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Chitosan nanoparticles and microspheres for the encapsulation of natural antioxidants extracted from *Ilex paraguariensis*

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ABSTRACT

The use of chitosan for the encapsulation of active components has gained interest in the last years due to its mucous adhesiveness, non-toxicity, biocompatibility and biodegradability. The benefits of encapsulating active agents in a polymer matrix include their protection from the surrounding medium or processing conditions and their controlled release.

In this study chitosan nanoparticles and microspheres were obtained for the encapsulation of yerba mate. Nanoparticles were prepared by ionic gelation of chitosan hydrochloride and sodium tripolyphosphate. The active components were added to the sodium tripolyphosphate solution and this was added dropwise to the chitosan hydrochloride solution while stirring. Microspheres were prepared by the spray-drying method. In this case, antioxidants were added to the chitosan solution and the mixture was spray-dried. The effect of the encapsulating systems on the active compound stability and its release properties was analyzed.

The products obtained allowed to control the release of natural antioxidants and therefore these encapsulating methods are a promising technique for nutraceutical and cosmetic applications.

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1. Introduction

Chitosan is a polymer that is obtained from the deacetylation of chitin, a naturally occurring and abundantly available polysaccharide. Chitosan is receiving a lot of interest in the encapsulation of active compounds due to its biocompatibility, low toxicity and biodegradability (Grenha et al., 2007; Hirano, Seino, Akiyama, & Nonaka, 1990). The benefits of encapsulating active agents in a polymer matrix include their protection from the surrounding medium or processing conditions and their controlled release (Aranaz et al., 2009; Paños, Acosta, & Heras, 2008).

Phenolic compounds exhibit high potent antioxidant activity. Incorporation of antioxidant compounds in manufactured foods, nutraceuticals or cosmetic preparations is a growing area of research. Yerba mate (*Ilex paraguariensis*) is a tea-like beverage traditionally drunk in different countries of South America, although its intake is spreading to other countries, introduced as a substitute of tea. Yerba mate presents a high content of caffeoyl derivatives and other phenolics (Bravo, Goya, & Lecumberri, 2007). Some of the pharmacological properties attributed to yerba mate such as hepatoprotective, diuretic, hypocholesterolemic, anti-rheumatic, anti-thrombotic, anti-inflammatory, anti-obesity or anti-ageing among others have been related to this high content of polyphenols (Chandra & Gonzalez de Mejia, 2004; Filip, Lopez, Giberti, Coussio, & Ferraro, 2001).

The result of the combination of chitosan, a natural polymer, with copassengers such as antioxidants is a new system which has the properties of both components and which in addition can improve the stability of the antioxidants and control their release. Kosaraju, D'ath, & Lawrence (2006) included antioxidants from olive leaf into chitosan microspheres and Deladino, Anbinder, Navarro, & Martino (2008) encapsulated yerba mate lyophilised extracts in calcium alginate and calcium alginate–chitosan beads. To our knowledge there has been no study on chitosan microspheres and nanoparticles of polyphenolic compounds from *I. paraguariensis* extract (ILE) cross-linked with sodium triphosphate pentabasic (TPP).

The aim of this study was to obtain chitosan hydrochloride nanoparticles and microspheres for the encapsulation of yerba mate extract for cosmetic applications. Nanoparticles were prepared by ionic gelation and microspheres by spray-drying and they were characterized in terms of morphology, zeta potential and in vitro release. The effect of encapsulation on the antioxidant properties was also studied.

2. Materials and methods

2.1. Materials

Chitosan hydrochloride (HCS) was obtained from Novamatrix (Norway). The degree of deacetylation was 90% and the molecular

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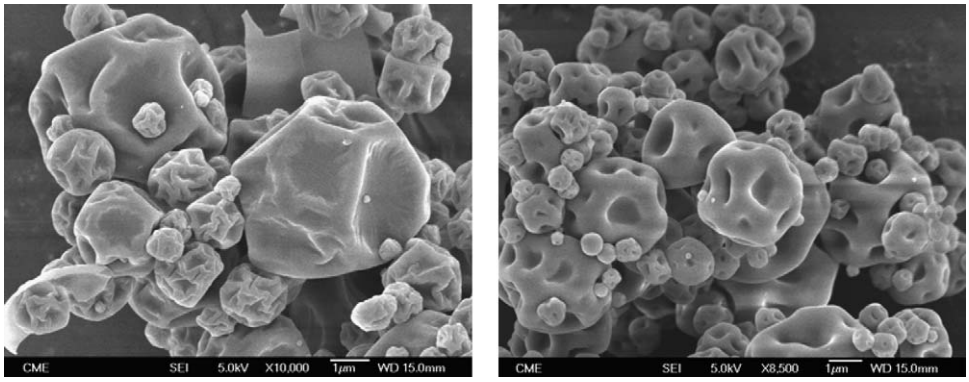

