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Preparation and characterization of curcumin loaded liposomes

Shuo Xu, Zihao Zhang, Geyu Pang, Shaoxuan Yu*
(School of Agricultural Engineering and Food Science, Shandong University of Technology, Zibo 255049,Shandong, China)
Corresponding Author: Shaoxuan Yu

Abstract: *Although curcumin (Cur)has a variety of pharmacological effects, its defects including low solubility, poor stability and low bioavailability, greatly limit its use in food and pharmaceutical products. In this work, we successfully encapsulated Cur in liposomes and the optimal amount of Cur, lecithin, and cholesterol was 2, 80, and 20 mg, respectively. The EE of the prepared liposomes was 95%, the particle size was about200 nm and zeta potential was -27.5mV. This work may provide a theoretical guidance forthe extensive and mature application of Cur.*

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I. Introduction

Curcumin (Cur) extracted from the rhizome of plants in the family of *Zingiberaceae* and *Araceae*, is a natural polyphenolic compound with a diketone structure [1]. Although Cur has a variety of pharmacological effects such as lowering blood lipids, preventing blood clots, anti-oxidation, anti-cancer, antibacterial, and anti-inflammatory [2, 3], more and more clinical results show that Cur has defects including low solubility, poor stability and low bioavailability,which greatly limit its use in food and pharmaceutical products[4,5].Liposomes are self-assembled spherical vesicles that have one or more concentric phospholipidbilayer layers and enclose a portion of the surrounding water-based medium.Therefore, liposomescan transport hydrophilic or hydrophobic active components and drugs by embedding in the internalaqueous phase or by incorporating lipid bilayer [6,7].Compared with other carrier systems, liposomesshow high stability, good biocompatibility, andcan control the releaserate ofloaded drugs [8].In this paper, curcumin loaded liposomes (CLip) were prepared and characterized, which may provide a theoretical guidance forthe extensive and mature application of Cur.

II. Materials and Methods

2.1 Experimental materials

Curcumin (Cur, >98%), soy lecithin, cholesterol,tween-80,and other common reagents were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Double distilled water (DDW) was used in all the experiments.

2.2 Preparation of CLip

Based on our previous study, CLip was prepared by a thin-filmhydration and ultrasonic combined method with some modifications [9]. Soy lecithin, cholesterol,tween-80,and Cur were firstly dissolved in ethanol at a mass ration of 8:1:1.6:0.1. Then the mixture was poured into a round bottom flask and rotationally evaporated at 50℃ and -0.1 MPa for 30min.A uniformfilmwas formed on the bottle wall. Phosphate buffer solution (PBS, 0.01 M, pH7.0) was added to the round bottom flask, which was rotated for 30min at 50℃. Afterthe film was eluted completely, the eluant was sonicated with a JYD-250 ultrasonic cell crusher (Xinbiaotengda, Beijing, China) at a pulse mode of 1s/1s (200W) in an ice bath for 10min. After ultrasonic treatment, the solution was centrifuged at 3000 rpm for 10 min to remove large particles. Finally, 20 mL CLip solution was obtained, and it was stored at 4℃for further use.

2.3 Optimization of the preparation conditions of CLip

The preparation method of CLip was optimizedon the basis ofthe encapsulation efficiency(EE) of Cur.

2.3.1 Mass ratio of lecithin to cholesterol

To determinethe appropriate mass ratio of lecithin and cholesterol,the total amount oflecithin and cholesterolwasfixed at 100 mg,and empty liposomes wereprepared at the mass ratios(lecithin vs. cholesterol)of 1:1 and 4:1, respectively.

2.3.2 The supplemental amount of curcumin

The contents of lecithin and cholesterol were determined to be 80 mg and 20 mg,respectively.Then different

amount ofCur (1, 2, and 4mg) were added to the preparationsystem. The optimumamount of Curwas determined according to the EE of Cur in Clip.

2.4 Spectroscopic characterization of liposomes and CLip

2.4.1 Fluorescence spectrum

The prepared empty liposomes and CLip sampleswith1, 2, and 4mg Cur were diluted with PBS (0.01 M, pH 7.0) by ten times.The fluorescence spectra of the diluents were measuredat 450-600 nm by a fluorescence photometer with an excitation wavelength of 425 nm and an emission wavelength of 525 nm.

2.4.2 Ultraviolet-visible absorption spectra

The UV-vis spectrophotometer was used to scan the baseline with PBS at 425nm, and thebackground was deducted to measure the absorbance of the four samples after fluorescencespectra were recovered.

