Microencapsulation of Capsaicin by the Complex Coacervation of Gelatin, Acacia and Tannins

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ABSTRACT: With gelatin and acacia as walls and capsaicin as the core substance, microcapsules were prepared through the mixing of two solutions of oppositely charged polymers and were then treated with tannins. The processing factors included the stirring rate and the types and dosages of the surfactants used in the preparation of the microcapsules. The morphology and size distribution of the microcapsules were analyzed with optical microscopy, environmental scanning electron microscopy, and laser particle size analysis. The microcapsules had a mean diameter of 20–30 μm, a maximal drug loading content of 19.84%, and an encapsulation efficiency of 88.21% with a good dispersion, even distribution, and round shape. The influence of the tannins on the morphology and structures of the microcapsules was also investigated, and their interaction mechanism was examined. © 2003 Wiley Periodicals, Inc. J Appl Polym Sci 91: 2669–2675, 2004

Key words: microencapsulation; drug delivery systems; particle size distribution

INTRODUCTION

Nowadays, microencapsulation is widely applied in applications such as controllable drug release.[1–3] The polymeric materials used in microencapsulation have become one of the pivotal factors in the performance of microcapsules. Common materials include natural, seminatural, and synthetic polymers with the general requirements of mechanical strength, stability, and ease of processing.[4,5] Natural polymeric carriers such as gelatin and chitosan have unique advantages in microencapsulation because of their good safety, biocompatibility, and biodegradation[6–8] and the moderate processing conditions needed for modification or crosslinking.[9] With the electrostatic interactions of two solutions of oppositely charged polymers, such as gelatin and acacia, gelatin and sodium alginate, or chitosan and sodium alginate, microcapsules can be fabricated by complex coacervation.[10,11] Moreover, the interactions between the polymers are reversible and can be adjusted to special requirements before crosslinking.[5] More importantly, these carriers can be modified with other polar or magnetism-targeted groups to obtain selectivity and direction by physical or chemical methods.[12]

Capsaicin (the main pungent ingredient in hot peppers) and its synthetic derivatives have been extensively investigated for pharmaceutical,[13,14] neuroscience,[15,16] and antimicrobial drugs.[17] The antimicrobial properties of the capsicum species and their uses might date back to Mayan times.[17] Two pungent compounds found in the capsicum species (capsaicin and dihydrocapsaicin) have been tested for their antimicrobial effects. Plain and heated extracts exhibit various degrees of inhibition against microbes. Recently, researchers have shown that capsaicin can inhibit the growth of some food-borne pathogenic bacteria.[18] Capsaicin has also been assayed for activity against microbes and insects.[19] Capsaicin has a naturally occurring pungent odor that, benign to the environment, can prevent sea animals from attaching to marine structures.[19] With the method of microencapsulation, the release rate can be delayed, and the efficiency of these natural extracts of plants can be improved.

In this study, with gelatin A (positively charged) and acacia (negatively charged) as polymeric carriers and capsaicin as the core substance, microcapsules were prepared by complex coacervation and then treated with tannins. The processing factors for preparing the microcapsules were examined. The morphology, size distribution, and drug content of the microcapsules were also investigated.

EXPERIMENTAL

Materials used in the microcapsule preparation

Gelatin A [isoelectric point (IEP) = 8.86] was purchased from Tianjin Third Chemical Factory (Tianjin,

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China). Acacia was obtained from Shanghai Chemical Co. (Shanghai, China). Capsaicin (6.6%) was supplied by Nanjing Tianshu Biological Co. (Nanjing, China). Hydroxylethyl cellulose (HEC), poly(vinyl alcohol) (PVA), and poly(vinyl pyrrolidone) (PVP) were provided by Jilin Chemical Corp. (Jilin, China). A 5% (w/v) glutaraldehyde aqueous solution was supplied by Tianjin Fine Chemical Co. (Tianjin, China). The sodium hydroxide, glacial acetic acid, and tannins were analytical-grade. All aqueous solutions were prepared from deionized water.