Fig. 1. Scanning electron micrographs of ILE microspheres and M1 (HCS–TPP–ILE) microspheres.

weight (Mw) was 200 kDa. All other reagents were all commercially available and used without any modification.

2.2. Preparation of microspheres and nanoparticles

Chitosan microspheres were prepared by spray-drying (Harris, Paños, Acosta, & Heras, 2008): M1, with 0.5% (w/v) HCS solution and 0.1% (w/v) TPP, and M2 with 1% (w/v) HCS and 0.2% (w/v) TPP. The active component (30%, w/w) was added to the TPP solution. ILE microspheres without chitosan and TPP were also obtained by spray-drying.

Ionic gelation of tripolyphosphate pentasodium (TPP) and chitosan hydrochloride (HCS) was used to prepare chitosan nanoparticles as described by Fernández-Urrusuna, Calvo, Vila-Jato, and Alonso (1999). Two different concentrations of HCS and TPP were used and two lots of nanoparticles were obtained: NP1, with 0.15% (w/v) HCS and 0.084% (w/v) TPP, and NP2, with 0.3% (w/v) HCS and 0.168% (w/v) TPP. The active component (10%, w/w) was encapsulated in nanoparticles by mixing it with the TPP solution before nanoparticle formation with constant stirring.

2.3. Morphology

The shape and surface of microspheres and nanoparticles were observed by scanning electron microscopy (SEM). The samples were examined using a scanning electron microscope (JEOL JSM-6400, JEOL, Tokyo, Japan).

2.4. Zeta potential

Zeta potential measurements were made by microelectrophoresis using a Malvern Zetasizer Nanoseries Nano ZS (Malvern Instruments, Herrenberg, Germany) (Müller, 1996).

2.5. Determination of total polyphenols content and antioxidant activity

Total polyphenols content was spectrophotometrically quantified at 750 nm using the Folin–Ciocalteu reagent (Montreau, 1972) and gallic acid as standard.

Antioxidant activity was determined by the ferric reducing/antioxidant power (FRAP) assay at 595 nm (Benzie & Strain, 1999; Pulido, Bravo, & Saura-Calixto, 2000) using a water-soluble vitamin E analogue, trolox, as standard. A GBC UV/Vis 920 spectrophotometer (GBC Scientific Equipment PTY LTD, Melbourne, Victoria, Australia) was used in both methods.

2.6. Stability of polyphenolic compounds and encapsulation efficiency

The polyphenols encapsulated in microspheres were quantified by Folin–Ciocalteu method after dissolving 30 mg of microspheres in 20 mL of deionized water. This procedure was done after the obtention of microspheres and 3 months later in order to evaluate the stability of the encapsulated polyphenols over time.

2.7. In vitro polyphenol release

Microspheres (150 mg), inside a cellulose dialysis bag (dialysis tubing, Mw cut off 12,000 Da, Sigma–Aldrich) were suspended in buffers with different pH values (pH 6.5 and 5.7). The dialysis bag was placed in a glass with 100 mL of the release medium at 37 °C and 100 rpm (Rotabit horizontal shaker, Selecta, Barcelona, Spain). Nanoparticle suspensions were aliquoted in 1 mL tubes, centrifuged at 15,000 rpm during 30 min, resuspended in buffers at a pH of 6.5 or 5.7 and incubated at 37 °C and 100 rpm. The release of the polyphenols was quantified using Folin–Ciocalteu method. All the experiments were carried out in triplicate.

3. Results and discussion

3.1. Morphology

Fig. 1 shows the morphology of ILE microspheres and HCS microspheres cross-linked with TPP and loaded with ILE extract. All microspheres prepared were spherical in shape. Their size was less than 5 μm. Indentations appeared on the surface of microspheres due to the rapid evaporation of the solvent. Microspheres with a higher chitosan and TPP concentration had a similar morphology.

