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# Polysaccharide-based nanoparticles by chitosan and gum arabic polyelectrolyte complexation as carriers for curcumin

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ABSTRACT

This study developed the polysaccharide-based nanoparticles by the polyelectrolyte complexation between chitosan (CS) and gum arabic (GA) as novel delivery systems for curcumin. Optimization of parameters affecting the formulation of such nanocarriers was performed by means of dynamic light scattering and fluorescence analysis. It was demonstrated that at pH 4.0 and 1:1 mixing ratio of CS to GA, the two biopolymers can form hydrophilic, monodisperse and highly positively charged colloidal nanoparticles. Fourier-transform infrared and X-ray diffraction further confirmed their electrostatic interaction. Subsequently, the formulations and stability of nanoparticles loaded with curcumin were compared. The optimum formulation was found to be Tween 80/egg yolk phospholipid (1:1, w/w), curcumin/emulsifier (0.5:5, w/w) and curcumin concentration initially prepared (4%, w/w). The developed nanoparticles showed the average diameter in the range of 250–290 nm. The curcumin encapsulation efficiency and loading content respectively exceeded 90% and 3.8%, with a retention rate higher than 85% during storage. Additionally, whatever the antioxidant model was, the antioxidant activities of curcumin were significantly enhanced by nanoencapsulation. Furthermore, compared to emulsion without biopolymer coating, CS-GA nanoparticles can improve the stability and delay the release of curcumin in a simulated gastrointestinal environment. These findings suggested that CS-GA nanoparticles could be used as an ideal carrier to deliver hydrophobic bioactive ingredients like curcumin in functional foods.



## 1. Introduction

The utilization of curcumin as a nutraceutical ingredient is limited by its high hydrophobicity and low bioavailability (Yen, Wu, Tzeng, Lin, & Lin, 2010). Because of the inherent chemical reactivity, curcumin is also sensitive to the environmental stresses, such as oxygen, light and heat. Therefore, the challenge now is to protect such promising molecules from deteriorative agents and increase their physiological benefits (Sari et al., 2015).

Nanoparticle delivery system have outstanding advantages on improving biological efficiencies, controlling delivery and preventing side effect of drugs (Ensign, Cone, & Hanes, 2012). Polysaccharides are the most popular natural materials for drug delivery (Liu, Jiao, Wang, Zhou, & Zhang, 2008). In recent years, polysaccharide-based nanoparticles have shown huge potential in biological, pharmaceutical and food applications. The use of chitosan (CS), a natural polysaccharide produced by the deacetylation of chitin, is particularly promising owing to its biocompatibility and nontoxicity (Xia, Liu, Zhang, & Chen, 2011). At acid pH, its amino groups are ionized, thus, making it hydrosoluble and positively charged. These properties enable it to interact with oppositely charged polymers by intermolecular electrostatic interaction, forming polyelectrolyte complexation (PEC). Some negatively charged polysaccharides can complex with CS to generate PEC nanoparticles, including carboxymethyl cellulose (Kaihara, Suzuki, & Fujimoto, 2011), dextran sulfate (Anitha, Deepagan, et al., 2011), alginate (Das, Kasoju, & Bora, 2010), heparin (Lin et al., 2013; Shahbazi, Hamidi, & Mohammadi-Samani, 2013), and hyaluronan (Almalik et al., 2013). Recently, the chitosan-based polysaccharide nanoparticles applied for curcumin encapsulation have been found

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in chitosan-alginate-pluronic composite nanoparticles (Das et al., 2010), dextran sulfate-chitosan nanoparticles (Anitha, Deepagan, et al., 2011) and carboxymethyl chitosan nanoparticles (Anitha et al., 2012; Anitha, Maya, et al., 2011).

Gum arabic (GA), a biocompatible and biodegradable polysaccharide, is widely utilized in oral, topical pharmaceutical and food formulations as a suspending and emulsifying agent (Phillips & Phillips, 2011). At near neutral pH, its carboxyl groups will be largely dissociated and lead the molecule to be an open, highly charged and expanded structure. This feature contributes to its good surface activity and viscoelastic film-forming ability (Dickinson, 2009). Furthermore, compared to other polysaccharides, GA is demonstrated to have more interaction sites and negative charge for interaction with polycationic chitosan (Avadi et al., 2010). Therefore, GA when mixed with CS is very good at forming PEC. It is supposed that the electrostatic complex between GA and CS could form strong viscoelastic films around oil droplets and provide them with physical-chemical barrier against oxidation (Moschakis, Murray, & Biliaderis, 2010). The nanoformulations with CS-GA nanoparticles have already been reported for oral delivery of protein (Avadi et al., 2010). However, to the best of our knowledge, studies on such nanoparticles for delivering curcumin have not yet been reported in the literature.

The present study aimed to develop polysaccharide-based nanoparticles as novel delivery systems to encapsulate, stabilize and deliver curcumin effectively. Especially, the relationship between nanoparticle structure and stabilization of incorporated molecules is crucial for the development of nutraceutical. The developed nanoparticles were characterized using dynamic light scattering, fluorescence, Fourier-transform infrared and X-ray diffraction. Afterwards, the formulations were optimized in the preparation of curcumin-loaded nanoparticles. In addition, knowledge of the effects of encapsulation on the antioxidant activity and release behavior of curcumin in the simulated human gastrointestinal fluid was achieved.

