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Development of tannic acid cross-linked hollow zein nanoparticles as potential oral delivery vehicles for curcumin

Siqi Hu, Taoran Wang, Maria Luz Fernandez, Yangchao Luo*

Department of Nutritional Sciences, University of Connecticut, Storrs, CT, 06269, USA

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ABSTRACT

Sodium carbonate was proposed as a sacrifice template and tannic acid was used as a natural cross-linker to prepare hollow zein nanoparticles (HZN/T). The formulation of nanoparticles, including the amount of water, zein and sodium carbonate, were optimized by surface response methodology (Box-Behnken design). The optimized HZN/T exhibited a small dimension of 87.93 nm with a PDI of only 0.105 and a zeta potential of −39.70 mV, indicating the nanoparticles were homogenous and formed stable colloidal dispersion. Then curcumin was used as a model lipophilic nutrient to explore the encapsulation and delivery potentials of HZN/T, in comparison with hollow zein nanoparticles without tannic acid (HZN/NT) and solid zein nanoparticles with tannic acid (SZN/T) prepared under the same conditions. Generally, the encapsulation efficiency of HZN/T or HZN/NT was significantly higher than that of SZN/T. Interestingly, encapsulation of curcumin dramatically increased particle size of SZN/T by 50 nm, while it did not induce any expansion of the dimension of HZN/T due to its hollow structure. The molecular interactions between curcumin and zein nanoparticles were investigated by Fourier transform infrared spectroscopy and fluorescent spectrophotometer. The in vitro stability and release profile of nanoparticles were evaluated under the simulated gastrointestinal conditions. Although all types of zein nanoparticles showed a sustained release of curcumin, cross-linking via tannic acid played an important role to make zein nanoparticles more resistant against simulated intestinal digestion. Therefore, compared with traditional SZN/T, the HZN/T developed in this study has promising features as a potential oral delivery system for lipophilic nutrients/drugs.

1. Introduction

Curcumin, a member of the polyphenol family, is a hydrophobic compound found in the herb *Curcuma longa*. Previous studies supported that curcumin possesses anti-oxidant (Weber, Hunsaker, Abcouwer, Deck, & Vander Jagt, 2005), anti-inflammatory (Satoskar, Shah, & Shenoy, 1986), anti-microbial (De et al., 2009), and anti-carcinogenic properties (Limtrakul, Lipigorngoson, Namwong, Apisariyakul, & Dunn, 1997). Nevertheless, the compromised bioavailability, including poor absorption and rapid clearance from human body, obstructed curcumin to be applied in therapeutics and functional foods (Anand, Kunnumakkara, Newman, & Aggarwal, 2007). In order to increase its bioavailability, nanotechnology has been employed to develop delivery systems for curcumin. Thus far, a variety of nanoparticles have been studied as potential vehicles to deliver curcumin through different routes, either injection (Sun et al., 2013) or oral administration (Shaikh, Ankola, Beniwal, Singh, & Kumar, 2009). Although oral administration has been a preferred route over injection, many delivery systems of curcumin are prepared from either synthetic polymers and/or surfactants, which are often associated with potential risk of toxicity. Alternatively, food biopolymers, including polysaccharides and proteins, have received increasing attention for their encapsulation and delivery potentials for nutrients (Abd El-Salam & El-Shibiny, 2012; B. Hu & Huang, 2013; McClements, 2015), due to their naturally-occurring status with biodegradability and biocompatibility.

Zein nanoparticles are one of the recently studied biodegradable polymeric nanoparticles and have been applied in drug delivery (Lai & Guo, 2011), (Regier, Taylor, Borcyk, Yang, & Pannier, 2012), nutrient delivery (Luo, Zhang, Whent, Yu, & Wang, 2011) and tissue engineering (Dong, Sun, & Wang, 2004; Paliwal & Palakurthi, 2014).

* Corresponding author. Department of Nutritional Sciences, University of Connecticut, 3624 Horsebarn Road Extension, U-4017, Storrs, CT, 06269-4017, USA.
E-mail address: yangchao.luo@uconn.edu (Y. Luo).

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Zein is a prolamine protein found as storage protein of maize and is generally regarded as safe (GRAS) as a food additive (Janes, Kooshesh, & Johnson, 2002). Compositional analysis indicated that zein contains two thirds of non-polar amino acids (Righetti, Gianazza, Viotti, & Soave,), and one third of polar amino acids (Geraghty, Peifer, Rubenstein, & Messing, 1981; Luo & Wang, 2014). Zein nanoparticles are commonly prepared by liquid-liquid anti-solvent precipitation method and such-prepared nanoparticles are often reported to have a dimension of 200–300 nm with a solid internal core. Recently, Xu and colleagues reported a novel method to prepare hollow zein nanoparticles (HZN) by introducing sodium carbonate as a sacrifice template (Helan Xu, Jiang, Reddy, & Yang, 2011). The HZN had a particle size around 60 nm and exhibited a significantly higher loading capacity than solid zein nanoparticles (SZN). This is considered as a novel methodology to prepare protein-based hollow nanoparticles, as the core template sodium carbonate is removed simultaneously during the formation of zein nanoparticles by anti-solvent process, while thermal or chemical treatment is usually required in the preparation of many other hollow nanoparticles (Peng & Sun, 2007; Zeng et al., 2008). Nevertheless, for a new methodology, the comprehensive optimization, including fabrication procedures and nanoparticle formulations, is needed to prepare HZN for delivery of nutrients.

