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Release kinetics and antibacterial activity of curcumin loaded zein fibers

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ABSTRACT

The aim of the present study was to evaluate the release kinetics and antibacterial activities of curcumin (CUR) loaded in zein (zein-CUR) electrospun fibers towards *Escherichia coli* (*E. coli*, 8099) and *Staphylococcus aureus* (*S. aureus*, ATCC 6538). CUR was successfully loaded in zein fibers by electrospinning technique, and the encapsulation efficiency was close to 100% and the encapsulated CUR still retained its antioxidant capacity. The morphology and size of fibers strongly depended on CUR contents. The interaction between CUR and zein matrix was confirmed to be hydrogen bonding, and the addition of CUR caused a slight increase in the T_g of zein matrix. The electrode process of CUR electrooxidation was diffusion-controlled and could be preceded by chemical reaction. The predominant release of CUR from fibers was Fickian diffusion, and First-order model and Hixson-Crowell model could well describe this diffusion behavior. The zein-CUR fibers showed good antibacterial activity towards *S. aureus* and *E. coli*, and the inhibition efficiency increased with the increase of CUR contents. However, the antibacterial activity towards *S. aureus* was better than that towards *E. coli*. The study displayed that the zein-CUR fibers might have potential as a promising material for antimicrobial applications to inhibit bacterial growth and propagation in active food packaging.

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1. Introduction

As a representative active food packaging, antimicrobial packaging has attracted more attention in controlling infectious food borne outbreaks (Alkan et al., 2011). It is convenient to achieve the antimicrobial activity by incorporating natural bioactive agents such as antimicrobial enzymes (Wang et al., 2015a,c; Wang, Yue, & Lee, 2015), bacteriocins (Cleveland, Montville, Nes, & Chikindas, 2001; Deegan, Cotter, Hill, & Ross, 2006), essential oils (Salarbashi et al., 2013; Wen et al., 2016), and phenolic compounds (Alkan et al., 2011; Neo, Swift, et al., 2013) into packaging materials. Among them, phenolic compounds have attracted particular interest owing to their good antibacterial and antioxidant activity in food systems (Coma, 2008; Crespy & Williamson, 2004; Komes, Horžić, Belščak, Ganić, & Vulić, 2010). Hence, curcumin (CUR) was used as an antimicrobial agent in the present study.

CUR, an extract from *Curcuma longa*, is a low molecular weight natural yellow-orange polyphenol compound, which is widely used in wound healing, diabetes and cardiovascular ailments (Goel, Kunnumakkara, & Aggarwal, 2008) owing to its good anti-inflammation (Sharma, Steward, & Gescher, 2007), antiviral (Sharma, Gescher, & Steward, 2005) and anti-cancer (Bar-Sela, Epelbaum, & Schaffer, 2010; Dhillon et al., 2008) activities. Besides, CUR is also an antioxidant (Tomren, Masson, Loftsson, & Tønnesen, 2007) and antibacterial agent (Chattopadhyay, Biswas, Bandyopadhyay, & Banerjee, 2004). Nevertheless, CUR can be easily oxidized exposure to oxygen during processing or storage. Therefore, as antimicrobial agent used in active food packaging, an effective encapsulating and controlled release technology is necessary.

Electrospinning is a simple, versatile and low cost technique for culturing cell (Wang, Castro, et al., 2012) and encapsulating functional agents e.g., anti-oxidation agent (Wang et al., 2016), antibacterial agent (Qi et al., 2013; Wang, Zheng, et al., 2012; Zheng et al., 2013), and drug (Chou, Carson, & Woodrow, 2015; Wang

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et al., 2015a,c) into the nanofibrious matrixes to achieve the sustainable controlled release effectiveness. Various studies have confirmed electrospinning is a promising method for stabilization of antioxidants (e.g., β-carotene (Fernandez, Torresginer, & Lagaron, 2009), fish oil (Moomand & Lim, 2015), tea polyphenols (Shao et al., 2011) and CUR (Sun, Williams, Hou, & Zhu, 2013). In addition, CUR has been successfully incorporated into electrospun fibers as anticoagulation agent (Chen, Lin, Fei, Wang, & Gao, 2010), antitumor agent (Guo et al., 2011), drug (Brahatheeswaran et al., 2012; Suwantong, Opanasopit, Ruktanonchai, & Supaphol, 2007), and antibacterial agent (Dhurai et al., 2013). In the present work, CUR was incorporated into zein fibers *via* electrospinning technique to develop active food packaging. Zein is a promising material in food packaging owing to its excellent oxygen barrier property and relatively high thermal resistance (Neo, Ray, et al., 2013; Shukla & Cheryan, 2001).

Recently, two systems of CUR loaded zein (zein-CUR) electrospun fibers have been reported: one is zein-CUR membrane for iron(III) ions by Saithongdee, Praphairaksit, and Imyim (2014), the other is hybrid fluorescent zein-CUR electrospun nanofibrous scaffold for biomedical applications by Brahatheeswaran et al. (2012). Different from them, our work was focused on the investigation of the kinetics and antioxidant capacity of CUR in zein-CUR electrospun fibers by cyclic voltammetry and the evaluation of antibacterial activities of the fibers. The morphology, structure, thermal property, and encapsulation efficiency of zein-CUR fibers were also explored. In order to assess the antibacterial activity of zein-CUR fibers, *Escherichia coli* (*E. coli*, 8099) and *Staphylococcus aureus* (*S. aureus*, ATCC 6538) were tested as Gram-positive and Gram-negative model bacteria in general antibacterial experiment.

