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PREPARATION OF CHITOSAN WITH CONTROLLED NANOPARTICLES SIZES

Márcia R. de Moura[1,2], Fauze A. Aouada[1,2], Luiz H.C. Mattoso[2]

[1]Depto. de Quimica - UFSCar, 13560-905, São Carlos/SP, Brazil
[2]Embrapa Instrumentação Agropecuária, 13560-970, São Carlos/S, BraziL, mattoso@cnpdia.embrapa.br

Chitosan (CS) has recently gained more interest due to its applications in food and pharmaceuticals. In this work, chitosan nanoparticles were prepared by template polymerization of methacrylic acid in chitosan solution using different concentration of chitosan. The nanosize and morphology of chitosan nanoparticles were studied using Fiber Optic Quasi Elastic Light Scattering and Transmission electron microscopy measurements at different pHs. Results demonstrated that by increasing the amount of CS in the preparation medium the particle size decreases. Furthermore, the pH at which the nanoparticles are equilibrated has a strong effect on swelling and aggregation of the nanoparticles. The ionic interaction between COO^- group of poly(methacrylic acid) and NH_3^+ group of chitosan, investigated by FT-IR spectra, is also discussed.

Introduction

Nanotechnology is of great interest in most fields, since it allows the manipulating of matter at the nanoscale level [1-2] and improving on properties of the final product. As developments in nanotechnology continue to emerge, its applicability to food industry, such as in food packaging and edible films seems to be clearly increasing [3].

Chitosan (CS), a polysaccharide derived from crustacean chitin, is of particular interest in the food packaging area, since it is biodegradable, bioresorbable and exhibits bioactive properties either in its polymeric or oligomeric form [4-5].

In this work, chitosan nanoparticles were prepared by template polymerization using poly(methacrylic acid) (PMAA) The influence of chitosan concentration and pH on particle size was investigated.

Experimental

Preparation of CS-PMAA nanoparticles

Nanoparticles were obtained by polymerization of methacrylic acid (MAA) in CS solution in two-step process. In the first step, chitosan was dissolved in MAA aqueous solution (0.5 v:v-%) for 12 h under magnetic stirring. The CS concentrations used in synthesis were 0.2, 0.5, and 0.8 (wt %). In the second step, 0.2 mmol of $K_2S_2O_8$ was added in the solution with continued stirring. Then, the polymerization was carried out at 70 ^{0}C under magnetic stirring. This system was kept at 70 ^{0}C for 1 h leading to the formation of CS–PMAA nanoparticles which was then cooled in an ice bath.

Determination of Particles Size

A Fiber Optic Quasi Elastic Light Scattering (FOQELS) (Brookhaven, USA) was used to measure the particle size of the nanoparticles. All FOQELS analyses were done with laser diode (10 mW), operating at a wavelength of 671 nm. The measurements of particles size were carried out after equilibrating the prepared samples in several pHs values (3.0-8.0) at 25 °C. All analysis was performed in triplicate.

Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) (Philips CM200) was used to observe the morphology and size of the CS–PMAA nanoparticles. Samples were placed onto copper grill covered with nitrocellulose. They were dried at room temperature, prior to the TEM analysis.

FT-IR analysis

FT-IR spectra of CS, and NPs were taken on a Perkin Elmer Spectrum model Paragon 1000 in the range from 4000 to 400 cm^{-1}. The FT-IR spectra were used to determine the chemical interaction between CS and PMAA. The CS–PMAA nanoparticles were frozen by liquid nitrogen and lyophilized by a freeze dryer system to obtain dried CS–PMAA nanoparticles. Powdered samples were prepared using KBr to form pellets.

Results and Discussion

Nanoparticles were successfully produced through polymerization of methacrylic acid in the

presence of chitosan solution, as show the results from Table 1. It can be observed that the size of CS-PMAA nanoparticles decrease with increase in the content of chitosan within the feeding solution used. It should be pointed out that the possibility of producing nanoparticles with controlled particle size is rightly desirable, in order to be able to optimize, for instance, the final properties of nanoparticles reinforced polymer films.

Table 1 -Particle size values of CS–PMAA nanoparticles prepared at pH 4.0.with several contents of chitosan

chitosan (wt %)	Particle Size (nm)
0.2	111 ± 4
0.5	82 ± 2
0.8	60 ± 4

The effect of pH on particle size is shown in Fig. 1. One can observe that particle size increases from 99 to 218 with an increase in pH from 3.0 to 8.0. Possible reasons for this behavior are two. Firstly, also an increase in pH leads to an increase on the ionization degree and charge density of the PMAA molecules. As a consequence, the electrostatic repulsive forces of inter- and intra-PMAA molecules increased, resulting in an increase of the swelling degree of PMAA and therefore an increase of the mean size of CS–PMAA nanoparticles. Secondly, as the pH increases the solubility of, chitosan decreases, what may lead to aggregation of nanoparticles. Figure 2 shows that both aggregation and swelling effects are possible, althought under the dried conditions under which the TEM were carried out an opposite effect to swelling was noticed, as expected, with particle size decreasing from 180 to around 70 nm.