2.5 EE of CLip

Solutions of empty liposomes and CLip sample (5 mL) were centrifuged at 20,000 r/min for 10 min to collect the supernatants. Then 1 mL of the supernatants was dilutedby anhydrous ethanol for 10 times. The absorbance values of unencapsulated Cur in the supernants were measuredat 425 nm by UV-vis spectrophotometer after background was deducted from the correspondingblank solution.According to the standard curve, the concentrations of Cur in thesupernatants were calculated and the mass of unencapsulated curcumin was obtained. Finally, theEE of Cur was calculated by the following formula.

$$EE\ (\%) = (1\text{-}W/W0) \times 100\%$$

Where W0 is the total amount of Curused in preparation and W isthe amount of unencapsulated Cur in the supernatant.

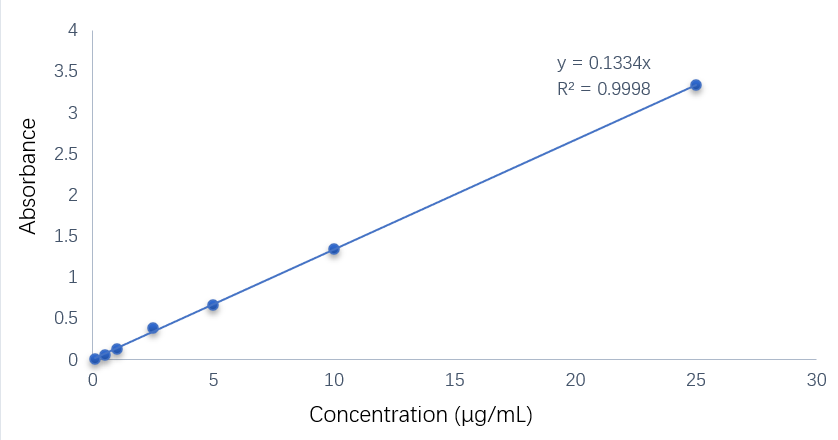

Fig. 1 Standard curve of curcumin.

2.6 Morphology of liposomes and CLip with 2 mg Cur

The morphology was characterized according to previous studies [10,11]. Briefly, a thin film of a sample wasprepared on a silicon chip and subjected toscanning electron microscope (SEM) (S-4800,Hitachi High-Tech, Japan).

III. Results and Discussion

3.1 Optimization of the preparation conditions of CLip

3.1.1 Optimization of the mass ratio of lecithin to cholesterol

To improve the stability of the phospholipid bilayer in liposomes, the fluidity of the membrane can be regulated by adding acertain proportion of cholesterol during the preparation [6].Therefore, the effect of the mass ratio of lecithin to cholesterol on the preparation of empty liposomeswas first analyzed in this study. When the mass ratio of lecithin tocholesterol was 1:1, the hydration time was significantly extended, and the film on the wall of theround-bottom flask could not be completely eluted. After 10 min of ultrasonic treatment by the cell breakage instrument, the solution was cloudyandflocculent precipitation formed (Fig. 2A).When the mass ratio of lecithin to cholesterol reached4:1, a clarified liposome solution was obtainedas shown in Fig. 2B.Therefore, themass ratio of lecithin to cholesterol in the subsequent work was 4:1.

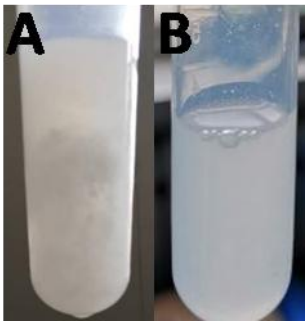

Fig. 2 Pictures of the solutions of empty liposomes prepared at 1:1 (A) and 4:1 (B) mass ratios of lecithin to cholesterol.

3.1.2Optimization of the amount of Cur

The influence of different amount of Cur (1, 2 and 4 mg) on the preparation of CLip was studied and thephotographs of the obtained CLip solutions were shown in Fig. 3.With the increase of Cur amount, thecolor of the solution gradually deepened, changing from light yellow to orange. Solutions of CLip with 1 and 2 mg Curshowedno visible precipitation and were always in a uniform andtransparent stateduring the storage at 4℃,but CLip with 4 mg Cur presented obvious precipitation.In addition, there were a lot of yellow solids remaining on thewall of the round-bottom flaskduring the hydration process of CLip with 4 mg Cur. Thisindicated that a lot ofCur was not embedded in liposomes at thisaddition level.

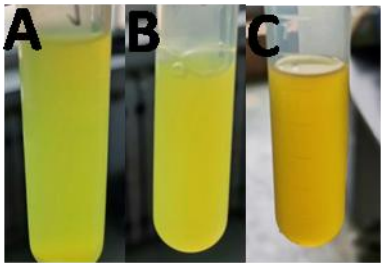

Fig. 3 Photographs of CLip solutions with 1 (A), 2 (B), and 4 (C) mg Cur.

