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N,N,N-trimethyl chitosan nanoparticles as a vitamin carrier system

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ABSTRACT

There is considerable interest in incorporating stabilized vitamins into biopolymeric nanoparticles, especially in the development of carriers and active systems for pharmaceutical and food applications. Amongst biopolymer, chitosan is highly desirable owing to its good biocompatibility, biodegradability and ability to be chemically modified. In this paper, nanoparticles from three kinds of water-soluble derivative chitosan (N,N,N-trimethyl chitosan, TMC) have successfully been synthesized by ionic gelation with tripolyphosphate (TPP) anions. Combinations of concentrations of TMC and TPP have resulted in nanoparticles with varying sizes for which the capability for loading with vitamins was investigated. Zeta potential measurement and particle size analysis demonstrated that the size of the nanoparticles was optimized (196 ± 8 nm) when the lowest TMC and TPP amounts were used, *i.e.*, 0.86 mg mL^{-1} and 0.114 mg mL^{-1} respectively. As the TMC and/or the TPP concentrations increase, the resulting size of the nanoparticles increases considerably. Three different vitamins (B9, B12 and C) were tested as additives and the final system characterized in relation to size, morphology, spectroscopic and zeta potential properties. In general, the incorporation of vitamins increased all the TMC–TPP original nanoparticle sizes, reaching a maximum diameter of 534 ± 20 nm when loaded with vitamin C. The presence of vitamins also decreases the zeta potential, with one exception observed when using vitamin C. The preliminary results of this study suggested that all TMC/TPP nanoparticles can be successfully used as a stable medium to incorporate and transport vitamins, with potential applications in foodstuffs.

1. Introduction

N,N,N-trimethyl chitosan (TMC) is a cationic polyelectrolyte obtained by extensive methylation of chitosan parent polymer (de Britto & Assis, 2007a). The resulting derivative is a water-soluble polysaccharide useful for a variety of applications and particularly suitable for nanoparticle processing (Xu, Du, Huang, & Gao, 2003). TMC is a non-toxic and biocompatible polymer from which particles with sizes in the range of 100–200 nm can be easily obtained via an ionic gelation process, using sodium tripolyphosphate (TPP) as a counterion. For these, efficiencies of up to 90% can be attained to drug entrapment, according to the analysis of bovine serum albumin as a model drug, as performed by Xu et al. (2003). Several others applications have emerged for TMC nanoparticles including a nasal and oral vaccine delivery system (Slütter & Jiskoot, 2010), protein carrier (Luo, Zhang, Cheng, & Wang, 2010), insulin controlled release (Sadeghi et al., 2008) and applications as a food additive (Chen & Subirade, 2005).

Particularly in foodstuffs, the use of edible nanoparticles as a carrier or release system has many potential applications (Matalanis, Jones, & McClements, 2011). Flavors, antioxidants, enzymes, antimicrobials and vitamins are able to be encapsulated or immobilized on nanoparticles, thus retaining activity. Vitamins, for example, are sensitive and unstable compounds that lose their functionality when exposed to inappropriate temperatures, oxygen, light or humidity (Ottaway, 1993). The encapsulation of vitamins may partially help to reduce some of these limitations, thus providing a prolonged shelf life and stability in a foreign medium. Encapsulated vitamins have particularly interesting applications as nutritional supplements for the enrichment of processed products in certain foods. In beverages for example, the encapsulation can act positively in masking the flavor of the vitamins and minerals, providing a tastier product for consumers (Chen & Wagner, 2004). Nanoparticles can also be associated with a polymeric matrix, providing an alternative way to fortify coatings or edible films thus envisaging the development of active packaging (Coma, 2008; Garcia, Forbe, & Gonzalez, 2010; Imran et al., 2010; Kerry, O'Grady, & Hogan, 2006; Sekhon, 2010). The incorporation of nutrients in food employing nanoparticles is undoubtedly

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emerging as one of the fastest growing technologies in the alimentary field with potentially many applications. However the control of nanoparticle processing parameters and ensuring reproducibility still require further development.

The purpose of the present study was to produce TMC chitosan-based nanoparticles with controllable sizes by TPP crosslinking and then evaluate the loading efficiency of these nanoparticles with vitamins. The resulting structure of these nanoparticles was analyzed by solid-state ^{13}C NMR spectroscopy and by Fourier Transform Infrared techniques. The morphological characterization was carried out by Atomic Force Microscopy and Scanning Electron Microscopy. In addition the stability of the nanoparticles was evaluated through zeta potential analysis.

2. Experimental

2.1. Materials

Sodium tripolyphosphate (TPP) and medium molecular weight chitosan (80% deacetylated) were purchased from Aldrich Chemical Company Inc. (USA), and used as delivered. Dimethylsulfate was obtained from Vetec (Brazil) and the other chemicals from Synth (Brazil). Vitamins of pharmaceutical grade were acquired in a local drugstore with following specifications, according to the manufacturer: vitamin C, white powder, assay = 99%, heavy metal < 10 ppm, ash = 0.03%, oxalic acid < 0.3%; vitamin B9 (folic acid), orange yellowish powder, assay = 97.11%, ash = 0.3%; vitamin B12 (cyanocobalamin), powder deep pink, assay = 99.49%.

