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Fabrication of novel core-shell hybrid alginate hydrogel beads

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Abstract

Novel hybrid alginate hydrogel beads with shells of porous $CaCO_3$ microparticles were fabricated by templating water-in-oil emulsion and subsequent in situ gelation. Porous $CaCO_3$ microparticles were self-assembled at interfaces of water-in-oil emulsion. Water droplets containing alginate in the emulsion were subsequently in situ gelated by Ca^{2+} released from $CaCO_3$ through decreasing pH with slow hydrolysis of D-glucono-δ-lactone (GDL). The resulting hybrid beads with alginate gel cores and shells of porous $CaCO_3$ microparticles were called colloidosomes. The packed density of $CaCO_3$ microparticles in the shell increased with increasing the ratio of the $CaCO_3$ microparticle weight to the water phase volume M_p/V_w and decreased with addition of NaCl into water. The size of the produced colloidosome beads was independent of M_p/V_w. Increasing the volume fraction of water Φ_w to 0.5, some colloidosome beads deformed to nonspheral shape and even broken. Brilliant blue (BB) as a drug model was loaded into the colloidosome beads by being dissolved in the alginate aqueous solution before gelation. The BB release from the colloidosome beads was slowed down because of the formation of the shells of $CaCO_3$ microparticles. The colloidosome beads may find applications as delivery vehicles for drugs, cosmetics, food supplements and living cell.

Keywords: Alginate hydrogel bead; Porous $CaCO_3$ microparticle; Self-assembly; Water-in-oil emulsion; Controlled release

1. Introduction

Hydrogels are significant materials used for biomedical applications due to their bio-similarity, aqueous inner environment, porous structure, and facility of conjugation with various biological macromolecules, such as proteins (Peppas, 1987; Tabata and Ikada, 1998; Hoffman, 2002; Omidian et al., 2005; das Neves and Bahia, 2006; Wang et al., 2007a,b,c,d). Alginate is a hydrogel-forming polysaccharide consisting of (1 → 4) linked β-D-mannuronate (M) and its C-5 epimer α-L-guluronate (G) residues (Moe et al., 1995; Liew et al., 2006). The most attractive property of alginate is the versatility of gel formation simply induced by various divalent cations. Alginate hydrogel cross-linked by Ca^{2+} is the most typical one and has been widely utilized in tissue engineering and drug delivery (Chretien and Chaumeil, 2005; Pasparakis and Bouropoulos, 2006; Sriamornsak and Kennedy, 2006; Pongjanyakul and Puttipipatkhachorn, 2007).

Artificial conjugation of hydrogels and inorganic compounds is interesting for preparing novel functional materials. Hybrid composite hydrogels possess both advantages of hydrogel and inorganic compound. Grassmann and Löbmann (2004) investigated the biomimetic nucleation and growth of $CaCO_3$ in polyacrylamide hydrogel. Hydrogel cylinders were placed into a U-tube connecting aqueous solutions of $CaCl_2$ and $NaHCO_3$. As the consequence of diffusion of the reactants, $CaCO_3$ precipitation occurs within the gels. Ogomi et al. (2005) fabricated $CaCO_3$ layer covered poly(vinyl alcohol) hydrogel by repeated soaking of the hydrogel into the corresponding salt solutions alternately until achieving desired layer thickness for controlled release. Kuang et al. (2005) prepared poly(*N*-isopropylacrylamide-co-(4-vinylpyridine)) hydrogel spheres containing $CaCO_3$ by in situ mineralization of $CaCl_2$ reacting with CO_2 vapor.

Recently, some super-structured spheres or emulsions fabricated by assembly of colloidal particles at liquid–liquid interfaces were reported (Velev et al., 1996; Dinsmore et al., 2002; Lin et al., 2003; Binks et al., 2005; Croll et al., 2005; Hong et al., 2006; Schmid et al., 2006). On the base of this technique, Noble et al. (2004) and Cayre et al. (2004) fabricated novel hydrogel microspheres with shells consisting of coagulated or

