Formation of cinnamon essential oil/xanthan gum/chitosan composite microcapsules basing on Pickering emulsions

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Abstract
Cinnamon essential oil (CNO) is a natural and renewable antibacterial agent. However, CNO is highly volatile and unstable, which limits its practical application as a long-term and wide antibacterial agent. In order to improve the CNO stability, we have microencapsulated CNO into composite microcapsules basing on Pickering emulsion stabilized by silica (SiO₂) nanoparticles. The CNO-loaded composite microcapsules possess the hybrid microcapsule shell including SiO₂, xanthan gum and chitosan. Moreover, the results show that the microcapsules have spherical appearance. Microencapsulation technique effectively promotes the CNO stability, and the loaded CNO is slowly released from microcapsules. The antibacterial test indicates that the minimal inhibitory concentration of microcapsules was 2 mg mL⁻¹ against Escherichia coli and Staphylococcus aureus, and the microcapsules can play an effective long-term antibacterial effect. Thus, Pickering emulsion templates is a convenient and effective technique to construct antibacterial essential oil-contained microcapsules, which can be used as long-term antibacterial agents.

Keywords Antibacterial microcapsules · Chitosan · Cinnamon essential oil · Emulsion templating · Xanthan gum

Introduction
With the visible development of society and the gradual improvement of living standards, people not only pursue economic growth, but also increasingly pay attention to their health, sanitation and environmental condition [1]. It is worth noting that in the survival environment people get inevitably exposed to various pathogenic microorganisms such as bacteria, molds and viruses, which can proliferate and spread rapidly under appropriate environmental conditions and in turn clearly threaten human health [2].

Therefore, the research and development of efficient antibacterial products have attracted a great amount of attention, especially after the outbreak of 2019 novel coronavirus infection [3, 4].

Recently, antibacterial materials originated from natural sources, for instance plant essential oils, have become the hot raw materials for the development of antibacterial agents, because of their favourable antibacterial property, biosafety, degradability and renewability [5–7]. Plant essential oils usually contain a variety of compounds with biological activity such as phenols, olefins, terpenoids and aldehydes and ketones, which can play an important role in extensive bacteriostasis [8, 9]. Thus, the use of plant essential oils as natural antibacterial agents has been widely concerned as promising substitutes for unfriendly synthetic antibacterial agents. Among various antibacterial plant essential oils, cinnamon essential oil (CNO) is a volatile aromatic oil extracted from the bark, branches or leaves of cinnamon, which has been widely used as raw material in research and industry fields [10, 11]. CNO has shown the effective and broad spectrum antimicrobial ability to resist bacteria, molds and yeast, as well as displayed favourable nontoxicity, antioxidant and insect repellent functional activities. Therefore, CNO can be served as a highly effective

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and environmentally friendly antibacterial agent [12, 13]. However, CNO is highly unstable when it is exposed into the undesirable environment in the presence of air, heat and light [14]. This above issue of CNO tends to shorten the shelf life and reduce the functional antibacterial activity effect and finally leads to the limitation of practical application as antibacterial agents [15, 16]. Moreover, CNO carries a strong smell, which also would prohibit its wide application as antibacterial agents. Therefore, it is obviously necessary to explore effective ways to improve the poor stability and alleviate the strong odor of CNO, and in turn broaden the potential antibacterial application fields of CNO.

