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Chitosan-pectin hybrid nanoparticles prepared by coating and blending techniques

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ABSTRACT

The preparation of chitosan nanoparticles in combination with pectins, as additional mucoadhesive biopolymers, was investigated. Pectins from apple and from citrus fruit were considered; polygalacturonic acid was taken as a reference. Tripolyphosphate was used as an anionic cross-linker. Two different techniques were compared, namely the coating and the blending. Coated nanoparticles (NPs) in the ratio pectin:NPs from 2:1 to 5:1 evidenced that the size of NPs increased as the amount of pectin (both from apple and citrus fruit) was increased. In particular, for NPs coated with pectin from citrus fruit the size ranges from 200 to 260 nm; while for NPs coated with pectin from apple the size ranges from 330 to 450 nm. A minimum value of Z-potential around −35 mV was obtained for the ratio pectin:NPs 4:1, while further addition of pectin did not decrease the Z-potential. Also blended NPs showed a dependence of the size on the ratio of the components: for a given ratio pectin:tripolyphosphate the size increases as the fraction of chitosan increases; for a low ratio chitosan:pectin a high amount of tripolyphosphate was needed to obtain a compact structure. The effect of the additional presence of loaded proteins in chitosan-pectin nanoparticles was also investigated, since proteins contribute to alter the electrostatic interactions among charged species. FT-IR and DSC characterization are presented to confirm the interactions between biopolymers. Finally, the biocompatibility of the used materials was assessed by the chorioallantoic membrane assay, confirming the safety of the materials.

1. Introduction

Microspheres and microcapsules based on chitosan were developed for pharmaceutical applications and the importance of mucoadhesive properties for site specific drug delivery were described (He et al., 1998). The great potentiality of the use of chitosan as drug carrier is amply recognized. Various techniques have been described in literature for the preparation of chitosan microcarriers, as well as the parameters affecting drug release (Sinha et al., 2004). Different forms of chitosan based nanomaterials have been also reported in recent reviews (Borgogna et al., 2011; Shukla et al., 2013). The nanotechnological approach for the development of nanoparticle-based drug delivery systems has gained increase attention in the recent years (Desai, 2012). Several examples are reported in literature on the use of chitosan nanoparticles. More recently, rivastigmine loaded chitosan nanoparticles were investigated for intranasal delivery in case of Alzheimer's disease (Fazil et al., 2012); verapamil HCl loaded chitosan microspheres were studied for intranasal administration (Abdel Mouez et al., 2014); gemcitabine loaded in chitosan NPs was studied for oral delivery by Derakhshandeh and Fathi (2012).

One of the widely used techniques for the preparation of chitosan nanoparticles (NPs) is the ionic gelation, which is relatively simple and mild; it allows the successful encapsulation of labile molecules, such as proteins, since it avoids the use of organic solvents and high temperatures (Al-Qadi et al., 2012; Berger et al., 2004; Nasti et al., 2009; Xu and Du, 2003). The preparation of chitosan NPs following the method described by Calvo et al. (1997), was recently investigated focusing the attention on the effect of the ratio between chitosan and TPP (tripolyphosphate) on the loading of protein, being all charged species (Rampino et al., 2013). This study and other literature results (Papadimitriou et al., 2008; Bagre et al., 2013) show that small nanoparticles are obtained with a ratio of chitosan to TPP of 5:1. Moreover, chitosan is widely used for the preparation of several carriers due to its bioadhesive properties, its film forming abilities and low toxicity as well as its abundance in nature. Chitosan can interact with the constituents of the mucosal glycoprotein layer thus prolonging the residence time at the absorption

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site, increasing drug bioavailability (Sogias et al., 2012). The basic mechanisms of mucoadhesion have been reported and extensively commented (Andrews et al., 2009; Serra et al., 2009).

Pectin is another natural polysaccharide of pharmaceutical interest, whose properties mainly depend on the esterification degree. Its increasing use in the pharmaceutical field is due to its high availability in nature, its low or non toxicity nature and above all its mucoadhesive properties together with resistance to degradation by proteases and amylases. Such features make pectin attractive for the formulation of drug delivery carriers for many administration routes (Marras-Marquez et al., 2015). In particular, the correlation between the mucoadhesive properties and the degree of methoxylation of different types of pectin has been investigated by Hagesaether et al. (2008). The same authors paid attention also to investigate the effect of formulation, i.e., when specific ions are added to the pectin solution. Indeed, on one side cross-linking can reduce the polymer mobility, therefore hampering its diffusion and interpenetration within mucin molecules; on the other side, particles of very small sizes have better potential to penetrate the mucus layer. In general, optimal size characteristics are required in order to achieve mucoadhesion by mean of the increased residence time and closer contact with mucosa. The possibility of obtaining hybrid nanoparticles, containing not only chitosan and TPP, but also other poly- or oligo-saccharides has been described in literature. Such hybrid systems are characterized by improved physical properties and better performances when used as drug delivery carriers (Goycoolea et al., 2009 and references therein).

The aim of this work was the investigation of two techniques, named coating and blending, for the preparation of hybrid pectin-chitosan nanoparticles.

