Nanoencapsulation of Capsaicin by Complex Coacervation of Gelatin, Acacia, and Tannins

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ABSTRACT: Using gelatin and acacia as wall and capsaicin as core substance, nanocapsules were prepared by mixing two solutions of oppositely charged polymers, and then treated by hydrolysable tannins. The morphology and size distribution of the nanocapsules were analyzed by transmission electron microscope and laser particle size analyzer, respectively. The nanocapsules had a mean diameter of 300–600 nm, with mean drug loading content (20.81%) and encapsulation efficiency (81.17%) with good dispersion and spherical morphology. The interaction between gelatin and tannins is discussed in the article. Moreover, the addition of hydrolysable tannins in the system had an important influence on the morphology and particle distribution of the nanocapsules because of the synergistic actions of hydrogen bonding and hydrophobic effects. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 96: 2225–2229, 2005

Key words: gelatin; nanoencapsulation; complex coacervation; capsaicin; tannins

INTRODUCTION

Polymeric nanocapsules, defined as capsules with a size in the range of 10–1000 nm, consist of natural or synthetic polymers, which are a promising method in controllable delivery of drugs. Nanocapsules are vesicular systems in which the drugs are confined to a cavity surrounded by a unique polymer membrane.[1] Nanocapsules may be prepared using a variety of methods, such as solvent evaporation,[2] phase inversion,[3] spray drying,[4] and self assembly,[5] due to different physical and chemical properties of model drugs and polymeric materials. Many kinds of polymers have been applied in drug delivery as they can effectively release the drugs to a specific target while protecting vulnerable molecules from degradation in a given environment. Natural polymeric materials used as potential drug carriers have unique advantages in nanoencapsulation because of their excellent safety, biocompatibility, and biodegradation. Using their electrostatic interactions of two solutions of oppositely charged polymers, nanocapsules were fabricated by complex coacervation.[6,7] Moreover, these carriers could be modified with other polar or magnetism-targeted groups to get selectivity and direction by physical or chemical methods.[8]

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Capsaicin (the main pungent ingredient in hot peppers) and its synthetic derivatives have been extensively investigated in pharmacy,[9] neuroscience,[10] and antimicrobial drugs.[11] The plain and heated extracts from peppers were found to exhibit various degrees of inhibition against microbes. So far, researchers have investigated that capsaicin could inhibit the growth of some foodborne pathogenic bacteria, such as Listeria monocytogenes, Staphylococcus aureus, Salmonella typhimurium, and Bacillus cereus.[11] Capsaicin was also studied for activity against microbes and insects.[12] Capsaicin had a naturally occurring pungent odor that could prevent sea animals from attaching to marine structures while being benign to the environment. Using the method of nanoencapsulation can delay the release property as well as improve the efficiency of these natural extracts of plants.

In this article, using gelatin A (positively charged) and acacia (negatively charged) as polymeric carriers, and capsaicin as core substance, nanocapsules were prepared by complex coacervation, and then treated by tannins. The morphology, size distribution, and drug content of the nanocapsules were investigated, and the interaction between tannins and gelatin is also reported in the article.

METHODS

Materials used in nanocapsule preparation

Gelatin A (isoelectric point IEP = 6.80) was purchased from Tianjin Third Chemical Factory (Tianjin, China).

Acacia was obtained from Shanghai Chemical Company (Shanghai, China). Capsaicin (6.6%) was supplied by Nanjing Tianshu Biological Company (Nanjing, China). Hydrolysable tannins were provided by Tianjin Jiangtian Chemical Company. 5% (w/v) glutaraldehyde aqueous solution was supplied by Tianjin Fine Chemical Company (Tianjin, China). Sodium hydroxide and glacial acetic acid were of analytical grade. All aqueous solutions were prepared from distilled water.