2.2. Synthesis of TMC–TPP nanoparticles

The TMC was obtained by methylation of chitosan with dimethylsulfate at 70 °C. The basic reaction sequence comprised a suspension of 1.0 g of chitosan (0.005 mol) in 16 mL of dimethylsulfate and 4 mL of deionized water. Details of the methylation process and the derivative characterization are available in the literature (de Britto & Assis, 2007a).

The nanoparticles were obtained by the procedure reported by Moura et al. (2009) but with different relations of TMC–TPP (Table 1). The process consists of a continuous addition of sodium TPP (at a rate of 1 mL/min) into TMC aqueous solution. The mixture was mixed at room temperature using a homogenizer (Polytron PT 3000 – Brinkmann) at 6000 rpm. The zone of opalescent suspension was further examined as nanoparticles.

2.3. Incorporation of vitamins into TMC–TPP nanoparticles

The preparation of TMC–TPP nanoparticles with incorporated vitamins B9, B12 and C was performed in a similar way. Previously, aqueous solutions of the vitamins were prepared by dissolving an amount of vitamin corresponding to 15 wt% of TMC weight. The aqueous solution of vitamin was then added to the TMC solution. Nanoparticles were then obtained as described above for the synthesis of TMC–TPP nanoparticles. For these experiments, a TMC–TPP concentration relation of 0.86–0.144 was chosen since this combination resulted in particles with a smaller size (Table 1).

Table 1
Influence of TMC and TPP concentration on the nanoparticles size and zeta potential.

TMC concentration (mg mL^{-1})	TPP concentration (mg mL^{-1})	Particle size (nm)	Zeta potential (mV)
0.86	0.114	196 ± 8	31.6 ± 0.6
1.14	0.114	302 ± 15	24.7 ± 1.0
1.07	0.214	606 ± 25	12.7 ± 0.4

2.4. TMC–TPP and vitamin-loaded nanoparticles characterization

2.4.1. Fourier transform infrared (FTIR)

Films of TMC and TMC–TPP nanoparticles were prepared by solution casting onto acrylic Petri dishes. Solvents were allowed to evaporate at room temperature and the films peeled off the dishes (thickness ≅ 50–100 μm). Fourier Transformed Infrared (FTIR) spectra were obtained for both films using a Perkin Elmer spectrometer model Paragon 1000, in the regions of 400–4000 cm^{-1}.

2.4.2. Morphological investigation

Morphological analyses were performed directly on the cast film surface by Atomic Force Microscopy (AFM) (Dimension V from Veeco) and by Scanning Electron Microscopy (SEM), model LEO 440 from Leica–Zeiss. AFM images were acquired in contact-mode in random areas of 5.0 μm × 5.0 μm using a conventional silicon-nitrite pyramidal tip with spring constant of 0.06 N/m (Veeco). For this, a diluted suspension of nanoparticles (~1.0 mg mL^{-1}) was deposited on a cleaned microscope glass slide (10 × 10 mm) and cast in a desiccator. The morphological aspects and nanoparticles size were estimated by SEM using an accelerating voltage of 15 keV. In a similar way as that for AFM, cast nanoparticles on microscope slide was fixed in specimen stub and coated with an ultrathin coating of gold by low vacuum sputter coating to avoid the accumulation of electrostatic charge.

2.4.3. Particle size and zeta potential measurements

A Malvern Zetasizer nano ZS (model Zen 3600) instrument, based on light scattering technique, was used to measure the hydrodynamic average size distribution and zeta potential of the nanoparticles. The size distribution in fluids was evaluated using the photon correlation spectroscopy method. All analysis was performed in triplicate at 25 °C and individual measurements were calculated from the average of the three readings. The results were expressed as the mean size ± SD.

2.4.4. Solid-state CP-MAS ^{13}C NMR

Structural analysis of TMC–TPP and TMC–TPP–vitamins loaded were investigated by ^{13}C Nuclear Magnetic Resonance spectroscopy performed on a Varian Unity Inova 400 spectrometer operating at 400 MHz for ^{1}H frequency. Combined techniques of proton dipolar decoupling (DD), magic angle spinning (MAS) and cross-polarization (CP) were used to obtain well-resolved shift correlation spectra. Contact time was 1 ms, acquisition time 51.2 ms and the recycle delay 4 s. The proton pulse width was 6 ms and an 18 kHz spectral window was used. Typically 2000 scans were acquired for each spectrum.

3. Results and discussion

According to Agnihotri, Mallikarjuna, and Aminabhavi (2004), drug loading in micro/nanoparticulate systems can be attained by two basic methods: *i*) by incorporation, in which charge is carried during the preparation of the particles and *ii*) by incubation, in which the charge is carried after the formation of the particles. In the first methodology, the drug is dissolved or dispersed into the polymer solution, being physically entrapped into the matrix during the particle formation. In the second, the drug is adsorbed onto the surface and is able to migrate to the interior of the particles. Evaluation of drug incorporation processes in polysaccharides-based nanoparticles (Liu, Jiao, Wang, Zhou, & Zhang, 2008) have showed loading efficiencies as high as 95% achieved in water-soluble chitosan derivatives. Additionally, the process of incorporation when