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Construction, in vitro release and rheological behavior of apigenin-encapsulated hexagonal liquid crystal

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ABSTRACT

For the purpose of encapsulating apigenin, hexagonal liquid crystal was constructed in Tween 80/water-souble chitosan (WCS)/ethyl oleate (EtOL)/H_2O systems at different WCS content. After introducing WCS, the hexagonal liquid crystal underwent a transition from viscoelastic fluid to gel-like fluid as determined by rheological measurement. The WCS enhanced the stability of apigenin-encapsulated hexagonal liquid crystal as indicated by high elastic modulus and prolonged the release time of apigenin effectively. At relatively high WCS content, the elastic modulus of hexagonal phase decreased with an increase in temperature, showing different stability. While the release rate of apigenin increased and then decreased with increasing temperature. It was worth noting that after introducing WCS, the apigenin-encapsulated hexagonal phase showed pH-dependence, where the higher the pH, the slower the resulting apigenin release. These results suggested that the hexagonal liquid crystal is promising drug carrier to deliver apigenin.

1. Introduction

Apigenin, as one of natural polyphenolic compounds, was found in many fruits and vegetables. It exhibits various biological activities, including antioxidant [1], anti-inflammatory [2] and anti-cancer [3]. However, the solubility of apigenin in water (about 2.16 μg/mL) is low [4], which limits its practical application. Therefore, in order to effectively exert the beneficial activities of apigenin, encapsulating apigenin in drug carriers can improve its solubility and achieve its slow release [5].

Recently, some drug delivery systems including microemulsions [6], liposomes [7], hydrogel [8], emulsions [9] and lyotropic liquid crystal [5] were constructed to encapsulate apigenin. Among them, lyotropic liquid crystal was easy to prepare and possessed good viscoelasticity and stability [10]. It can improve the solubility of drugs and achieve the sustained release of drugs [5]. Based on our previous research, the lyotropic liquid crystal formed in oleyl polyoxyethylene ether surfactant (Brij 97) and sodium deoxycholate (NaDC) mixtures increased the solubility of apigenin and showed sustained release effect. The cumulative percent release of apigenin increased at body temperature (37 °C) [5].

It has been attracted wide attention to construct biocompatible drug carriers to encapsulate drugs. Chitosan (CS), as a natural macromolecule polysaccharide, was widely used to construct to drug carriers because of its nontoxicity, good biocompatibility and biodegradability [11,12]. One study reported that the nanoparticles formed in CS and negatively charged gum arabic (GA) mixture (1:1), can exhibit high curcumin encapsulation efficiency, loading content and physical stability [13]. Moreover, the CS based aggregates usually exhibited pH sensitive due to the fact that the pKa of CS was 6.0–6.5 in aqueous media [14–16]. Therefore, compared to our previous work [5,17], the CS based lyotropic liquid crystal was constructed to encapsulate drugs by introducing CS, which may show better stability and pH-dependence.

In this paper, the water-souble chitosan (WCS) based hexagonal liquid crystal was constructed in Tween 80/WCS/ethyl oleate (EtOL)/H_2O system to encapsulate apigenin. The rheological

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properties of apigenin-encapsulated hexagonal liquid crystal and in-vitro release of apigenin in hexagonal liquid crystal were evaluated at different conditions, such as different temperature or different pH values.

2. Materials and methods

2.1. Materials

Water-soluble chitosan (WCS) with deacetylation degree of 85% was purchased from Jinan Haidebei Marine Bioengineering Co. Ltd. (Jinan, China). Polyoxyethylene (20) sorbitan monooleate (Tween 80) and ethyl oleate (EtOL) were obtained from Sinopharm Chemicals Reagent Company (Shanghai, China). Apigenin (95%) was purchased from Nanjing Zelang Medical Technology Co., Ltd. China. All chemicals were used directly without further purification. Deionized water was used after double-distilled.

2.2. Phase diagram determination

Ternary phase diagram was measured according to the previous method described by Wang et al. [18]. First, the surfactant mixture of Tween 80 and WCS at constant weight ratio was prepared. Second, the different amount of oil was added in the surfactant mixture with weight ratio varying from 0:10 to 10:0 and stirred well at 700 rpm for 1 min located about 60–70 °C to be homogenized. Third, the water phase was added drop by drop with the interval of 2% (mass ratio) and mixed using a vortex mixer. Fourth, the samples were kept in a water bath at 25 °C to achieve phase equilibrium. The phase boundaries were identified by visual observation in normal light. The samples were stored at six days for further investigation. The concentration of apigenin in hexagonal liquid crystal was calculated by measuring the absorbance at a wavelength of 340 nm. The nomenclature, compositions of hexagonal samples and apigenin content in them were shown in Table S1 (supplementary materials).