Preparation of nanocapsules

The method used for preparing the nanocapsules was complex coacervation. In this work, the gelatin–acacia pair in an equal concentration (w/v) was coacervated at pH 4.2. The procedure was as follows: 0.30g capsaicin was dispersed in 40 mL of 0.2% (w/v) acacia solution with 10 mL of 0.5% (m/m) hydroxylethyl cellulose (HEC) solution as a surfactant and sonicated for 30 min (power of the ultrasonic instrument is 200 W) to form a stable oil-in-water emulsion. Then the resulting emulsion was poured into 40 mL of 0.2% (w/v) gelatin solution at 50°C. The coacervation procedure was maintained about 1 h after adding 5% (v/v) aqueous solution of glacial acetic acid to adjust the pH to 4.2, and then treated with 0.5% (w/v) aqueous solution of tannins for about 12 h at 30°C. After several washings with distilled water, the nanocapsules were crosslinked by using 0.5% (w/v) glutaraldehyde solution at the temperature of 5–10°C and pH of 8–9. The resulting nanocapsules were rinsed several times by distilled water, and then centrifugalized at 8000 rpm (model MR 18.22, Jouan Company). Finally, the nanocapsules were lyophilized in a freeze-dryer for 24 h (model Alpha 2–4, Martin Chaist Company).

Basic parameters of gelatin and acacia involved for complex coacervation

The isoelectric point (IEP = 6.80) of gelatin A and hydrolysable tannins (IEP = 3.22) were measured by a Zeta potential analyzer (model: BI-90plus, Brookhaven Instruments Corp.).

The number average molecular weight of hydrolysable tannins (1.52×10^3), gelatin (4.00×10^4), and acacia (1.04×10^5) were measured by GPC (model 410, Waters Company; PEO was used as the standard sample and $NaNO_3$ as the flowing phase).

Particle size and distribution

The particle size and distribution of the nanocapsules were analyzed by a laser particle size analyzer (model Ms17, Malvern Instruments Corp., U.K., and model BI-90plus, Brookhaven Instruments Corp.).

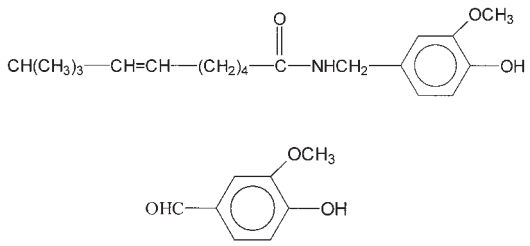

Figure 1 The chemical structure of capsaicin (a) and vanillin (b).

Transmission electron microscope studies

The morphology of the nanocapsules was observed by transmission electron microscope (model 100LX, Phillips Company, Holland).

Drug content analysis and release behavior

The capsaicin content of the nanocapsules was determined by vanillin-sodium nitrite colorimetry,[13] which was based on sodium nitrite colorimetry. The basic principle was that both vanillin and capsaicin had the functional group -OCH_3 (their chemical structures are illustrated in Fig. 1), which could react with sodium nitrite to obtain complex compounds under acidic condition using a molybdenum ion as a catalyst. These two compounds had similar absorbent peaks at 420nm in a UV-VIS spectrophotometer (model 2101, Shimadzu Company, Japan), so vanillin-sodium nitrite colorimetry was an alternative method.

The percentage encapsulation efficiency of nanocapsules was calculated as follows[13]:

Actual drug content (mg) = Total drug content − Drug in the solution (mg)

% Encapsulation efficiency (E) = (Actual drug content) / (Total drug content) × 100%

% Drug loading content (D) = (Actual drug content) / (Total mass of nanocapsules) × 100%

RESULTS AND DISCUSSION

The isoelectric point (iep) of the gelatin

The gelatin was a type of amphoteric polymer, which showed cationic properties at the acid condition and anion properties at the alkaline environment. When it reached the isoelectric point, the charged number of cations and anions was in balance to keep neutralization of the gelatin solutions.

The IEP of the gelatin solution, which determined the quantitative interaction between gelatin and acacia in the coacervation process, was measured through a Zeta potential analyzer on the basis of the relation

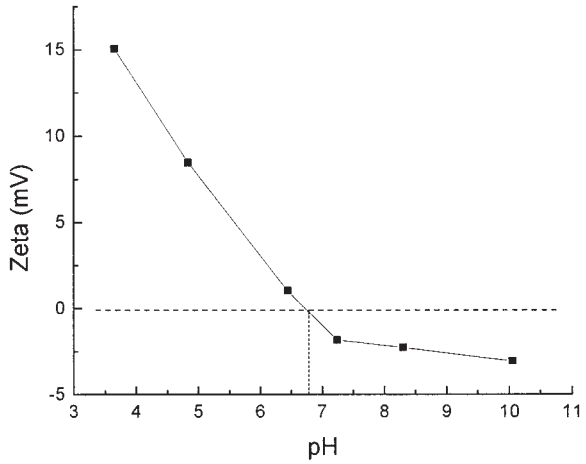

Figure 2 The relationship between Zeta potential and pH value of gelatin solutions (0.10%).

between the Zeta potential and pH in the solutions. The result is shown in Figure 2.