2. Materials and methods

2.1. Materials

Zein from maize (≥97%, CAS number 9010-66-6) was obtained from Sigma-Aldrich (St Louis, MO, USA). Curcumin (CUR), Gallic acid (GA), Folin-Ciocalteu's reagent, sodium hydroxide, L-tartaric acid, sodium chloride and ethanol were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) and absolute ethanol was used as solvent. Sodium carbonate was procured from Aladdin Chemical Reagent Co., Ltd. (Shanghai, China). All the chemicals and reagents were of analytical grade. All solutions were prepared with Milli-Q water from a purification system (Millipore, Bedford, MA, USA). *Escherichia coli* (*E. coli*, 8099) and *Staphylococcus aureus* (*S. aureus*, ATCC 6538) were provided by the China Center of Industrial Culture Collection (Beijing, China). Agar-agar, peptone and beef extract were purchased from Beijing ordered Star Biological Technology Co., Ltd. (Beijing, China). All the chemicals were used as received.

2.2. Preparation of CUR loaded zein fibers

The preparation and electrospinning of the zein and zein-CUR solutions are similar to our previous studies (Wang et al., 2016). Zein solutions (25%, w/w) were prepared by dissolving zein powder in 80% ethanol aqueous solutions (ethanol/deionized water = 4/1, w/w) assisted with magnetic stirring at 25 °C for 1 h. The required CUR was added into above solution to obtain electrospinning solutions (CUR contents: 20, 30, and 40%, all based on the weight of zein), the corresponding fibers were coded as zein-CUR 20, zein-CUR 30, and zein-CUR 40, respectively. The CUR loaded zein fibers were fabricated on an electrospinning device consisting of a syringe, a needle (0.41 mm internal diameter), a copper sheet, a ground electrode and a high-voltage power supply (DW-P403-1ACCC, Tianjin Dongwen, China). On the basis of experimental data, electrospinning processing was carried out at room temperature with relative humidity (RH) at 50%. The supplied voltage was kept at 15 kV and tip-to-collector distance (TCD) was kept at 12 cm. The as-spun fibers were dried overnight in a vacuum oven at 25 °C.

2.3. Methods

2.3.1. Characterization of electrospinning solutions

Conductivity and viscosity of electrospinning solutions were measured at 25 °C to evaluate their influences on the morphology of fibers. Conductivity was tested by DDL-801 conductivity meter (Shanghai, China) equipped with a DJS-1 platinum conductance electrode coated with platinum black. Viscosity was tested by NDJ-5S rotational viscometer (Shanghai, China), and the measurements were reported at a spindle speed of 60 rpm.

The voltammetric experiments were performed in a three-electrode electrochemical cell using a potentiostat (CHI 660D, Chenhua, Shanghai). Before each measurement, the working glassy carbon electrode (MF-2012, 3.0 mm diameter) was cleaned by polishing with 0.05 μm alumina (CF-1050) for 1 min between runs, rinsed thoroughly with Milli-Q water and then sonicated for 2–3 min to remove alumina residues. A BAS Ag/AgCl reference electrode (+207 mV vs. SHE) was used in conjunction with a platinum wire counter electrode. Fig. 1A shows the cyclic voltammograms of GA standards in the electrolyte solution (12% v/v ethanol, 0.033 M L-tartaric acid, 0.1 M NaCl, adjusted to pH 3.6 by NaOH). According to Fig. 1A, the GA standard calibration curve (Fig. 1B) was obtained by plotting the total charge (Q_t) of anodic oxidation peaks *versus* GA concentrations (0.53–2.48 mM), and the corresponding regression equation was presented as: $y = 0.0149408x - 5.35391 \times 10^{-4}$, $R^2 = 0.99889$ (Fig. 1B), herein, the results were expressed as mM GA equivalents per mM of the CUR in sample.

A weight of 0.16 g of representative zein-CUR 30 fibers was added into Milli-Q water (44 mL) and placed on a shaker platform (120 rpm) at 25 °C for 3 h to assure the release equilibrium. After removed away the fibers, we prepared 50 mL testing electrolyte solution described above by adding ethanol, L-tartaric acid, NaCl and NaOH. The testing solutions were measured by cyclic voltammetry at 25 °C, and the cyclic voltammograms were obtained by scanning the potential from 0 to 1.0 V at six scanning rates (0.01–0.36 Vs^{-1}).

2.3.2. Encapsulation efficiency

The encapsulation efficiency of CUR in fibers was determined by first washing 0.01 g of the sample with 20 mL Milli-Q water to remove the surface CUR, and then dissolving in 50 mL of 80% ethanol aqueous solution. The amount of CUR remaining the solution was measured using the Folin-Ciocalteu method with some modifications (Atoui, Mansouri, Boskou, & Kefalas, 2005; Rebelo, Rego, Ferreira, & Oliveira, 2013; Turkmen, Sari, & Velioglu, 2006; Wright, Mphangwe, Nyirenda, & Apostolides, 2000). The encapsulation efficiency (*EE*) of CUR in fibers was calculated as follows:

$$EE(\%) = \frac{Actual\ CUR\ concentration}{Theoretical\ CUR\ concentration} \times 100 \quad (1)$$

Actual CUR concentrations were determined from regression equation ($y = 4.58x + 0.0538$, $R^2 = 0.99$) (detailed method in S1). Absorbance was measured at 765 nm using a UV-754 PC spectrophotometer (Shanghai Jinghua, China).

2.3.3. Characterization of CUR-zein fibers

The morphologies of samples were observed with scanning

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