Preparation of the microcapsules

The microcapsules were prepared by complex coacervation, which classically occurs through the mutual interaction of two oppositely charged polymers. In this work, the gelatin–acacia pair in an equal concentration (w/v) was coacervated at pH 4.2. The procedure can be summarized as follows: 0.8 g of capsaicin was dispersed in 40 mL of a 5% (w/v) acacia solution with 15 mL of a 1% (m/m) HEC solution as a surfactant, and the mixture was sonicated for 30 min to form a stable oil-in-water emulsion (the power of the ultrasonic instrument was 50 W, and its model number was KQ-50; Kunshan, China). Then, the resulting emulsion was poured into 40 mL of a 5% (w/v) gelatin solution at a stirring rate of 350 rpm at 50°C. The coacervation procedure was continued for 1 h to obtain small aggregates after the addition of a 5% (v/v) aqueous solution of glacial acetic acid to adjust the pH to 4.2. Afterward, the temperature was lowered to 5–10°C and the pH was adjusted to 8–9 through the addition of a 5% (m/m) aqueous solution of sodium hydroxide. The small aggregates were then crosslinked with a 5% (w/v) glutaraldehyde solution. After several washings with deionized water, the microcapsules were treated with a 10% (w/v) aqueous solution of tannins for about 10 h at room temperature. Finally, the microcapsules were washed several times with deionized water and dried in vessels at room temperature.

Basic parameters of gelatin and acacia involved in complex coacervation

The IEP value (8.86) of gelatin A was obtained with an electrical conductivity instrument (DDS-11A, Shanghai Electromagnetic Instrument, Shanghai, China) to determine the inflexion of the curve. With the addition of the gelatin solution, the electrical conductivity increased because there were more charges in the system. When the solution reached an IEP, the charged groups were in balance, so there was an inflection point in the curve.

The number-average molecular weights of gelatin (4.00×10^4) and acacia (1.04×10^5) were measured by gel permeation chromatography [model 410, Waters Co.; poly(ethylene oxide) was used as a standard sample, and $NaNO_3$ was used as a flowing phase].

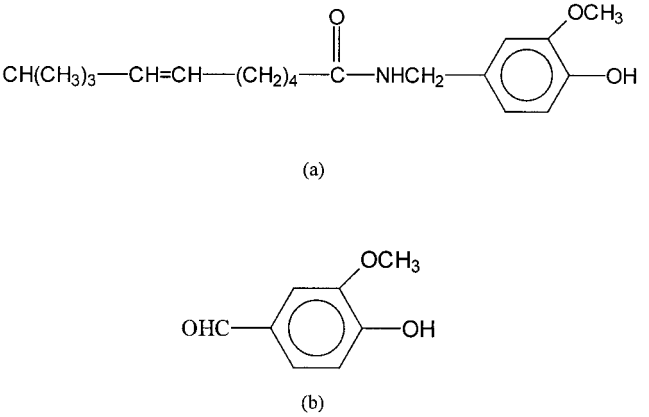


Figure 1 Chemical structures of (a) capsaicin and (b) vanillin.

Particle size and particle size distribution

The particle size and particle size distribution of the microcapsules were analyzed with a laser particle size analyzer (model Ms17, Malvern Instrument, United Kingdom).

Optical microscopy and environmental scanning electron microscopy studies

The internal and external morphology of the microcapsules was observed with an optical microscope (model BX51, Olympus Co., Japan) and an environmental scanning electron microscope (model XL30, Philips Co., Holland). The wet samples were fixed on the glass substrate and then observed with the optical microscope with transmitted light at magnifications of 400× and 1000×.

Drug content analysis

The capsaicin content of the microcapsules was determined by vanillin/sodium nitrite colorimetry (based on sodium nitrite colorimetry)[20] because it is hard to obtain pure capsaicin in China. 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 yield complex compounds under acidic conditions with molybdenum ion as a catalyst. These two compounds were shown to have similar absorbance peaks at 420 nm with an ultraviolet–visible spectrophotometer (model 2101, Shimadzu Co., Japan), so vanillin/sodium nitrite colorimetry was an alternative method.