The concentration of gelatin and acacia on the particle size and distribution

Four kinds of concentration of gelatin and acacia solutions were chosen to investigate their effects on the particle size of the nanocapsules. The effective diameter of the nanocapsules at different concentrations is shown in Figure 3. With the increase in concentration of the system, the effective diameter of nanocapsules also increased to a certain extent. The higher the concentration of the solutions, the larger the effective diameter of nanocapsules, but when the concentration of the gelatin solutions reached above 1%, the particles in the system turned into microparticles. The increase of concentration in the gelatin and acacia solutions may lead to change of viscosity in the systems, which caused the microparticles to conglutinate easily in the coacervation on a large scale.

The influence of tannins on the morphology of the nanocapsules

Hydrolysable tannins were employed to treat wet nanocapsules after coacervation because of the interaction between tannins and gelatin. Tannins were plant polyphenols that had a unique protein precipitating ability owing to the function of hydrogen bonding and hydrophobic effects.[14] More recent studies have highlighted the intrinsic complexity of the molecular recognition processes that occur between tannins and gelatin in aqueous media.[15] There is not a static matching of binding groups in the host and the guest; rather, there is dynamic polydentate association.

The interaction mechanism between tannin and gelatin belongs to the "hand–glove" model. This kind of

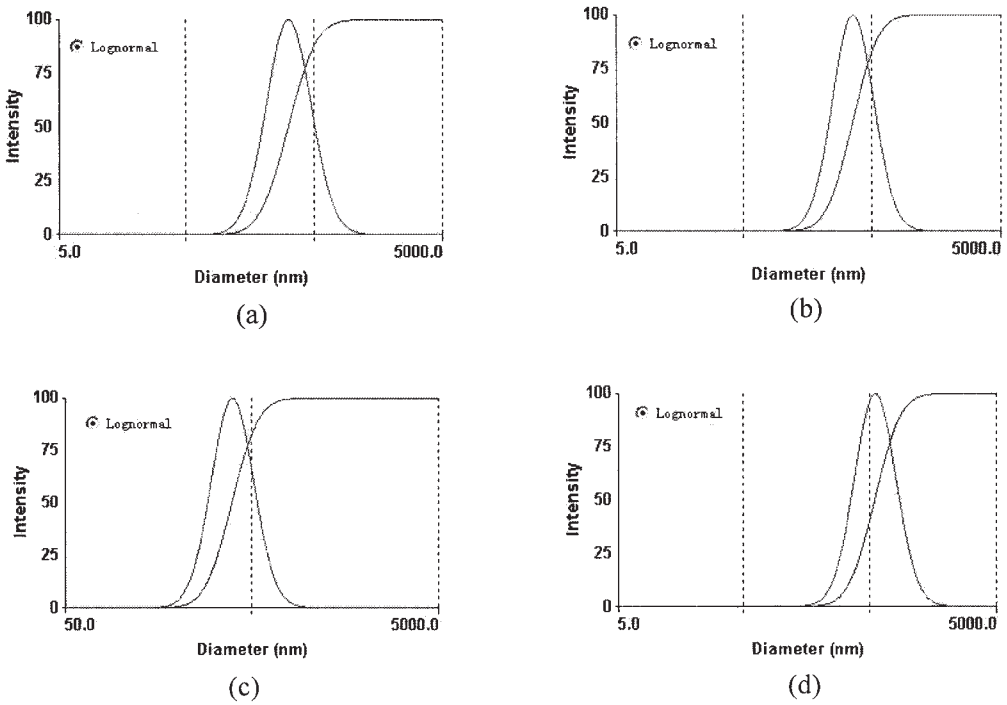

Figure 3 Particle size and distribution of the nanocapsules prepared under different concentrations of gelatin and acacia solutions: (a) 0.05%; (b) 0.10%; (c) 0.20%; (d) 0.50%.