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partially fused colloid particles by templating water-in-oil emulsion and subsequently gelation of the aqueous core. Hot aqueous solution of agarose was emulsified in oil in the presence of solid colloid particles, such as epoxy resin microrods (Noble et al., 2004) and polystyrene latex particles (Cayre et al., 2004) to produce particle-stabilized water-in-oil emulsion and then the system was cooled down to set the agarose gel. The hydrogel microspheres with the shell of different colloid particles are called *colloidosomes*. Duan et al. (2005) prepared magnetic hybrid agarose colloidosome with Fe_3O_4 nanoparticle shell via the same technique, which provided a novel new hybrid hydrogel microspheres with inorganic shells. It has been found that the colloidosome shell offer a great potential to control the release rate of encapsulated species (Dinsmore et al., 2002; Duan et al., 2005). The major advantage is that the shell pore size can be adjusted by varying the particle size and by controlling the degree of fusion or coagulation. Therefore, the colloidosomes may find applications as delivery vehicles for drugs, cosmetics, food additives and living cell (Dinsmore et al., 2002; Duan et al., 2005).

In this work, we have fabricated novel hybrid alginate hydrogel beads with shells of porous $CaCO_3$ microparticles by templating water-in-oil emulsion and subsequent in situ gelation of alginate solution as the core. Porous $CaCO_3$ microparticles were emerging as a new category of biomaterials due to their large specific surface area, inclusion capability to various drugs, cell compatibility, and protein-adhesive property (Davis, 2002; Tosheva and Valtchev, 2005) and played two roles of the cross-linker for alginate and the stabilizer for the water-in-oil emulsion. Porous $CaCO_3$ microparticle shells also endowed the hybrid alginate hydrogel beads new loading capability. For example, one bioactive substance can be loaded into hydrogel cores and another can be loaded into porous $CaCO_3$ microparticle shells. Therefore, double functional beads can be obtained. This fabrication technique that involves templating water-in-oil emulsion stabilized by colloid particles and subsequent gelation of the aqueous phase provides a novel and facile way to create new biocompatible colloidosome beads with shells of bioactive inorganic particles for biomedical applications. Few hydrogel beads with inorganic shells were reported up to now. Brilliant blue (BB) was loaded into the hybrid collodosome hydrogel beads as a model drug to observe the release behavior with fluorescence spectra.

2. Materials and methods

2.1. Materials

Sodium alginate (Kimitsu Chemical Industries Co., Japan, MW 120 000), sunflower oil (CQS Foods Co. Ltd., China), D-glucono-δ-lactone (GDL, Sigma), sodium poly(styrenesulfonate) (PSS, Aldrich, MW 70 000), $CaCl_2{\cdot}2H_2O$, Na_2CO_3 (Guangzhou Chemical Factory, China), Rhodamine B isothiocyanate (RITC, Sigma), Coomassie brilliant blue G250 (BB, Fluka) were used without further purification. Porous $CaCO_3$ microparticles with an average diameter of 5 (m were prepared as described in our previous paper (Wang et al., 2006). All other reagents were of analytical grade and used as received. Water used in all experiments was purified by deionization and filtration with a Millipore purification apparatus to the resistivity higher than 18.0 MΩ cm.

2.2. Fabrication of colloidosome hydrogel beads

In a typical fabrication, 0.04 g porous $CaCO_3$ microparticles were dispersed in 4 mL sunflower oil through stirring for 1 h. Aqueous solution of 1 mL with 1 wt% sodium alginate was added into oil and water-in-oil emulsion was formed by stirring for several minutes. Freshly prepared 0.2 g/mL GDL aqueous solution of 100 μL was added to gelate the alginate core. The emulsion was shaken for two hours and then left for 48 h. After collection by centrifugation, followed by three times of wash with ethanol, the obtained colloidosome hydrogel beads were redispersed in water.

The BB-loaded colloidosome hydrogel beads were fabricated in the same way as described above except that the sodium alginate aqueous solution containing 1 g/L BB. BB-loaded pure alginate gel spheres were prepared in the way similar to that for the BB-loaded colloidosome preparation except that no $CaCO_3$ microparticles were used as the stabilizer.

