

## VERSATILE AND EFFICIENT FORMATION OF NANO-PARTICLES OF POLYSACCHARIDE-BASED POLYELECTROLYTE COMPLEXES.

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### Abstract

The present contribution focuses on the formation of submicronic particles from the complexation of chitosan and dextran sulfate that are respectively a weak polybase and a strong polyacid. The purpose of these biocompatible particles is the *in vivo* delivery of biomolecules such as DNA, or proteins. The complexation of chitosan and dextran sulfate allows the formation of both cationic and anionic particles through the variation of the molar charge mixing ratio ( $n^+/n^-$ ).

Among the structural parameters of the polymers affecting the hydrodynamic diameter of the PEC particles, the molar mass is the most influent. Other parameters related to the medium of the complexation such as ionic strength or the molar concentration of polyelectrolytes are less significant. The mode of addition of components proved an interesting parameter of the complexation. Traditionally, PECs were formed by adding dropwise the solution of polyelectrolyte to the oppositely charged one. However, in our case, an important limitation was encountered, namely the irreversible flocculation of PECs as the molar mixing ratio was close to 1. In order to bypass this difficulty, the idea was to go beyond the flocculation point as quickly as possible to achieve a fast charge inversion which would allow maintaining the colloidal stability. Hence, the one-shot addition process was investigated.

Some results tend to prove that PEC particles featured a core/shell structure, the hydrophobic core resulting from the segregation of complexed segments whereas excess component in the outer shell ensured the colloidal stabilization against further coagulation. Finally, the stoichiometry of the complexes was investigated and it differed according to the structure of the polymer used in excess.

### Introduction

Polyelectrolyte complexes are formed in solution by electrostatic interactions of two polyelectrolytes of opposite charge. The complexation is entropy driven due to the release of small counterions, initially bound to the polyelectrolyte.

Water-soluble polyelectrolyte complexes can be formed with polyelectrolytes having weak ionic groups and large differences of molecular dimensions, when they are mixed in non-stoichiometric ratios.

Polyelectrolyte complexes at the colloidal level can be obtained with synthetic polyions if they are of similar molar mass and/or bare strong ionic groups. Several investigations have reported applications of fully synthetic colloidal complexes. Less detailed works reported the use of naturally occurring polymers for the formation of colloidal complexes. A variety of poly anions were used, DNA, dextrans sulfate<sup>1,2</sup>, poly- $\gamma$ -glutamic acid<sup>3</sup>, carboxymethyl konjac glucomannan<sup>4</sup>, or carboxymethyl cellulose<sup>5</sup>. The most widely used poly cation is chitosan and this is mainly motivated by its outstanding biological properties like biodegradability<sup>6</sup>, biocompatibility<sup>7</sup> and bioactivity<sup>8</sup>. In this paper we report on the synthesis of particles in the sub-micron range by complexation of dextran sulfate with chitosan. We investigated the impact of internal (molar mass, DA for chitosan) and external (polymer charge density and mode of addition) parameters on the formation of colloidal polyelectrolyte complexes,

## Material and Methods

Chitosans were purified according to<sup>1</sup>. Dextran sulfate was from Aldrich and used as such. Chitosan was dissolved in water with a stoichiometric amount of hydrochloric acid to ensure complete dissolution. Dextran sulfate was dissolved in water. The pH of the solutions was fixed to 4 and the ionic strength to 50 mM

*Formation of colloids by the dropwise addition.* First, polyelectrolyte solutions were added dropwise at a flow rate of 20 mL.h<sup>-1</sup> to oppositely charged polyelectrolyte solutions of same ionic strength and pH. A constant magnetic stirring of 500 rpm was maintained during all the titration. At defined molar mixing ratios ( $n^+/n^-$ ) between cationic and anionic charged units, aliquots of 2mL were collected. Chitosan and dextran sulfate concentrations in the mixture were re-calculated to deduce the required amount of dextran sulfate solution to get the next  $n^+/n^-$  ratio.

*Formation of colloids by the one-shot addition.* complexations were performed by the one-shot addition of a polyelectrolyte solution to the starting one at identical ionic strength, under a constant magnetic stirring of 1250 rpm. The final volume of particle dispersion was 30 mL with solid content of 0,1% W/W.