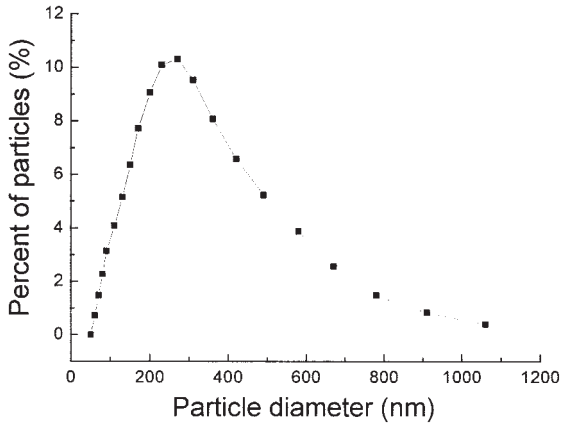

Figure 4 Particle size and distribution of the nanocapsules (mean diameter = 530 nm, span = 1.625).

model requires that donors and receptors have the ability to enable multi-binding sites between tannins and gelatin stable. Hydrophobic effects were seen as the main driving forces for the interaction between tannins and gelatin. The polyphenolic structure of tannins could interact by hydrogen bond with polar groups of the gelatin, such as peptide and carbonyl groups. The tannin–gelatin coprecipitation mechanism is a two-stage mechanism, which involves an initial complexation stage of tannins with gelatin and subsequent precipitation of the complexes. Environmental factors, such as pH, temperature, and ionic strength, mainly affect the second precipitation stage, while the gelatin concentration mainly affects the initial complexation stage.[16,17] In the precipitation stage, the precipitability of the complexes increases with an increase in the molar ratio between the tannins and gelatin.

The utilization of tannins could form a rigid hydrophobic film surrounding the wet prenanocapsules to make the subsequent drying nanocapsules have good dispersion and shape. The morphology, particle size, and distribution are demonstrated in Figures 4 and 5.

Drug content of nanocapsules and release behavior

The encapsulation efficiency and drug loading content were calculated by using the above-mentioned method. The results are listed in Table I. The mean efficiency was 81.17% (maximum 85.46%), and the mean drug content was 20.81% (maximum 22.73%). The drug loss in the emulsion could be decreased by choosing a suitable gelatin concentration, proportion between gelatin and capsaicin, and processing parameters, so the nanocapsules had relatively high encapsulation efficiency and drug content with good dispersion and even distribution.

TABLE I
Encapsulation Efficiency and Drug Loading Content of Nanocapsules

Samples	1	2	3	4	5	6
E%	82.92	80.05	85.46	79.88	78.32	80.38
D%	21.26	20.09	22.73	20.40	19.85	20.55

E: encapsulation efficiency; D: drug loading content.

The release behavior of nanocapsules in the distilled water is shown in Figure 6, which obviously reveals the burst release effect at the beginning of the drug delivery, but remained stable after 40h.

CONCLUSION

In this article, nanocapsules were prepared by complex coacervation of gelatin, acacia, and tannins The nanocapsules had a mean diameter of 300–600nm, mean drug loading content (20.81%), and encapsulation efficiency (81.17%), with good dispersion and spherical morphology. The addition of tannins in the system had an important influence on the morphology and particle dispersion of the nanocapsules because of the synergistic actions of hydrogen bonding and hydrophobic effects.

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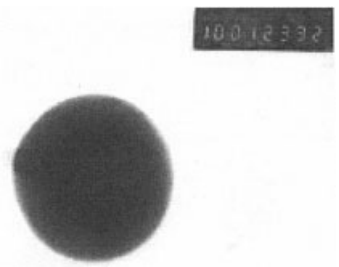

Figure 5 TEM photographs of the nanocapsule.

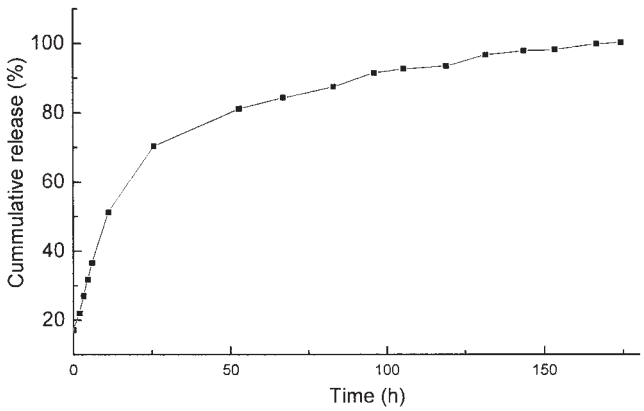

Figure 6 The release behavior of the nanocapsules.

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