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Development of microcapsules using chitosan and alginate via W/O emulsion for the protection of hydrophilic compounds by comparing with hydrogel beads

Yuxiao Wang [a,b,c,*], Chen Tan [b], Seyed Mohammad Davachi [b], Peilong Li [b], Philip Davidowsky [b], Bing Yan [b]

[a] College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, China
[b] Department of Food Science, Cornell University, NY 14853, United States
[c] State Key Laboratory of Animal Nutrition, Ministry of Agriculture Feed Industry Centre, China Agricultural University, Beijing 100083, China

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ABSTRACT

It is a critical challenge to protect hydrophilic compounds in food or pharmaceutical applications due to their strong tendency to leak out of the capsules into the external aqueous phase. In this work, we developed an encapsulation system that can protect hydrophilic ingredients using polyelectrolyte complexes prepared with chitosan and alginate via water-in-oil (W/O) emulsion. Unlike the traditional preparation of hydrogel beads, in which one material was added dropwise to another that had an opposite charge, we prepared microcapsules by electrostatic interaction between the positively charged $-NH_3^+$ groups of chitosan and the negatively charged $-COO^-$ groups of alginate by W/O emulsion via ultrasonication, which prevented the formation of large complexes. The preparation conditions were optimized at an ultrasonic power of 375 W and alginate/chitosan ratio of 7:5, in which the alginate/chitosan microcapsules presented a good polydispersity index of 0.26 and zeta potential of −44.6 mV. The SEM and TEM images showed the microcapsule contained multiple, irregular, conglutinated spheres with a core and shell structure. High encapsulation efficiency and retention efficiency showed its potential to protect hydrophilic components from harsh environments. This method provides a simple route that can efficiently encapsulate a wide range of food or pharmaceutical hydrophilic ingredients.

1. Introduction

Protecting and delivering hydrophilic, bioactive ingredients is challenging, because these ingredients are easy to degrade under certain environmental conditions, resulting in low bioavailability [1]. Many studies have explored different strategies to encapsulate hydrophobic ingredients [2], however, there have been few effective methods for encapsulating hydrophilic compounds due to their strong tendency to leak out of the capsules into the external aqueous phase. W/O/W emulsions [3] easily suffer from degradation into a single phase because of unfavorable environmental and processing conditions. Solid lipid nanoparticles [4] require high concentrations of the emulsifier to reduce the surface tension and facilitate the particle partition during homogenization, but also increase the risk of toxic side effects. Using polymer and cross-linking agent in hydrogels [5,6] also cause the concerns about the toxicity and excretion for oral drug delivery applications. These methods to encapsulate hydrophilic compounds include multiple emulsions, solid lipid nanoparticles, and hydrogels are limited by thermal instability and the utilization of non-food grade components.

Among the ingredients that can deliver hydrophilic compounds, polysaccharides (e.g., alginate, chitosan, carrageenan, and arabic gum) are attractive, because they are recognized as food-grade safe materials [7,8]. Alginate and chitosan are natural polysaccharides that have been used to encapsulate bioactive compounds [2,9–12], bacteria [13], and serve as edible coatings in the packaging [14,15]. Alginate hydrogel beads are another common delivery system for hydrophilic compounds. The method involves the injection of the alginate/ingredients mixture into the Ca^{2+} solution, by a fluidic device, with or without chitosan to form hydrogel beads [9,11,16]. The contact with Ca^{2+} immediately induces ionic cross-links between alginate, thus forming encapsulated hydrogel beads. It is well accepted that a higher network density can be achieved at the droplet surface with the high G (guluronic) alginate, thus yielding a higher encapsulation efficiency [17]. Hydrogel beads have great potential for improving the stability and efficacy during storage and gastrointestinal transit [13]. However, the size of the resulting hydrogel beads is commonly over 100 μm depending on the inner diameter of fluidic devices. Such large particles limit this application in the food industry because the hydrogel beads could change the food texture. Meanwhile, the Ca^{2+} ions first cross-link the capsule surface

* Corresponding author at: College of Food Science and Nutritional Engineering, China Agricultural University, Box 303, No. 17 Tsinghua East Road, Beijing 100083, China.
E-mail address: xuanhuirou@hotmail.com (Y. Wang).

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making to a highly cross-linked surface and a less well cross-linked interior, which could lead to aggregation and instability [18]. As a result, there is still an urgent demand to develop an effective strategy for hydrophilic bioactive delivery.

To meet this need, we developed a facile strategy for encapsulation of hydrophilic ingredients using polyelectrolyte complexes formed by water-in-oil (W/O) emulsion via ultrasonication which did not require synthetic polymers or the use of fluidic devices. In our previous work [1], we have successfully made the chondroitin sulfate and bovine serum albumin as wall materials to enable the formation of shell-cross-linked nanocapsules enclosing in W/O emulsions. Ultrasonication has been extensively used as a novel technique for emulsification and to enhance chemical reactions [6,7]. W/O emulsions prepared with ultrasonication can efficiently contain hydrophilic ingredients without leakage or damage. Using the combination of alginate and chitosan as shell materials, the hydrophilic compound (anthocyanin) was dispersed in a chitosan solution as the aqueous phase, followed by the addition of corn oil as the oil phase. To prevent the formation of large polyelectrolyte complexes of alginate and chitosan, the W/O emulsion was made by ultrasonication of the mixture of chitosan and anthocyanin before the addition of alginate. Then, the polyelectrolyte complexes, with an aqueous core loaded with the anthocyanin, formed in the emulsion by subsequently adding the alginate solution during continuous ultrasonication. Then, the loaded microcapsules could be easily collected after removing the oil phase by centrifugation. Our strategy provides a simple route that can efficiently encapsulate a wide range of hydrophilic bioactive ingredients to prevent the formation of large electrostatic complexes. We have successfully demonstrated this method in the application of anthocyanin.