The encapsulation efficiency of the microspheres was calculated as follows:[9]

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

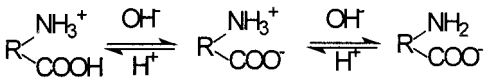
Encapsulation efficiency (%) = (Actual drug content)/(Total drug content) × 100%

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

RESULTS AND DISCUSSION

IEP of gelatin

Gelatin is an example of an amphoteric polymer, showing cationic properties under acid conditions and anionic characteristics in an alkaline environment. An IEP can thus be found, corresponding to the pH for which the polymer has an equal number of positively and negatively charged groups, according to the following balance:



The IEP determines the quantitative complexation relationship of gelatin and acacia in the coacervation and is measured through the inflection point of an electrical-conductivity/pH curve. Here, 40 mL of a 5% (w/v) gelatin solution was kept at a constant temperature (45.0 ± 0.1°C) in a vessel, and 0.2 mL of a 1% (m/m) NaOH solution was added to the vessel at each time to measure the relationship between the conductivity of the gelatin solution and the corresponding pH. Figure 2 shows the inflection point mentioned. From it, we found an IEP value of approximately 8.86.

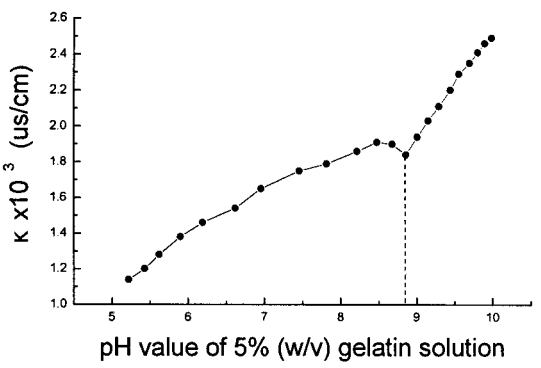


Figure 2 Relationship between the conductivity (κ) and pH value of gelatin solutions.

TABLE I
Particle Size and Distribution of Microcapsules Prepared at Different Stirring Rates

	Sample			
	1	2	3	4
Stirring rate (rpm)	250	300	350	400
Mean diameter (μm)	28.43	25.61	23.99	20.22
$D(V, 0.1)$ (μm)	12.68	13.12	13.28	14.22
$D(V, 0.5)$ (μm)	25.12	23.30	21.53	19.48
$D(V, 0.9)$ (μm)	48.75	40.66	35.20	27.17
Span	1.44	1.18	0.94	0.66

$D(V, 0.1)$ refers to 10% microparticles below the specified size, $D(V, 0.5)$ 50%, and $D(V, 0.9)$ 90%: Span = $[D(V,0.9) - D(V,0.1)/D(V,0.5)]$. 40 mL of 5% (w/v) gelatin and acacia solution, 0.8 g of capsaicin, and 15 mL of 1% (w/v) hydroxylethyl cellulose were used. pH = 4.2. Coacervation was for 1 h at 50°C, and then the sample was treated 30 with mL of a 10% aqueous solution of tannins.

Influence of the stirring rate on the morphology and particle size distribution of the microcapsules

While constant coacervation conditions were maintained, including the pH, temperature, and type and dosage of the surfactant, the microcapsules were prepared at different stirring rates for the determination of the optimal parameters based on the yield, particle size, and morphology. The particle size and particle size distribution of the microcapsules were found to depend on the stirring rate. The results showed that with an increase in the stirring rate, the particles of the microcapsules became smaller and their distributions became narrower (see Table I and Fig. 3). However, increasing the stirring rate could destabilize the oil-in-water emulsion, resulting in the distortion or even fragmentation of the microcapsules. This, in turn, reduced their drug loading content and encapsulation efficiency. The morphology of the microcapsules at different stirring rates is shown in Figure 4. A rate of 350 rpm was the optimal stirring rate, as determined from comprehensive considerations of later experiments.

