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Impact of molecular weight on the formation of electrosprayed chitosan microcapsules as delivery vehicles for bioactive compounds

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ABSTRACT

The molecular weight of chitosan is one of its most determinant characteristics, which affects its processability and its performance as a biomaterial. However, information about the effect of this parameter on the formation of electrosprayed chitosan microcapsules is scarce. In this work, the impact of chitosan molecular weight on its electrosprayability was studied and correlated with its effect on the viscosity, surface tension and electrical conductivity of solutions. A Discriminant Function Analysis revealed that the morphology of the electrosprayed chitosan materials could be correctly predicted using these three parameters for almost 85% of the samples. The suitability of using electrosprayed chitosan capsules as carriers for bioactive agents was also assessed by loading them with a model active compound, (−)-epigallocatechin gallate (EGCG). This encapsulation, with an estimated efficiency of around 80% in terms of preserved antioxidant activity, showed the potential to prolong the antiviral activity of EGCG against murine norovirus via gradual bioactive release combined with its protection against degradation in simulated physiological conditions.

1. Introduction

Micro- and nanoencapsulation, processes in which a compound is embedded within a protective matrix (Jiménez-Martín, Gharsallaoui, Pérez-Palacios, Carrascal, & Rojas, 2014) which is organized in the form of micro- or nanosized structures, have attracted increasing research interest for the protection of sensitive bioactive compounds (Pérez-Masiá, López-Nicolás et al., 2015) and address current concerns related to their formulation, bioavailability or their delivery to specific sites (Zaki, 2014).

Among the different techniques used for microencapsulation, electrohydrodynamic spraying (electrospraying) is rapidly emerging as a promising technology for the production of polymeric microparticles containing bioactive molecules (Bock, Dargaville, & Woodruff, 2012), as it overcomes some of the limitations of conventional methods. Electrospraying can generate microencapsulation structures in a one-step process (Chakraborty, Liao, Adler, & Leong, 2009) under mild conditions (López-Rubio & Lagaron, 2012; Sosnik, 2014) and in the absence of organic/toxic solvents (Tapia-Hernández et al., 2015), limiting inactivation of the bioactive compounds (Zamani, Prabhakaran, & Ramakrishna, 2013), being adequate for both hydrophilic and hydrophobic drugs or ingredients (Gómez-Mascaraque & López-Rubio, 2016) and generally achieving high loading efficiencies (Sosnik, 2014; Zamani et al., 2013). Therefore, it has found a number of potential applications in various fields, including the pharmaceutical, cosmetic and food industries (Jaworek & Sobczyk, 2008). It basically consists on subjecting a polymer solution (containing the bioactive to be encapsulated) to a high voltage so that the electric field deforms the interface of the liquid drop and breaks it into fine charged droplets, which are ejected towards a collector while the solvent evaporates, generating dry polymeric microparticles (Anu Bhushani & Anandharamakrishnan, 2014; Sosnik, 2014).

Biopolymers are preferred as encapsulating matrices for most applications because of their biocompatibility, biodegradability and non-toxicity (Ghorani & Tucker, 2015). Specially, chitosan is a biorenewable, biocompatible and biodegradable polysac-

Abbreviations: ABTS2, ,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; DDA, degree of deacetylation; DFA, Discriminant Function Analysis; EGCG, (−)-epigallocatechin gallate; FT-IR, Fourier transform infrared spectroscopy; GRAS, Generally Recognised As Safe; MEE, microencapsulation efficiency; MNV, murine norovirus; Mw, molecular weight(s); PBS, phosphate buffer saline; RSA, radical scavenging activity; SEM, scanning electron microscopy.

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charide considered a GRAS food additive by the FDA (Luo & Wang, 2016). Moreover, it has many attributed functional and bioactive properties, including antioxidant and lipid-lowering capacities, antimicrobial activity, wound healing and antiangiogenic effects, prevention of renal failure, etc. (Luo & Wang, 2013; Park, Saravanakumar, Kim, & Kwon, 2010; Ribeiro et al., 2009). For these reasons chitosan and its derivatives have been widely used in the pharmaceutical (Badwan, Rashid, Omari, & Darras, 2015; Cheung, Ng, Wong, & Chan, 2015), biomedical (Anitha et al., 2014; Ishihara, 2015), cosmetic (Anumansirikul, 2007; Jimtaisong & Saewan, 2014) and food industries (Fathi, Martin, & McClements, 2014; Zivanovic, Davis, & Golden, 2014), and is considered a good candidate for the encapsulation of bioactive compounds (Estevinho, Rocha, Santos, & Alves, 2013; Varshosaz, 2007). However, the electrohydrodynamic processing of chitosan is complex due to its particular behaviour in solution and its polycationic nature (Homayoni, Ravandi, & Valizadeh, 2009), consequence of its structure consisting of β-1,4 linked 2-acetamido-2-deoxy-β-D-glucopyranose units and 2-amino-2-deoxy-b-D-glucopyranose units (cf. Fig. S1 of the Supplementary material) (Khor & Lim, 2003).