Microencapsulation is a coating technology that generally uses film-forming matrix as the wall material and active functional material as the core material [17]. The active functional material is wrapped as microcapsule core by the packaging effect of microcapsule wall, which can prevent the active functional material against exposing in the external environment and in turn improve the stability of the active functional material, reduce the release rate of active functional material, prolong the shelf life of the active functional material and decrease the odor of the active functional material, which assist in expanding the application fields of the active functional material [18, 19]. Therefore, microcapsule technology can be used to prepare CNO-loaded microcapsules to prompt the practical application of CNO as antibacterial agents. Among a variety of microcapsule preparation method, emulsion template approach is a simple and effective way to prepare active functional material-loaded microcapsules. Therefore, the preparation of plant essential oil-contained microcapsules by emulsion template method has attracted much attention due to its simplicity and versatility [15, 20]. Particularly, compared with traditional emulsions stabilized by surfactants, Pickering emulsions use solid particles as particle emulsifiers, which are almost irreversibly adsorbed on the oil–water interface to endow Pickering emulsion with excellent stability [21, 22]. Hence, Pickering emulsion is considered as an ideal template for preparing microcapsules loading plant essential oils. In this aspect, in our previous work [23], we effectively constructed the CNO-loaded poly(melamine formaldehyde) composite microcapsules by in situ polymerization of CNO in water Pickering emulsion templates. The prepared composite microcapsules displayed the improved thermal stability, the controlled CNO release and visibly long-term antimicrobial effects. However, melamine and formaldehyde were used as the building blocks of the microcapsule shells for fabricating the CNO-loaded poly(melamine formaldehyde) composite microcapsules. The melamine and formaldehyde were non-biocompatible material, and their multipolymers namely poly(melamine formaldehyde) had the potential to release formaldehyde into the surrounding environment, which greatly limits the use fields of their related antimicrobial microcapsule products. Therefore, it is very strongly necessary to employ the biocompatible materials as the building blocks of the microcapsule shells of the CNO-loaded antimicrobial microcapsules in order to broaden the microcapsule potential antimicrobial applications.

For the formation of microcapsules, the raw materials used for microcapsule wall are diverse materials including synthetic and natural polymers [24]. Among various natural polymer materials, chitosan is the only basic and cationic polysaccharide derived from renewable chitin abundantly existing in crabs and shrimp shells [25]. Moreover, chitosan has the favourable characteristics of film-forming ability, anti-inflammatory, bactericidal performance, biodegradability, biocompatibility, which has been regarded as the raw material of microcapsule wall. In addition, xanthan gum is a kind of anionic extracellular polysaccharide derived from microorganisms, which has the advantages of low toxicity, high stability and good biocompatibility [26, 27]. The complex coacervation by the electrostatic interaction between the positive charges of chitosan and negative charges of xanthan gum can achieve the coacervation of the xanthan gum and chitosan polymer mixture at the interface of the emulsion droplets and then undergo the crosslinking process of the xanthan gum and chitosan polymer mixture to form the polymer microcapsule shells [28, 29]. However, there are few studies on the combination of chitosan and xanthan gum for the preparation of microcapsules by Pickering emulsion template.

In this work, the CNO-contained antibacterial composite microcapsules with hybrid shell of silicon dioxide (SiO2) nanoparticles/xanthan gum/chitosan were constructed basing on Pickering emulsions stabilized by SiO2 nanoparticles. Figure 1 displays the schematic diagram of the preparation of composite microcapsules in this work. Specifically, for the formation of Pickering emulsion, SiO2 nanoparticles were acted as the particle emulsifiers, and CNO was used as oil phase. Moreover, CNO was added into the water phase of acetic acid suspension including chitosan and SiO2 nanoparticles and then was emulsified to obtain CNO in water Pickering emulsion. Subsequently, the newly prepared emulsion was added to the xanthan gum solution, and then electrostatic interaction between chitosan and xanthan gum caused the composite coacervation of xanthan gum and chitosan at the interface of the emulsion droplets. Afterwards, the interface shell of the emulsion droplets was fixed by the crosslinking reaction between the amino group of chitosan and the aldehyde group of glutaraldehyde to obtain the CNO-loaded composite microcapsules. The morphology, thermal property and CNO loading capacity of the composite microcapsules were studied. Furthermore, the release behavior of CNO in vitro and the antibacterial
activity of microcapsules against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) were also discussed, respectively.

**Experimental**

**Materials**

Chitosan was provided by Bio Science & Technology Co., Ltd. (Shanghai, China). Xanthan gum was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). CNO was kindly offered by Jiangxi Jishui Lvxian Spice Refinery (Jishui, China). Silicon dioxide (*SiO₂*) nanoparticles (Trade name N20) were supplied from Wacker Chemie (Munich, Germany), and the mean particle size was about 22 nm. Agar was purchased from Biosharp (Hirono, Japan). Beef extract and peptone were obtained from Guangdong Huankai Microbial Technology Co., Ltd. (Guangzhou, China). Glutaraldehyde and glacial acetic acid were offered by Tianjin Damao Chemical Reagent Factory (Tianjin, China). All reagents were used as received without any further treatments.