2. Materials and methods

2.1. Materials

Chitosan of low molecular weight (Mw = 50–190 kDa, 75–85% of deacetylation, viscosity 20–300 cP), sodium alginate of low viscosity (viscosity 4–12 cP), and Span 80 were purchased from Sigma-Aldrich (St Louis, MO, USA). 100% pure corn oil (Mazola, ACH Food Companies, Inc., Cordova, TN) was obtained from a local supermarket. The anthocyanin extracted from blueberry was purchased from Bulk Supplements. Deionized water (18.2 MΩ cm) was purified using a Milli-Q system (Millipore, Billerica, MA, USA). Unless otherwise noted, the chemicals and reagents used in this experiment were of analytical grade.

2.2. Preparation of microcapsules via ultrasonication by comparing with hydrogel beads

The traditional alginate hydrogel beads were prepared following the procedure illustrated in Fig. 1a [11] with some modifications. Here, anthocyanin (1% w/v) was used as the hydrophilic compounds. The anthocyanin was first dissolved in deionized water and filtered with a 0.22 μm syringe filter to remove insoluble particles. 100 μL of the anthocyanin solution was then mixed with 2 mL alginate solution (1% w/v), followed by the dropwise addition of 2% w/v $CaCl_2$ solution, with or without chitosan, to produce the hydrogel beads. The appropriate concentration of alginate must be controlled in the range of 1–3%. Low alginate concentration (<1%) brings on the dilute gelling resulting in no outer layer of the bead. On the contrary, high alginate concentration (>3%) leads to high viscosity, which prevents the formation of beads [11].

The chitosan/alginate microcapsules we developed to deliver hydrophilic compounds were synthesized by the W/O emulsion template (see Fig. 1b). In order to optimize the microcapsule preparation method, we first fabricated blank microcapsules without loading anthocyanin. The ratio of alginate to chitosan (from 1:5 to 10:5) and the ultrasonic power (150, 225, 300, 375, and 450 W) were varied to optimize the preparation conditions. After this step, we used the optimum conditions to load the anthocyanin into the microcapsules. 0.5% w/v chitosan solution was prepared by dissolving chitosan with deionized water and adjusting the pH to 4.75 using acetic acid. 100 μL of the anthocyanin solution (1% w/v) was then mixed with 2 mL of the chitosan solution in a glass vial. 8 mL of corn oil with 0.12 g Span 80 was added as the oil phase. The W/O emulsion was prepared by high-intensity ultrasound using a 750 W ultrasonic processor with a high-power sonic tip operated at 20 kHz frequency (VC 750, Sonics vibra-cell, Sonics & Materials, Newtown, CT, USA). The bottom of the 13 mm diameter ultrasonic probe was immersed at the oil-water interface, and the system was sonicated for 1 min at an acoustic intensity of 300 W (5 s on, 2 s off). It has been previously reported that pulsed ultrasound is superior to continuous ultrasound regarding energy consumption and antioxidant activities of bioactive compounds [19]. Then, 2 mL of alginate solution (0.1–1% w/v) was added dropwise into the emulsion that we continued to ultrasonicate for another 1 min. The preparation process was kept in an ice bath, allowing the formation of polyelectrolyte complexes at low temperature. Some fragments with large diameter microcapsules still formed by the electrostatic interaction of chitosan and alginate. So, the fine emulsion was transferred into a centrifuge tube and centrifuged at 1000*g* for 1 min to remove the oil phase and fragments. The final microcapsules were washed twice with deionized water and centrifugated (15,000*g*, 5 min) to remove the residual oil. All the samples were stored at 4 °C in the dark before analysis.

2.3. Physicochemical characteristics

The average particle size and zeta potential were determined using a zeta-sizer (Nano-ZS90, Malvern Instruments Ltd., U.K.) as described previously [20]. Scanning electron microscopy (SEM) was used to characterize the morphology of the samples which were dropped onto an SEM steel stub, air-dried, and sputter-coated with iridium. Observations were performed at an accelerating voltage of 1 kV using a Gemini SEM 500 (Zeiss). For transmission electron microscopy (TEM), the samples were stained with 1.5 wt% uranium acetate and inspected at 120 kV accelerating voltage with a transmission electron microscopy (FEI T12, Hillsboro, OR, USA). Fourier transform infrared (FTIR) spectra were recorded on an IRAffinity-1S spectrometer equipped with a single-reflection attenuated total reflectance accessory (Shimadzu Corp., Kyoto, Japan). The FTIR spectra were scanned between 400 and 4000 cm^{-1}, with a resolution of 4 cm^{-1}.