2.3. Small angle X-ray scattering (SAXS)

SAXS measurements were performed in a SAXSess high-flux SAXS instrument (Anton-Paar, Austria) with a Cu Kα radiation (0.1542 nm) operating at 40 kV and 50 mA. The distance from samples to the detector was 264.5 nm. Measurements were carried out under vacuum to prevent air scattering. The temperature was kept using a temperature controller. The structures of liquid crystals were determined from the relative positions of the SAXS diffraction peaks.

2.4. Rheological measurements

Rheological measurements were performed with an American RS-2000ex rheometer (TA Instruments, New Castle, DE, USA) using a cone sensor (20 mm diameter, 2° angle). The sample thickness in the middle of the sensor was 0.5 mm. The plate was slowly elevated to its measuring position. Then, the sample was gently inserted on top of the plate of the sensor. The sample squeezed out from the sensor system was gently removed. After 10 min to allow for stress relaxation, measurements were carried out for every sample, which was consistent with our previous method [10].

First, the stress sweep measurements were carried out from 0.06 to 600 Pa at 1.0 Hz to determine the linear viscoelastic region. Second, the frequency sweep measurements were performed at a constant stress value with a range of angular frequency from 0.02 to 300 rad s^{-1}. Third, steady shear measurements were performed on all the samples at the range of shear rate from 0.01 to 1000 s^{-1}. Later, temperature ramp test were performed at the frequency of 1 Hz and a constant heating rate of 1 °C/min.

2.5. In-vitro release of apigenin

In vitro release of apigenin was investigated by using the dialysis method [5]. PBS buffer solution containing 30% (v/v) ethanol was used as release medium. Approximately 1.5 g of apigenin-encapsulated hexagonal liquid crystal was loaded into dialysis tube (molecular weight cut off of 8000–14000). Then, the dialysis tube was suspended in 45 mL PBS buffer solution with a stirring speed of 100 rpm using magneton (diameter × length, 6 × 10 mm). 3 mL of the release medium was removed at predetermined time intervals and the same volume of fresh medium was added to maintain the constant volume. The amount of released apigenin was determined at the wavelength of 340 nm by using UV-Vis spectroscopy analysis. The UV-Vis spectrum of apigenin and hexagonal phase without apigenin was shown in Fig. A1 (supplementary material).

3. Results and discussion

3.1. Phase behavior

The rough phase diagrams of the Tween 80/EtOL/H_2O and Tween 80/WCS/EtOL/H_2O systems at 25 °C was presented in Fig. 1(a and b) and Fig. A2 (supplementary material). The mass fraction of WCS (w_t) in the samples S_0, S_1, S_2 and S_3 was 0%, 5.0%, 7.5%, 9.9% respectively. One viscous liquid crystal was observed in every phase diagram. It can be noted that the region of liquid crystal became small and extended to the high water content with increasing WCS content. This was due to the fact that WCS was not solubilized completely at relatively low water content. As shown in Fig. 1(a and b), the H phase containing water between 17 and 58 w_t%, dissolving 24 w_t% of EtOL, while the H′ phase formed over the range of 41–58 w_t% water content, solubilizing 12 w_t% of EtOL.

The structure of the liquid crystal showed in Fig. 1(a and b) can be proved by SAXS described in Fig. 1(c). The sample S_0 without WCS exhibited three Bragg scattering peaks and the relative positions relationship was corresponding to $1 : \sqrt{3} : 2$, confirming the hexagonal liquid crystal structure (H_1) [18]. The sample S_2 possessed 7.5 w_t% WCS content was still kept the structure of hexagonal liquid crystal, as indicated by the similar relative positions of the Bragg peaks. The position of every component in the hexagonal phase was schemed in Fig. 2. The interfacial film of hexagonal liquid crystal was formed by surfactant Tween 80, which dissolved in oil water interface. Among them, the hydrophilic head of Tween 80 solubilized in water and hydrophobic tail solubilized in oil phase. The oil phase (EtOL) located in hydrophobic core. Water-souble chitosan mainly solubilized in outer aqueous phase, and several water-souble chitosan molecules dissolved in the “palisade” layer formed by the hydrophobic chains of Tween 80 molecules through the hydrophobic interaction and hydrogen bond. Moreover, the apigenin molecules tended to solubilize in the inner core and “palisade” layer. Therefore, we selected the hexagonal liquid crystal formed in Tween 80/EtOL/H_2O and Tween 80/WCS/EtOL/H_2O systems as the drug carriers to encapsulate apigenin for further study.

3.2. Effect of WCS

The SAXS pattern of the samples S_0 and S_2 was presented in Fig. 1(c). It can be observed that both samples possessed hexagonal liquid crystal structure whether containing WCS or not. After introducing WCS, the scattering vector of the first reflection of the