Preparation and characterization of chitosan microparticles modified with papain using crosslinking agents

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SUMMARY

In recent years, biodegradable and biocompatible polymeric microparticles have been widely studied as potential carriers for controlled delivery of drugs. Chitosan is a deacetylated derivate of chitin, present in crustaceans shells shuch as crab and shrimp. Due to its biocompatibility with human tissues and organs, this material has been considered for several biomedical and pharmaceutical aplications. Chitosan presents wound healing properties and the incorporation of other drugs can improve such qualities. Papain is an enzyme that presents anti-inflammatory and antibacterial properties. Therefore, it may act improving the healing of injured epithelial tissues. In the present work, chitosan microparticles were prepared using spraying and coagulation process. Chitosan microparticles were modified with papain and crosslinked with glutaraldehyde and sodium tripoliphosphate. The objective of this work was to evaluate papain immobilization in chitosan microparticles using different crosslinking agents. Morphology and spectral structure of microparticles were studied using Fourier transform infrared spectroscopy (FTIR-ATR), scanning electron microscopy (SEM) and the amount of release papain in pH 7.4 phosphate buffer was measured with (UV) spectrophotometer .
Keywords: biopolymers; chitosan; microparticles; morphology; crosslinking agents

1. Introduction

Chitosan, derived from chitin by deacetylation, is the second most abundant naturally occuring biopolymer (after cellulose) and is found in the exoskeleton of crustaceans such as crab and shrimp.

Due to its biodegrability, low toxicity and biocompatibility, chitosan has been widely investigated for many biomedical and pharmaceutical applications. It is insoluble in water, but becomes soluble and cationic in aqueous acidic solution (pH<6.5) and it could be readily into films, spheres and sponges (Chandy and Sharma, 1990; Sinha, 2004).

Chitosan also presents wound healing properties and its use together with other drugs could improve such qualities.

Papain is an enzyme that presents anti-inflammatory and antibacterial properties and can be used in the treatment of burns and in wound healing.

Chitosan microspheres have been extensively studied as carriers for drugs and controlled drug release systems (Mi et al., 2001; Park et al., 2002). Several works concerning controlled release of antibiotics, antihypertensive agents, antic carcinogenic, protein, peptide drugs and vaccines can be found in literature (Sinha, 2004; Queen et al., 2002; Calvo et al., 1997; Mi et al., 1999c).

Chemical crosslinking agents such as glutaraldehyde, ethyleneglycol diglycidyl ether and poly(ethyleneglycol), could be used to enhance controlled release of drugs from chitosan microparticles (Jameela and Jayakarishnan, 1995; Lim et al., 1997; Mi et al., 1999c; Blanco et al., 2000). However, the addition of these substances chemical can be limited due to its toxicity (Lim et al., 1997; Mi et al., 2001; Shu et al., 2001).

Other crosslinking mechanisms, such as ionic interaction, can be applied to overcome the disvantage of chemical crosslinking.

Tripolyphosphate (TPP) is a nontoxic salt, obtained from triple condensation of groups PO4. It acts by increasing the pH and ionic strength of the solution, forming gel and promoting ionic interaction between amino groups of chitosan and anionic groups of TPP (Aral and Akugba, 1998; Mi et al., 1999b; Shu and Zhu, 2000, 2001).

It has been shown that chitosan microparticles produced by ionic crosslinking with tripolyphosphate(TPP) increased the drug loading efficiency and prolonged the drug release period (Bodmeier et al., 1989; Calvo et al., 1997; Shu and Zhu, 2000, 2001).

Several processes have been used for preparation of chitosan microparticles, including ionotropic gelation, wet phase inversion, coacervation, complex-coacervation and spray drying. Freeze-drying also can be used to microparticles drying.

The objective of this work was to evaluate which crosslinking agents are more suitable to be used with chitosan microparticles to immobilize papain and their influence in the morphological properties and spectral structure.

2. Materials and Methods

2.1. Materials

Chitosan was purchased from Sigma®, with a deacetylation degree of approximately 85%. Casein was purchased from Synth®. Glutaraldehyde was purchased from Nuclear®. Tripolyposphate was purchased from Synth®. All other reagents were of analytical grade, and used without further purification.

2.2. Methods

2.2.1. Preparation of microparticles

Chitosan microparticles were prepared using spraying and coagulation process. Chitosan solutions were prepared by dissolving it in a 3% acetic acid solution.