**Preparation of CNO in water pickering emulsion**

Firstly, chitosan solution with the content of 0.5 w/v% was prepared by adding chitosan power into 1 v/v% acetic acid solution and stirring the mixture by magnetic stirrer for 2 h. Afterwards, 0.2 g of *SiO₂* nanoparticles was added to 40 mL of chitosan solution and then magnetically stirred for 20 min to obtain the uniform suspension, which was served as the aqueous phase. Moreover, 2 mL of CNO used as oil phase was added to the aqueous phase, and the oil–water mixture was emulsified under a 4000 r min⁻¹ homogenizer for 2 min to prepare the CNO in water Pickering emulsion stabilized by *SiO₂* nanoparticles (namely CNO Pickering emulsion).

**Construction of CNO/xanthan gum/chitosan composite microcapsules**

Firstly, the weighed xanthan gum was added into distilled water and magnetically stirred at 50 °C for 1 h to obtain the xanthan gum aqueous solution with the content of 0.2 w/v%. Subsequently, the above prepared Pickering emulsion was added into 20 mL of xanthan gum aqueous solution and then stirred at 750 r min⁻¹ for 40 min. Then, the mixing system was cooled to approximately 5 °C, and the system pH value was changed to 6 ~ 7 with 10 w/v% NaOH solution. Moreover, 5 mL of 10 wt% glutaraldehyde solution was dropped into the system, and the mixture was stirred at low temperature (nearly 0 °C) for 30 min. Additionally, the mixture was heated to 50 °C and stirred for 4 h to obtain the composite microcapsule suspension. After the suspension was washed and centrifuged for 3 times, the residue was dried in a sealed dryer containing phosphorus pentoxide and saturated CNO for 48 h to obtain the composite microcapsule powder.

**Microscopic observation of pickering emulsion**

The micromorphology of the emulsion droplets was observed by a Phenix BMC 500 microscope (Phenix, China). In order to improve its transmittance, the Pickering emulsion was diluted with deionized water before microscope observation, and then the diluted emulsion sample was laid on a glass slide. And the microscope photos of the emulsion were collected by a digital camera, which was connected to the Phenix microscope.

**Scanning electron microscopy (SEM) characterization of composite microcapsules**

The microstructure morphology of composite microcapsule was viewed by a Zeiss EVO 18 SEM (Carl ZEISS, Germany) at 10-kV acceleration voltage. Before observation, the microcapsule samples were sprayed with a thin gold layer to prevent electrostatic accumulation.

**Thermal stability and loading capacity investigation of composite microcapsules**

The thermogravimetric analysis of CNO, microcapsule shell materials and composite microcapsules was carried out by a DTG-60 thermal analyzer (Shimadzu, Japan) to investigate
their thermal stabilities. The test was performed from 50 to 700 °C in a nitrogen atmosphere at a rate of 10 °C min⁻¹. Moreover, according to the results of thermogravimetric analysis, the loading content of CNO (x) in the composite microcapsules was calculated by the following formula:

\[ W_M = (1-x)W_L + xW_o \]  

(1)

In the formula, \( W_M, W_L \) and \( W_o \) are respectively the final residual mass percentages of composite microcapsules, microcapsule shell and CNO at 700 °C, and x means the loading content of CNO in composite microcapsules.

**Release assessment of CNO from composite microcapsules**

\( W_a \) g of the newly prepared dry microcapsules containing \( W_o \) g of CNO was weighed and placed in a constant temperature incubator. Then, the microcapsule sample was accurately weighed at the arranged time, and the remaining sample weight (\( W_r \) g) after CNO release was recorded. All samples were tested in parallel for 4 times, and the averaged value was obtained. Finally, the cumulative release rate of CNO from the composite microcapsules was calculated as follows:

**Cumulative release rate (%)**  

\[ R_C = 100\times \frac{(W_a - W_r)}{W_o} \]  

(2)