3.2Spectroscopic characterization of liposomes and CLip

3.2.1 Fluorescence spectra of liposomes and CLip with 1 and 2 mg Cur

The prepared empty liposomes and CLip samples were diluted ten times with0.01 M PBS, and their fluorescence spectra in the range of 450 to 600 nm were measured at theexcitation wavelength of 425 nm.As we all know, the fluorescence spectrum of Cur has amaximum emission peak at 525 nmdue to the presence of benzene ring andcarbon-carbon double bond in its molecular structure[3].However, when it was embedded inliposomes, the positionof the fluorescence emission peak shifted to 500 nm, as shown in Fig. 4. This indicated thatCur mainly distributed between the phospholipid bilayerof the liposomes, and the non-polar microenvironment around its molecules was enhanced. On the other hand, the fluorescence intensity of CLipdecreased obviously with the increase of Curamount from 1 to2 mg, suggesting that the concentration of Cur was very high and fluorescence quenching occurred. As a control, no emission peak wasfound in empty liposomes under this excitation condition.

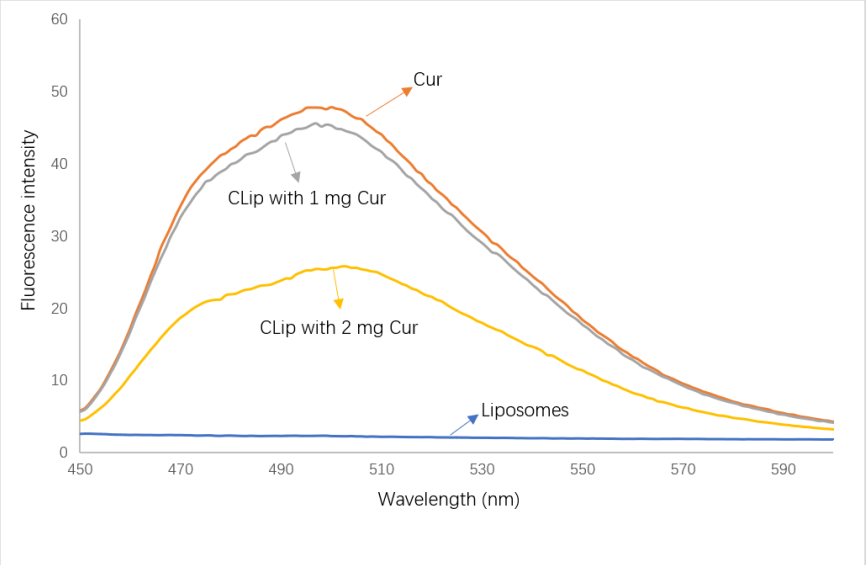

Fig. 4 Fluorescence spectra of liposomes, Cur and CLip with 1 and2 mg Cur.

3.2.2 Ultraviolet-visible absorption spectra of liposomes and CLipwith 1 and 2 mg Cur

As expected, the solution of empty liposomes had no obvious absorption peakin the given wavelength range, while the anhydrous ethanol solution ofCur showed an obviousabsorption peak at 425 nm, which is due to the existence of benzene ring, carbon-carbon doublebond and carbon-oxygen double bond chromophones in its molecular structure [3].As presented in Fig. 5, the UV-visabsorption spectra of CLipwith 1 and 2 mg Cur showed obvious absorption peaks at the same wavelength asCur, indicating that Cur was successfully embedded in liposomes.The EE of Cur in CLip with 1 and 2 mg Cur was 89.1±0.6% and 95.0±0.5%,respectively.In the follow-up experiment, CLip with 2 mg Cur was used as the sample.

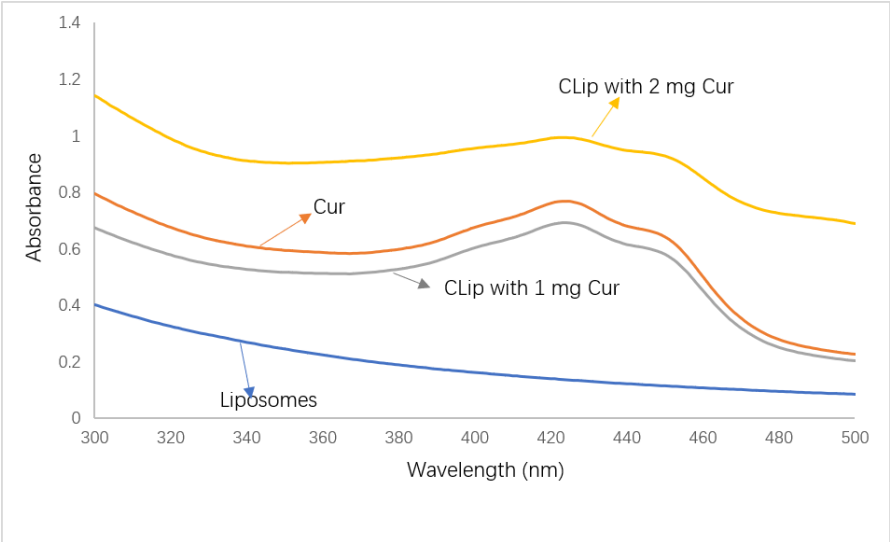

Fig. 5 UV-visible absorption spectra of liposomes, Cur and CLip with 1 and 2mg Cur.