2.4. Determination of yield and encapsulation efficiency

The microcapsule yield and encapsulation efficiency were determined using a previously reported method [1]. The weight (m_0) of the 2 mL centrifuge tubes was recorded, and then the microcapsule suspension (1 mL) was transferred to the tubes and centrifuged at 15,000*g* for 5 min, followed by the removal of the supernatant. The remaining microcapsule pellets were freeze-dried for 24 h and weighed again (m). The free amount (n_0) of anthocyanin was determined based on the UV–vis absorbance of anthocyanin at 519 nm with a multimode microplate reader (Spectramax iD3, Molecular Devices). The initial amount of anthocyanin (n) was recorded. The microcapsule yield and encapsulation efficiency were determined as follows:

$$Yield\ (\%) = \frac{m - m_0}{total\ shell\ material\ mass\ initially\ added} \times 100 \quad (1)$$

$$Encapsulation\ efficiency\ (\%) = \frac{n - n_0}{n} \times 100 \quad (2)$$

2.5. Stability assays

The anthocyanin loaded microcapsules were tested for stability due to the molecule's sensitivity to environmental conditions. The

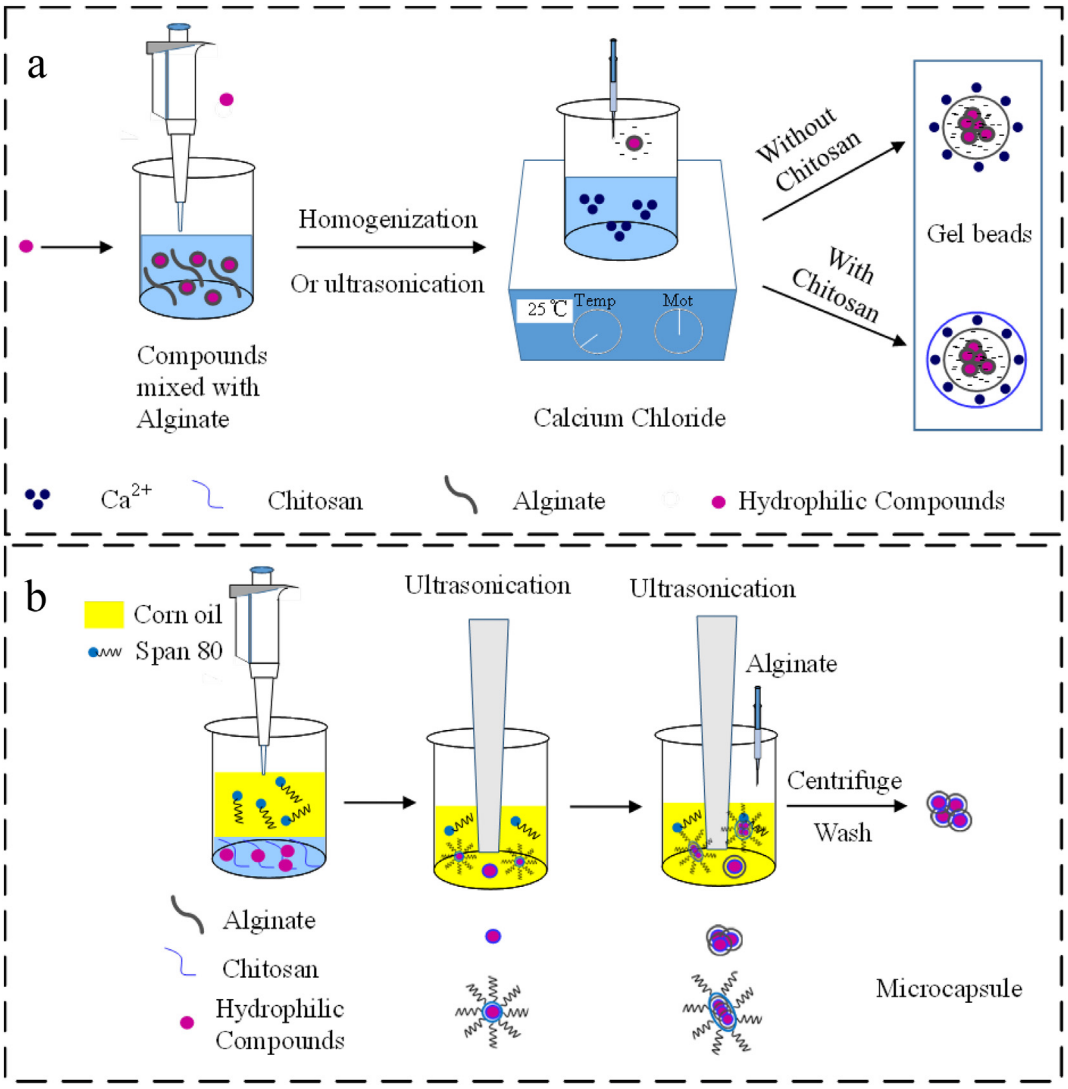

Fig. 1. Schematic illustration of the strategies for encapsulating hydrophilic compounds with (a) traditional hydrogel beads and (b) microcapsules prepared with water/oil emulsion via ultrasonication.

microcapsules containing anthocyanin were evaluated using an ascorbic acid assay and heating assay as described previously [1] with some modifications. The ascorbic acid assay was operated by dispersing the microcapsules in 1 mL of ascorbic acid (1 mg/mL) at room temperature for 10 h. The heating assay for the microcapsules loaded with anthocyanin was conducted in an oven at 80 °C for 10 h. Following the assays, the microcapsules were dried with a freeze dryer system (FreeZone, Labconco Corp., Kansas City, MO, USA) for 24 h. After freeze-drying, the anthocyanin was extracted with methanol. After filtering, the retention efficiency of anthocyanin was quantified as the amount that remained encapsulated after the treatment divided by the initial encapsulated amount in the microcapsules.

