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Temperature-dependent structure stability and in vitro release of chitosan-coated curcumin liposome

Yujia Liu [a], Dandan Liu [a], Li Zhu [a], Qian Gan [a], Xueyi Le [a,b,*]

[a] Department of Applied Chemistry, South China Agricultural University, Guangzhou 510642, China
[b] Institute of Biomaterial, South China Agricultural University, Guangzhou 510642, China

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ABSTRACT

Liposome, a promising delivery system with phospholipid bilayer, could improve the water solubility of curcumin, which is a polyphenolic natural product with many bioactivities. However, the instability of liposome limits its development in application. In this study, the chitosan was used to protect the structure stability of phospholipid bilayer of curcumin liposome and control the sustained release rate of curcumin further. The temperature stability and phase transition were studied by differential scanning calorimetry. When curcumin molecule was embedded in phospholipid bilayer, the pre-transition temperature (50.40 °C) and main phase transition temperature (61.47 °C) decreased about 10 °C compared to it without curcumin, and phospholipid bilayer fluidity was increased. The pre-transition temperature was disappeared after chitosan coated on the surface of curcumin liposome. The thermal stability test and X-ray diffraction also indicate that high temperature could change the structure of liposome and chitosan could protect the curcumin from damage and leak. The in vitro release study showed that the cumulative release rate got faster with temperature increased and chitosan could decrease the release rate. These results may guide the potential application of chitosan-coated liposome as a carrier of curcumin in nutraceutical and functional foods.

1. Introduction

Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-hepadiene-3,5-dione], a natural polyphenolic nutraceutical, is a major component of turmeric (*Curcuma longa*), which is commonly used as spice or food colorant in Asia. Scientific investigations of curcumin have found a booming trend in the past decade, with several prominent beneficial biological and pharmacological activities being linked to curcumin, such as antioxidant (Tikekar, Hernandez, Land, & Nitin, 2013), anticancer (Aggarwal, Kumar, & Bharti, 2003), anti-inflammatory (Imm, Zhang, Chan, Nitteranon, & Parkin, 2010) and wound healing characteristics (Kant et al., 2014). Curcumin has also been reported to have beneficial effects on antialcoholism (Nanji et al., 2003). Hydroxyl groups of the benzene rings, double bonds in the alkene part, and the diketone moiety were suggested to play crucial roles in the beneficial activities of curcumin (Ruby, Kuttan, Babu, Rajasekharan, & Kuttan, 1995).

Despite multiple advantages, low oral bioavailability of curcumin continues to be highlighted as a major challenge in developing formulations for application. One reason is that curcumin is poorly soluble in water under the condition of acidic or neutral pH. In order to improve its bioavailability and water solubility, various effective encapsulation approaches have been published, such as microemulsion (Wang, Chen, Zhang, Yang, & Zhai, 2012), chelation strategies (Chen et al., 2014), hydrogels (Martins et al., 2013) and liposome (Chen et al., 2012).

Liposome is a promising delivery system due to its phospholipid bilayer structure, which is similar as a biological membrane. Some researchers have reported that liposome could improve the bioactivity of curcumin, such as water solubility (Sun et al., 2012), stability (Niu, Ke, et al., 2012), gastrointestinal absorption and antioxidant activity (Takahashi, Uechi, Takara, Asikin, & Wada, 2009). Curcumin molecules are trapped in the phospholipid bilayer part of liposome, which physicochemical properties, such as structure and phase transition temperature, should be related to the stability and the bioactivity of curcumin liposome. Recently, some researchers found that binding of curcumin could induce the thinning of phospholipid bilayer and change the microstructural properties like the fluidity of the bilayer (Barry et al., 2009; Hung et al., 2008), which may lead the stability to reduce and curcumin to leak out. Therefore, others attempted to modify the surface by biopolymers for protecting the structure of phospholipid bilayer and

* Corresponding author at: Department of Applied Chemistry, South China Agricultural University, Guangzhou 510642, China. Tel./fax: +86 020 85280319.
E-mail address: lexyfu@163.com (X. Le).

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prolonging the retention time in gastrointestinal tract (Karewicz et al., 2013; Xia, Xu, & Zhang, 2006).

Chitosan is always used as a coating polymer material for foodstuff because of its nontoxic, biocompatible, and biodegradable characteristics (Qin et al., 2013). Moreover, chitosan was a naturally occurring basic polysaccharide which coated on the surface of liposome due to the electrostatic interactions between positively charged chitosan and negatively charged phosphates (Shin, Chung, Kim, Joung, & Park, 2013).

The present work was intended to elucidate the effects of temperature on the structure stability of chitosan-coated curcumin liposome (CS-Cur-Lip) and the in vitro release of curcumin. The ethanol injection method was used to prepare curcumin liposome (Cur-Lip) and mean particle size was determined by laser light scattering. In addition, the interaction between chitosan and liposome was represented by FT-IR spectroscopy. Especially, the structure transformation and phase transition of phospholipid bilayer were discussed through DSC and X-ray diffraction techniques. The release and release kinetics in vitro were investigated for the purpose of improving the curcumin bioavailability in functional foods.

2. Materials and methods

2.1. Materials

Chitosan (molecular weight, 28,000; degree of deacetylation, 89%) was obtained from BioTech Co. Ltd. (Shandong, China). Phosphatidylcholine (PC) was purchased from Aladdin (from soybean, >98%, Guangzhou, China). Cholesterol (≥95%) and curcumin (98% pure) were purchased from China Medicine (Group) Shanghai Chemical Reagent Co. (Shanghai, China).

