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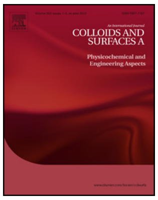

Physicochemical and morphological properties of size-controlled chitosan–tripolyphosphate nanoparticles

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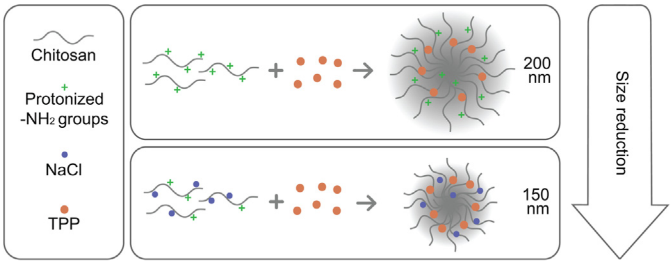
HIGHLIGHTS

- Chitosan–tripolyphosphate nanoparticles were prepared through ionic-crosslinking.
- Control of particle size by selecting appropriate conditions.
- Influence of initial pH and salinity of chitosan solution on nanoparticles formation.
- Separation of aggregates from nanoparticles by centrifugation.
- Ultra-sonication reduced particle size but caused fragmentation on their structure.

GRAPHICAL ABSTRACT



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ABSTRACT

Chitosan–tripolyphosphate nanoparticles have been extensively studied during the last decade because of their numerous applications. In this study, we describe conditions to optimize chitosan nanoparticles as potential nano-fillers in edible films. The ionic cross-linking between the cationic amino groups on the chitosan (CS) chain and the anionic phosphate groups of sodium tripolyphosphate (TPP) was verified via FTIR. Particle size, polydispersity index (PDI) and surface ζ-potential were controlled by chitosan's *Mw* and concentration, CS:TPP mass ratio, and external conditions such as pH and salinity of the initial chitosan solution. Post-processing methods such as centrifugation and ultra-sonication were used to further control particle size. We show that particle size can be controlled by selecting appropriate conditions. Particles with sizes below 120 nm were produced at different CS:TPP mass ratios depending on the CS concentration. Dilute NaCl was the optimal solution ionic composition that decreased the size by 25% and also resulted in a narrow particle size distribution. We show using UV–vis spectrophotometry that particles of different size, separated by centrifugation had different phosphorus content. Ultra-sonication can be used to reduce the size by 50% but long time caused fragmentation of the nanoparticles. Transmission electron microscopy (TEM) revealed the differences in the morphology of chitosan nanoparticles under various fabrication conditions.

1. Introduction

Chitosan (CS) is obtained by the deacetylation of the naturally occurring polysaccharide chitin, one of the most abundant biopolymers in nature. It is composed of β-(1-4)-linked D-glucosamine and *N*-acetyl-D-glucosamine units [1]. The degree of deacetylation (DD)

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of typical commercial chitosan is usually between 70% and 95%, and the *Mw* between 10 and 1000 kDa [2]. Chitosan is an ingestible food ingredient that is biologically renewable, biodegradable, biocompatible, non-antigenic, non-toxic and biofunctional [3]. In an acidic environment CS is positively charged and soluble due to protonation of the amino groups of glucosamine. Chitosan nanoparticle (CSNPs) formation is based on the simple and mild technique of ionotropic gelation where the CS protonated amino groups are cross-linked by inter- and intramolecular bonds by multivalent polyanions. Sodium tripolyphosphate (TPP) is a very popular polyanion because it is non-toxic and forms gels with desirable properties [4–6].

CSNPs because of their biodegradability, low toxicity and biocompatibility have been frequently developed as a delivery system for bioactive compounds such as, drugs [7–12], proteins [13–16], flavonoids [17], essential oils [18,19], and genes [5,20]. The formation conditions of the nanoparticle in these studies were examined in relation with the encapsulation efficiency and in tandem with the release profile or antimicrobial activity. Particles have been prepared in the size range of 50 to 300 nm with encapsulation efficiency reaching 95% showing that ionotropic gelation is an efficient method for CSNPs preparation. CSNPs have been reported to have broad antimicrobial properties. Their antibacterial activity can be higher than that of bulk chitosan due to their higher surface area and charge density [21,22].