2.6. Statistical analysis

All results obtained from experiments were performed in triplicate. The results were presented as the mean and standard deviation. Statistical comparisons were carried out using the least significant difference test of variance (ANOVA) to identify where differences occurred. A statistically significant difference was defined by a value of $p < 0.01$. The analyses were carried out using SAS (version 9.2) and GraphPad Prism (version 5.01).

3. Results and discussion

3.1. Formation of microcapsules via ultrasonication by comparing with hydrogel beads

We used the natural polysaccharides alginate and chitosan to encapsulate various hydrophilic bioactive compounds, such as anthocyanin. Two strategies for encapsulating anthocyanin were illustrated in Fig. 1, (a) traditional hydrogel beads and (b) microcapsules prepared with water/oil emulsion via ultrasonication. The preparation of traditional hydrogel beads involved mixing alginate with hydrophobic or hydrophilic ingredients together, which was then dropped into a Ca^{2+} solution with or without chitosan (see Fig. 1a). The contact between alginate and Ca^{2+} in the solution immediately induced ionic cross-links of alginate, thus forming encapsulated beads.

As an alternative, microcapsules composed of alginate and chitosan shell materials produced via W/O emulsion can efficiently encapsulate hydrophilic ingredients without leakage or damage. Ultrasound has been widely used to disperse and emulsify immiscible liquid compounds, demonstrating a little effect on the chemical structure and functional properties of the treated materials. Different types of chitosan (low, medium, and high molecular weight) and alginate (low and

medium viscosity) have been selected in our pre-experiments. We found the polyelectrolyte complexes would form regardless of what the types of chitosan and alginate were. The molecular weights of both the alginate and chitosan samples were the most significant parameters that influenced the particle size [21]. So, we selected the chitosan of low molecular weight and alginate of low viscosity to reduce the molecular weight and viscosity. We first made the W/O emulsion by dispersing the hydrophilic ingredient with chitosan by ultrasonication (see from Fig. 1b), then added alginate dropwise to form the polyelectrolyte complexes by electrostatic interaction between the positively charged $-NH_3^+$ groups of chitosan and the negatively charged $-COO^-$ groups of alginate.

We investigated the effects of the alginate/chitosan ratios on the average particle size, polydispersity index (PDI), and zeta potential (Fig. 2a–c). The ultrasonic power was initially set at 300 W. As shown in Fig. 2a, the average particle size increased for alginate/chitosan ratios of 1:5 to 4:5, but suddenly decreased at 5:5, then increased again from 5:5 to 8:5. For PDI (Fig. 2b), there was no significant difference between the alginate/chitosan ratios from 1:5 to 4:5 ($p < 0.01$). However, the ratio of 5:5 resulted in a PDI value of 0.668 which was significantly higher than other ratios ($p < 0.01$). A PDI value of <0.5 and an absolute zeta potential of over 30 mV are assumed to be good for colloidal suspension [22]. A higher PDI value is not good for colloidal suspensions. The zeta potential is most often used as an indicator of dispersion stability. We note that from the alginate/chitosan ratios of 1:5: to 4:5, the zeta potential of the materials was positive. However, at the ratio of 5:5, the zeta potential became negative, as shown in Fig. 2c. The negative zeta potential indicated a presence of more carboxyl groups, suggesting the positively charged chitosan had completely reacted with the alginate. According to Fig. 2a–c, we selected a ratio of 7:5 because it showed a smaller particle size, lower PDI, and desirable zeta potential.

We also investigated the effects of ultrasonic power on the average particle size, PDI, and zeta potential. As shown in Fig. 2d, the average particle size decreased as the ultrasonic power increased. This is expected as the increased energy helps disperse the emulsion. However, the average particle size and zeta potential showed no significant difference from 300 to 450 W ($p < 0.01$). The PDI also decreased as the ultrasonic power was increased (Fig. 2e) giving a more desirable particle size distribution. However, even though the PDI continued to decrease, other factors, such as energy consumption, should also be considered. The highest absolute zeta potential value was obtained at 150 W (Fig. 2f) though it also showed the largest average particle size. Therefore, this power was not good for colloidal suspensions (Fig. 2d). Although the best PDI obtained occurred at 450 W, this condition resulted in higher solution temperature and higher energy input, both of which were bad for the sensitive compounds. Therefore, the formation of alginate/chitosan microcapsules by ultrasonication was optimized at an ultrasonic power of 375 W and alginate/chitosan ratio of 7:5. Under these conditions, the blank microcapsule particle size measured by the zeta-sizer was 1129 ± 24 nm with a PDI of 0.26 and zeta potential of −44.6 mV (see Fig. 2 and Supplementary Fig. 1a).

3.2. Morphological characteristics of the microcapsules

Fig. 3a and b displayed SEM images of the blank alginate/chitosan microcapsules. The microcapsules appeared to contain multiple, irregular, conglutinated spheres. The irregular spheres were formed due to the dispersion of the mixture of chitosan and blank microcapsule by ultrasonication, which was significantly different from the morphology of the hydrogel beads (Fig. 5a). The particle diameter of the microcapsule observed by SEM was ~150 nm, which did not match the particle size of 1129 nm determined by the zeta-sizer. This could be explained by the swelling of the alginate in water [2,11,22,23]. Kanokpanont [23] found that the water swelling ability of alginate/chitosan beads was over 2000%. A similar phenomenon could be also observed when the size of such beads decreased with the evaporation of water.