As all other protein nanoparticles, a major challenge for zein nanoparticles as oral delivery vehicles for nutrients is the stability under gastrointestinal conditions, where high concentration of salts, extreme pHs, and digestive enzymes are present. Although zein is relatively resistant to digestive enzymes, it has been shown that zein nanoparticles without any modification were rapidly hydrolyzed or aggregated (Luo, Teng, & Wang, 2012; Luo et al., 2011), resulting in a burst effect of encapsulated drugs/nutrients. Many strategies are available to possibly address this concern. For instance, surface coating with another polymer (Luo et al., 2012; Luo et al., 2013; Luo et al., 2011) has demonstrated promising effects to slow release rate of nutrients from zein nanoparticles. Cross-linking is another approach that may be helpful to stabilize zein nanoparticles and improve their delivery potentials. Particularly, citric acid has been recently reported as a non-toxic chemical cross-linker to significantly prolong in vivo residence time of HZN (H. Xu, Shen, Xu, & Yang, 2015). However, heating at 50 °C for 10 h is required in this process to create amide bonds between carboxylic groups of citric acid and amine groups of zein, which may negate its applications in encapsulating temperature-sensitive labile nutrients, such as curcumin. Previous studies have shown that binding proteins with tannins increase their stability and thus decreased digestibility (Taylor, Bean, Ioerger, & Taylor, 2007). Tannic acid belongs to the tannin family and contains abundant hydroxyl groups, allowing it to form hydrogen bonds with other compounds, including protein. The formation of tannic acid-protein complex was suggested to be based on non-covalent interactions between carbonyl groups of the protein and hydroxyl groups of the tannic acid (Van Buren & Robinson, 1969). Hydrophobic amino acids in zein, such as proline and phenylalanine, are potential binding sites for tannic acid (Jobstl, O'Connell, Fairclough, & Williamson, 2004). Therefore in order to increase the stability of zein nanoparticles in the gastrointestinal tract, tannic acid could be a possible option to make zein nanoparticles more stable.

The first objective of this study was to explore the main and interactive effects of nanoparticle fabrication conditions and to optimize the formulation of hollow nanoparticles using surface response methodology with Box-Behnken design. Tannic acid was included in the formulation as a non-covalent cross-linker to stabilize HZN/T. The second objective was to evaluate the potential of as-prepared HZN/T as an oral delivery vehicle for curcumin, with solid SZN/T as well as HZN without tannic acid (HZN/NT) studied as controls. The physicochemical properties, including morphology, intermolecular interactions, encapsulation efficiency, as well as stability and controlled release under simulated gastrointestinal conditions were comprehensively characterized.

2. Materials and methods

2.1. Materials

Zein, sodium carbonate (Na_2CO_3, purity $\geq$ 99.0%), and tannic acid (ACS reagent) were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). Curcumin (purity $\geq$ 98%) was obtained from ACROS Organics (Geel, Belgium). Other chemicals, including pepsin and pancreatin, were of analytical grade and obtained from Thermo Fisher Scientific (Pittsburgh, PA, USA).

2.2. Preparation of nanoparticles

HZN/T was prepared by using anti-solvent precipitation method with Na_2CO_3 used as sacrificing template (Helan Xu et al., 2011). A certain amount of zein powder (100–500 mg) was dissolved in 10 mL 70% v/v aqueous ethanol to form a stock solution. Sacrifice template was formed by pouring 0.7 mL of pure ethanol into 0.3 mL deionized water containing carbonate (2.5–7.5 mg). Then, 1 mL of zein stock solution was mixed with the template solution. Pre-established amount of deionized water (5–15 mL) with 2 mg tannic acid was pipetted gradually to the mixture under constant magnetic stirring. Particles were stirred for another 30 min for stabilization. HZN/NT and SZN/T were prepared in parallel as controls for comparison purpose. In the SZN/T preparation, carbonate was pre-dissolved in 1 mL water (no formation of sacrifice template), rather than 70% v/v aqueous ethanol solution.

2.3. Box-Behnken design

A Box-Behnken design with 15 runs, 3 factors and 3 levels, was utilized to optimize formulations of HZN/T. Design-Expert software (Trial Version 8.0.6, Stat-Ease Inc., MN) was used to develop a quadratic response surface, which was designed for testing main effects, quadratic effects of independent variables, and interactions between them. The software generated a quadratic model based on the following equation:

$$Y = A_0 + A_1x_1 + A_2x_2 + A_3x_3 + A_4x_1x_2 + A_5x_1x_3 + A_6x_2x_3 + A_7x_1^2 + A_8x_2^2 + A_9x_3^2$$

Y represents the response variable needs to be optimized; A_1–A_9 are regression coefficients of independent factors, showing their interactions and quadratic terms; X_1, X_2 and X_3 are the coded levels of explanatory variables. Selection of independent variables, i.e. amount of additional water (X_1), Na_2CO_3 (X_2) and zein (X_3), was based on previous study (Helan Xu et al., 2011), and the detail information was shown Table 1. Independent factors were divided into three levels: −1, 0 and 1, representing low, medium and high values, respectively. Table 2 presented the study design matrix established by the software. Dependent variables of this study

Table 1
Experiment factors and levels.

Factors	Level (−1)	Level (0)	Level (+1)
Water (mL), X_1	5	10	15
Sodium (mg), X_2	2.5	5	7.5
Zein (mg), X_3	10	30	50