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Structural analysis of chitosan hydrogels containing polymeric nanocapsules

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ABSTRACT

The incorporation of different concentrations of polymeric nanocapsule suspensions into chitosan hydrogels is proposed, in order to study the structure of a formulation with the properties of great tissue adhesion and controlled release of the nanoencapsulated drugs, represented here by capsaicinoids. The gels presented acceptable acid pH values and the nanoparticles were visually observed in the system. A transition from the micrometer to the nanometer scales suggested that the nanocapsules are initially agglomerated in the hydrogel. A sedimentation tendency of the nanocapsules in the system was observed and only physical interaction between the chitosan chains and polymeric nanocapsules was verified. The hydrogels, despite the presence of nanocapsules, presented shear-thinning properties and an elastic behavior under low and high frequencies, showing a very structured gel network. The observed variation in the elasticity of the hydrogels may arise from a decrease in the number of interactions and degree of entanglement between the chitosan chains, caused by the presence of nanoparticles.

1. Introduction

Chitosan, a cationic biopolymer, has been widely applied in the development of differentiated pharmaceutical forms, such as polymeric films, hydrogels and beads, which are useful in distinct research areas [1–3]. Its advantageous properties include biodegradability, bioadherence, wound healing promotion and a bacteriostatic effect [4,5], besides a possible effect on the tight junctions between epithelial cells [6], leading to enhancement in the permeation of active compounds into biological tissues. Chitosan hydrogels can be formed by simple acidification of the aqueous medium and further entanglement of the chitosan chains due to secondary interactions [2]. The gel characteristics are strongly dependent on the chitosan molecular weight and degree of deacetylation [7]. Moreover, the addition of crosslinkers brings new properties to the hydrogels, such as thermal and pH responses (physical crosslinking) or a very structured hydrogel network (chemical crosslinking) [2,3].

The incorporation of polymeric nanocapsules into chitosan hydrogels is a promising system recently proposed by our research group [8]. This innovative system combines the previously-mentioned chitosan properties with the advantages associated with nanocapsules, such as the control of the drug release [9,10], as observed in the controlled release of nanoencapsulated capsaicinoids [8]. The capsaicinoids found in chili peppers present topical analgesic action, due to depletion of P substances, and recently they have been used to treat chronic pain [11]. Since capsaicin and dihydrocapsaicin, which are the major capsaicinoids, are irritant substances and many applications are required to achieve the desired effect [11], their nanoencapsulation could increase the patient's compliance, by means of a decrease in the release rate [8] and in the irritation effect [12].

In the first work describing the association of polymeric nanocapsules and chitosan gel [8], the incorporation of cationic polymeric nanocapsules containing capsaicinoids into the referred gel led to a slight increase in the hydrogel viscosity, measured immediately after preparation, suggesting that the presence of the nanoparticles has an effect on the chitosan gel structure. In addition, the chitosan hydrogel containing nanocapsules did not show a further increase in viscosity during storage, as noted for the chitosan hydrogel containing pure water. Although interesting properties of the novel formulation has been already described such as controlled release [8], great skin adhesion [13] and decrease on the skin irritation [12], the elucidation of the hydrogel structure in the presence of nanocapsules has not been studied so far, and it is of great importance in order to explain such changes in the gel behavior.

The incorporation of liposomes [14] and lipid nanoparticles [15] into chitosan hydrogels, as well as a rheological study on these types of formulations, has been previously described. The presence of liposomes slightly increased the gelation rate and gel strength of chitosan thermosensitive hydrogels [14]. Also, Souto and co-workers [15] produced a 1% chitosan hydrogel containing solid lipid and nanostructured lipid

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nanoparticles. Their results showed that pure hydrogels presented a weaker and more sensitive structure when compared to nanoparticle-containing hydrogels.

Based on the exposed, the aim of this study was to, by adding different nanocapsule amounts to the hydrogels, investigate the structure of chitosan hydrogels containing capsaicinoid-loaded polymeric nanocapsules, as well as to determine the influence of the addition of nanoparticles on chitosan hydrogel networks.

2. Materials and methods

2.1. Materials

Chitosan (medium molecular weight, deacetylation degree of 75%) and Eudragit RS 100® were obtained from Degussa (Darmstadt, Germany) and Sigma-Aldrich (São Paulo, Brazil), respectively. Polysorbate 80, capric/caprylic triglycerides and lactic acid 85% were purchased from Labsynth (São Paulo, Brazil), Brasquim (Porto Alegre, Brazil) and Via Farma (São Paulo, Brazil). Acetonitrile was purchased from Tedia (Fairfield, USA). The capsaicinoids used as model drugs for nanoencapsulation were obtained from Deg (São Paulo, Brazil) (58% of capsaicin and 33% of dihydrocapsaicin). The acetone used was purchased from Vetec (Rio de Janeiro, Brazil) and was of analytical grade.

