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Two-step method for encapsulation of oregano essential oil in chitosan nanoparticles: Preparation, characterization and *in vitro* release study

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ABSTRACT

In this study, oregano essential oil (OEO) has been encapsulated in chitosan nanoparticles by a two-step method, *i.e.*, oil-in-water emulsion and ionic gelation of chitosan with sodium tripolyphosphate (TPP). The success of OEO encapsulation was confirmed by Fourier transform infrared (FT-IR) spectroscopy, UV–vis spectrophotometry, thermogravimetric analysis (TGA) and X-ray diffraction (XRD) techniques. The obtained nanoparticles exhibited a regular distribution and spherical shape with size range of 40–80 nm as observed by scanning electron microscopy (SEM) and atomic force microscopy (AFM). As determined by TGA technique, the encapsulation efficiency (EE) and loading capacity (LC) of OEO-loaded chitosan nanoparticles were about 21–47% and 3–8%, respectively, when the initial OEO content was 0.1–0.8 g/g chitosan. *In vitro* release studies showed an initial burst effect and followed by a slow drug release.

1. Introduction

Essential oils (EOs) are aromatic and volatile oily liquids obtained from plants. They are normally formed in special cells or groups of cells, found in leaves and stems, and commonly concentrated in one particular region such as leaves, bark or fruit (Oussalah, Caillet, Saucier, & Lacroix, 2006). EOs extracted from plants or spices are rich sources of biologically active compounds such as terpenoids and phenolic acids. The antibacterial and antifungal activities of EOs have long been recognized (Burt, 2004; Nychas, 1995), but the food industry has recently been paying more attention to their application as natural antimicrobials (Plooy, Regnier, & Combrinck, 2009). Within a great variety of EOs, oregano essential oil (OEO) extracted from *Origanum vulgare* L. is well known for its antioxidative and antimicrobial activity (Botsoglou, Grigoropoulou, Botsoglou, Govaris, & Papageorgiou, 2003). These activities are mainly due to the two phenols, carvacrol and thymol (major components of oregano essential oil) and the monoterpene hydrocarbons *p*-cymene and γ-terpinene (Baydar, Sagdic, Ozkan, & Karadogan, 2004) which present at lower concentration (Juliano, Mattana, & Usai, 2000).

However, like other EOs, OEO are volatile compound which easily evaporates and/or decomposes during food processing, drug formulation, and preparation of antimicrobial film, *etc.*, owing to direct exposure to heat, pressure, light or oxygen. In order to overcome the susceptibility and improve the stability of bioactive compounds during processing and storage, the emerging technology of nano-encapsulation has been recently applied in food and nutraceutical industries. Nanoencapsulation of bioactive compounds represents a viable and efficient approach to increase the physical stability of the active substances, protect them from the interactions with the food ingredients and enhance their bioactivity, because of the subcellular size (Donsì, Annunziata, Sessa, & Ferrari, 2011). In other words, encapsulation can reduce the loss of activity of the active compounds. In the case of antimicrobials, the nano-level encapsulation can increase the concentration of the bioactive compounds in food areas where microorganisms are preferably located, for example water-rich phases or liquid–solid interfaces (Weiss, Gaysinksy, Davidson, & McClements, 2009).

In the recent years, there has been considerable interest in developing biodegradable nanoparticles as effective lipophilic bioactive food components delivery systems. Chitosan is receiving a lot of interest in the encapsulation of bioactive compounds due to its biocompatibility, low toxicity and biodegradability (Donsì et al., 2011; Harris, Lecumberri, Mateos-Aparicio, Mengíbar, & Heras,

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2011; Hu et al., 2008; Luo, Zhang, Whent, Yu, & Wang, 2011; Muzzarelli, 2010). Among variety of methods developed to prepare chitosan nanoparticles, ionic gelation technique has attracted considerable attention due to this process is non-toxic, organic solvent free, convenient and controllable (Agnihotri, Mallikarjuna, & Aminabhavi, 2004). Ionic gelation technique is based on the electrostatic interaction between the positively charged primary amino groups of chitosan and the negatively charged groups of polyanion, such as sodium tripolyphosphate (TPP) (Calvo, Remũnán-López, & Vila-Jato Alonso, 1997; Dyer et al., 2002; Yang et al., 2011). The chitosan–TPP nanoparticles, composed of food-safe ingredients, has shown its capacity for the encapsulation and delivery of proteins (Avadi et al., 2009; Xu & Du, 2003), genes (Csaba, Köping-Höggård, & Alonso, 2009; Gan, Wang, & McCarron, 2005), hydrophilic and hydrophobic drugs (Ajun, Yan, Li, & Huili, 2009; Trapani et al., 2011; Wang et al., 2006), vitamins (Luo et al., 2011; Yoksan, Jirawutthiwongchai, & Arpo, 2010), and polyphenolic compounds (Bao, Xu, & Wang, 2009; Dudhani & Kosaraju, 2010; Keawchaoon & Yoksan, 2011). Keawchaoon and Yoksan (2011) revealed that the encapsulation of essential oil-derived bioactive compounds such as carvacrol into chitosan–tripolyphosphate particles could extend its shelf life and retain its functional properties. Generally, EOs possessing the strongest antibacterial properties are those that contain phenolic compounds such as carvacrol, eugenol, and thymol (Hirasa & Takemasa, 1998; Rota, Carraminana, Burillo, & Herrera, 2004). To our knowledge, the loading of OEO into chitosan particles at a nanolevel size has not been elucidated.

