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Fabrication and characterization of binary composite nanoparticles between zein and shellac by anti-solvent co-precipitation

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ABSTRACT

The anti-solvent co-precipitation method was applied to fabricate zein (Z) and shellac (S) composite nanoparticles with different mass ratios (Z:S, 50:1, 10:1, 5:1, 2.5:1, 1:1, 1:1.5 and 1:2.5) at pH 8.0. Measurement of particle size and turbidity, in combination with analyses of Fourier transform infrared spectroscopy (FTIR), circular dichroism (CD), differential scanning calorimetry (DSC), fluorescence spectroscopy, and atomic force microscope (AFM) were performed to characterize Z–S composite nanoparticles. Results showed that hydrogen bonding and hydrophobic attraction were involved in the interactions between zein and shellac, leading to the changes in secondary structure and thermal stability of zein. At low levels of shellac (Z:S, from 50:1 to 2.5:1), a compact structure of Z–S composite nanoparticles was formed, which had smaller particle sizes, higher turbidity value and better thermal stability. At high levels of shellac (Z:S, from 2.5:1 to 1:2.5), a cross-linked structure of Z–S composite nanoparticles was generated, which exhibited larger particle sizes, lower turbidity value, and poorer thermal stability. The potential mechanism of a two-step process was proposed to explain the formation of Z–S composite nanoparticles. Findings in the present work will help further understand the interaction between alcohol-soluble biopolymers (e.g. zein and shellac) and provide a new insight into the development of potential carriers for bioactive compounds.

1. Introduction

Nanoparticles have particular physicochemical properties and functional attributes. They are increasingly being applied as delivery systems in the food industry in order to improve the stability and oral bioavailability of bioactive components, e.g. curcumin, β-carotene, and quercetin. Nanoparticles have an advantage over hydrogels, organogels, liposome, and microparticles due to their smaller particle size, higher encapsulation efficiency, more effective penetration ability and targetability (Wang et al., 2016). Nanoparticles are usually fabricated from varieties of natural polymers, mainly including food-grade proteins and polysaccharides, because they are biocompatible, biodegradable, and non-toxic properties (Joye and McClements, 2016), such as soy protein (Chen et al., 2016), lactoferrin (Bollimpelli et al., 2016), gelatin (Gómez-Estaca et al., 2015), chitosan (Facchi et al., 2016), alginate (Hu and McClements, 2015), and β-cyclodextrin (Moorthi et al., 2013).

Protein-based nanoparticles are usually formed with water-soluble proteins, such as soy protein and lactoferrin, as well as alcohol-soluble proteins. Zein, the main storage protein in corn seeds, contains over 50% hydrophobic amino acids, and it can be easily converted into spherical colloidal nanoparticles by the method of anti-solvent precipitation

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(ASP) (Joye and Mcclements, 2013). Compared with zein nanoparticles, the composite nanoparticles prepared by zein and biopolymers have some advantages. Liang et al. (2015) revealed that zein-quaternized chitosan composite nanoparticles provided a better protection for curcumin than zein nanoparticles. Hu and McClements (2015) reported that zein–alginate composite nanoparticles had good stability to pH (from pH 3.0 to 8.0) and temperature (90 °C, 120 min). Hu et al. (2015) prepared core–shell nanoparticles, using zein as the core and pectin as the shell at pH 4.0 and the nanoparticles showed a higher loading efficiency (>86%) of curcumin. Nevertheless, most previous studies focused on the development of complexes using zein and hydrophilic biopolymers, such as alginate (Hu and McClements 2015), chitosan (Liang et al., 2015), pectin (Hu et al., 2015), and caseinate (Pan and Zhong, 2016). Because of many bioactive components, e.g. curcumin, β-carotene, and quercetin were hydrophobic, they might tend to be encapsulated in the hydrophobic delivery system. Yet little information was available on the composite nanoparticle systems consisting of zein and hydrophobic biopolymers.

Shellac is a resinous secretion of the female insect *Kerrialacca*, which is a natural hydrophobic biopolymer, and mainly contains the polyesters of hydroxy fatty acid and sesquiterpene acids. It has a good film-forming capacity, high gloss, and low permeability to gases, acid and water vapor, which allow it for coating purposes in foods and drugs (Wei et al., 2015a). Al-Gousous et al. (2015) studied the disintegration properties of shellac-based enteric coatings, and regarded shellac as a promising coating material, especially for use in colon-targeted drug delivery since it could resist the stomach acid environment and achieve a timed enteric or colonic release (Wang et al., 2015). Therefore, shellac was chosen to fabricate composite nanoparticles with zein in the present study.

Previous studies mainly focused on the formation of protein and biopolymer composite nanoparticles commonly resulting from electrostatic interactions between oppositely charged macromolecules (De Kruif et al., 2004). It is worthy to mention that zein and shellac, have similar electric charges in acidic or alkaline environment. A hypothesis is proposed that zein and shellac might form a special structure.