In addition, in order to discuss the release mechanism of CNO, the first-order dynamics equation ln(100-\( Q_t \))=\( K_1 t + C_1 \), Hixson-Crowell dynamics Eq. 100\(^{1/3}\) - (100 - \( Q_t \))\(^{1/3}\) = \( K_2 t + C_2 \), Higuchi dynamics equation \( Q_t = K_3 t^{1/2} + C_3 \) and zero-order kinetic equation \( Q_t = K_4 t + C_4 \) were used to fit the release curves of CNO from composite microcapsules at different temperatures. Herein, \( Q_t \) is the cumulative release rate of CNO at time t; \( K_1, C_1, K_2, C_2, K_3, C_3, K_4, C_4 \) are the correlation constants of the corresponding dynamics model. \( R_1^2, R_2^2, R_3^2 \) and \( R_4^2 \) are the correlation coefficients of the corresponding dynamics models. \( R^2 \) values of the fitting equations for each model were used to determine the best fitting model for the CNO release.

**Antibacterial property evaluation of composite microcapsules**

The antibacterial property of composite microcapsules against *E. coli* and *S. aureus* was investigated qualitatively and quantitatively by inhibition zone method and minimum inhibition concentration assay. The concentration of the employed bacterial suspension was \( 10^7 \) CFU mL⁻¹.

The inhibition zone method was used for the qualitative antibacterial evaluation of composite microcapsules, and the specific steps were shown as follows. 0.1 mL of bacterial suspension was evenly smeared on the surface of nutrient agar plate with a coater. Then, a sterile filter paper (6-mm diameter) soaked in 32 mg mL⁻¹ microcapsule broth suspension was equably pasted on the surface of the agar plate. Then, the tested sample was stored in a 37 °C constant temperature incubator, and afterwards the bacterial growth condition on the agar plate was observed at the arranged period. In this aspect, the antibacterial performance of composite microcapsules against *E. coli* and *S. aureus* was intuitively observed and captured by a digital camera. In addition, the sterile filter paper soaked in broth was used as the control sample.

Minimum inhibition concentration assay was applied for the quantitative antibacterial assessment of composite microcapsules, and the detail steps were displayed as follows. Five milliliters of microcapsule broth suspension with a concentration of 32 mg mL⁻¹ was put into the first test tube, then 5 mL of broth culture medium was added and mixed with microcapsule broth suspension evenly. Afterward, 5 mL of the mixture derived from the first test tube was taken into the second test tube, then 5 mL of broth culture medium was added and mixed into the second test tube. In this way, tubes containing 5 mL of microcapsule broth suspensions with bacteria content of 16, 8, 4, 2, 1, 0.5 and 0.25 mg mL⁻¹ were obtained by tube double dilution method. Herein, the test tube containing 5 mL sterile broth medium was used as control sample. Moreover, 0.1 mL of bacterial suspension and 0.1 mL of 2,3, 5-triphenyltetrazole chloride aqueous solution (0.1 w/v%) were added to each test tube, respectively. Therewith, all test tubes were placed in a shaking apparatus with the shaking speed of 150 rpm under 37 °C. After 18-h storage period, it was observed the color of the suspension in the test. If the suspension in the test tube turned its color into red, it indicated that the antibacterial property of microcapsules in the test tube cannot completely inhibit the bacterial growth. The minimum microcapsule concentration contained in the tested suspension without turning red color was regarded as the minimum inhibitory concentration of composite microcapsules.

**Results and analysis**

**Preparation and characterization of Pickering emulsion**

In this study, SiO₂ nanoparticles were selected as emulsifiers to construct the stable CNO Pickering emulsion. Herein, SiO₂ nanoparticles were evenly dispersed in chitosan solution to serve as the water phase, and the oil phase CNO was added into the water phase and then homogenized to prepare CNO Pickering emulsion. The prepared Pickering emulsion presented milky white (see Fig. 2a), and the Pickering emulsion underwent centrifugation at 5000 rpm for 10 min at room temperature without the appearance of
stratification or demulsification, which showed that the Pickering emulsion had favourable stability. The morphology result of the emulsion droplets observed by Phenix BMC500 optical microscope was shown in Fig. 2b. As can be seen from Fig. 2b, the droplets of CNO Pickering emulsion were approximately spherical shape, and the particle size distribution of droplets presented polydispersity in the range of several to dozens of microns. In addition, the type of prepared Pickering emulsion was evaluated by droplet drop test, and the result showed that Pickering emulsion was easy to evenly disperse in water, but aggregate and deposit at the bottom of the beaker containing CNO (see Fig. 2c, d). The droplet drop test confirmed that the CNO Pickering emulsion prepared in this study was the oil in water type.

