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Chitosan/carrageenan nanoparticles: Effect of cross-linking with tripolyphosphate and charge ratios

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ABSTRACT

Chitosan/carrageenan/tripolyphosphate nanoparticles were prepared by polyelectrolyte complexation/ionic gelation, the latter compound acting as cross-linker. The incorporation of the three components in the nanoparticle matrix was assessed by analytical techniques (FTIR, XPS and TOF-SIMS).

Using chitosan/carrageenan nanoparticles as control, the effect of the cross-linker in the particles properties was studied. A decrease in size (from 450–500 nm to 150–300 nm) and in zeta potential (from +75 – +85 mV to +50 – +60 mV), and an increase in production yield (from 15–20% to 25–35%), and in stability (from one week to up to 9 months) were observed. Also, a correlation between positive to negative charge ratios in the formulations and the above characteristics was established.

The small size and high positive surface charge make the developed chitosan/carrageenan/tripolyphosphate nanoparticles potential tools for an application in mucosal delivery of macromolecules.

1. Introduction

Polymeric nanoparticles have been used increasingly in various fields, such as drug delivery, imaging and tissue engineering, the first being, by far, the most reported application. The main reason justifying the widespread use of polymeric nanoparticles relies on the displayed high surface-to-volume ratio which improves the loading capacity of the selected molecule, while providing its protection. In addition, increased drug absorption might be attained by the capacity of nanoparticles to reduce epithelial resistance to transport (de la Fuente, Csaba, Garcia-Fuentes, & Alonso, 2008; Rawat, Singh & Saraf, 2006; Reis and Ribeiro, 2006).

Many polymers have been used to prepare these vehicles, but those of natural origin are often preferred because, as compared to synthetic counterparts, they comply more easily with the requisites of biocompatibility, biodegradability and absence of toxicity that are mandatory in any biomedical application (Liu, Jiao, Wang, Zhou & Zhang, 2008; Malafaya, Silva & Reis, 2007). Chitosan (CS) and carrageenan (CRG) are two marine-derived polymers which belong to the above mentioned class, and have demonstrated in a previous study the ability to assemble into nanoparticles of 400–600 nm (Grenha et al., 2010). CS is a cationic polysaccharide composed of repeating units of *N*-acetylglucosamine and D-glucosamine that are β-(1–4)-linked (Fig. 1), and presents well-documented favorable properties for drug delivery such as biocompatibility, biodegradability, low toxicity (Dornish, Hagen, Hansson, Peucheur, Vedier & Skaugrud, 1997; Hirano, Seino, Akiyama & Nonaka, 1988) and mucoadhesiveness (Lehr, Bouwstra, Schacht & Junginger, 1992). CRG is another polysaccharide, extracted from red seaweed (van de Velde, Knutsen, Usov, Rollemay, & Cerezo, 2002) and composed of galactose and anhydrogalactose units, linked by glycosidic bonds (Fig. 1) (Lim, Gwon, Choi, Shin & Nho, 2010). Due to its half-ester sulfate moieties, carrageenan displays a strong ionic nature and exhibits a high capacity to react with proteins (Malafaya et al., 2007; Mohamadnia, Zohuriaan-Mehr, Kabiri, Jamshidi & Mobedi, 2007). There are two types of carrageenan that evidence gel-forming ability, *k*- and *i*-, *k*-carrageenan gels being more firm than those obtained with *i*-carrageenan, which are more elastic and soft (Bixler, 1993). The assembly of the referred CS/CRG nanoparticles was mediated by polyelectrolyte complexation (Grenha et al., 2010), a method that uses very mild conditions, avoiding harmful organic solvents or high shear forces. Therefore, it has the general capability of protecting the encapsulated molecules and retaining their activity during the encapsulation, which are its principal advantages (Mohanraj and Chen, 2006; Saboktakin, Tabatabaie, Maharramov & Ramazanov, 2010; Grenha, 2012). This methodology involves the interaction between a chitosan with high degree of protonation and a polyanion, permitting the rapid formation of

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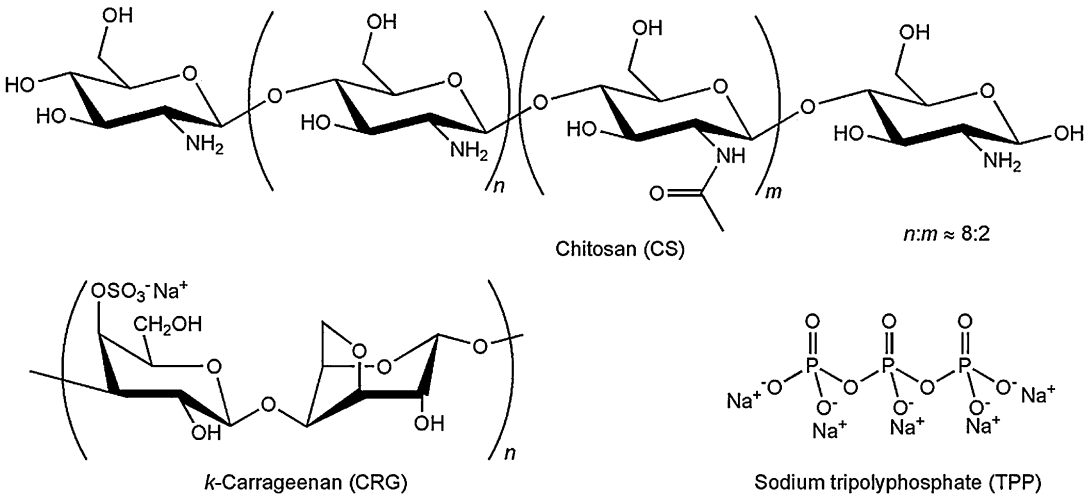