2.2. Production of capsaicinoids-loaded nanocapsules

The capsaicinoid-loaded nanocapsules were prepared through the interfacial deposition of the preformed polymer [16], as previously described [8]. Briefly, an organic phase containing 100 mg of Eudragit RS 100®, 5 mg of capsaicinoids, 27 mL of acetone and 330 μL of capric/caprylic triglyceride was injected, applying a controlled rate, into an aqueous phase containing 76 mg of polysorbate 80. After mixing for 10 min, the solvents were eliminated using a reduced-pressure rotary evaporator (Büchi) for the adjustment of the final volume (10 mL). Eudragit RS 100®, the selected polymer, has already been used for the obtainment of nanocapsules for the cutaneous [8], vaginal [17] and ocular [18] administration routes.

2.3. Incorporation of nanocapsules into the chitosan hydrogel

In order to obtain chitosan hydrogels containing nanocapsules, different percentages of nanocapsule suspension and/or water (Table 1) were added to the chitosan, which was present at a final concentration of 3%. Prior to use, the chitosan was sieved (sieve number 355) to guarantee a homogeneous system. The dispersion was acidified through the addition of lactic acid to give a final concentration of 1%. Manual mixing led to the obtainment of hydrogels. The formulations presented theoretical final concentrations of capsaicinoids between 0.15 (hydrogel B) and 0.5 $mg \cdot g^{-1}$ gel (hydrogel D).

2.4. Characterization of the chitosan hydrogels containing nanocapsules

2.4.1. pH value

The pH values of the hydrogels were determined by potentiometry (potentiometer B474, Micronal) after the dilution of the semi-solid formulations in Milli Q® water (1:10 w/v). Data are represented as the mean of three independent experiments.

2.4.2. Particle size distribution

The hydrogels were characterized in terms of particle size distribution by laser diffraction (Mastersizer®, Malvern), after their dispersion in distilled water, using 1.38 and 1.52 as the refraction indexes of Eudragit RS 100® (Gels B, C and D) and chitosan (Gels A, B, C and D), respectively. Both refraction indexes were used for the data analysis in order to observe the behavior of the nanoparticles and also of the chitosan hydrogel microdomains.

2.4.3. Zeta potential

The zeta potential values of the final formulations were determined after the dispersion of the hydrogels in a filtered 10 mM NaCl solution (20 mg of gel in 10 mL, vortex mixing). The data are represented as the mean of three different measurements.

2.4.4. Physical stability

The physical stability of the hydrogels was determined by the multiple light scattering techniques (Turbiscan LAB®, Formulation, France). Each sample (around 15 g) was placed into a cylindrical glass cell (25 mm diameter, 55 mm height) at 25 °C. The glass cells were completely scanned at time intervals of 5 min for 1 h immediately after the preparation of the samples, and also after 5, 10 and 30 days under storage in the same glass cells at 25 °C. The transmitted and backscattered light as a function of the cell height was obtained during this time interval.

2.4.5. Oscillatory rheology

In order to determine the rheological behavior of the chitosan hydrogels, preliminary strain-sweep experiments were carried out to guarantee the linear viscoelastic regime. Oscillatory frequency-sweep measurements were then performed at angular frequencies ranging from 10^{-2} to 103 Hz at a constant temperature (23.0 ± 0.2 °C) to record the elastic storage modulus (G′), and the viscous loss modulus (G″). All experiments were conducted in a rheometer (model MCR 101, Anton-Paar Physica, Germany), with a cone-plate geometry (25 mm in diameter). Steady shear flow curves were also obtained for all of the chitosan hydrogel formulations under the same temperature conditions.

2.4.6. Fourier transform infrared (FT-IR) spectroscopy

The hydrogels under investigation were cast into petri dishes and dried to produce films suitable for FT-IR spectroscopy analysis. The spectra for the films were acquired in a Perkin-Elmer PC-16 spectrophotometer, and recorded with 4 cm^{-1} of resolution (16 scans) in the range of 4000 to 400 cm.

2.4.7. Differential scanning calorimetry (DSC)

The thermal properties of the hydrogels were evaluated using a differential scanning calorimeter (DSC series Q1000 — TA Instruments), operating at a heating rate of 10 °C min^{-1} from −10 to 300 °C under N_2 atmosphere. The weight of each sample was around 9 mg. After the first heating cycle, the samples were cooled to 0 °C and scanned again with heating up to 300 °C at the same heating/cooling rate. DSC curves of each hydrogel were obtained from the second heating run at

Table 1
Composition of chitosan hydrogels (the percentages are related to the liquid phase used to formulate each gel).

Hydrogels	Water (% of liquid phase)	Nanocapsule suspension (% of liquid phase)	Nanocapsule concentration ($\times 10^{13}$ nanoparticles$\cdot g^{-1}$)	Capsaicinoids concentration ($mg \cdot g^{-1}$)
A	100%	0%	–	–
B	70%	30%	0.81	0.15
C	50%	50%	1.35	0.25
D	0%	100%	2.70	0.5