Various amounts of chitosan microparticles were prepared and cross-linked with glutaraldehyde solution 0.75% v/v and others were crosslinked with Tripolyphosphate solution 10% w/v. The crosslinking time were of 2 h. After the crosslinked time, microparticles were whashed with Milli-Q water repeatedly. The microparticles were adsorbed with papain 1% w/v for 12h, after adsortion time, the microparticles were washed and dried.

Freeze-drying was the chosen method. The microparticles were suddenly frozen through immersion in

liquid nitrogen (-195°C), and then freeze-dried at –62°C and pressure of 10^{-2} Torr, using a manifold freeze-dryer.

2.2.2. Morphology

The dried microparticles obtained by freeze-drying was examined using scanning electron microscopy (SEM). Samples were covered by a thin gold layer (10nm) using a sputter coater SCD 050 (Baltec, Liechenstein) and observed on a JEOL JXA-840^{A} field emission microscope (25kV)(Jeol, Japan).

2.2.3.Fourier transform infrared spectroscopy

Spectroscopic structural study of chitosan microparticles was done by FTIR-ATR (Nicolet Protegé 460).

2.2.4.*In vitro* release studies

For the *in vitro* release studies, microparticles (10mg) were suspended in 40 ml of phosphate-buffered saline (PBS) (pH 7,4) contained in a glass bottle, and maintained at 37°C, the shaker kept the agitation of 100 rpm, during 24h-period. In determined times, 2ml of supernatant were taken from the solution to measure the amount of released papain. EDTA, Cistein, Casein and phosphate-buffer were added to the 2ml-samples. The resulting mixture was left at 37°C for 30 min and after this time, 6ml of tricloroacetic acid were added in order to stop the enzymatic reaction. Centrifugation was applied on samples for 15 min and the amount of released tyrosine was measured using UV-VIS spectrophotometer (λ=280nm).

3. Results and Discussion

3.1.Scanning electron microscopy

The surface morphology of freeze-dried chitosan microparticles revealed porosity on those crosslinked with glutaraldehyde. On the other hand, the microparticles crosslinked with tripolyphosphate showed a surface that

appeared to be dense, but when broken, revealed the internal macropores (Fig. 1-a).

