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Tannic acid cross-linked gelatin–gum arabic coacervate microspheres for sustained release of allyl isothiocyanate: Characterization and *in vitro* release study

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ABSTRACT

Tannic acid cross-linked gelatin–gum arabic coacervate microspheres, capable of sustained release of allyl isothiocyanate (AITC) with high encapsulation efficiency, were developed for safe and efficient oral delivery of AITC. The microspheres were spherical in shape and clustered. Statistical screening and optimization studies revealed that a maximum AITC encapsulation efficiency could be obtained when the microspheres were prepared with 4.5% total biopolymer, 6.5% oil, and 5.9% AITC in oil phase. Release studies showed that the sustained release performance of the optimized microspheres was greatly enhanced by using more tannic acid without loss in the encapsulation efficiency. The microspheres optimized with 1.5% tannic acid, having an encapsulation efficiency of 83.75% and a mean cluster size of 116.80 μm, released 46% of encapsulated AITC after 2 h in pepsin-containing simulated gastric fluid (pH 1.2), followed by releasing additional 48% in 6 h after being transferred to pancreatin-containing simulated intestinal fluid (pH 7.5).

1. Introduction

Allyl isothiocyanate (AITC), one of the most abundant isothiocyanates found in cruciferous plants such as cabbage and broccoli, has long been used in foods as a pungent flavoring agent and well known for its outstanding antimicrobial activity (Delaquis & Sholberg, 1997). Recent studies revealed that AITC also possesses multiple health-promoting properties, such as chemopreventive, gastric legion preventive, and anti-*Helicobacter pylori* activities (Hwang & Lee, 2006; Matsuda, Ochi, Nagatomo, & Yoshikawa, 2007; Shin, Masuda, & Naohide, 2004). AITC, therefore, has a high potential to be used as an active component of nutraceutical formulations. However, the direct use of AITC in the products is limited because it is highly volatile, strongly pungent, poorly soluble, and easily decomposed by nucleophilic reactions, especially in an aqueous environment (Chacon, Buffo, & Holley, 2006; Kim, Chung, Shin, Yam, & Chung, 2008; Pecháček, Velíšek, & Hrabcová, 1997).

A promising strategy to overcome these limitations is to encapsulate AITC molecules within a biopolymer vehicle in the form of capsule or matrix. This approach, furthermore, could enable the sustained release of AITC during digestion so that AITC can be more efficiently absorbed by human body. So far, a few biopolymer systems have been investigated to encapsulate AITC for antimicrobial packaging applications, which include gum arabic amorphous matrix by traditional flavor microencapsulation technique (Chacon et al., 2006), α- and β-cyclodextrin molecular inclusion complexes (Li, Jin, & Wang, 2007), and electrospun soy protein and poly(lactic acid) fibers (Vega-Lugo & Lim, 2009). For oral applications, alginate hydrogel matrix reinforced with chitosan was demonstrated to have a high potential, however, it was suggested by the authors that its relatively low encapsulation efficiency of about 56% and the initial sudden release mode observed in simulated digestive fluids need to be improved (Kim et al., 2008).

Microencapsulation by complex coacervation of two oppositely charged biopolymers, especially of gelatin and gum arabic due to their abundance, biocompatibility, biodegradability, and safety, has been widely investigated to encapsulate hydrophobic, volatile food and pharmaceutical actives (Chang, Leung, Lin, & Hsu, 2006; Dong, Touré, Jia, Zhang, & Xu, 2007; Junyaprasert, Mitrevej, Sinchaipanid, Boonme, & Wurster, 2001; Prata, Zanin, Ré, & Grosso, 2008; Xing, Cheng, Yang, & Ma, 2004; Yeo, Bellas, Firestone, Langer, & Kohane, 2005). The two biopolymers undergo associative electrostatic interactions followed by phase separation to create the layer of complex coacervates surrounding active-containing oil-in-water emulsion droplets. The liquid coacervate layer can be transformed to a rigid membrane by gelatin cross-linking. Formaldehyde or glutaraldehyde, which can form Schiff's base with the free amino groups of gelatin, is commonly used as a cross-linking agent (Xing et al., 2004). However, such aldehyde agents are considered to be toxic to human body, which is one of the major limitations of using the complex coacervation to produce microspheres in food and pharmaceutical industries (Dong et al., 2007; Huang, Cheng, Yu, Tsai, & Cham, 2007). Tannins, widely distributed plant polyphenols, could be employed as a safe alternative cross-linker because of their ability to complex with conformationally open proteins, such as gelatin, primarily through hydrogen bonding and hydrophobic interactions (Hagerman & Butler, 1981; Xing et al., 2004). Xing et al. (2004) reported that the

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membrane of capsaicin-loaded gelatin–gum arabic microspheres cross-linked with glutaraldehyde became more rigid after treatment with tannins. However, little further information is available yet on the properties of coacervate microspheres cross-linked with tannins.

The present study aimed to develop and characterize gelatin–gum arabic coacervate microspheres strengthened by the tannic acid-induced cross-linking process, which are capable of sustaining AITC release with high encapsulation efficiency, as a device for safe and efficient oral delivery of AITC. Tannic acid is a hydrolysable tannin having a glucose core esterified with an average of 9 to 10 gallic acid residues (Osawa & Walsh, 1993). The study is composed of the following three phases: firstly, the key factors influencing microsphere characteristics were identify using Plackett–Burman screening analysis, secondly, AITC encapsulation efficiency was maximized by optimizing the key factors using response surface methodology, and finally, the optimized microspheres were further improved for better sustained release performance.