Influence of the types of surfactants on the morphology and particle size

Three kinds of surfactants (PVA, HEC, and PVP) were investigated with respect to their effects on the morphology and particle size of the microcapsules. The mean diameter of the microparticles prepared by the different surfactants demonstrated some differences: 20.22 μm for PVA, 23.99 μm for HEC, and 30.25 μm for PVP. The relevant morphology is shown in Figure 5. The microcapsules with HEC as the surfactant had

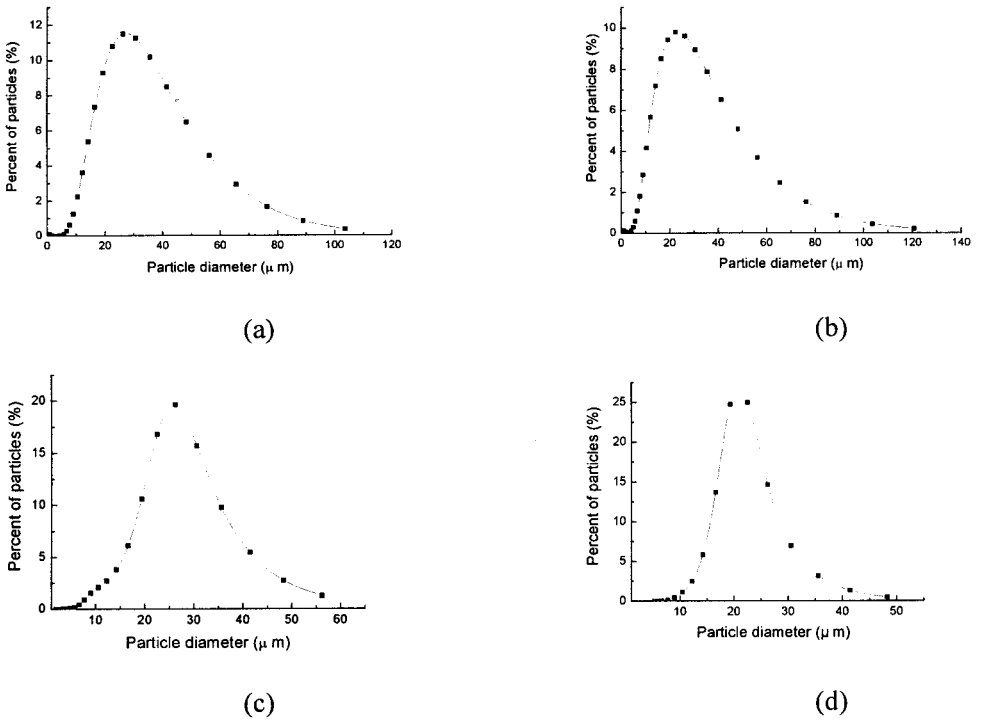


Figure 3 Particle size and particle size distribution of microcapsules prepared at different stirring rates: (a) 250, (b) 300, (c) 350, and (d) 400 rpm. The preparation conditions are described in Table I.

relatively smooth surfaces, a spherical shape, and a lower degree of aggregation, whereas those with PVA and PVP as surfactants had some degree of distortion in the shape and a higher extent of aggregation; this might be due to the basic structure and hydrophilic properties of those surfactants. PVP had a few positive