*Particle characterization.* Particle mean size and size distribution were determined by photon correlation spectroscopy using a Malvern Zetasizer HS3000. Electrophoretic mobilities were determined on the same equipment.

*Chitosan Assays.* The stoichiometry of the complex was determined by the depletion method based on the quantification of the residual polymers in the supernatants after complexation.

The residual concentration of chitosan was quantified by titration using Orange II, an anionic dye which can interact with ammonium groups of chitosan as established by Roberts et al<sup>9</sup>.

*Dextran Sulfate Assay.* The residual amount of dextran sulfate was determined by the titration of sulfate moieties with cationic dye Toluidine blue. The absorbance was measured at 584 nM.

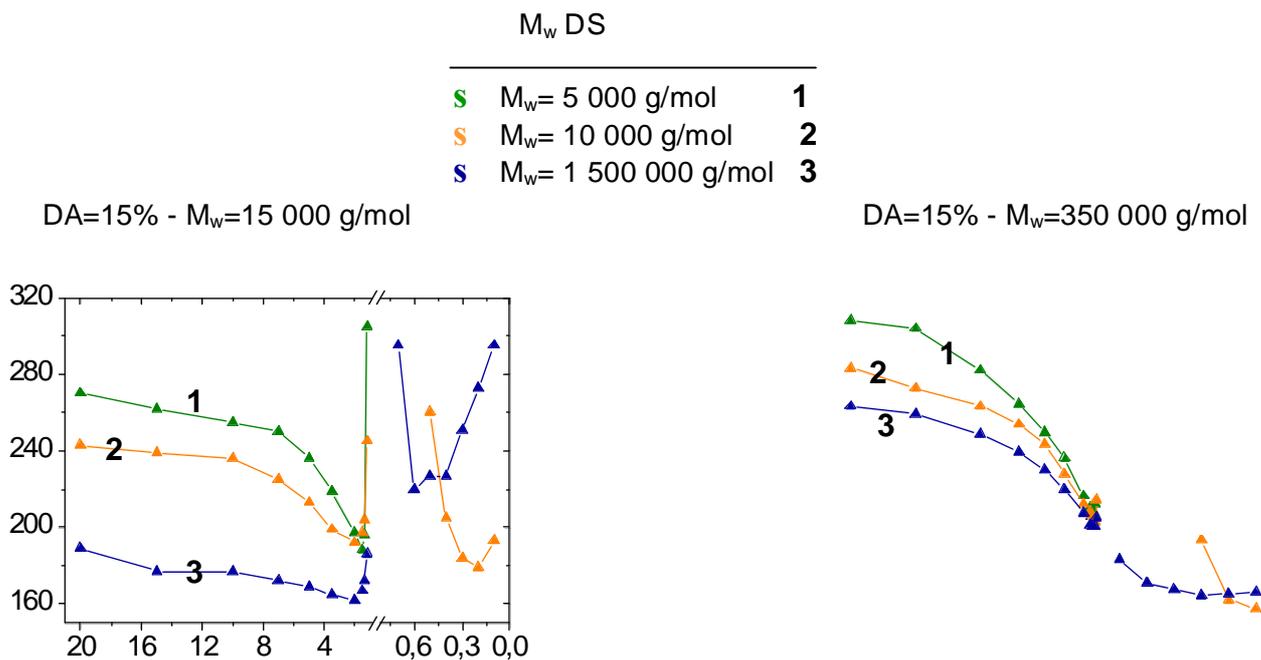
Both assays required calibration curves established before each experiment