**Fig. 2** a Digital photo and b optical micrograph of Pickering emulsion. Digital photos of Pickering emulsion added into c water and d CNO

**Morphology observation of microcapsules**

The CNO-loaded composite microcapsules with SiO$_2$ nanoparticles/xanthan gum/chitosan (inorganic and organic) hybrid shells were effectively constructed after complexly coacervating and curing at the interface shell of the emulsion droplets. Specially, the electrostatic interaction between the positive charge of chitosan and the negative charge of xanthan gum prompted the coacervation of the xanthan gum and chitosan polymer mixture on the interface of the emulsion droplets, and the polymer mixture and SiO$_2$ nanoparticles wrapped CNO together. Moreover, the interface shell of the emulsion droplets was fixed due to the crosslinking reaction between the amino group of chitosan and the aldehyde group of glutaraldehyde. Thus, the prepared microcapsule shell was composed of SiO$_2$ nanoparticles and chitosan/xanthan gum composite polymers. The hybrid shell of microcapsules was beneficial to improve the stability of core material CNO. The microstructure appearance of the composite microcapsules was observed by SEM. As shown in Fig. 3, the prepared composite microcapsules were approximately spherical shape, and the particle size was mainly distributed in the range of 5 to 10 μm. There were no observable cracks or holes on the microcapsule shell. The intact shell of microcapsules was conducive to protect the core material CNO for the improved stability, as well as reduce the release rate of CNO for realizing the long-term antibacterial effect.

**Fig. 3** SEM photos of the prepared composite microcapsules: a low magnification, b high magnification

**Thermal stability and loading content determination of microcapsules**

The thermal stabilities of CNO, microcapsule shell and composite microcapsule were investigated by thermogravimetric analyzer, and the results are shown in Fig. 4. As observed, the weights of all three samples decreased with the increase of the test temperature. Particularly, the temperature ranges for the rapid weight loss phase of CNO, composite microcapsule and microcapsule shell were 80–200 °C, 130–260 °C and 270–330 °C, respectively. As seen from the
thermogravimetric curve result of CNO, the residual amount of CNO was only 10.25 wt% at 200 °C. Moreover, almost all of CNO was pyrolyzed away at 700 °C. However, at 700 °C, the final residual weights of microcapsule shell and composite microcapsule were 71.0 wt% and 35.4 wt%, respectively. The results of thermogravimetric analysis showed that the thermal stability of CNO was poor, and the main decomposition temperature and residual mass of composite microcapsules containing CNO were significantly higher than those of CNO. Thus, the thermal stability of CNO contained in the composite microcapsules was significantly improved via the microencapsulation technology. In addition, the calculation results derived from the thermogravimetric curves displayed that the loading content of CNO in the composite microcapsules was 50.1%, which indicated that the composite microcapsules had loaded CNO with the content of 50.1% to the respect of their own weights.

**In vitro CNO release study**

This study had investigated the CNO release from composite microcapsules in an incubator under constant temperature. Figure 5 shows the cumulative release CNO in vitro with release time at different ambient temperatures (25, 37 and 50 °C). As can be seen, the cumulative release tendency of CNO was highly similar at various ambient temperatures. Specifically, during the study period of CNO release, the initial release rate of CNO was fast, but slowed down gradually with the extension of time. The rapid release of CNO was mainly caused by the easy release of CNO near the inner surface of microcapsule shells, while the subsequent slow release of CNO might be ascribed to the decrease of CNO concentration difference between the inner and outer environment of composite microcapsules. In addition, it can also be seen that with the increase of ambient temperature, the cumulative release amount of CNO from composite microcapsules increased significantly, which was mainly because the increase of ambient temperature prompted an obvious increase in the volatility of CNO, in turn accelerating the release of CNO from composite microcapsules.