2.3. In vitro release

For release study, the supernatant solution was collected from 2 mL suspension of about 0.1 g BB-loaded colloidosome beads or BB-loaded pure alginate gel spheres including ca. 1×10^{-4} g BB at pH 7.4 by centrifuging after desired dipping interval. The released quantity of BB was estimated upon the fluorescence intensity at 383 nm of the separated supernatant solution. This release process was repeated until the BB quantity in the supernatant became indetectable. In order to determine the BB quantity remaining in the colloidosome, the hybrid hydrogel beads after the release test were treated repeatedly with acid and ultrasonication. All supernatant solutions after centrifugation were collected and the quantity of unreleased BB in the hybrid hydrogel beads was evaluated with fluorescence intensity. The relationship between fluorescence intensity and concentration of BB is linear in our experimental range.

2.4. Characterization

The specific surface area, porous volume, and porous size distribution of $CaCO_3$ microparticles were determined following the Brunauer–Emmett–Teller (BET) method of nitrogen adsorption/desorption at −196 °C with an ASAP2010 surface area analyzer (Micromeritics Instrument, USA).

The morphology of $CaCO_3$ microparticles and dry colloidosome hydrogel beads was observed by scanning electron microscopy (SEM) with a Philips XL 30 at the acceleration voltage of 15 kV. Samples were prepared by dropping the microparticle or colloidosome suspension on a quartz wafer, dried overnight, then sputtered with gold.

The wet hybrid hydrogel beads were observed with an optical microscope (Carl Zeiss, German). The size and size distribution of the wet colloidosomes were estimated on counting 500 hybrid hydrogel beads with the optical microscope.

The confocal micrograph was taken with a Leica TCS-SP2 confocal laser scanning microscope (CLSM) with a 20× objective with a numerical aperture of 1.4. The colloidosome hydrogel beads were visualized by RITC-labeled $CaCO_3$ microparticles at excitation wavelength of 543 nm.

Fluorescence emission intensity at 383 nm was measured with a Hitachi F-4500 fluorescence spectrometer at 338 nm excitation and excitation/emission slits of 5 nm for BB quantification.

3. Results and discussion

3.1. Optimization of colloidosome fabrication

The emulsion stabilized only with colloidal particles has been well documented (Binks, 2002; Aveyard et al., 2003; Zeng et al., 2006). Herein, colloidosome hydrogel beads are fabricated by templating water-in-oil emulsion stabilized with colloidal particles and subsequent in situ gelation of the aqueous phase. This strategy is illustrated in Fig. 1. Aqueous solution of sodium alginate was emulsified in sunflower oil containing porous $CaCO_3$ microparticles to produce the water-in-oil emulsion. The self-assembled cover layer of porous $CaCO_3$ microparticles at liquid–liquid interfaces acted as the stabilizer for the emulsion. D-Glucono-δ-lactone (GDL) aqueous solution was added to lower the pH value of the water phase gradually through its slow hydrolysis (Lu et al., 2005, 2006) and Ca^{2+} cations were released from $CaCO_3$ to cross-link the alginate chains in the water phase to form gel core. This in situ formed alginate gel core supported the $CaCO_3$ particle shell and endowed the colloidosome hydrogel bead enough stiffness for separation from the oil phase by centrifugation. Here, porous $CaCO_3$ microparticles play two roles: stabilizer for the water-in-oil emulsion and cross-linker for the alginate. Porous $CaCO_3$ microparticles at the shell also endow the colloidosome hydrogel bead new loading capacity due to their versatile inclusive property to various species. This gelation process can be carried out at low temperature to minimize heat effects on the bioactivity of encapsulated materials.

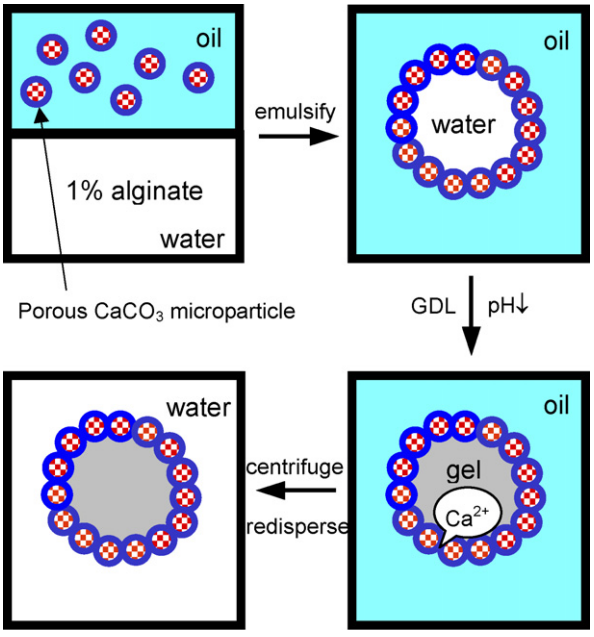

Fig. 1. Schematic illustration of the process for preparing colloidosome based on self-assembled $CaCO_3$ microparticles at the interface of the water droplet including 1 wt% alginate in sunflower oil.