## Results and Discussion

### *Effect of the molar masses of the polymer on the particle size*

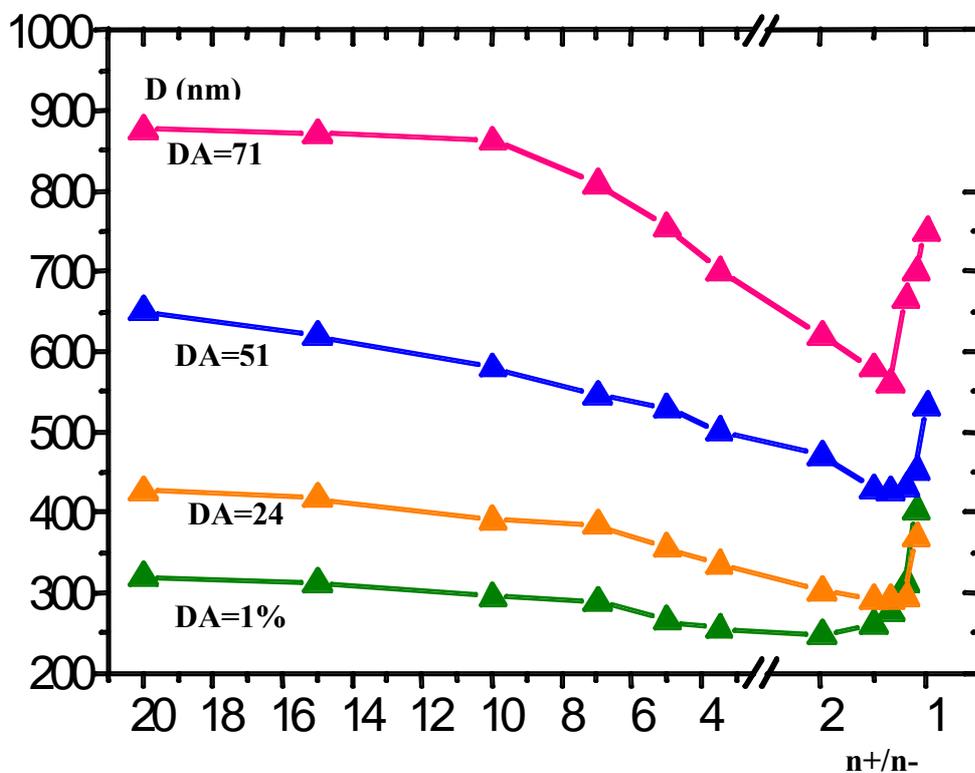
In Figure 1 are reported the variations of the hydrodynamic diameter as a function of the molar charge ratio  $R=n^+/n^-$ . An increase in the molar mass of the polymer in excess resulted in increased particle sizes (particle diameter obtained with the higher chitosan are always above 1  $\mu$ m in diameter), this was particularly true for the cationic particles ( $R>1$ ). Once the molar mass of chitosan was set, increasing the molar mass of dextran sulfate resulted in a decrease of the mean diameter. The variations of the average hydrodynamic diameter with decreasing  $R$  (for positively charged particles) can be divided into three regimes: i) a constant diameter (more obvious for the particles obtained with the low molar mass chitosan for  $20<R<8$ ), ii) a decrease in average size (for

8 < R < 1.5 with the lower molar mass polysaccharide), iii) a sharp increase as the charge ratio gets close to 1 with a flocculation of the system for complete charge neutralization.



**Figure 1 :** Effect of molar mass of the polysaccharides on the particle size

Effect of charge density of chitosan on the particle size



**Figure 2 :** Effect of the density of chitosan on the average diameter of the colloids

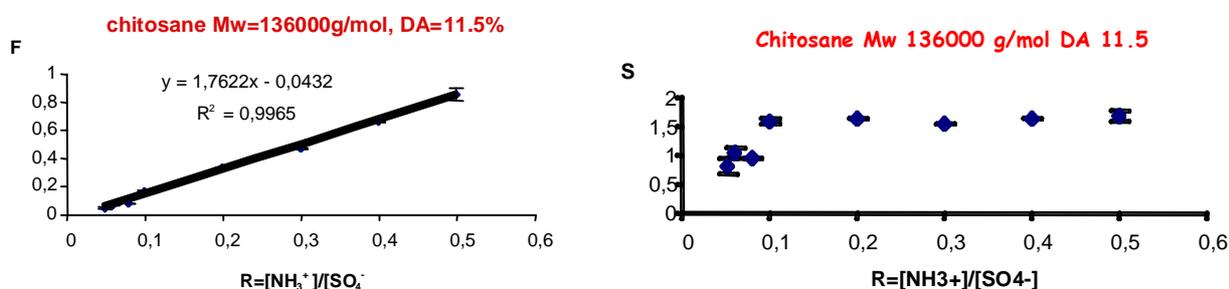
The charge density of chitosan can be varied by increasing the degree of acetylation (DA), which is the fraction of N-acetylated primary amines in the polymer chain. An increase in DA, i.e. a charge density reduction, resulted in an increase in particle diameter. This can be explained by the fact that the complexes are less dense, more hydrated as a result of a less favored charge neutralization.