The TEM images showed the microcapsule contained multiple, irregular spheres with a core and shell structure (Fig. 3c and d). The single core was about 347.8 nm, and the shell was about 17.9 nm. In Fig. 3d, the spheres appear conglutinated together. The most desirable structure for these microcapsules, created using ultrasonication, was a single sphere. However, the conglutinated structures of our experimental

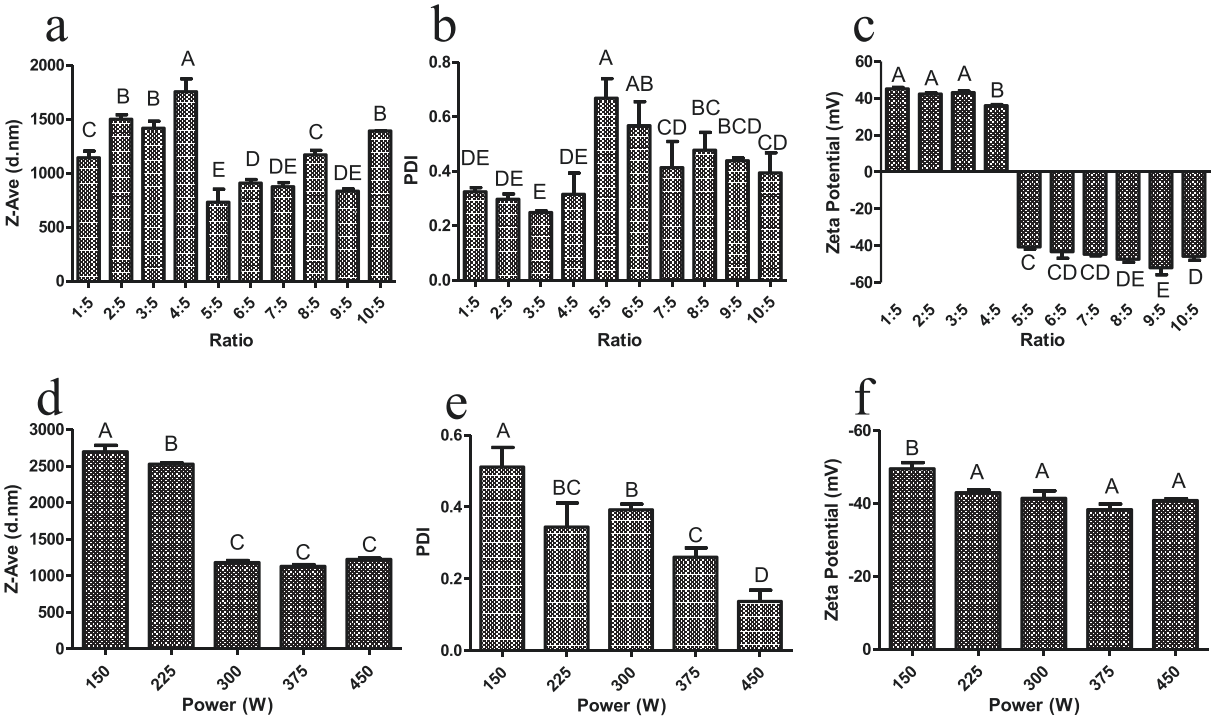

Fig. 2. Optimization of the formation of microcapsules by ultrasonication. Results using: alginate/chitosan ratio (g/g) from 1:5 to 10:5 at constant power of 300 W (a, b, c), and ultrasonic power from 150 to 450 W at constant ratio of 7:5 (d, e, f). Means indicated by different letters differed significantly with a value of $p < 0.01$.

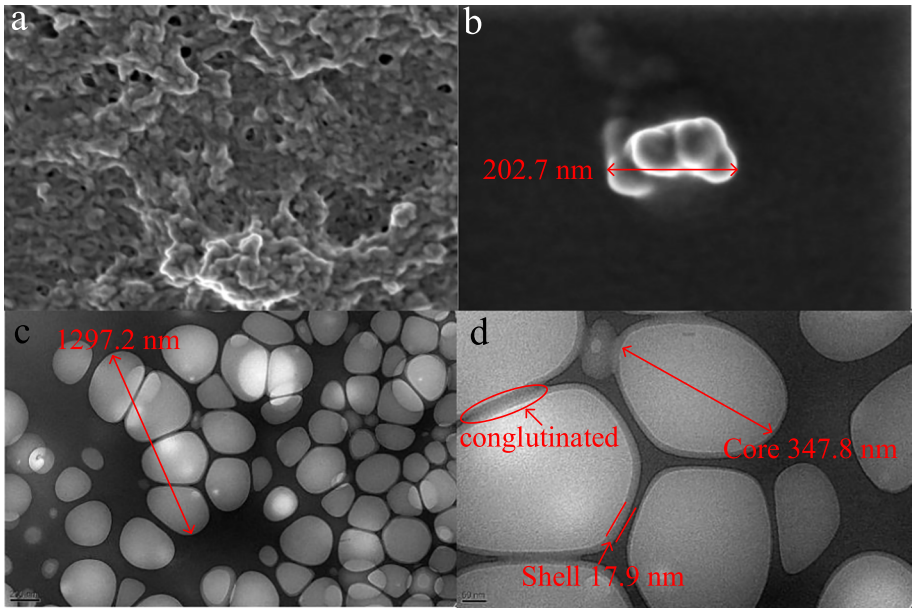

Fig. 3. The (a, b) SEM, and (c, d) TEM images of the alginate/chitosan microcapsules synthesized at a reactant ratio of 7:5 and ultrasonication power of 375 W.

