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Chitosan/fucoidan multilayer nanocapsules as a vehicle for controlled release of bioactive compounds

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ABSTRACT

Hollow multilayer nanocapsules were successfully prepared through layer-by-layer assembly of two bioactive polysaccharides, chitosan and fucoidan. The stepwise adsorption of 10 chitosan/fucoidan layers and the consequent formation of a multilayer film on polystyrene nanoparticles (used as templates) were followed through ζ-potential measurement and the removal of the polystyrene core was confirmed by FTIR analysis. The chitosan/fucoidan nanocapsules morphology and size were evaluated by SEM and TEM, which showed that after the core removal, the nanocapsules maintained their spherical shape and a decrease of size occurred. A cationic bioactive compound, poly-L-lysine (PLL), was chosen to evaluate the loading and release behaviour of the nanocapsules. The chitosan/fucoidan nanocapsules showed a good capacity for the encapsulation and loading of PLL, which shows to be influenced by the initial PLL concentration and the method of encapsulation used. The results of fitting the linear superimposition model to the experimental data of PLL release suggest an anomalous behaviour, with one main polymer relaxation. The PLL release was found to be pH-dependent: at pH 2 relaxation is the governing phenomenon and at pH 7 Fick's diffusion is the main mechanism of PLL release.

Chitosan/fucoidan nanocapsules is a promising delivery system for water soluble bioactive compounds, such as PLL, showing a great potential of application in food and pharmaceutical industries

1. Introduction

Nanoencapsulation systems exhibit high potential as carriers of bioactive substances. Their subcellular size allows relatively higher intracellular uptake and their permanence in circulation for longer, therefore extending their biological activity compared to micro-sized systems (Mora-Huertas, Fessi, & Elaissari, 2010). Also, nanoencapsulation may be beneficial regarding improved stability and protection capability of labile substances against degradation factors (Preetz, Rübe, Reiche, Hause, & Mäder, 2008). Layer-by-layer (LbL) deposition technique is one of the most powerful methods to create multilayer nanocapsules, which can be specially engineered with controlled sizes, composition, porosity, stability, surface functionality and colloidal stability and can be used as carriers for bioactive compounds. Also, the step-wise formation of multilayer nanocapsules allows introducing multiple functionalities (Johnston, Cortez, Angelatos, & Caruso, 2006). LbL assembly is based on the electrostatic interaction between oppositely charged polyelectrolytes alternatively adsorbed onto an appropriate template (Decher & Schlenoff, 2003).

Core–shell nanoparticles can be produced through the deposition of polyelectrolyte layers onto colloidal nanoparticles; by subsequently removing the core (by dissolution or decomposition) from the core–shell structure, it is possible to obtain hollow nanocapsules with different properties, depending on the polyelectrolytes used (Cuomo, Lopez, Miguel, & Lindman, 2010). Solid particles such as polystyrene, silica and $CaCO_3$ are the most often used sacrificed templates for formation of capsules (Szczepanowicz et al., 2010). In addition, diameter, membrane thickness and permeability of hollow capsules prepared by LbL assembly can be easily controlled (Caruso, Caruso, & Möhwald, 1998). Different bioactive compounds can be encapsulated in the hollow inner cavities of these nanocapsules and they can be released in a well-controlled manner (Li, Guo, Wen, & Zhang, 2013).

Therefore, multilayer nanocapsules have promising applications in the release of bioactive compounds in the pharmaceutical and food industries; however for these applications to be possible,

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biofunctionality and the use of non-toxic materials are the most fundamental conditions to be met. Therefore, natural polyelectrolytes such as chitosan and fucoidan are competitive candidates as materials for the formation of multilayer nanocapsules. Chitosan is a cationic polysaccharide obtained by deacetylation of chitin which is the major constituent of exoskeleton of crustaceous animals. Chitosan is nontoxic, biodegradable, biocompatible and has intrinsic antimicrobial activity, inhibiting the growth of a wide variety of bacteria (Helander, Nurmiaho-Lassila, Ahvenainen, Rhoades, & Roller, 2001; Shahidi, Arachchi, & Jeon, 1999). Due to its promising properties, chitosan has been one of the most popular biopolymers for development of bioactive compounds delivery systems for a wide range of applications (Luo & Wang, 2014). Fucoidan, an anionic sulphated polysaccharide found in brown algae display diverse biological activities, including antioxidant, anticoagulant, antibacterial, antiviral, anti-inflammatory and antitumor effects (Kusaykin et al., 2008).

Several techniques have been proposed to incorporate bioactive compounds within nanocapsules (Johnston et al., 2006). In general, the bioactive compound can be loaded during the preparation of capsules (adsorption of the bioactive compound to the surface of a particle that can be subsequently coated using the LbL technique) (Shu et al., 2010) or after the formation of the capsules (diffusion of bioactive compound from the surrounding medium in which the capsules are dispersed into the capsule) (Ye, Wang, Liu, & Tong, 2005).