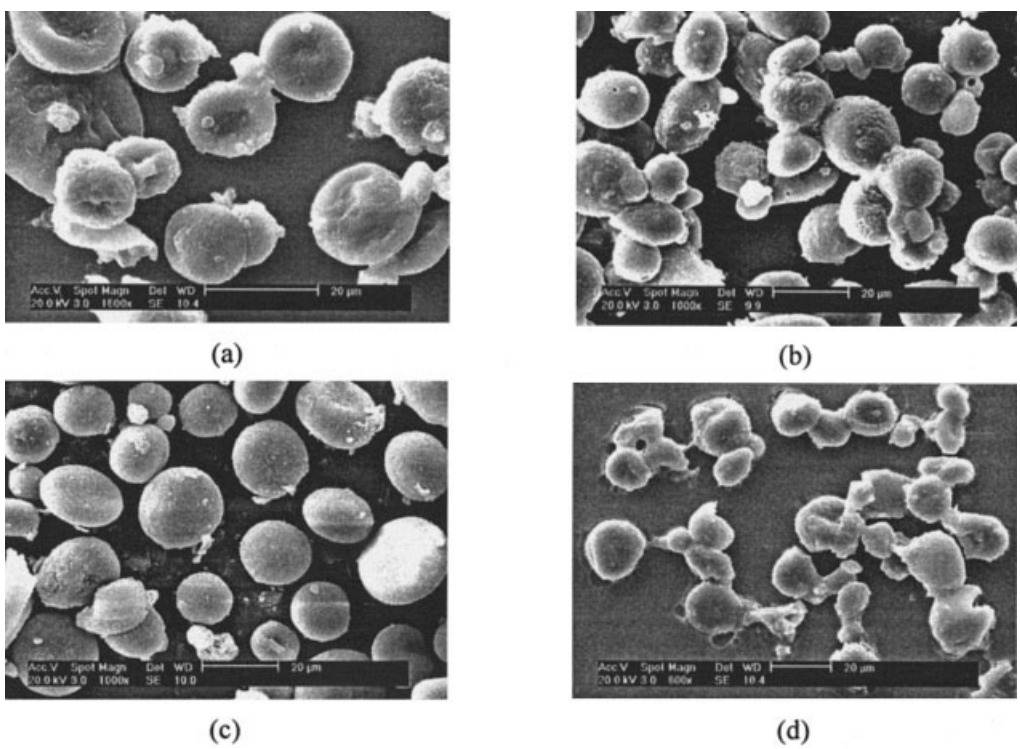


Figure 4 Scanning electron microscopy photographs of microcapsules prepared at different stirring rates: (a) 250, (b) 300, (c) 350, and (d) 400 rpm. The preparation conditions are described in Table I.

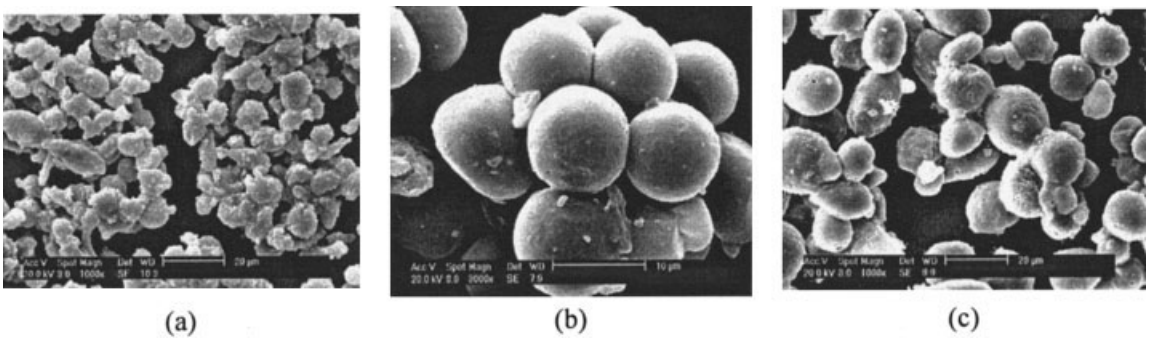


Figure 5 Scanning electron microscopy photographs of microcapsules prepared with different kinds of surfactants: (a) PVA, (b) HEC, and (c) PVP. The preparation conditions were as follows: 40 mL of a 5% (w/v) gelatin and acacia solution; 0.8 g of capsaicin; 15 mL of 1% (w/v) HEC, PVA, or PVP; a pH of 4.2; coacervation for 1 h at 50°C; and subsequent treatment with 30 mL of a 10% aqueous solution of tannins.

charges, which could interact with wall materials to result in aggregation, which made the dispersion worse. PVA had relatively large viscosity and hydrophilic properties, which led to the poor dispersion of the microcapsules. HEC produced a good dispersion because it had a relatively rigid structure and the function of hydrogen bonds for the formation of stable microcapsules.