#### Determination of the complex stoichiometry

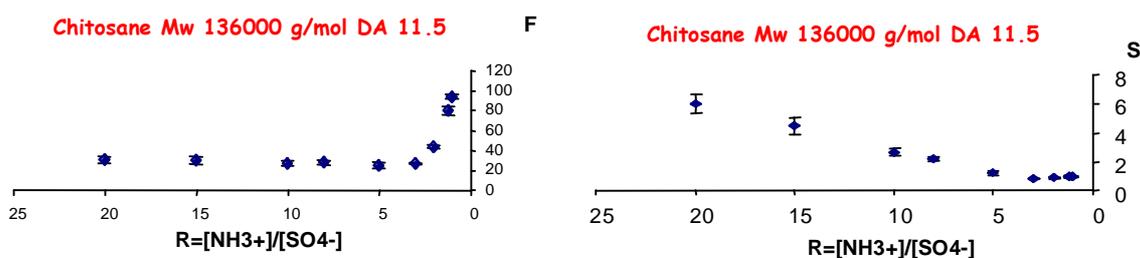
Two ratios were defined, F the fraction of ionic group in excess complexed to the counterpart in default and S the stoichiometry ratio i.e. the experimental composition of the complexes. With Dextran sulfate used in excess, F was the ratio of complexed sulfate moieties to the overall sulfate concentration available in the starting solution and S the amount of the complexed sulfate groups to the amount of ammonium moieties added. Hence, the two ratios defined could write as:

$$F = [\text{SO}_4^-]_{\text{complexed}} / [\text{SO}_4^-]_{\text{initial}} \quad \text{and} \quad S = [\text{SO}_4^-]_{\text{complexed}} / [\text{NH}_3^+]_{\text{introduced}}$$

When chitosan was used in excess:  $F = [\text{NH}_3^+]_{\text{complexed}} / [\text{NH}_3^+]_{\text{initial}}$  and  $S = [\text{NH}_3^+]_{\text{complexed}} / [\text{SO}_4^-]_{\text{introduced}}$ . F represents the fraction of ammonium moieties involved in the complex and S shows the amount of ammonium groups necessary to bind one sulfate counterpart.



**Figure 3 :** Complexation factor (F) and stoichiometry (S) of the complexes obtained with dextran sulfate as polymer in excess



**Figure 4 :** Complexation factor (F) and stoichiometry (S) of the complexes obtained with chitosan as polymer in excess

In Figures 3 and 4 the difference of behavior according to the polymer used in excess is striking. In the presence of excess dextran sulfate, the variation of F was linear, i.e. the consumption of the sulfate groups remained constant during the whole process, and the stoichiometry was in between 1.5 and 1.85 (the higher value was for the lowest molar mass chitosan). This suggests that not all the sulfate moieties were accessible for complexation with the primary ammonium groups of chitosan. When chitosan was used in excess, the consumption of the ammonium groups was not linear: an initial consumption of ca 20% of the initial amount was observed from  $R=20$  down to a critical charge ratio  $R = 4$ . For R values decreasing from 4 to 1, the consumption increased up to completion of the ammonium groups available. As a consequence of this mechanism, the stoichiometry was initially high (between 6 and 4 depending on the internal parameters of chitosan) and decreased regularly to 1 when the critical value of R was reached.

The difference in the formation of the complexes described above can be attributed to the properties of the polymer in excess: dextran sulfate is a flexible macromolecule with sulfate groups which are strong anions whereas chitosan is stiffer<sup>2</sup> and a weak polycation. The excess of strong polyanion may favor a residue-to-residue complexation

## Conclusion

We demonstrated that colloidal particles could be obtained by the formation of polyelectrolyte complexes of polysaccharide of opposite charges. The internal parameters that control the formation of particles are the molar mass of the polymer in excess and its charge density. The main pertinent external parameters are the polymer concentration and a moderate ionic strength of no more than 50 mM. Finally, the complex stoichiometry is controlled by the nature of the excess polymer: when dextran sulfate (a strong polyanion) was in excess, the composition of the complex was constant throughout the formation process, but in the case of an excess of chitosan, the stoichiometry, decreased to reach the limit value of one. Interestingly, the one-shot addition of reactants allowed the synthesis of colloids either positively or negatively charged, independently of the order of addition (excess to default or vice and versa).

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