Porous $CaCO_3$ microparticles with an average diameter of 5 (m were prepared as described in our previous paper (Wang et al., 2006). The specific surface area, pore volume, average pore diameter of $CaCO_3$ microparticles were 40 m^2/g, 0.23 cm^3/g and 22.3 nm, respectively, as determined by BET method of nitrogen adsorption/desorption.

3.1.1. Emulsion type

First, we fixed the volume fraction of water phase Φ_w at 0.2 and emulsions were tested in which the $CaCO_3$ particles were initially dispersed in either the water or oil phase. When the particles were initially dispersed in water, the produced water-in-oil emulsion was anti-Bancroft type with particles in the droplets (Golemanov et al., 2006). After GDL was added, water droplets containing alginate with the $CaCO_3$ microparticle outer layer rapidly coalesced into a macroscopic gel with some $CaCO_3$ microparticles inside. We could not obtain the colloidosome hydrogel beads from templating the anti-Bancroft emulsion. It indicated that adding aqueous solution of GDL to the water-in-oil emulsion could lower the pH value of the water phase through its slow hydrolysis and sufficient Ca^{2+} cations could be released from $CaCO_3$ to cross-link the alginate chains in the water phase to form gel. When the particles were initially dispersed in sunflower oil, the produced water-in-oil emulsion was Bancroft type with particles in the continuous phase (Bancroft, 1913). After adding GDL, water droplets containing alginate with the $CaCO_3$ microparticle outer layer were slowly gelated into micro-hydrogels without coalescence. After set for 48 h, the colloidosome hydrogel beads sank to the bottom due to gravity. We successfully fabricated the colloidosome hydrogel beads with templating the Bancroft water-in-oil emulsion.

Fig. 2 represents typical microscopical photos of the colloidosome beads with $CaCO_3$ microparticle shell and alginate gel core in sunflower oil (a, c and d) or in water (b) produced from 0.04 g of $CaCO_3$ microparticles and 1 mL of 1 wt% alginate solution at $\Phi_w = 0.2$. In this case, the ratio of the $CaCO_3$ microparticle weight to the water phase volume W_p/V_w was 0.04 g/mL. The $CaCO_3$ microparticle shell of the hydrogel bead can be observed from Fig. 2c and especially from Fig. 2d under the polarized light. These $CaCO_3$ microparticles were close-packed on the surface of the alginate hydrogel bead. The colloidosome hydrogel beads could be redispersed in water as seen from Fig. 2b, appearing similarly to those dispersed in oil from Fig. 2a. $CaCO_3$ microparticles were not lost from the colloidosome during the washing and centrifugation processes because of strong adhesive force of the alginate gel core.