Influence of the dosage of HEC on the morphology and particle size

Different amounts of the surfactant (HEC) for the preparation of the microcapsules were investigated for the determination of a suitable HEC dosage that could yield a narrow size distribution and a spherical shape for the products. The morphology of the microcapsules is shown in Figure 6. Insufficient HEC contents (the addition of 10 mL of 1% HEC) were not enough to form stable oil-in-water emulsions, and this demonstrated some distortion in the morphology and a lower drug content of the resultant products; however, an excessive addition (20 mL) of the surfactant could also cause adverse effects, provoking easy aggregation of the microparticles in the coacervation process. Moreover, with the increasing addition of the surfactant, the mean diameter of the microparticles decreased to some extent.

Influence of the tannins on the morphology of the microcapsules

Glutaraldehyde is a well-known chemical crosslinking agent. Crosslinking occurs through the chemical coupling of the aldehyde groups with the free amino functions of the gelatin; this is called Schiff's base reaction.[9] However, the dried crosslinked microcapsules had a poor dispersion and showed a tendency to aggregate, so tannins were used to treat wet microcapsules because of the interaction between tannins and gelatin. Tannins are plant polyphenols with a unique protein precipitating ability. The binding mechanism of the coprecipitation involved the synergistic actions of hydrogen bonding and hydrophobic effects.[21] More recent studies have highlighted the intrinsic complexity of the molecular recognition processes that occur between tannins and gelatin in aqueous media.[22] Molecular recognition between tannins and gelatin polyphenols can be described by the metaphor of a hand in a glove.

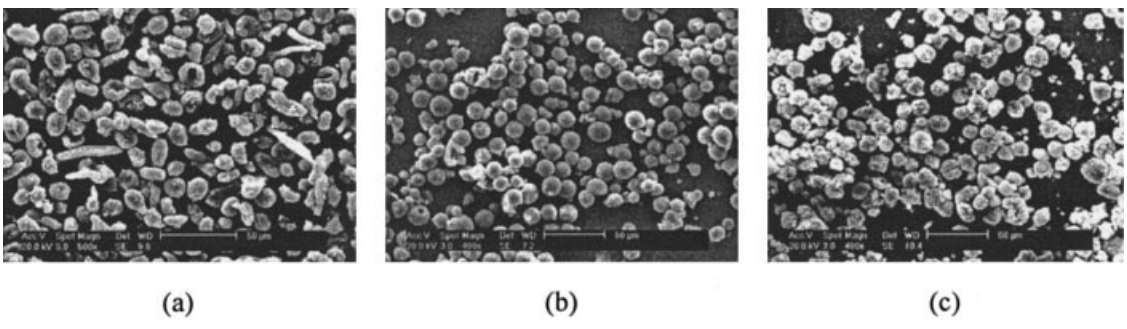


Figure 6 Scanning electron microscopy photographs of microcapsules prepared with different amounts of HEC as a surfactant: (a) 10, (b) 15, and (c) 20 mL. The preparation conditions were as follows: 40 mL of a 5% (w/v) gelatin and acacia solution; 0.8 g of capsaicin; 15 mL of 1% (w/v) HEC; a pH of 4.2; coacervation for 1 h at 50°C; and subsequent treatment with 30 mL of a 10% aqueous solution of tannins.