microcapsules could be due to the interaction of the remaining amino groups of chitosan and carboxyl groups of alginate. The aggregation of the spheres depended on the preparation conditions such as the concentration, ultrasonic power, and so on. The correlation coefficient of prepared microcapsules also suggested the large size and a significant proportion of aggregation (see Supplementary Fig. 1b). The particle size

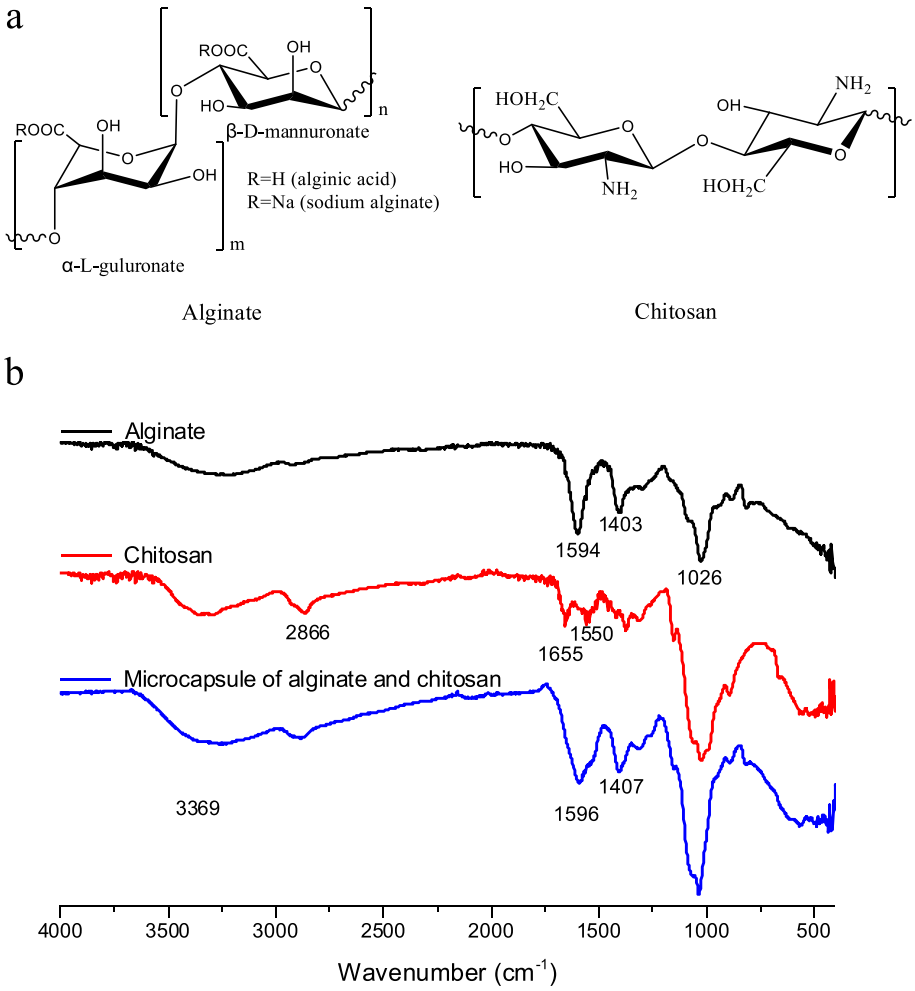

Fig. 4. The (a) molecular structures of alginate and chitosan. (b) The FTIR spectra of alginate, chitosan, and alginate/chitosan microcapsules.

containing four conglutinated spheres was 1297.2 nm, which was consistent with the particle size of 1129 nm determined by zeta-sizer.

3.3. FTIR analysis of the alginate/chitosan microcapsules

As shown in Fig. 4a, alginate is a naturally occurring anionic polymer that consists of linear chains of α-L-guluronate and β-D-mannuronate residues joined by 1,4-glycosidic linkages. Meanwhile, chitosan is a cationic natural linear polysaccharide consisting of copolymers of D-glucosamine and *N*-acetyl-D-glucosamine units linked by β-(1-4)-glycosidic linkages [16].

The FTIR spectra of chitosan, alginate, and the blank microcapsule of chitosan/alginate (Fig. 4b) exhibited a broad, intense hydroxyl group stretching band at 3363 cm^{-1} [20]. The peaks in the alginate spectra at 1594 and 1403 cm^{-1} are associated with asymmetric and symmetric stretching vibration of the COO^- groups, respectively [22]. The absorptions of all samples at 1026 cm^{-1} could be attributed to the stretching vibrations of C-OH side groups [24]. Meanwhile, the peaks in the chitosan spectra at 1655 and 1550 cm^{-1} are caused by asymmetric and symmetric bending vibration of NH^{3+} groups in chitosan [25,26]. The peaks at 1655 and 1550 cm^{-1} in chitosan disappear due to the interaction between the negatively charged -COO^- groups and the positively charged -NH^{3+} groups, which shows the electrostatic interaction between alginate and chitosan [27]. The sharp peak at 1596 cm^{-1} confirms the electrostatic interaction, which is in agreement with the reported interaction between amino groups of chondroitin sulfate and carboxyl groups of alginate at 1604 cm^{-1} [20].