Characterization of PEC properties in terms of size, charge, and surface morphology, shows the strict dependence on the macromolecular parameters of the polyions used (in addition to concentration, ionic strength, pH) and on the operative mixing conditions (interaction under resting or streaming mixing). For linear polymers the charge density (number of charges per unit length) defines not only the value for counterion condensation, but also the conditions for chain pairing. These comments may help the understanding of pectin-chitosan interaction with the co-presence of TPP, under the hypothesis of thermodynamic equilibrium. The practical effects of addition of pectin are investigated following the coating technique (post-synthesis of NPs) or the blending technique (during synthesis of NPs).

On one side the coating technique allows to create a core consisting of a polymer and a protein, thus ideally the protein is more protected from the outside environment due to the presence of an outer shell obtained by subsequent coating with a polymer. This is not presumably obtained by a blending technique where the protein is dispersed throughout the polymeric matrix. On the other side one of the main advantages of the blending technique is the possibility of the one-step formulation, that is not possible with the coating technique.

Thus, it has been possible to exploit the well known capability of chitosan to form nanoparticles, to obtain a drug delivery system based on the combination of two classes of mucoadhesive biopolymers.

2. Materials and methods

2.1. Materials

Low molecular weight (LMW) chitosan (MW 150 kDa; $[\eta] = 2.37$ dL/g; degree of acetylation DA = 13%), polygalacturonic acid from orange, MW 18 kDa, degree of esterification (DE) 10,6% (Cesàro et al., 1982), pectin from citrus fruit (MW 17 kDa, DE 22%), pectin from apple (MW 30–100 kDa, degree of esterification 71%), bovine serum albumin (BSA), albumin from chicken egg albumen (OVA), technical grade pentasodium tripolyphosphate (TPP), sodium acetate, sodium hydroxide, and sodium chloride were all purchased from Sigma-Aldrich Co. (St. Louis, Missouri, USA). Acetic acid and hydrochloric acid were obtained from Carlo Erba Reagents (Carlo Erba, Milan, Italy). All other chemicals were of the highest purity grade commercially available and used without further purification.

The commercial chitosan sample was purified and characterized as reported elsewhere (Donati et al., 2005). The intrinsic viscosity of chitosan was measured by employing a Schott–Geräte AVS/G automatic apparatus and an Ubbelohde type viscometer (in acetate buffer 0.25 M, pH 4.7), as reported in the previous paper (Rampino et al., 2013).

2.2. Methods

2.2.1. Nanoparticles preparation

Chitosan nanoparticles (NPs) were prepared using the ionotropic gelation method (Calvo et al., 1997; Rampino et al., 2013). A 0.25% *w*/*v* chitosan solution was prepared by dissolving LMW chitosan in 0.05% *v*/*v* acetic acid solution for 24 h under stirring. The pH of the solution was adjusted to 5.5 with a sodium hydroxide solution while deionized water was added to obtain the desired final concentration. TPP was dissolved in deionized water at a concentration of 0.25% (*w*/*v*) and subsequently diluted to obtain solutions at different concentrations.

The TPP and chitosan solutions were filtered through a 0.45 μm mixed cellulose esters membrane (Millipore, Massachusetts, USA) to remove any insoluble matter. TPP solution was added drop wise to the chitosan solution under magnetic stirring at room temperature (Rampino et al., 2013).

Chitosan NPs have been loaded with two different model proteins: BSA and OVA, whose isoelectric points are 4.8 and 4.7, respectively. The protein were dissolved in deionized water (concentration 4 mg/mL) and added directly to the chitosan solution under magnetic stirring. Batches with different theoretical loading were prepared by adding different volumes of the protein stock solution, to obtain final protein concentrations of 200, 400, and 600 μg/mL. The solution, containing chitosan and protein, was then diluted to a final volume of 5 mL using deionized water. After dropping the TPP solution, the dispersion was left under constant stirring for 30 min at room temperature. The suspension was centrifuged for 2 h at 3270 RCF to remove the excess of chitosan and protein. The supernatant was collected separately while the sedimented particles were re-dispersed in deionized water, analyzed for their size and surface charge and then lyophilized.

2.2.2. Pectin-chitosan nanoparticles

Each pectin sample was dissolved in deionized water adjusting the pH between 6 and 7, thus forming the sodium salt. Hybrid pectin-chitosan NPs were prepared following two different procedures named coating (Borges et al., 2005) and blending (Alonso-Sande et al., 2006).

2.2.2.1. Coating. LMW Chitosan NPs suspension was added to a pectin solution drop wise at different ratios under magnetic stirring at room temperature. The suspension of coated particles was centrifuged for 2 h at 3270 RCF and the supernatant was discarded. Particles were re-dispersed in deionized water, characterized and freeze dried. Protein loaded NPs were prepared according to the procedure reported for sole chitosan NPs (Rampino et al., 2013). Pectin-coated NPs were prepared by using sodium polygalacturonate, pectin from citrus fruit and pectin from apple.

2.2.2.2. Blending. Chitosan and TPP solutions were prepared as previously described. TPP solution was mixed under magnetic stirring to pectin solution at different volume ratios. NPs formed spontaneously upon drop wise addition of the cationic solution to the anionic one under stirring. NPs were isolated by centrifuging at 3270 RCF for 2 h, the supernatant was discarded and the pellet re-dispersed in deionized water, characterized and freeze dried. Loaded NPs were prepared dissolving the model protein directly in the anionic solution containing TPP and