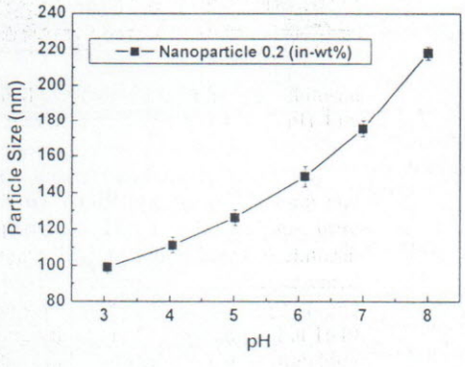

Figure 1 – Dependence of particle size on the pH at which chitosan nanoparticle, prepared with 0.2 of chitosan (wt%) at pH 4, were equilibrated after nanoparticle preparation.

TEM microphotographs of chitosan nanoparticle prepared using 0.5 of chitosan (wt%) at pH 4.0 are depicted in Fig. 3. The TEM images indicate a spherical shape of chitosan nanoparticles and also confirm the de-swelling effect, showing a decrease from 82 nm to around 40 nm upon drying.

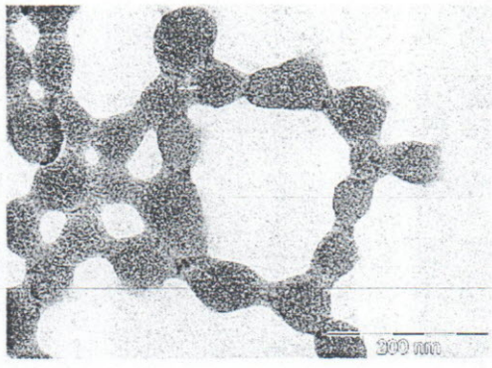

Figure 2 – TEM microphotographs of chitosan nanoparticle prepared with 0.2 of chitosan (wt%) at pH 4 and equilibrated at pH 7.0 after the nanoparticle preparation.

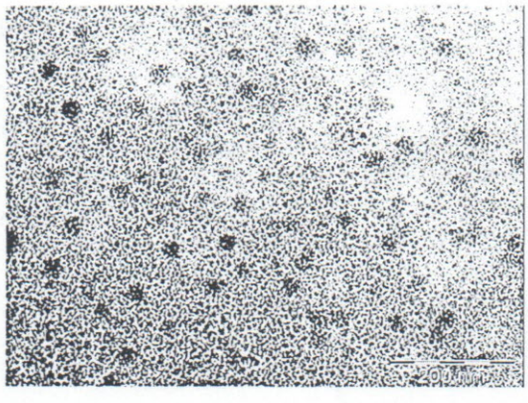

Figure 3 – TEM microphotographs of chitosan nanoparticle prepared using 0.5 of CS (wt%) at pH 4.0.

Fig. 4 shows FT-IR spectra of chitosan and CS-PMAA nanoparticles. The FT-IR analyses were utilized to investigate the interaction between chitosan and PMAA. The FT-IR spectrum of chitosan presented characteristics peaks at: 3435 cm^{-1} assigned to NH_2 and OH group stretching vibration; C=O of amide I at 1649 cm^{-1}; 1083–1020 cm^{-1} reflecting C-O stretching vibration and at 620 cm^{-1} reflecting pyranosidic ring stretching vibration. It can be observed from the FT-IR spectrum of the nanoparticle that the C=O band at 1649 cm^{-1} disappears, and two news bands at 1638 cm^{-1} (COO^- groups) and 1545 cm^{-1} (NH_3^+ groups) were present, indicating a ionic interaction between PMAA and CS during nanoparticles formations. The bands at 1703 and 1264 cm^{-1} (C=O) indicate the presence of PMAA [6] within the nanoparticles.

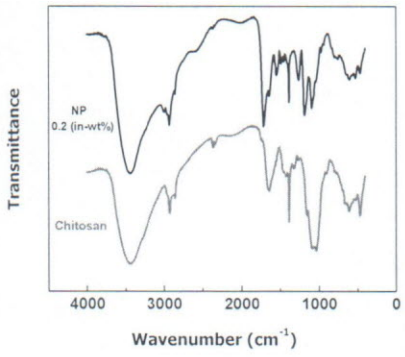

Figure 4 – Representative FT-IR transmittance spectra of chitosan and chitosan nanoparticles.

Conclusions

The CS–PMAA nanoparticles can be successfully prepared by polymerizing methacrylic acid into chitosan solution. The particle size is dependent on the chitosan concentration used in the synthesis. The nanoparticles solution obtained are also pH-sensitive, due to swelling and aggregation of the nanoparticles, which present a predominantly spherical shape. It is proposed that the carboxylic groups of PMAA are dissociated into COO^- groups which complex with protonated amino groups of CS through ionic interaction to form a polyelectrolyte complex during the polymerization, which leads to the formation of nanoparticles. This system might find interesting applications in food packaging.

Acknowledgements

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