

Chitosan-based nanoparticles prepared by template polymerizationP. Pereira¹, FM. Gama¹¹ IBB-Institute for Biotechnology and Bioengineering, Centre for Biological Engineering, Minho University, Campus de Gualtar 4710-057, Braga, Portugal.

INTRODUCTION: Chitosan (CS) /poly(acrylic acid) (PAA) nanoparticles (NPs) have recently been obtained by template polymerization¹. In this technique, the NPs are produced upon polymerization of an acrylic monomer next to the chitosan backbone. Due to the electrostatic interaction, the negatively charged acrylic monomers align along the chitosan molecules. These physic interactions leads to self-assembled particles. The molecular weight and deacetylation degree of chitosan affect the solubility, size, and stability of the particles.

METHODS: The CS/PAA NPs were synthesized by polymerization of AA in a CS solution. The CS was firstly dissolved in 50mL of AA solution (3mmol), in molar ratio of the 1:1, [aminoglucoside units]: [AA], under magnetic stirring until the solution became clear. In order to induce the polymerization of acrylic monomer, an initiator ($K_2S_2O_8$, 0,1mmol) was added to the solution. The pH value of the system was maintained at about 4,0. The mixture was placed at 70°C, under magnetic stirring and nitrogen atmosphere. After two hours, the solution became opaque and the reaction was stopped in an ice bath. The milky solution was filtered to eliminate any aggregates. To remove the initiator and the acrylic monomers that not reacted, the solution was dialysed against deionised water, in a 12-14KDa cut-off membrane, for three days. The CS/PAA NPs characterization was carried out by dynamic light scattering (DLS) to determine de size distribution and zeta potential, cryo-field emission scanning electron microscopy (Cryo-FESEM) to visualize the size and shape of the particles and size exclusion chromatography (SEC) to verify the extension of the acrylic monomer polymerization.

RESULTS: The polymerization of acrylic acid in the presence of chitosan is showed in Fig. 1. The size distribution of the nanoparticles was evaluated with a Malvern Zetasizer instrument, and showed an average size of 360nm (Fig. 2a). The CS/PAA NPs have size stability even after seven months. However, the NPs exhibited a pH-sensitive behaviour and their size increases with increasing of pH. The CS/PAA NPs appeared spherical in Cryo-FESEM micrographs (Fig. 2b).

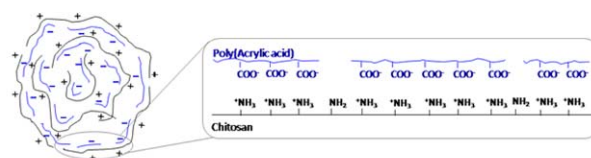


Fig. 1: Schematic representation of electrostatic interactions that allows the self-assembling.

The surface charge of the NPs at acid pH is quite positive, about 40mV. However, at pH 7.4 the zeta potential decreases to near neutrality. The average molecular weight of PAA in CS solution, measured using SEC, was about 800Da.

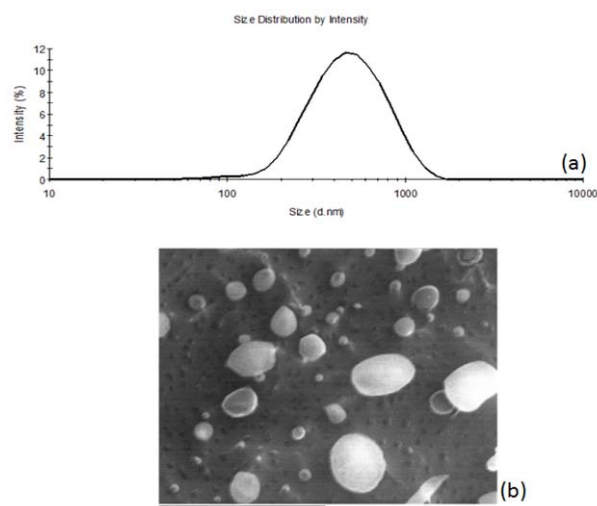


Fig. 2: Size distribution by intensity (a) and Cryo-FESEM image (b) of CS/PAA NPs.

DISCUSSION & CONCLUSIONS: The electrostatic interaction between poly(acrylic acid), negatively charged, and chitosan backbone with positive charge promotes the self-assembly of NPs. The size profile of CS/PAA NPs observed by Cryo-FESEM was consistent with that measured in the DLS, ranging from 200nm to 1000nm. As the surface charge, at pH 7.4 the zeta potential decreases to near neutrality, because the pka of chitosan is 6.5 and therefore the amines are no longer protonated. In short, these NPs can be successfully produced under very mild conditions.

REFERENCES: ¹ Y. Hu, X. Jiang, Y. Ding, et al (2002) *Biomaterials* **23**: 3193-201.

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