3.4. Microencapsulation of hydrophilic compounds

The particle size of the traditional hydrogel beads is usually correlated with the initial drop size, the viscosity of the chitosan solution, the concentration of alginate, and can vary between micrometer size to 4 mm [28]. The average particle size of the microcapsules carrying anthocyanin ranged from 1.1 to 1.5 μm (Fig. 5c), which was smaller than the hydrogel beads which ranged from 2.8 to 4.4 mm (Fig. 5a and b). Prior to observation, the hydrogel beads loading anthocyanin were air-dried on the SEM steel stub. It showed an irregular spherical structure (Fig. 5b). The cavities formed between two adjacent guluronates in the poly-guluronic acid or poly-mannuronic acid blocks were cooperatively binding with Ca^{2+} to form the compact egg-box structure [9,23]. Compared with the wet hydrogel beads (Fig. 5a), the fold surface with concave and convex structure was due to alginate losing the swelling water [23].

The PDI of the microcapsules carrying anthocyanin was 0.34 ± 0.04 showing good dispersity. The zeta potential was −43.53 ± 2.67 mV, suggesting good stability. A zeta potential value lower than −30 mV is generally considered to have sufficient repulsive force to attain better physical colloidal stability [29]. If the absolute value of the zeta potential is small, it could result in particle aggregation and flocculation due to the van der Waals attractive forces [29]. The microcapsules carrying anthocyanin showed a good yield of 72.52 ± 1.56% and an encapsulation efficiency of 80.69 ± 4.86%. The average particle size, PDI, zeta potential showed no significant difference between the blank and containing anthocyanin microcapsules ($p < 0.01$). This indicated that these microcapsules formed by W/O emulsion could be used as a general carrier for hydrophilic compounds.

The visual appearance of microcapsules loaded with anthocyanin was shown in Fig. 5c. After centrifugation at 15,000*g* for 5 min, we could see the microcapsules loading with anthocyanin existing at the bottom of the centrifuge tube (Fig. 5c). The hydrophilic ingredients would not leak out of the capsules during the process of washing as the dense shell. After washing and centrifuge, we could still see the microcapsules loading with anthocyanin at the bottom of the centrifuge tube. The dense shell formed by the polyelectrolyte complex possessed improved structural strength and mechanical stability [9]. The morphology of the microcapsules containing anthocyanin (Fig. 5d) was observed by SEM. Similar to the blank microcapsule (Fig. 3b), the anthocyanin-

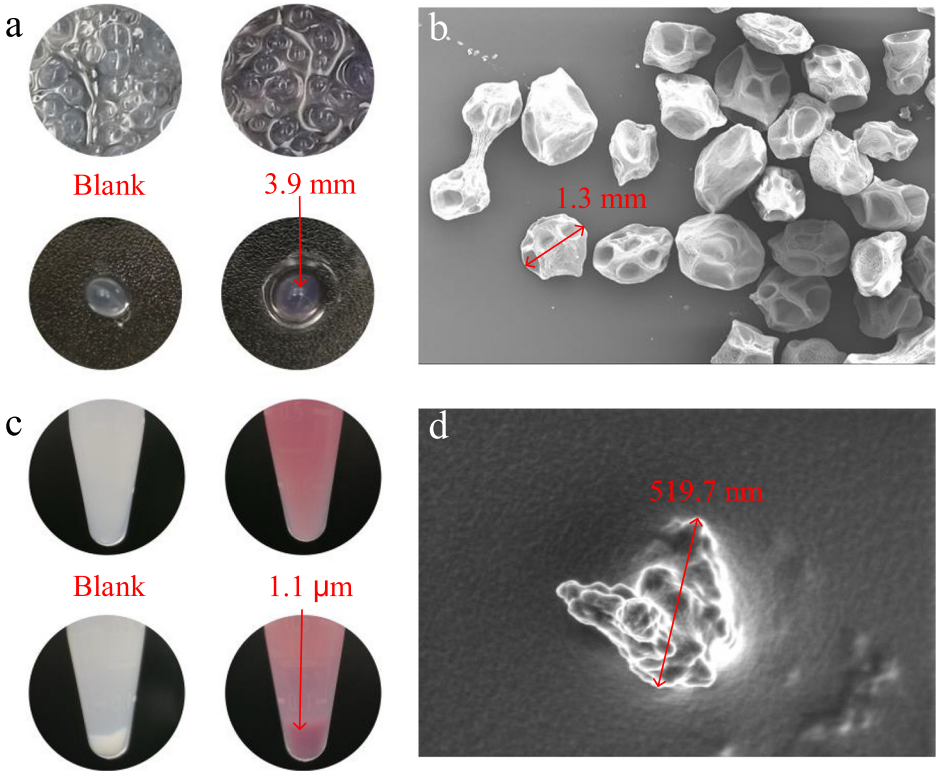

Fig. 5. The morphology of the hydrogel beads and microcapsules of alginate/chitosan prepared by W/O emulsion. Results showed (a) traditional hydrogel beads of blank and anthocyanin, (b) SEM image of hydrogel beads loading anthocyanin, (c) microcapsules of blank and anthocyanin, and (d) SEM image of microcapsules loading anthocyanin.