Other authors have also reported the presence of wrinkles and indentations on the surface of spray-dried microspheres (Desai & Park, 2005; Kosaraju et al., 2006; Martinac, Filipovic-Grcic, Voinovich, Perissutti, & Franceschinis, 2005).

The nanoparticle size (hydrodynamic radius) distribution is shown in Fig. 2.

3.2. Zeta potential

Zeta potential of microspheres is shown in Table 1. The zeta potential values of microspheres and nanoparticles were all positive which indicates the presence of amino groups of chitosan on the surface. The zeta potential of nanoparticles was higher than that of microspheres. These delivery systems have mucoadhesive potential and absorption enhancement properties (Bernkop-Schnürch, 2005).

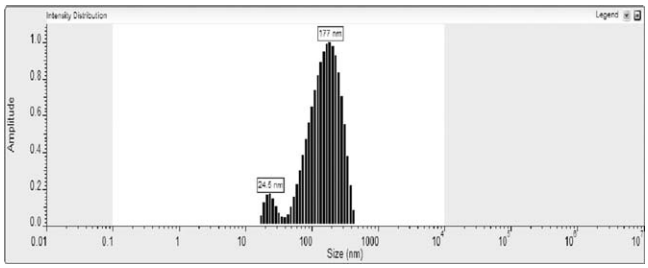

Fig. 2. Nanoparticle size distribution of chitosan hydrochloride–TPP nanoparticles.

Table 1
Composition and zeta potential of ILE microspheres, M1 (0.5%, w/v HCS, 0.1%, w/v TPP), M2 (1%, w/v HCS, 0.2%, w/v TPP), NP1 (0.15%, w/v HCS, 0.084%, w/v TPP) and NP2 (0.3%, w/v HCS, 0.168%, w/v TPP).

Delivery systems	Zeta potential ± SD (mV)
ILE	−6.68 ± 3.24
M1	+17.6 ± 3.93
M2	+15.3 ± 4.07
NP1	+26.9 ± 4.51
NP2	+29.4 ± 4.69

3.3. Stability of polyphenolic compounds and encapsulation efficiency

The total polyphenol content in the obtained chitosan hydrochloride microspheres was measured to determine the encapsulation efficiency. In addition, this was also calculated after 3 months to study the stability of the encapsulated polyphenols over time. The encapsulation efficiency was near 100% for M1 and M2. After 3 months the polyphenol content was 87 and 88% for M1 and M2, respectively. Therefore, chitosan microspheres maintained the stability of the polyphenols over time and are a good vehicle for the encapsulation of these compounds. Kosaraju et al. (2006) also observed that the encapsulation of olive leaf extract by the spray-drying process did not lead to the inactivation of polyphenolic compounds.

3.4. Release studies

Although the pH of the skin is 5.5, the pH of cosmetic formulations can range between 5.5 and 7 (Meyer Rosen, 2005). The release studies of ILE microspheres and M1 and M2 microspheres were performed in two buffers with different pH values (pH 5.7 and 6.5). Release profiles at pH 5.7 and 6.5 are shown in Fig. 3A and B, respectively. In both cases, 90% of polyphenols was delivered from ILE microspheres after 4 h, while M1 and M2 slowed down the release, being at this same time 60% in pH 5.7 and between 40 and 45% in pH 6.5. The higher release in pH 5.7 was due to the solubility of chitosan hydrochloride at this pH. Deladino et al. (2008) prepared chitosan–alginate beads for the encapsulation of yerba mate. Their studies showed that beads without chitosan released around 50% of the polyphenol content and beads with chitosan released around 35% at 3.5 h, which is significantly lower than the released amount in the present study.

