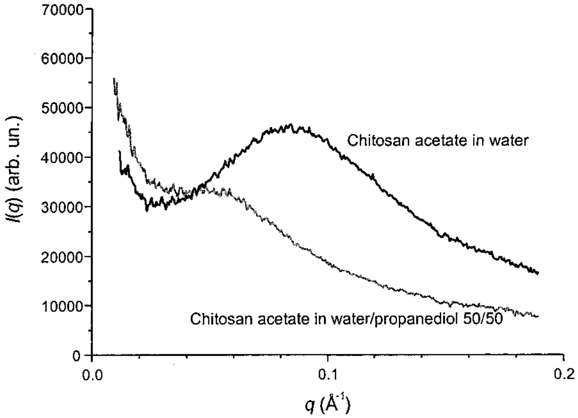

Figure 4. Evolution of the polyelectrolyte peaks for chitosan acetate in water (black line) and in water/propanediol for a concentration of 1.5% and a *DA* of 2.6%

Conclusion

Chitosan is a versatile and unique natural cationic polyelectrolyte, enabling the fundamental study of polyelectrolyte solutions ordering in various experimental conditions. The role of DA is to change the apparent charge density that in turn controls the structure of chitosan solutions. We observed the polyelectrolyte peak in the ionic condensation regime and out of the condensation regime: the scaling exponent α yielding the location of the polyelectrolyte peak $q_{\max}$ as a function of polymer concentration changes from 1/2 to 1/3 by increasing the degree of acetylation from below 28% to above 28%, *i.e.* the Manning parameter from above 1 to below 1. This transition[13] is rather gradual, and in the vicinity of the limit between the two ordering regimes, the width of the polyelectrolyte peak is the largest, as a result of the coexistence of different arrangement types. Such an effect is qualitatively observed if the solution medium is enriched in propanediol, thus inducing a lower dissociation of the acid, and thus, again, a lower charged fraction of the polyelectrolyte.

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