However, there have been limited reports on the use of the nanoparticles as a modifier of mechanical and physicochemical properties of biopolymer films. The present study investigated the physical characteristics of CSNPs for use as a potential nanocomposite modifier. CSNPs have been incorporated in edible or biodegradable films [4,23–25] and demonstrated very good dispersability in biopolymers and showed potential antimicrobial activity in the films [26]. Nanoparticles improve mechanical and barrier properties by occupying the empty spaces within the film matrix which induces the collapse of the pores [27]. The purpose of this study was to optimize CSNPs intended as bio-nanocomposites for film applications and focused on the fabrication of compact nano-sized particles with narrow polydispersity index (PDI). The behavior of the particle as a function of size, PDI and surface charge (ζ-potential) was examined by changing key parameters such as, CS molecular weight (*Mw*), CS concentration, CS:TPP mass ratio and external conditions such as, pH and salinity of the initial CS solution. In processes where a mixture of nanoparticles and aggregates were formed, centrifugation and ultra-sonication were applied to isolate nanoparticles from large particles and aggregates. Transmission electron microscopy (TEM) was used in order to observe morphological changes in the structure for various fabrication processes. The conditions for the production of narrow PDI nanoparticles and of a specific size range would be useful for improved edible and biodegradable film production.

2. Materials and methods

2.1. Materials

Chitosan of *Mw* 50 and 100 kDa and DD of 90% derived from crab shells were obtained from Golden-Shell Biochemical Co., Ltd. (Hangzhou, China). Sodium tripolyphosphate was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Other reagents were all commercially available and used as received. Distilled water was used in all experiments.

2.2. Preparation of CSNPs

CSNPs were produced according to the procedure first reported by [13], based on the ionic gelation of CS with TPP anions. CS (0.5 mg/mL) was dissolved in 1% (v/v) aqueous acetic acid and the pH was adjusted using 10 M NaOH. A 0.7 mg/mL aqueous solution of TPP was added drop-wise to the CS solution under vigorous magnetic stirring at room temperature. CSNPs formed spontaneously. In order to use different mass ratios of CS:TPP and CS:NaCl, the initial concentration of CS was kept constant while the volume of TPP and NaCl added was varied. All samples were prepared in triplicate and immediately subjected to further analysis, centrifuged at 10,000 × *g* for 30 min at room temperature in order to separate the nanoparticles from large particles or aggregates or freeze dried to obtain powder samples. After centrifugation sediment and supernatant were separated by carefully removing the supernatant layer.

2.3. Characterization of CSNPs

The measurements of particle diameter, PDI and ζ-potential of nanoparticles were performed at 25 °C on a Zetasizer Nano-ZS (Malvern Instruments, Worcestershire, UK) on the basis of dynamic light scattering (DLS). The pH of the particle suspensions was measured at room temperature using a Mettler Toledo EL20 (Shanghai, China).

The morphological characteristics of nanoparticles were examined by a high-performance digital imaging TEM machine (JEOL H-7650, Hitachi High-Technologies Corp., Tokyo, Japan). One drop of the nanoparticle solution was spread onto a carbon-coated copper grid and stained with 2% (w/v) phosphotungstic acid. After drying at room temperature the samples were placed for TEM analysis using accelerating voltage of 100 kV.

FTIR spectra of CS, TPP and CSNPs were determined using an infrared spectrometer (FTIR) (Thermo Fisher Scientific Inc., Nicolet iS10, USA). The spectra were obtained in the range of 500 and 4000 cm^{-1}, using 16 scans at a resolution of 2 cm^{-1}. Powdered CSNPs were obtained by freeze drying and the samples were prepared using KBr to form pellets.

2.4. Determination of phosphorus in CSNPs

The determination was based on the method reported by [28] with modifications. Powdered samples of CSNPs were prepared by freeze drying. The samples were weighed and placed into Kjeldahl flasks and digested by a mixture of sulfuric acid, perchloric acid and nitric acid. The hydrolyzed solution was cooled to room temperature and transferred to 100 mL flasks by rinsing the Kjeldahl flasks several times with distilled water. 2 mL of the obtained solutions were transferred into 20 mL tubes and 2 mL solution of ammonium molybdate, 1 mL solution of sodium sulfite and 1 mL hydroquinone were added and mixed. At last, distilled water was added to reach the mark of the tube and left 30 min at room temperature to ensure colour development. The same procedures were used for blanks.

Monopotassium phosphate (KH_2PO_4) was used as the standard. For the P determination in both standard and working solutions the absorbance was determined by absorption at 660 nm using a UV-1600 Spectrophotometer (Shanghai, China). All samples were analyzed in triplicate and the content of phosphorus was calculated using the following equation:

$$X = \left(\frac{M_1}{M}\right) \times \left(\frac{V_1}{V_2}\right) \times 100 \qquad (1)$$

X = the content of phosphorus in the sample (mg/100 g); M_1 = the content of phosphorus in the sample solution (from the standard curve or regression equation (mg)); V_1 = the total volume of sample digestion liquid (mL); V_2 = the volume of sample digestion liquid for determination (mL); M = the weight of the sample (g).