Fig. 1. Chemical structures of materials composing the matrix of nanoparticles: chitosan, *k*-carrageenan and sodium tripolyphosphate.

nanoparticles. Their size, as well as other characteristics, might be modulated by adjusting formulation parameters like the type of materials composing the particles matrix, their concentration and mass ratios, amongst others (Calvo, Remuñán-López, Vila-Jato, & Alonso, 1997a; Grenha, 2012).

In many cases, for instance if the nanoparticles are to be applied in mucosal delivery, it is important to ensure that their size will permit the contact with the epithelial surface, an effect that is maximised for particles between 50 nm and 500 nm (Desai, Labhasetwar, Amidon & Levy, 1996; Jani, Halbert, Langridge & Florence, 1990). Preparing nanoparticles in this size range is facilitated by the use of adequate cross-linking agents. Tripolyphosphate (TPP) is a non-toxic polyanion (Fig. 1) known for its capacity to cross-link chitosan, a reaction mediated by electrostatic forces, resulting in the formation of ionic cross-linked networks (Janes, Calvo & Alonso, 2001; Mi, Sung, Shyu, Su & Peng, 2003).

The objective of this work was to produce CS/CRG nanoparticles, including in the formulation TPP as cross-linking agent, and to evaluate the effect of the presence of this polyanion on the properties of nanoparticles, namely concerning size, surface charge and stability. To do so, different amounts of cross-linker were used and formulations with different polymeric mass ratios were tested. Reduced size and strong positive surface charge would improve the nanoparticles contact with mucosal epithelial surfaces, which is very positive when considering an application in mucosal drug delivery.

2. Experimental

2.1. Materials

Chitosan (low molecular weight, deacetylation degree = 75–85%), pentasodium tripolyphosphate, glycerol and glacial acetic acid were supplied by Sigma Chemicals (Germany). *k*-carrageenan and potassium bromide (KBr) were obtained from FMC Biopolymer (Norway) and Riedel-del-Haën (Germany), respectively. Ultrapure water (Milli-Q Plus, Millipore Iberica, Spain) was used throughout.

2.2. Nanoparticles preparation

CS/CRG/TPP nanoparticles were prepared by a modification of a previously described methodology (Grenha et al., 2010), based on the polyelectrolyte complexation of CS with CRG and additional ionic gelation of chitosan with TPP anions. Briefly, CS was dissolved in 1% (w/w) acetic acid to obtain a solution of 1 mg/mL and CRG and TPP were dissolved in purified water to obtain stock solutions of 2.5 and 10 mg/mL, respectively. Different volumes of the latter solutions were mixed in order to obtain volumes of 0.8 mL of solutions with the required concentrations of both components. The spontaneous formation of nanoparticles occurs upon incorporation, under gentle magnetic stirring at room temperature, of the aforementioned solutions into 2 mL of the CS solution, corresponding to final theoretical CS/CRG/TPP ratios varying from 4/1/0 to 7/1/1 (w/w).

Nanoparticles were concentrated by centrifugation at 16,000 × *g* on a 10 μL glycerol layer for 30 min at 15 °C (centrifuge 5804R, Eppendorf, Germany). The supernatants were discarded and nanoparticles were ressuspended in 200 μL of purified water.

2.3. Nanoparticles physicochemical characterization

The production yield of nanoparticles was calculated by gravimetry. Fixed volumes of nanoparticle suspensions were centrifuged (16,000 × *g*, 30 min, 15 °C), and sediments were freeze-dried over 24 h at −34 °C, followed by a gradual increase in temperature until 20 °C, using a Labconco freeze dryer (Labconco, USA) ($n = 3$).

The process yield (P.Y.) was calculated as follows: P.Y. (%) = (nanoparticle sediment weight/total solid weight) × 100.

The morphological examination of CS/CRG/TPP nanoparticles was conducted by transmission electron microscopy (TEM) (JEM-1011, JEOL, Japan). The samples were stained with 2% (w/v) phosphotungstic acid and placed on copper grids with Formvar® films for TEM observation.

Measurements of nanoparticle size and zeta potential were performed on freshly prepared samples by photon correlation spectroscopy and laser Doppler anemometry, respectively, using a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK). For the analysis of particle size and determination of the electrophoretic mobility, each sample was diluted to the appropriate concentration with ultrapure water and placed in the electrophoretic cell. Each analysis was performed at 25 °C. Three batches of each formulation were analyzed ($n = 3$).

2.4. Nanoparticle stability study

Aliquots of nanoparticle formulations with and without TPP (formulations 5/1/1 and 5/1/0, respectively) were stored at 4 °C. Nanoparticle sizes and zeta potentials were monitored as a function of time for 250 days, using the technique described above ($n = 3$).