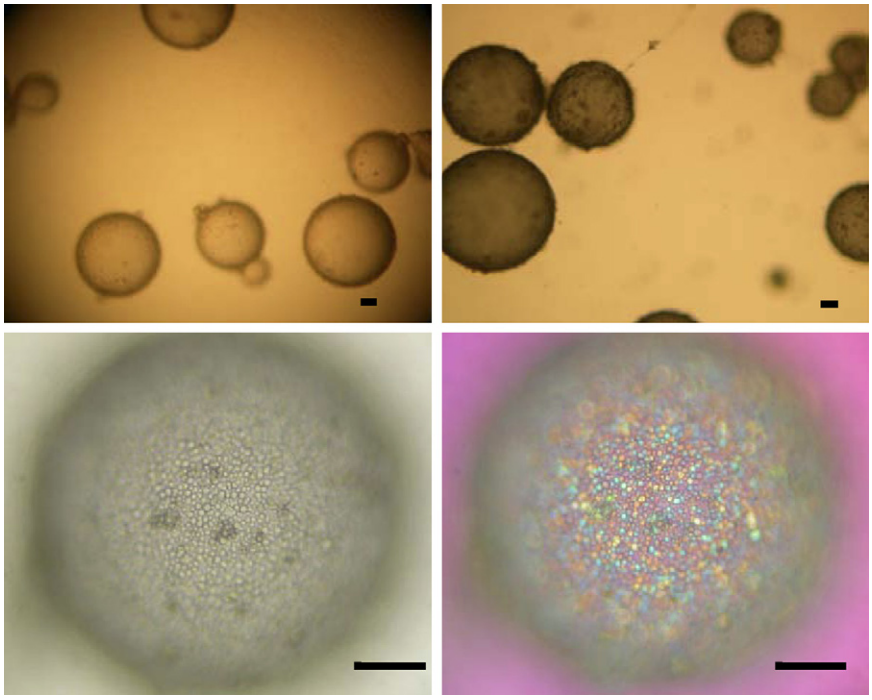

Fig. 2. Optical micrographs of the colloidosomes with $CaCO_3$ microparticle shell and alginate gel core in sunflower oil (a) and in water (b–d); d is the photo under crossed polarizer and analyzer of c. Black bars are 50 μm.

3.1.2. Ratio of particle weight to disperse phase volume (W_p/V_w)

Three W_p/V_w values of 0.03, 0.04, and 0.05 g/mL (i.e., 0.03, 0.04 and 0.05 g $CaCO_3$ microparticles in 4 mL sunflower oil, respectively) were selected to stabilize 1 mL of 1 wt% alginate solution at $\Phi_w = 0.2$ and at the same stirring speed to test the effect of W_p/V_w on the colloidosome formation. The appearance of the colloidosome is showed in Fig. 3. When $W_p/V_w = 0.03$ g/mL, the $CaCO_3$ microparticles were not closely packed and the alginate gel core was not completely covered with $CaCO_3$ microparticles (Fig. 3a). When $W_p/V_w = 0.04$ g/mL, almost close-packed shell of $CaCO_3$ microparticles was formed

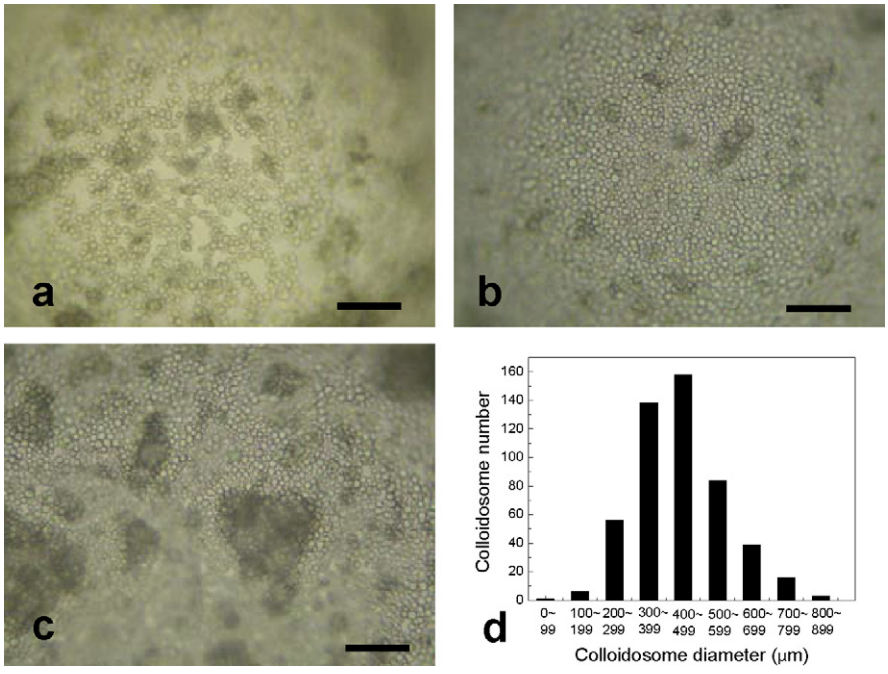

Fig. 3. Optical micrographs of the colloidosomes prepared at $W_p/V_w = 0.03$ g/mL (a), 0.04 g/mL (b), 0.05 g/mL (c) and diameter distribution histogram (d) of the colloidosome at $W_p/V_w = 0.04$ g/mL. Black bars are 50 μm.