The electrohydrodynamic spinning (electrospinning) of chitosan for the production of nanofibers from non-toxic solvents has been extensively studied (Sun & Li, 2011), and the impact of different processing parameters, solution properties and/or the molecular weight of the polymer on the morphology of the obtained fibers have been addressed (Geng, Kwon, & Jang, 2005; Homayoni et al., 2009). However, as the focus of these works is the manufacture of fibers, the range of explored conditions does not cover the production of nano/microparticles. The use of electrospraying for the production of dry (Arya, Chakraborty, Dube, & Katti, 2009; Zhang & Kawakami, 2010) or gelled (Pancholi, Ahras, Stride, & Edirisinghe, 2009; Wang et al., 2015; Yunoki, Tsuchiya, Fukui, Fujii, & Maruyama, 2014) chitosan micro- and nanospheres has also been reported, however, all these works use only one particular grade of chitosan, with a fixed molecular weight. Given that the molecular weight of chitosan is one of its key characteristics, which can affect not only its processability but also its performance as a delivery vehicle (Arya et al., 2009), the focus of this work was to study the influence of the molecular weight on the sprayability of chitosan, and to assess the suitability of selected electrosprayed capsules as delivery vehicles for a model bioactive compound: (−)-epigallocatechin gallate (EGCG). EGCG is the most abundant and bioactive compound in green tea (Barras et al., 2009) and possesses many attributed health benefits (Singh, Shankar, & Srivastava, 2011), including protective effects against infections (Steinmann, Buer, Pietschmann, & Steinmann, 2013), cardiovascular and neurodegenerative diseases (Fu et al., 2011), inflammation and arthritis (Singh, Akhtar, & Haqqi, 2010) and cancer (Larsen & Dashwood, 2009, 2010). In the present work, its antioxidant (Fu et al., 2011) and antiviral (Dhiman, 2011; Xiao, 2008) activities were assessed before and after encapsulation within the chitosan electrosprayed capsules.

2. Materials and methods

2.1. Materials

Chitosans with reported degree of deacetylation of 85 ± 2.5% and different molecular weights, ranging from 25 to 300 kDa, were purchased from Heppe Medical Chitosan GmbH. (−)-Epigallocatechin gallate (EGCG), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulfate ($K_2O_8S_2$) and spectroscopic grade potassium bromide (KBr) were obtained from Sigma-Aldrich. 96% (v/v) acetic acid was supplied by Scharlab.

2.2. Preparation of chitosan solutions

Chitosan solutions of different concentrations, i.e. from 0.5 to 8% (w/v), were prepared by dissolving the polysaccharide in acetic acid at room temperature under magnetic agitation overnight. Different acetic acid concentrations were used for this purpose, from 20 to 90% (v/v).

2.3. Characterization of the solutions

The surface tension of the solutions was measured using the Wilhelmy plate method in an EasyDyne K20 tensiometer (Krüss GmbH, Hamburg, Germany) at room temperature.

The electrical conductivity of the solutions was measured using a conductivity meter XS Con6 (Labbox, Barcelona, Spain) at room temperature.

The rheological behaviour of the solutions was studied using a rheometer model AR-G2 (TA Instruments, USA), with a parallel plate geometry, and the method described in (Gómez-Mascaraque, Lagarón, & López-Rubio, 2015). Briefly, continuous shear rate ramps were performed from 0.1 to 200 s^{-1} during 15 min at 25 ± 0.1 °C using a stainless steel plate with a diameter of 60 mm and a gap of 0.5 mm. All measurements were made at least in triplicate.

2.4. Electrohydrodynamic processing of the solutions

The solutions were processed using a homemade electrospinning/electrospraying apparatus, equipped with a variable high-voltage 0–30 kV power supply. Solutions were introduced in a 5 mL syringe and were pumped at a steady flow-rate (0.15 mL/h) through a stainless-steel needle (0.9 mm of inner diameter). The needle was connected through a PTFE wire to the syringe, which was placed on a digitally controlled syringe pump. Processed samples were collected on a grounded stainless-steel plate placed at a distance of 10 cm from the tip of the needle in a horizontal configuration. A voltage of 17 kV was applied to the solutions as selected in preliminary trials.

2.5. Morphological characterization of the particles

Scanning electron microscopy (SEM) was conducted on a Hitachi microscope (Hitachi S-4800) at an accelerating voltage of 10 kV and a working distance of 7–10 mm. As prepared samples were sputter-coated with a gold-palladium mixture under vacuum prior to examination.

2.6. Fourier transform infrared (FT-IR) analysis of the particles

Samples (ca. 1–2 mg) of selected chitosan capsules, both unloaded and EGCG-loaded, were grounded and dispersed in about 130 mg of spectroscopic grade potassium bromide (KBr). A pellet was then formed by compressing the samples at ca. 150 MPa. FT-IR spectra was collected in transmission mode using a Bruker (Rheinstetten, Germany) FT-IR Tensor 37 equipment. The spectra was obtained by averaging 10 scans at 1 cm^{-1} resolution.

2.7. Antioxidant activity of free and encapsulated EGCG

The $ABTS^{+\bullet}$ radical scavenging assay (Re et al., 1999) was performed in order to quantify the antioxidant activity of both free and encapsulated EGCG, following the protocol described in a previous work (Gómez-Mascaraque et al., 2015). Briefly, a stock solution of $ABTS^{+\bullet}$ was prepared by reacting ABTS 7 mM with potassium persulfate 2.45 mM, both in distilled water, and allowing the mixture to stand in the dark at room temperature for 24 h. The stock solution was then diluted with acetic acid 20% v/v to an