## 2. Materials and methods

### 2.1. Materials

Chitosan (CS) with average molecular weight of 100,000 and deacetylation degree of 93% was a gift from Jinhu Crust Product Co., Ltd. (Shandong, China). Gum arabic (GA) and polyoxyethylene sorbitan monooleate (Tween 80) were obtained from China Medicine (Group) Shanghai Chemical Reagent Co. (Shanghai, China). Curcumin (95% purity) was purchased from Zelang Medical Technology Co., Ltd. (Jiangsu, China). Pyrene and 2, 2′-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Egg yolk phospholipid (EYPC) was purchased from Chemical Reagent Plant of East China Normal University (Shanghai, China). All other chemicals used were of analytical grade.

### 2.2. Preparation of CS-GA nanoparticles and curcumin-loaded CS-GA nanoparticles

CS-GA nanoparticles were prepared as previously described (Avadi et al., 2010) with a slight modification. Briefly, CS was dissolved in acetic acid solution (1%, v/v) with high speed stirring until completely dissolved. The solution was filtered (filter pore size 30–50 μm) to remove the insoluble substance. GA solution was prepared by dissolving it in the distilled water, adjusting the same pH to CS solution. Afterwards, GA solution was added dropwise to CS solution at equal volume at 800 r/min for 30 min using an IKA RW 20 digital overhead stirrer (IKA Works Guangzhou). When evaluating the influence of pH on the formation of nanoparticles, the mixing ratio of CS and GA was fixed at 1:1; the pH values were adjusted to 3.0, 3.5, 4.0, 4.5, and 5.0, respectively. When evaluating the influence of biopolymers mixing ratio (CS/GA, w/w), the pH value was fixed at 4.0; the mixing ratios were adjusted to 3:1, 2:1, 1:1, 1:2 and 1:3, respectively.

CS-GA nanoparticles loading curcumin were prepared using the ethanol injection method. Curcumin was first dissolved in ethanol together with Tween 80 and EYPC at a certain weight ratio. The initial concentration of curcumin ($IC = m_{curcumin}/m_{polysaccharides}$, % w/w) in the formulations were selected at 2%, 4%, 6%, 8% and 10%, respectively. After dissolution, the mixture was dispersed into the CS solution followed by the addition of GA, as described for CS-GA nanoparticles preparation. The system was then attached to a rotary evaporator for removing the ethanol. The final samples were transferred in vials under nitrogen bed and stored in the refrigerator (at 4 °C in the dark) until use.

Curcumin emulsion was prepared by replacing the CS and GA solution with distilled water. Other procedures were in the same way as described above. The obtained curcumin emulsion was stored in the refrigerator (at 4 °C in the dark) until used in the assays of antioxidant activity and *in vitro* release. Curcumin powder suspension was prepared to be served as a control in the antioxidant study. Curcumin powder was first dissolved in the ethanol and spread out in a beaker. Then, a steady stream of nitrogen gas was used to remove the ethanol, followed by redispersing in distilled water. The concentration of curcumin in powder suspension and emulsion was adjusted to the same concentration as the nanoparticles.

### 2.3. Characterization of CS-GA nanoparticles

#### 2.3.1. Particle size and zeta potential analysis

Particle size was carried out using a commercial zeta-sized (NanoZS90, Malvern Instrument Ltd., UK) with a He/Ne laser ($\lambda = 633$ nm) at a fixed scattering angle of 90° at temperature of ($25 \pm 0.1$) °C. Before analysis, aliquots of 1 mL samples were diluted to 10 mL to avoid multiple scattering phenomena (Tan et al., 2013). The diluted sample was immediately transferred into the polystyrene cuvette for size determination or capillary cells for zeta potential, and then the *z*-average diameter and particle size distribution (polydispersity index, PDI) were recorded by dynamic light scattering (DLS). The zeta potential values were automatically calculated form the electrophoretic mobility, based on the Smouluchowski model (Fukui & Fujimoto, 2009). The determinations were repeated three times and the results given were average.

#### 2.3.2. Fluorescence analysis

A small quantify of fluorescence probe pyrene was dissolved in ethanol with the concentration of $1 \times 10^{-4}$ mol/L. One hundred microliters of the pyrene stock solution was placed into empty vials and the solvent was evaporated under vacuum. Then, 5 mL of nanoparticles was added into the vials and gently stirred, followed by incubation at 37 °C for 30 min. The final concentration of pyrene was $2 \times 10^{-6}$ mol/L. Such a low concentration was selected to minimize the influence of pyrene on the formation and/or the stability of hydrophobic domains (Henni-Silhadi et al., 2007). The fluorescence emission spectra ranging from 350 to 550 nm was detected by a fluorescence spectrometer (Hitachi F-7000, Tokyo, Japan). The excitation wavelength was set at 338 nm with the emission and excitation slit at 2.5 nm. The relative micropolarity surrounding the pyrene environment was determined from the fine structure of pyrene monomers fluorescence, namely the ratio $I_1/I_3$ corresponding to the monomers peaks at 373 nm ($I_1$) and 383 nm ($I_3$) (Purohit, Kulkarni, & Somasundaran, 2012).