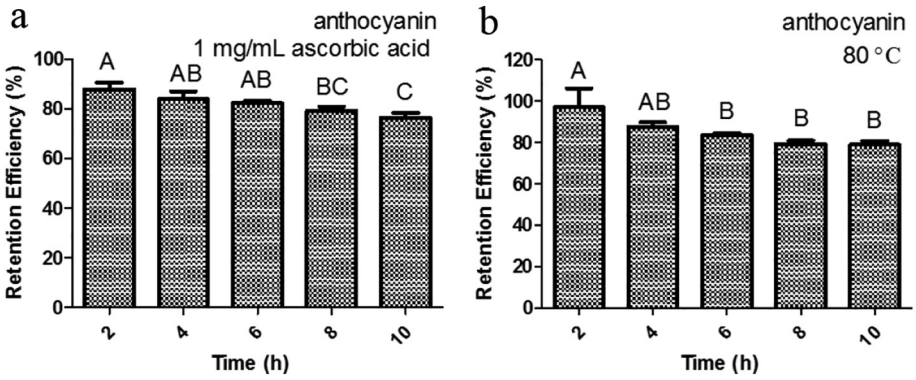

Fig. 6. Stability analysis of the microcapsules loaded with anthocyanin. The retention efficiency of the microcapsules containing anthocyanin (a) in the presence of ascorbic acid, and (b) conducted at 80 °C for 10 h. Means indicated by different letters differed significantly with a value of $p < 0.01$.

loaded microcapsules had a structure with multiple, irregular, agglomerated spheres. The particle size was measured to be ~150–200 nm, which was similar to the blank microcapsule (see Fig. 3d). The different particle size observed by zeta-sizer and SEM may also be attributed to the water swelling ability of the alginate [23].

3.5. Stability of anthocyanin loaded microcapsules

We investigated the stability of the microcapsules loaded with anthocyanin after exposure to harsh environments (Fig. 6). For the ascorbic acid treatment, the retention efficiency of anthocyanin decreased slowly (Fig. 6a). Specifically, the microcapsules retained 87.91 ± 2.54% of the loaded anthocyanin after 2 h incubation, with no significant difference with the retention efficiency at 4 and 6 h ($p < 0.01$). In our previous work [1], the retention efficiency of anthocyanin was only 20% in the presence of ascorbic acid. When anthocyanin was encapsulated in the nanocapsules prepared with polysaccharide and protein, the retention efficiency could be increased up to 62.8%. The stabilizing effect could be attributed to the higher penetration barrier provided by the dense shell formed with alginate and chitosan.

When the microcapsules were exposed to heat, the retention efficiency of anthocyanin decreased slowly from 2 to 10 h. The retention efficiency of anthocyanin retained 78.93 ± 1.80% after 10 h incubation at 80 °C (Fig. 6b), with no significant difference with the retention efficiency at 6 and 8 h ($p < 0.01$). These results demonstrated that the alginate/chitosan microcapsules were able to protect the anthocyanin from degradation by ascorbic acid and heat, respectively. Besides the stability of encapsulated hydrophilic ingredients, other studies have investigated the stability of the microcapsule itself formed by the polyelectrolyte complex [21]. Increasing the pH above 7 led to a significant increase in particle size, while the polyelectrolyte complex was very stable over a temperature range of 4–37 °C. Increasing the ionic strength (0.15 M NaCl) led to the polyelectrolyte complex with less reproducible structures, and the polyelectrolyte complex had a reduced zeta potential. All these findings suggest this delivery system could decrease the exposure of the loaded hydrophilic compounds to harsh aqueous environments.

4. Conclusions

Traditional encapsulation methods for hydrophilic ingredients are still largely ineffective resulting in poor encapsulation efficiency and retention ability. Therefore, we developed a new platform for the encapsulation of hydrophilic ingredients. Microcapsules of alginate/chitosan were formed through successive electrostatic interaction of carboxyl and amino groups triggered via ultrasonication in the W/O emulsion template. W/O emulsions prepared with ultrasonication can efficiently encapsulate hydrophilic ingredients without leakage or damage. The preparation conditions were optimized at an ultrasonic power of 375 W and alginate/chitosan ratio of 7:5, in which the alginate/chitosan microcapsules presented a good PDI of 0.26 and zeta potential of −44.6 mV. The structure of these microcapsules contained multiple, irregular, conglutinated spheres. The prepared microcapsules could effectively protect the internal functional components from harsh environments. This strategy presents good encapsulation efficiency and could be applied to a wide variety of food or pharmaceutical hydrophilic ingredients.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijbiomac.2021.02.089.

CRediT authorship contribution statement

Yuxiao Wang: Funding acquisition, Investigation, Visualization, Writing - Original draft, Writing - Reviewing & editing. **Chen Tan:** Investigation. **Seyed Mohammad Davachi:** Software, Data curation. **Peilong Li:** Investigation, Visualization. **Philip Davidowsky:** Writing - Reviewing & editing. **Bing Yan:** Formal analysis.

Declaration of competing interest

The authors declare no competing financial interest.

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