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Encapsulation of the flavonoid quercetin with chitosan-coated nano-liposomes

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ABSTRACT

A facile electrostatic deposition method was proposed to encapsulate quercetin within chitosan/lecithin polymeric nanocapsules, with the aim to protect quercetin against degradation and enhance its biocompatibility. Quercetin loaded polymeric nanocapsules (Q-NPs) were characterized in terms of size, morphology, encapsulation efficiency, storage stability, anti-oxidant and anti-proliferative activities. The obtained homogeneous and spherical Q-NPs have small particle size and high encapsulation efficiency (71.14%). The storage stability and antioxidant activity was improved compared with native quercetin. MTT assay and trypan blue exclusion assay showed the inhibitory effect of Q-NPs on HepG2 cells were comparable with that of free quercetin. This novel nanocapsule may provide a new system for the protection and delivery of a range of hydrophobic chemicals in vivo and food products.

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1. Introduction

With the increasing cost of healthcare, there is an increasing consumer demand for functional food in recent years (Betoret, Betoret, Vidal, & Fito, 2011). Functional foods are food products fortified with special ingredient that possess positive effect to human body, including improve the general conditions of the body (e.g., vitamins and probiotics), and modify risk factors for coronary heart disease, cancer, type 2 diabetes, obesity, osteoporosis and periodontal disease (Bigliardi & Galati, 2013). Formulation and blending are simply and cheap technology to develop functional foods, which have been successfully applied in the enhancement of vitamins A and D (FAO & WHO, 2001, 2006), iodine (Marine & Kimball, 1920) and iron et al. Combination of bioactive compounds is always difficult using traditional technology, due to their low stability, incompatibility or unpleasant taste (Celli, Ghanem, & Brooks, 2015). Some specific technologies are also applied in the manufacture of functional food to prevent the deterioration of physiologically active compounds, including microencapsulation, edible films and coatings and vacuum impregnation.

Encapsulation is the process to envelop core material or active agent in a coating, based on the embedding effect of a polymeric matrix (Betoret et al., 2011). It has attracted considerable attentions in recent years. With the benefits of nontoxic, biodegradable and nonimmunogenic, encapsulation is a good option to protect and improve biocompatibility of bioactive components (Fukui & Fujimoto, 2009; Teramura & Iwata, 2009). Various effective encapsulation approaches have been published, such as micromulsion (Jang & Lee, 2002), chelation strategies (Cheng et al., 2012), hydrogels (Hoffmeister et al., 2012) and liposomes (Jiang, Mo, Bellotti, Zhou, & Gu, 2014). Among them, liposome has been widely used as a release-on-demand carrier system for both hydrophilic and hydrophobic functional compounds such as antimicrobials, antioxidants and other bioactive compounds (Laye, McClements, & Weiss, 2008). However, traditional liposome have limited applications in food industry, mainly due to organic residues, instability and leakages during storage (Celli et al., 2015; Zhao, Temelli, Curtis, & Chen, 2015). Chitosan, known as a polycationic polymer, can form polyelectrolyte complexes with oppositely charged macromolecules by intermolecular electrostatic deposition. It can be used as coating material to overcome those drawbacks of liposomes (Madrigal-Carballo et al., 2008; Szoka &

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Papahadjopoulos, 1980).

Quercetin (3,3',4,5,7-pentahydroxyflavone) is a naturally occurring flavonoid found mostly in fruits like apples, grape and vegetables like onions (C. H. Wu, Shieh, Wang, Huang, & Hsia, 2015). It has received extensive studies in recent years, since its numerous biological activities including antioxidant, anti-inflammatory, anti-tumor, hepatoprotective activity, protection to DNA damage, and anti-proliferative effects on various human cancer cell lines (Ghosh et al., 2012; Rogerio et al., 2010; T.-H.; Wu et al., 2008; Yuan et al., 2006). Epidemiological researches suggest that a diet rich in quercetin may be associated with decreased cardiovascular damages and cancer risks (Kobori et al., 2015). However, a regular diet cannot provide adequate amount of quercetin, thus making quercetin-enhanced foods or supplements of great interest (Russo, Spagnuolo, Tedesco, Bilotto, & Russo, 2012). However, as a drawback, quercetin has poor aqueous solubility and easy degradable, which limits its application in hydrophilic food systems (Frenzel & Steffen-Heins, 2015) .

In the present study, using quercetin as an example, we proposed a novel electrostatic deposition method to encapsulate quercetin in chitosan-coated lipid nanoparticles by electrostatic interactions between positively charged chitosan and negatively charged phosphates (Fig. 1). The stability and antioxidant activity were tested and anticancer efficacy of Q-NPs was also observed in vitro. To the best of our knowledge, chitosan coated nano-liposomes as quercetin delivery carriers have not yet been reported. This nanocapsulation system has enormous potential to be applied in food industry, which can be also extended to protect and deliver other hydrophobic chemicals.