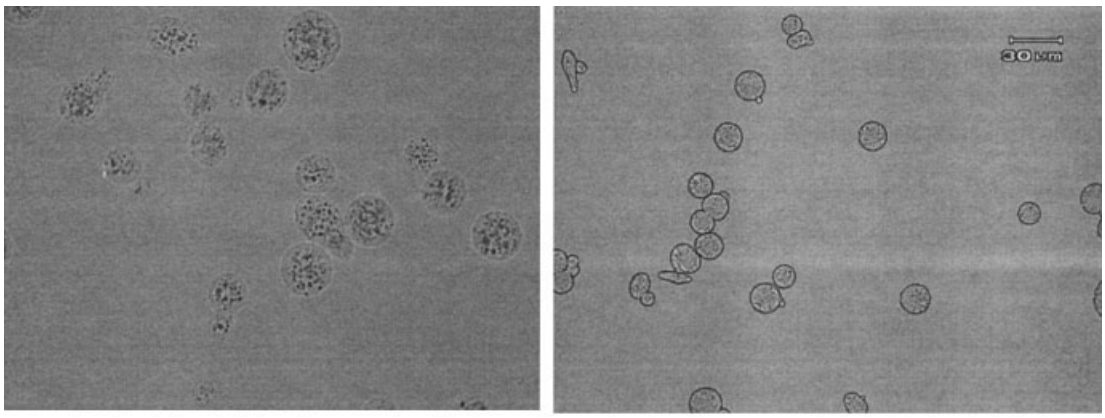


Figure 7 Optical photographs of microcapsules (a) before and (b) after treatment with tannins (1000× magnification). The preparation conditions were as follows: 40 mL of a 5% (w/v) gelatin and acacia solution; 0.8 g of capsaicin; 15 mL of 1% (w/v) HEC; a pH of 4.2; coacervation for 1 h at 50°C; and subsequent treatment with 30 mL of a 10% aqueous solution of tannins.

In the initial stages, the process is driven strongly by hydrophobic effects. There is no static matching of binding groups in the host and the guest, but instead there is a dynamic polydentate association.[23]

The effectiveness of tannins on microcapsule synthesis is shown in Figure 7, which presents a rigid hydrophobic film surrounding the wet premicrocapsules. The dried microcapsules had a good dispersion and a round shape (see Fig. 8).

Drug content of the microcapsules

The encapsulation efficiency and drug loading content were calculated with the aforementioned method. The results are listed in Table II. The mean efficiency was 78.92% (maximum = 88.21%), and the mean drug content was 18.69% (maximum = 19.84%). The drug loss in the emulsion could be reduced with suitable processing conditions, so the microcapsules had a relatively high encapsulation efficiency and a relatively high drug content with a good dispersion and an even distribution and shape.

In this study, the contents of the surfactants had a greater influence on the encapsulation efficiency and drug loading content, whereas the types of surfactants had a smaller influence, although they exerted an important effect on the morphology of the microcapsules.

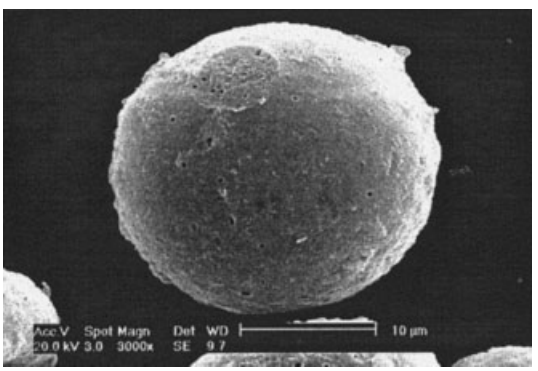


Figure 8 Scanning electron microscopy photograph of microcapsules after treatment with tannins. The preparation conditions were as follows: 40 mL of a 5% (w/v) gelatin and acacia solution; 0.8 g of capsaicin; 15 mL of 1% (w/v) HEC; a pH of 4.2; coacervation for 1 h at 50°C; and subsequent treatment with 30 mL of a 10% aqueous solution of tannins.

CONCLUSIONS

In this article, the preparation of microcapsules by the complex coacervation of gelatin, acacia, and tannins has been discussed. The optimum parameters, including the stirring rate and types and dosage of surfactants, have been investigated. The microcapsules had a mean diameter of 20–30 μm, a high drug loading content of 19.84%, and a high encapsulation efficiency of 88.21% with a good dispersion, an even distribution, and a spherical morphology. The addition of tannins to the system had an important effect on the morphology and particle dispersion of the microcapsules because of the synergistic actions of hydrogen bonding and hydrophobic effects.

TABLE II
Encapsulation Efficiency (*E*) and Drug Loading Content (*D*) of Microcapsules

	Sample							
	1	2	3	4	5	6	7	8
E(%)	74.02	79.83	88.21	70.94	74.56	77.82	84.59	81.37
D(%)	17.38	18.85	19.84	16.75	18.32	18.93	19.61	19.42

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