The method of anti-solvent co-precipitation (ASCP) was applied to prepare Z–S composite nanoparticles, which was obviously different from the traditional anti-solvent precipitation method (Joye and Mcclements, 2013; Davidov-Pardo et al., 2015; Luo et al., 2011; Yadav and Kumar, 2014). Sun et al. (2017) used the ASCP method to fabricate zein and propylene glycol alginate (PGA) composite nanoparticles, and investigated the interaction mechanism between zein and amphiphilic biopolymer (e.g. PGA). Nevertheless, shellac is a hydrophobic biopolymer, the interaction mechanism between zein and shellac, and characteristics of Z–S binary composite nanoparticles prepared by ASCP method were investigated. The purpose in present study was to explore the formation mechanism and the physical, structural, thermal and morphological characteristics of Z–S composite nanoparticles. Results from present work might play a promoting role in developing novel carriers for bioactive compounds, which would have potential application for nutraceutical delivery systems.

2. Materials and methods

2.1. Materials

Shellac (wax-free) was purchased from Sigma–Aldrich (St. Louis, MO, USA). Zein with a protein content of 95% (w/w) was obtained from Gaoyou Group Co., Ltd. (Jiangsu, China). Ethanol (99.9%) was purchased from Eshowbokoo Biological Technology Co., Ltd. (Beijing, China). All other chemicals were analytical grade unless stated otherwise.

2.2. Preparation of Z–S composite nanoparticles

Zein and shellac were separately dissolved in 500 mL 80% ethanol aqueous solution containing 0.1 M phosphate buffer saline (PBS). The zein solution (10 mg/mL) and shellac solution (10 mg/mL) were stirred by a constant magnetic stirring at 600 rpm for 2 h at room temperature, and then were adjusted to pH 8.0. Shellac solution was diluted by 80% ethanol aqueous solution to acquire seven different concentrations, and 10 mL of each shellac solution was mixed with zein solution at a fixed concentration in an equivalent volume on a vortex shaker at 1500 rpm for 30 s, which formed the resulting solutions with seven Z:S mass ratios (50:1, 10:1, 5:1, 2.5:1, 1:1, 1:1.5, 1:2.5). The mixed solutions were held for 2 h at room temperature.

Afterwards, Z–S composite nanoparticles were formed by the ASCP method described in the previous literature with some modifications (Sun et al., 2017). Briefly, 20 mL zein and shellac ethanol aqueous solution was slowly injected into 60 mL PBS (pH 8.0) using a syringe with a constant stirring at 600 rpm for 20 min. To acquire aqueous dispersions, three quarters of ethanol aqueous solution were removed under reduced pressure (−0.1 MPa) by rotary evaporation at 45 °C. The composite nanoparticle dispersions of Z–S were acquired. Individual zein or shellac dispersion was prepared by the same way as controls. The colloidal nanoparticle dispersions were stored in the refrigerator at 4 °C for further analysis, and part of the dispersions were freeze-dried for 48 h with Alpha 1–2 D Plus freeze-drying apparatus (Marin Christ, Germany) to acquire dry particles for solid state characterization analysis.

2.3. Particle size and zeta-potential measurements

Particle size of Z–S composite nanoparticles was determined by dynamic light scattering (DLS), using a Zetasizer Nano-ZS90 (Malvern Instruments, Worcestershire, UK). The intensity of light scattered was monitored at a 90° angle. All the liquid samples were equilibrated for 60 s at 25 °C inside the instrument before analysis, and then data were collected over 10 sequential readings. Each sample was analyzed in triplicate and the results were collected as cumulative mean diameter (size, nm) for particle size. Zeta-potential values of zein and shellac dispersions were determined in triplicate, and the data were calculated by the instrument using the Smoluchowski model.

2.4. Turbidity measurement

Nephelometry measurements were performed in a HACH 2100N laboratory turbidimeter (Loveland, USA), and the turbidity of Z–S composite nanoparticle dispersions was evaluated as described by Yang et al. (2014a). The optical apparatus was equipped with a tungsten-filament lamp with three detectors: a 90° scattered-light detector, a forward-scatter light detector and a transmitted light detector. The calibration was performed using a Gelex Secondary Turbidity Standard Kit (HACH, Loveland, USA), which consists of stable suspensions of a metal oxide in a gel. All measurements were performed in triplicate.

2.5. Fourier transform infrared (FTIR) spectroscopy

The infrared spectra of samples were measured with the potassium bromide (KBr) pellet method described by Sun et al. (2015) using a Spectrum 100 Fourier transform spectrophotometer (PerkinElmer, U.K.) in the range of 400–4000 cm^{-1}, with a resolution of 4 cm^{-1}. Potassium bromide was used as a reference. For each measurement, 11 scans were taken. All the samples analyzed were freeze-dried from evaporated disper-