Poly-L-lysine (PLL) is a short cationic polypeptide composed of 27–33 identical L-lysine residues and is industrially produced by fermentation using *Streptomyces albulus* ssp. *Lysinololymerus* (Najjar, Kashtanov, & Chikindas, 2007). This polypeptide exhibits a strong antimicrobial activity, is stable at high temperatures and under both acidic and alkaline conditions and is safe for human consumption (Najjar et al., 2007; Yoshida & Nagasawa, 2003); therefore it is widely used as natural food preservative (Yamanaka & Hamano, 2010). PLL inhibits the growth of a wide spectrum of microorganisms including Gram-positive and Gram-negative bacteria; it also has anti-phage activity (Hamano et al., 2007). Its mechanism of action against microbial growth is the electrostatic adsorption to the cell surface and subsequent interference with cell membranes (Najjar et al., 2007).

In this work, biodegradable hollow nanocapsules were developed through LbL assembly of chitosan and fucoidan. Nanocapsules were built through the alternate deposition of 10 chitosan/fucoidan layers on polystyrene (PS) nanoparticles (diameter ≈ 100 nm), used as templates, followed by removal of the PS core. PLL was entrapped in the nanocapsules and its loading and release behaviour was evaluated.

2. Materials and methods

2.1. Materials

Dispersion of polystyrene nanoparticles with a diameter of 93.8 ± 1.5 nm and a ζ-potential of −44.8 ± 0.3 mV was obtained from Polysciences, Inc. (Polybead® Polystyrene 0.10 μm Microspheres, Warrington, PA, USA). Fucoidan with a M_W = 57,260 was obtained from Sigma-Aldrich (Fucoidan from *Fucus vesiculosus*, St. Louis, MO, USA) and chitosan (deacetylation degree ≥95%) was purchased from Golden-Shell Biochemical Co., Ltd. (Zhejiang, China). Lactic acid (90%) was obtained from Acros Organics (Geel, Belgium) and ethanol, methanol and sulphuric acid were obtained from Panreac (Spain). Tetrahydrofuran (THF), 1,1-diphenyl-2-picrylhydrazyl (DPPH), hydrochloric acid and poly-L-lysine hydrobromide (M_W 1000–5000 Da) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Determination of polysaccharides composition

Fucoidan sample was hydrolyzed with H_2SO_4 1 mol L^{-1} at 100 °C during 2.5 h according to other authors (Selvendran, March, & Ring, 1979). Neutral sugars were converted to alditol acetates, as previously described (Coimbra, Delgadillo, Waldron, & Selvendran, 1996) and uronic acids were quantified through the 3-phenylphenol colorimetric method (Blumenkrantz & Asboe-Hansen, 1973), modified by Coimbra et al. (1996).

Chitosan sample was hydrolyzed with HCl 6 mol L^{-1} at 100 °C during 20 h, followed by glucosamine conversion to alditol acetate by reduction with sodium borohydride and acetylation, as previously described (Coimbra et al., 1996). Alditol acetate derivatives were analysed by gas chromatography with a 30 m column DB-225 (J&W Scientific, Folsom, CA, USA) with i.d. and film thickness of 0.25 mm and 0.15 μm, respectively and using a flame ionization detector. The oven temperature program used was: initial temperature 200 °C, a rise in temperature at a rate of 40 °C min^{-1} until 220 °C, standing for 7 min, followed by a rate of 20 °C min^{-1} until 230 °C and maintained at this temperature for 1 min. The injector and detector temperatures were, respectively, 220 and 230 °C. The flow rate of the carrier gas (H_2) was set at 1.7 mL min^{-1}.

2.3. Antioxidant activity of fucoidan

The free-radical scavenging capacity of fucoidan was analysed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) test according to the method of Blois (1958), with some modifications. BHT and BHA were used as positive controls. Briefly, 0.2 mL of MeOH and 0.3 mL of the sample dissolved in MeOH (concentrations ranging from 0.05 to 1.0 mg mL^{-1}) were mixed in a 10 mL test tube. DPPH (2.5 mL of 75 μmol L^{-1} in MeOH) were then added to achieve a final volume of 3.0 mL. The solution was kept at room temperature for 30 min and the absorbance was measured at 517 nm (Blois, 1958).

The DPPH scavenging effect was calculated as follows:

$$\text{scavevenging effect}\,(\%) = \frac{A_0 - (A - A_b)}{A_0} \times 100 \quad (1)$$

where A_0 is the absorbance at 517 nm of DPPH without sample, A is the absorbance at 517 nm of sample and DPPH and A_b is the absorbance at 517 nm of sample without DPPH. The absorbance measurements were performed in Elisa Biotech Synergy HT (Biotek, USA).

2.4. Antimicrobial activity of chitosan

The antibacterial activity of chitosan was tested against two bacterial strains: *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) by the disc agar diffusion test according to other authors (Bauer, Kirby, Sherris, & Turck, 1966). Briefly, the chitosan solution (1 mg mL^{-1}) was absorbed in sterilized filter paper discs (0.6 cm in diameter) and placed on the lawn cultures of *S. aureus* and *E. coli*. The agar plates were incubated for 24 h at 37 °C and diameters of the inhibitory zone of clearance (cm) surrounding the discs were measured to estimate the antimicrobial activity. Sterile distilled water at the pH of the chitosan solution was used as control.

2.5. Evaluation of the interactions between chitosan and fucoidan

2.5.1. Quartz crystal microbalance

The adsorption behaviour of fucoidan and chitosan was evaluated using a quartz crystal microbalance (QCM 200, purchased from Stanford Research Systems, SRS, USA), equipped with AT-cut quartz crystals (5 MHz) with optically flat polished chrome/gold electrodes on contact and liquid sides.