3.3 Particle size and zeta potential of liposomes and CLip with 2 mg Cur

Table 1 Average particle size and zeta potential of liposomes and CLip with 2 mg Cur

Samples	Average diameter (nm)	Zeta potential (mV)
Liposomes	197.2±0.95 [a]	- (27.5±4.6) [a]
CLip with 2 mg Cur	186.7±1.8 [b]	- (27.6±2.2) [a]

Note: For the same indicator, the same lowercase letters in the table indicate no significant difference between

the data, and different lowercase letters indicate a significant difference between the data at the $P < 0.05$ level.

We measured the diameter and zeta potential of empty liposomes and CLip with 2 mg Cur with the Zetasizer Nano ZS nanoparticle potentiometer. From Table 1, it was found that the average diameter ofempty liposomes and CLip with 2 mg Cur were 197.2±0.95 nm and 186.7±1.8 nm, respectively.Theparticle size ofliposomes decreased when they wereprepared by adding Cur, and there is a significantdifference between them ($P < 0.05$).This indicated thatthe insertion of Cur into thephospholipid bilayer made the binding between lecithin molecules more tightly, and the particle sizeof the liposome formed after ultrasound was slightly reduced.After reviewing the literature, we canknow that the particle size of general Curloaded liposomes is about 80 nm [10]. The obvious difference may result fromthefollowing aspects: (1) tween as a cosolvent was not used in this work; (2)the massratio of lecithin to cholesterolwas different.

For zeta potential, there was no significant difference between empty liposomes and CLip with 2 mg Cur.Therefore, we speculated that Curwas mainly embedded in the bilayer, rather than adsorbed on the surface of the liposomes.

3.4 Morphology of liposomes and CLip with 2 mg Cur

Fig. 6 shows the images of empty liposomes and CLip with 2 mg Curobtained with a scanning electron microscope (SEM).As we can see, both liposomes and CLip with 2 mg Curwere aggregated particles with irregular shape. The size of the smallest particle was about 100nm.Itis speculated that the aggregation of the particles was caused by the preparation conditions of SEM samples which were air dried at room temperature.

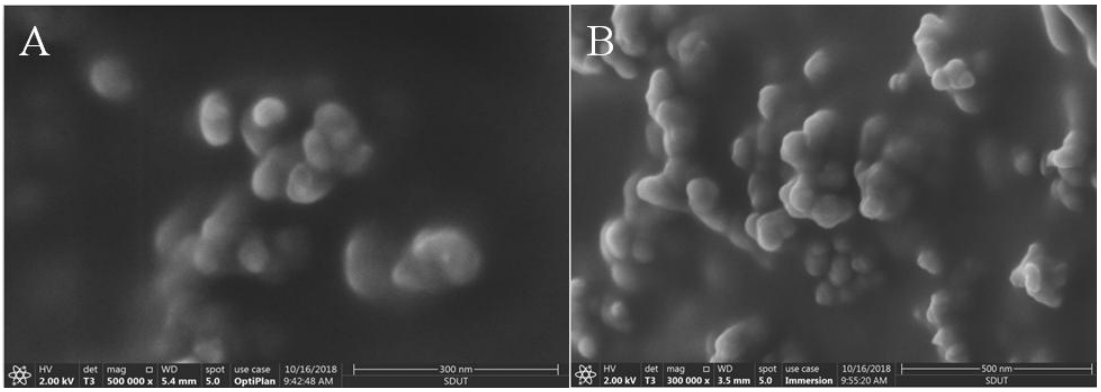

Fig. 6 SEM images of liposomes and CLip with 2 mg Cur.

IV. Conclusion

The optimal preparation conditions of Curloaded liposomes were as follows: the amount of Cur, lecithin, and cholesterol was 2, 80, and 20 mg, respectively; the temperature ofrotary vacuum evaporating was 50℃. The EE of the prepared liposomes was 95%, the particle size was about200 nm and zeta potential was -27.5mV.This work provideda theoretical guidance forthe extensive and mature application of Cur.

Acknowledgements

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