Drug release studies were also performed with ILE loaded nanoparticles in both buffers. Nanoparticles, in both pH values released 100% of ILE-polyphenols loaded after 15 min. Kim et al. (2006) showed that chitosan nanoparticles maintain their characteristics and this confers them valuable properties, as protective and moisturizer, for the encapsulation of active agents for cosmetic applications. Therefore, nanoparticles could be an eligible vehicle

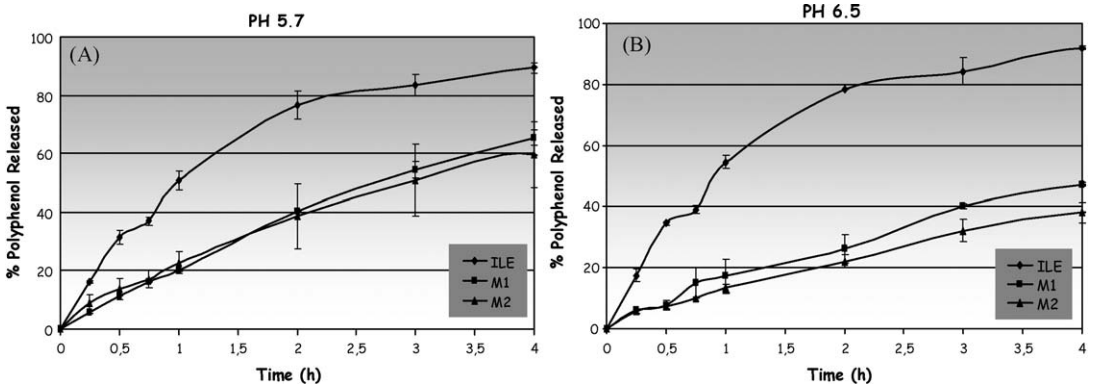

Fig. 3. Release profiles of ILE extract from ILE microspheres (ILE) and chitosan hydrochloride microspheres with 0.5% (w/v) HCS and 0.1% (w/v) TPP (M1) and with 1% (w/v) HCS and 0.2% (w/v) TPP (M2), in buffers pH 5 (A) and pH 6.5 (B).

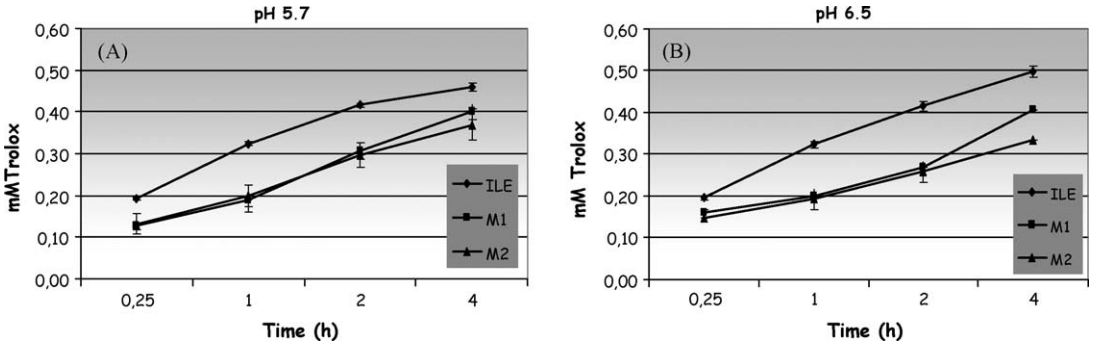

Fig. 4. Antioxidant activity of polyphenols released from ILE microspheres, M1 (0.5%, w/v HCS, 0.1%, w/v TPP) and M2 (1%, w/v HCS, 0.2%, w/v TPP) microspheres in buffers pH 5.7 (A) and pH 6.5 (B).

to encapsulate antioxidants and protect them, but further studies should be done to obtain nanoparticles that control the release of ILE active agents.

3.5. Antioxidant activity

The antioxidant activity at different time intervals of the release experiments was measured for ILE microspheres, M1 and M2 at both pH values (5.7 and 6.5).

As can be observed in Fig. 4A and B, in microspheres, the ferric reducing antioxidant power in the medium increased with time in the same way as polyphenol release. Based on these results it can be concluded that the processes carried out during the release experiments of the microspheres did not affect the antioxidant activity of phenolic compounds.

4. Conclusions

Chitosan hydrochloride–TPP microspheres and nanoparticles have proved to be adequate vehicles for the encapsulation of natural antioxidants because they maintain the antioxidant activity of ILE-polyphenols. The release of the active agents was regulated by encapsulation in chitosan hydrochloride–TPP microspheres. More studies have to be done to further control the release from these microspheres and nanoparticles for their use in cosmetic applications.

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