In addition, in order to study the in vitro release mechanism of CNO, the first-order, Hixson-Crowell, Higuchi and zero-order dynamics equations were used to fit the CNO release curves. And the fitted dynamics model with the supreme correlation coefficient ($R^2$) was regarded as the CNO release dynamics model. The relevant results are shown in Fig. 6. The $R^2$ values obtained by fitting CNO release curve using Higuchi kinetic equation were the highest values, which indicated that Higuchi kinetic model could be used to describe the release mechanism of CNO from microcapsules and the release mechanism of CNO abided by Fickian diffusion.

**Antibacterial performance assessment of microcapsules**

In this study, the antibacterial properties of microcapsules against *E. coli* and *S. aureus* were investigated qualitatively and quantitatively by inhibition zone method and minimum inhibition concentration assay. Figure 7 shows the antibacterial results of the sample filter paper soaked with the CNO-loaded microcapsules and blank filter paper, which were obtained by inhibition zone method. As shown, there was no obvious antibacterial zone around the blank filter paper, indicating that the control filter paper had almost no antibacterial performance. However, the sample filter paper
containing the CNO-loaded microcapsules had obvious antibacterial zone, which verified favourable antibacterial performance of the CNO-loaded microcapsules against \textit{E. coli} and \textit{S. aureus}. At the same time, we had also observed from Fig. 7 that the size of inhibition zone around the microcapsule-contained filter paper had no clearly change

\textbf{Fig. 6} Fitting results for the release curves of CNO from microcapsules by different kinetic equations

\textbf{Fig. 7} Antibacterial result photos of blank filter paper disk (Blank) and microcapsule-contained filter paper disk (Sample) against \textit{E. coli} (a) and \textit{S. aureus} (b)
between 1 and 7 days of culture period, indicating that the prepared microcapsules could play a long-term antibacterial effect, and in turn further showing that antibacterial CNO could achieve slow release from the composite microcapsules. Moreover, Table 1 shows the quantitative antibacterial results of composite microcapsules against E. coli and S. aureus. As shown, the minimum inhibitory concentrations of the composite microcapsules against E. coli and S. aureus were 2 mg mL\(^{-1}\). Based on the above results of inhibition zone method and minimum inhibition concentration assay, the composite microcapsules prepared in this study could achieve effective and long-term antibacterial effect against E. coli and S. aureus.

**Table 1** The minimum inhibitory concentrations of the composite microcapsules

| Code       | Bacteria types | Concentration (mg mL\(^{-1}\)) |
|------------|----------------|-------------------------------|
|            | E. coli        | 16  | 8  | 4  | 2  | 1  | 0.5 | 0.25 |
| Microcapsules | S. aureus     |  +  | +  |  + |   |  |  |  |
| Blank control | E. coli       |    |  + |    |   |  |  |  |
|             | S. aureus     |    |    |    |  + |  |  |  |

“-” means to the bacteriostatic rate of 100%; “+” means that the bacteriostasis rate is less than 100%

**Conclusion**

In this study, CNO/xanthan gum/chitosan composite microcapsules with the antibacterial performance were successfully formed via templating SiO\(_2\) nanoparticles-stabilized CNO in water Pickering emulsion. The prepared composite microcapsules showed nearly spherical structures. CNO was successfully loaded into composite microcapsules, which effectively improved the thermal stability of CNO. The in vitro release of CNO from microcapsules presented a slow-release behavior, and the release amount of CNO can be regulated by adjusting the release ambient temperature, and the release mechanism study had shown that the release of CNO followed Fickian diffusion. In addition, in vitro antibacterial assays showed that the composite microcapsules had obvious effective and long-term antibacterial effects against E. coli and S. aureus. Based on the above research results, it could be seen that it was feasible to prepare the antibacterial CNO-loaded composite microcapsules using templating oil in water Pickering emulsion stabilized by SiO\(_2\) nanoparticles, and the constructed composite microcapsules have broad application prospects as antibacterial materials in textiles, coatings, biopesticides and other fields.

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**Declarations**

**Competing interests** The authors declare no competing interests.

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