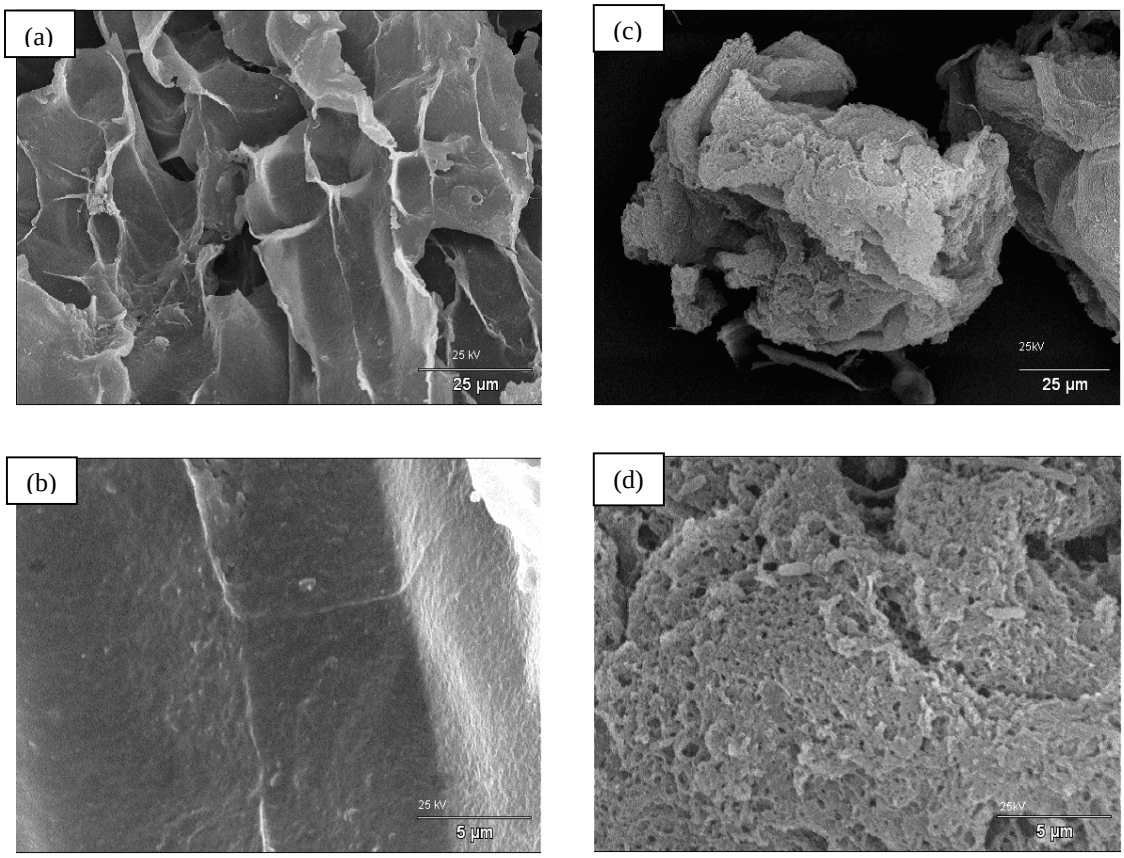

Fig.1: (a)TPP-crosslinked chitosan microparticles surface;(b)fracture surface of TPP-crosslinked chitosan microparticles; (c) and (d) glutaraldehyde-crosslinked chitosan microparticles surface.

3.2. Fourier transform infrared spectroscopy

FTIR-ATR spectra of chitosan microparticles are depicted in figure 2: (a) the spectra of natural chitosan according to the literature, showed peaks at 1100 cm^{-1}- aliphatic amines; 1650 cm^{-1}-N-H group; 1750 cm^{-1}-C-O-O group; (b) the spectra of chitosan-TPP-papain showed a peak at1150 cm^{-1} due to P=O bond; (c) the spectra of chitosan crosslinked with glutaraldehyde showed

typical aliphatic groups from glutaraldehyde at 1100 cm^{-1} and 1650 cm^{-1} due to N=C bond; 1560 cm^{-1} attributed to C=C bond; 1720 cm^{-1} free aldehyde function.

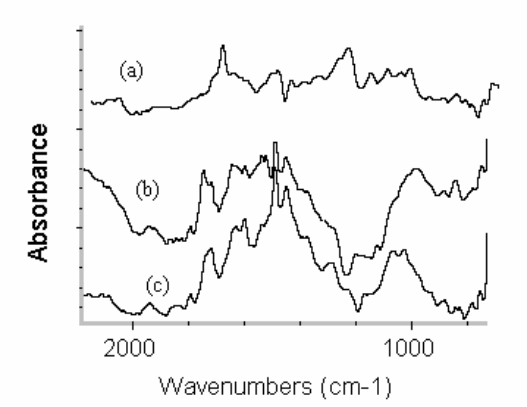

Fig. 2: FTIR-ATR spectra of pure chitosan microparticles(a) and chitosan microparticles crosslinked with TPP and papain (b) and chitosan microparticles crosslinked with glutaraldehyde and papain (c).

3.3.*In vitro* release studies

The papain release studies from chitosan microparticles crosslinked with TPP and glutaraldehyde, observed in (Fig. 3 and 4), showed a stabilization in the drug release, with low variation of tyrosine concentration in the experiment during 25 h. This is indicating an efficient immobilization of papain in the chitosan microparticles using the crosslinking.

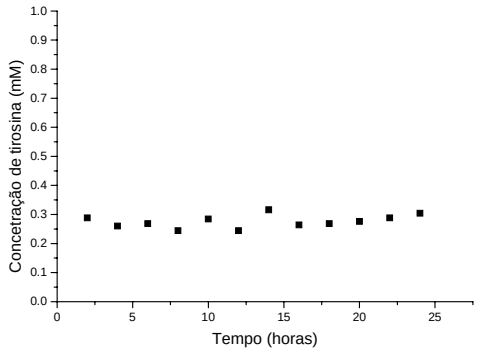

Fig. 3: Papain release from chitosan microparticles crosslinked with TPP.

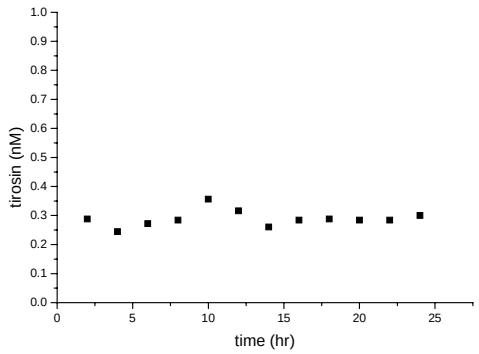

Fig. 4: Papain release from chitosan microparticles crosslinked with glutaraldehyde.

4. Conclusion

The use of crosslinking agents to chitosan microparticles showed to be efficient for controlled papain release. The chitosan microparticles crosslinked with glutaraldehyde presented meso-porosity while the microparticles crosslinked with TPP showed macro-porosity. However, TPP will preferably be chosen as it presents lower toxicity in our further studies.

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