2. Materials and methods

2.1. Materials

Allyl isothiocyanate (AITC, CH_2=CH—CH_2—N=C=S, molecular weight = 99.16, >98% purity) was purchased from Fluka (Steinheim, Germany). Gelatin B (type B from bovine skin, gel strength 225 Bloom) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Gum arabic (from Acacia tree) and tannic acid were purchased from Carl Roth GmbH (Karlsruhe, Germany). The viscosity average molecular weights of gelatin and gum arabic, determined in our preliminary experiments, were 72 and 382 kDa, respectively. Olive oil and Tween 80 were obtained from Sam Chun Chemical Co. Ltd. (Gyeonggi, Korea) and Bio Science Inc. (East Markham, Ontario, Canada), respectively. Pepsin (800–2500 units/mg) from porcine gastric mucosa and pancreatin (4× USP specifications) from porcine pancreas were purchased from Sigma Chemical Co. All other reagents were of analytical grade purity.

2.2. Preparation of microspheres

Aqueous solutions of known concentrations of gelatin and gum arabic were prepared. An appropriate amount of olive oil containing a known amount of AITC was sonicated in 30 g of gelatin solution at a given temperature and power for 1 min using a XL2020 ultrasonic processor (Misonix Inc., Farmingdale, NY, USA) to form an oil-in-water emulsion. An appropriate amount of gum arabic solution containing a known amount of Tween 80 was slowly added to the emulsion at the same temperature with the mass ratio of gelatin to gum arabic as 1:1. The pH of the mixture was adjusted to 4.0 with 10% (v/v) acetic acid, followed by stirring the mixture at 400 rpm in an ice bath for 15 min until its temperature reached 4 °C. An appropriate volume of 10% (w/w) aqueous solution of tannic acid was added to the mixture and stirred for a given time at room temperature (22 ± 1 °C). The microspheres sunk to the bottom of the mixture were washed twice by decanting with distilled water and collected by centrifugation at 4000 *g* for 3 min (centrifuge 5810R, Eppendorf, Hamburg, Germany).

2.3. Experimental design and data analysis

An eight-factor, twelve-run Plackett–Burman screening design was employed to identify the main factors influencing microsphere characteristics. The eight factors examined include: X_1 = total biopolymer concentration (% w/w), X_2 = concentration of olive oil (% w/w), X_3 = concentration of AITC in olive oil (% w/w), X_4 = concentration of Tween 80 (% w/w), X_5 = concentration of tannic acid (% w/w), X_6 = cross-linking time (min), X_7 = ultrasonic power for emulsification (W at 20 kHz), and X_8 = temperature for emulsification and complex coacervation (°C). The four microsphere characteristics investigated include: Y_1 = morphology, Y_2 = mean size (μm), Y_3 = size distribution, and Y_4 = AITC encapsulation efficiency (%). The relationship between X_i and Y_i (except Y_1) was examined using the following first-order polynomial model:

$$Y_i = b_o + \sum_{i=1}^{8} b_i X_i \quad (1)$$

where b_o and b_i are model intercept and linear regression coefficient, respectively.

Three factors, X_1, X_2, and X_3, determined as the main factors affecting Y_4 by the above analysis (see section 3.1.4 for a detailed discussion), were optimized for a maximum Y_4 using a three-factor, five-level central composite design consisting of twenty experimental points, including fractional 2^3 factorial points, six star points, and six replicates at the center point. The relationship between X_i and Y_4 was modeled using the following nonlinear quadratic equation:

$$Y_4 = \beta_o + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j>i}^{3} \beta_{ij} X_i X_j \quad (2)$$

where β_0, β_i, β_{ii}, and β_{ij} are model intercept, linear, quadratic, and interactive regression coefficients, respectively. Design-Expert 7.0.0 (Stat-Ease Inc., Minneapolis, MN, USA) was used for statistical analyses of experimental data.

2.4. Morphology

The morphology (Y_1) of microspheres was observed with an optical microscope (BX51, Olympus Inc., Tokyo, Japan) at a magnification of 1000×. For the screening analysis, the value of 0 or 1 was assigned to characterize the morphology because the morphology is a categorical response, where Y_1 = 0 or 1 indicates the tendency to form microspheres with smooth or rugged surface, respectively.

2.5. Size analysis

The mean size (Y_2) and size distribution (Y_3) of microspheres were determined using a laser scattering particle size analyzer (Mastersizer 2000, Malvern Instruments Ltd., Worcestershire, UK). The mean diameter was expressed as volume weighted mean diameter ($d_{4,3}$) in micrometer. The size distribution was expressed as the value of span, which is a measure of the width of the size distribution:

$$Y_3 = \frac{d_{v,90} - d_{v,10}}{d_{v,50}} \quad (3)$$

where $d_{v,10}$, $d_{v,50}$, and $d_{v,90}$ are microsphere diameters below which 10, 50, and 90% of the volume of microspheres lie, respectively.

2.6. Determination of encapsulation efficiency

Accurately weighed amounts (about 0.3 g) of microspheres were dispersed in 3.5 mL of hexane in a 4.2 mL gas-tight glass vial and then crushed by sonication for 20 min at room temperature, during which the mixture was vortexed for 2 min at every 5 min interval. The concentration of AITC in the mixture was determined as described by Kim et al. (2008) and Zhang, Kim, Park, and Chung (2010) with a slight modification. The mixture was heated at 60 °C for 10 min in a water bath and placed at refrigerated temperature for 2 h until complete phase separation. An aliquot of 1 μL of the hexane layer was injected to a gas chromatograph (Clarus 500, Perkin-Elmer Inc., Las Vegas, NV, USA) equipped with a flame ionization detector (FID) and a HP-Innowax capillary column (30 m × 0.32 mm i.d., 0.5-μm film thickness, Agilent Technologies, Inc., Palo Alto, CA, USA). The oven temperature was programmed from 50 to 250 °C at a rate of 15 °C/min with initial and