2. Materials and methods

2.1. Materials

All reagents were of analytical grade or better. Quercetin and soy lecithin were obtained from Sinopharm Chemical Reagent Co., Ltd. Chitosan and 1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma (St. Louis,USA). MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazoliunbromide) (purity C93%), RPMI 1640 cell cultures, fetal bovine serum (FBS) and trypan blue were obtained from Wolsen Biotechnology, Ltd (Xian, China). All reagents were used without further purification and deionized water was used in all experiments.

2.2. Liposome preparation and encapsulation of quercetin

Soybean lecithin liposomes were prepared using thin film hydration method as previously reported with some modifications (Szoka & Papahadjopoulos, 1980). In brief, 60 mg of soybean lecithin and 2 mg of quercetin were dissolved in 15 mL mixture of chloroform and methanol (2:1, mL:mL). A thin film was obtained by rotary evaporation of the organic solvents under vacuum at 30 °C. Then, the thin film was dried under nitrogen and redissolved with PBS (pH 7.4). Finally, the mixture was probe sonicated (JY92-IIDN, Ningbo Scientz Biotechnology Co., Ltd., China) for 5 s (2 cycles, 30% of maximum output) in ice bath to obtain smaller sized liposomes (Tsumoto, Matsuo, Tomita, & Yoshimura, 2009). The liposomal suspensions were stored in 4 °C prior to further use.

2.3. Preparation of chitosan-coated liposomes

Fresh solution of chitosan (CS, pH 6.0) in the concentration of 1 mg/mL was prepared in acetic acid (5.0 mL/L). Two milliliter of uncoated liposome suspension was injected dropwise (syringe with internal diameter of 0.38 mm) into chitosan under softly magnetic stirring to obtain CS coated nano-liposomes suspension. The CS coated nano-liposomes were then separated by ultracentrifugation at 16,000 × g at 4 °C for 30 min and lyophilized for further research. The chitosan-coated nano-liposome and quercetin loaded chitosan/liposome nanoparticles were named Np and Q-NPs, respectively.

2.4. Characterization of Q-NPs

The encapsulation efficiency (EE) of quercetin was determined by an indirect method using ultracentrifugation technique. The concentration of quercetin in the ultrafiltrate was measured by UV/Vis spectrophotometry (UV-2550, Shimadzu, Japan) at 370 nm. The quercetin encapsulation efficiency of quercetin was then calculated according to Equation (1):

$$EE\% = \frac{W_{total} - W_{free}}{W_{total}} \times 100 \tag{1}$$

Where W_{total} is the total quercetin weight in liposomes suspension; W_{free} is the weight of free quercetin.

The shape and surface morphology of the Q-NPs were visualized by field emission scanning electron microscope (SEM, S-4800, Hitachi High-Tech). Particle size and zeta potential were assessed by dynamic light scattering (DLS) and electrophoretic light scattering (ELS) using Zetasizer Nano ZS instrument (ZEN3600, Malvern Instruments). UV/Vis absorption spectra of nanocapsule (NPs), Q-NPs and native quercetin were obtained in a UV-2550 spectrophotometer (Shimadzu, Japan). Fourier transform infrared (FTIR) spectra were recorded in a FTIR spectroscopy (TENSOR27, Bruker, Germany) in the scanning range of 400–4000 cm^{-1} at 1 cm^{-1} resolution.

2.5. Relative storage stability

Native quercetin and Q-NPs was stored at 4 °C and 37 °C in dark or natural light for 14 days. The stability was determined by monitoring the absorbance of solution at 370 nm every day.

2.6. Antioxidant activity evaluation

DPPH scavenging capacity of Q-NPs was evaluated as follow. Typically, 400 μL of varying concentrations of sample solution (2, 4, 6, 8, 10 μg/mL) were added into 400 μL of DPPH solution (100 μmol/L, in ethanol). The mixing solutions were incubated in dark at room temperature for 30 min and the absorbance at 517 nm was recorded by a spectrophotometer. All determinations were carried out in triplicate.

The radical scavenging rate was calculated by Equation (2):

$$\text{DPPH scavenging rate}(\%) = \frac{A_0 - A_i}{A_0} \times 100 \tag{2}$$

Where A_0 is the absorbance of blank (0.1 mmol/L DPPH reagent), A_i is the absorbance of the sample.

Hydroxyl radical scavenging capacity of Q-NPs was also determined. Briefly, 500 μL of varying concentrations of sample solutions were mixed with 100 μL of $FeSO_4$ (6 mmol/L) and 50 μL of H_2O_2 (7.5, mL/100 mL). After 10 min' standing, 350 μL of salicylic acid (6 mmol/L) was added and the mixture was incubated at 37 °C for 10 min. The absorbance was then measured at 530 nm against a reagent blank. The hydroxyl radical scavenging rate was calculated using Equation (3):

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