The present research thus focused on the fabrication and characterization of chitosan–TPP nanoparticles loaded with OEO by a two-step process: oil-in-water (o/w) emulsification, and ionic gelation. We also clarified the successful encapsulation by UV–vis spectrophotometry, FT-IR spectroscopy, TGA and XRD techniques, and determined the shape, morphology and mean particle size by SEM, AFM, and laser light scattering (LLS). The effects of initial OEO content on loading capacity (LC), encapsulation efficiency (EE), and mean particles size were also investigated. In addition, the release profiles of OEO from chitosan nanoparticles were investigated.

2. Materials and methods

2.1. Materials

Medium molecular weight chitosan (75–85% degree of deacetylation, CAS # 9012-76-4), TPP (CAS # 7758-29-4) and Tween 80 (CAS # 9005-65-6) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Acetic acid (CAS # 64-19-7) was supplied by Merck Chemicals Co. (Darmstadt, Germany). OEO (100% pure, CAS # 8007-11-2) was obtained from New Directions Aromatics Inc. (Hampshire, UK). All chemicals were used as received without further purification.

2.2. Preparation of OEO-loaded chitosan particles

OEO-loaded chitosan nanoparticles were prepared according to a method modified from the ones described by Calvo et al. (1997) and Yoksan et al. (2010). Briefly, aqueous and oil phase solutions were produced. Chitosan solution (1% (w/v)) was prepared by agitating chitosan in an aqueous acetic acid solution (1% (v/v)) at ambient temperature (23–25 °C) overnight. The mixture was then centrifuged using a laboratory centrifuges (SIGMA 2-16KC, Germany) for 30 min at 9000 rpm; the supernatant was removed then and filtered through 1 μm pore size filters. Tween 80 (HLB 15.9, 0.45 g) was then added as a surfactant to the solution (40 mL) and stirred at 45 °C for 2 h to obtain a homogeneous mixture.

OEO (0.04, 0.08, 0.16 and 0.32 g) was dissolved separately in CH_2Cl_2 (4 mL) and then this oil phase is gradually dropped into the aqueous chitosan solution (40 mL) during homogenization (Ultra-Turrax T25 basic, IKA, Germany) at a speed of 13,000 rpm for 10 min under an ice-bath condition to obtain an oil-in-water emulsion. TPP solution (0.4% (w/v), 40 mL) was then added drop wise into the agitated emulsion. Agitation was continuously performed for 40 min. The formed particles were collected by centrifugation at 9000 × *g* for 30 min at 4 °C, and subsequently washed several times with deionized water. Finally, ultrasonication was performed by a sonicatior (Bandelin sonopuls HD3200, KE 76 probe, Germany) in an ice bath for 4 min with a sequence of 0.7 s of sonication and 0.3 s of rest, resulting in a homogeneous suspension. The suspensions were immediately freeze-dried at −35 °C for 72 h using Freeze Dryer (GAMMA 1-16 LSC, UK). Both chitosan nanoparticles and supernatant were stored at 4 °C until further analysis. Weight ratios of chitosan to oregano essential oils (Chitosan: OEO) of 1:0.1, 1:0.2, 1:0.4 and 1:0.8 were used for the present study.

2.3. Particle size and morphology of nanoparticles

Measurement of mean particle size of freshly prepared nanoparticles was determined by laser light scattering (SEMATECH, SEM-633, France) with 633 nm wavelength. Triplicate samples were analyzed and mean value was reported.

The morphology of the freeze-dried nanoparticles was studied by scanning electron microscopy (SEM) (VEGA II, TESCAN, Czech Republic). The frozen dried nanoparticles (1 mg) were dispersed in deionized water (20 mL) and sonicated for 4 min. One drop of the dispersion containing chitosan nanoparticles (loaded or unloaded with OEO) was placed on a glass plate and dried at room temperature. The dried nanoparticles were then coated with gold metal under high vacuum and then examined. Representative SEM images were reported.

Atomic force microscope (DualScope™ DS95-50, DME, Denmark) was used for morphological characterization and particle size and size distribution of both chitosan nanoparticles and OEO-loaded chitosan nanoparticles. A drop of diluted nanoparticle suspension (0.05 mg/mL) was deposited on the freshly cleaved clean glass surface, spread and dried at room temperature. The image measurement was performed in tapping mode using silicon probe cantilever of 230 μm length, resonance frequency of 150–190 kHz, spring constant of 20–60 N/m and nominal, 5–10 nm tip radius of curvature. The scan rate was used as 1 Hz. A minimum of 10 images from each sample were analyzed to assure reproducible results.

2.4. Instrumental analyses

FTIR analyses for pure chitosan, pure OEO and chitosan nanoparticles (loaded or unloaded with OEO) were recorded from wave number 400–4000 cm^{-1} by a Bruker Equinox 55 spectrometer (Equinox 55 Bruker Banner Lane, Coventry, Germany). Samples were prepared by grinding the dried nanoparticles with KBr and pressing them to form disks. For each spectrum, 16 scans at a resolution of 4 cm^{-1} were obtained.

TGA analysis was performed with a Perkin-Elmer PYRIS I Thermogravimetric Analyzer (USA). Each freeze-dried sample (10–15 mg) was placed in the TGA furnace and the measurements were carried out under nitrogen atmosphere with a heating rate of 10 °C/min from 25 to 600 °C.

XRD patterns were recorded over a 2θ range of 5–50° using a X-ray diffractometer (Siemens, model D5000) with a step angle of 0.04°/min.