2.2. Preparation of Cur-Lip and CS-Cur-Lip

Curcumin-loaded liposome was prepared according to the ethanol injection method (Shin et al., 2013): A weight ratio at 5:1 of PC and cholesterol was dissolved in the ethanol. PC and curcumin were mixed in a ratio of 10:1 (*w*/*w*). The resulting solution was injected evenly into the phosphate buffer solution (PBS) of pH 7.0 and stirred at 500 rpm for 60 min at room temperature. The mixed solution was transferred into a round bottom flask and was evaporated on a rotary evaporator at 40 °C for 30 min until the ethanol has been removed cleanly. Then the liposome was dispersed by ultrasonic treatment for 15 min. The final concentrations of PC, cholesterol and curcumin were kept at 10 mg/mL, 2 mg/mL and 1 mg/mL, respectively.

The Cur-Lip coated with chitosan was prepared according to the following method. The chitosan solution (1%, *w*/*v*) was prepared by dissolving chitosan in distilled water containing 0.1% (*w*/*v*) acetic acid and then added dropwise into bare Cur-Lip suspension at equal volume at 500 rpm for 60 min with a magnetic stirrer. The final concentration of chitosan was 0.5% (*w*/*v*).

2.3. Particle size distribution and zeta potential measurements

The particle size distribution and zeta potential of Cur-Lip and CS-Cur-Lip were determined using a laser particle size analyzer (BI-200SM, BrookHeaven, USA). 3 mL of samples dispersion was added to polystyrene latex cells, and the mean particle size and zeta potential were measured at 25 °C with a detector angle of 90°. The average values from at least five measurements were reported.

2.4. Differential scanning calorimetry (DSC)

DSC was performed on a differential scanning calorimeter (DSC8000, PerkinElmer, USA) for liposome without curcumin (blank liposome), Cur-Lip and CS-Cur-Lip. 50 μL sample was placed in the aluminum pan with the liposome concentration of 10 mg/mL. Then, the aluminum pan was sealed by a tablet press machine. The heating scan was carried out from 35 to 90 °C at a scanning rate of 5 °C/min under dry nitrogen. A baseline was recorded with blank hermetic aluminum pan.

2.5. FT-IR analyses

FT-IR analyses of free curcumin, Cur-Lip, pure chitosan and CS-Cur-Lip were recorded in the range of 400–4000 cm^{-1} by a FT-IR spectrometer (Equinox 55, Bruker, Germany). Samples were prepared by smearing solution samples on a KBr slice and drying 2 min with infrared drying lamp. For each spectrum, 16 scans at a resolution of 4 cm^{-1} were obtained.

2.6. X-ray diffraction

The X-ray diffraction pattern was characterized by X-ray diffraction (MiniFlex 600, Rigaku, Japan) with CuKa radiation generated at 15 mA and 30 kV. The scanning speed used was 5°/min from 10° to 60° diffraction angle (2θ) range. Samples were prepared at 23 °C and 80 °C water bath for 1 h, respectively.

2.7. Entrapment efficiency

The sample was centrifuged at 20,160 ×*g* at 4 °C for 1 h and 0.5 mL supernatant was diluted to 2 mL for examining the content of unencapsulated curcumin by a UV/Vis spectrophotometer (UV-2450, Shimadzu, Japan) at 425 nm, which was the characteristic absorption of curcumin and not found in liposome or chitosan UV/Vis spectra. The amount of curcumin encapsulated in liposome was expressed as encapsulation efficiency calculated as follows:

$$\text{Encapsulation efficiency}(\%) = [(C_0 - C_t)/C_0] \times 100 \quad (1)$$

where C_0 is the total amount of initial curcumin and C_t is the unencapsulated curcumin in the supernatant.

2.8. Thermal stability

The thermal stability of Cur-Lip and CS-Cur-Lip in phosphate buffer solution of pH 7.0 was investigated by recording the absorption spectrum at 425 nm of Cur-Lip and CS-Cur-Lip for 240 min at 4 °C and 23 °C. The stability of free curcumin in the same buffer solution was also tested for comparison at the same test condition.

In the study of storage stability of Cur-Lip and CS-Cur-Lip at 4 °C and 23 °C, the maximum absorption at 425 nm of the sample were recorded at 0, 1, 3, 5, 7, 15 and 40 days, respectively.

The effect of different temperature on the stability was investigated by recording the absorption spectrum at 20, 30, 40, 50, 60, 70, 80 and 90 °C, respectively. The samples were kept at a certain temperature for 10 min before each measurement and the free curcumin was also tested for comparison.

The stability of curcumin was calculated according to the formula:

$$\text{Stability of curcumin}(\%) = C_t/C_0 \times 100 \quad (2)$$

where C_0 and C_t represent the initial concentration and concentrations of different sampling time point of curcumin, respectively.

2.9. In vitro release

The in vitro release of curcumin from liposome was carried out by dialysis membrane method (Shaikh, Ankola, Beniwal, Singh, & Kumar, 2009). 1 mL sample (free curcumin, Cur-Lip or CS-Cur-Lip, containing equivalent to 1 mg/mL of curcumin, respectively) was transferred in dialysis bags with a molecular cut off of 14 kDa. The