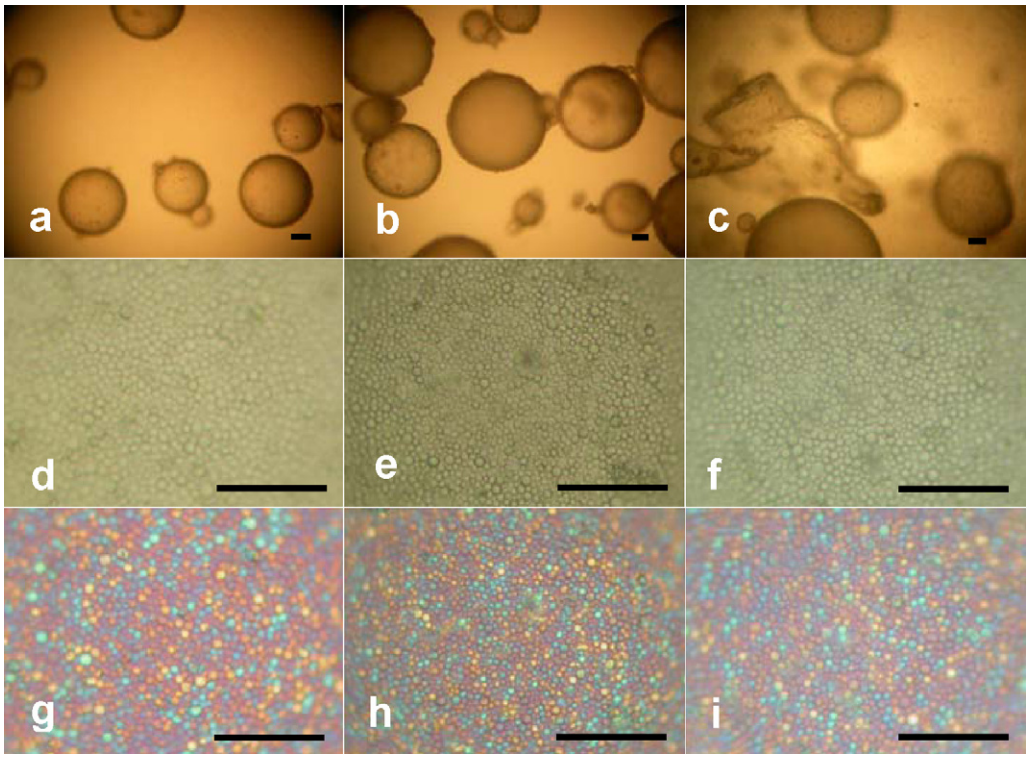

Fig. 4. Optical micrographs of the colloidosomes prepared at $\Phi_w = 0.2$ (a, d, g), 0.3 (b, e, h) and 0.5 g/mL (c, f, i); g, h and i are the photos under crossed polarizer and analyzer of d, e and f, respectively. Black bars are 70 μm.

(Fig. 3b). When $W_p/V_w = 0.05$ g/mL, a close-packed $CaCO_3$ microparticle shell was found with many $CaCO_3$ microparticle aggregates on the surface (Fig. 3c).

We have also determined the diameter distribution of these colloidosomes and the result of the colloidosome at $W_p/V_w = 0.04$ g/mL is presented in Fig. 3d for example. The colloidosome diameter ranged from tens to 900 μm with an average diameter of 430 μm. The other two colloidosomes (the data are not shown here) had the similar diameter distribution and similar average diameter: 440 μm for $W_p/V_w = 0.03$ g/mL and 435 μm for $W_p/V_w = 0.05$ g/mL. For the water-in-oil emulsion of Bancroft type, the size of the droplets is solely determined by the input of mechanical energy during emulsification (Kralchevsky et al., 2005) and is independent of W_p/V_w.

The diameter of $CaCO_3$ microparticles d used in our experiments (5 μm) is much smaller than the average diameter D of the colloidosome and all the $CaCO_3$ microparticles are roughly supposed to be evenly adsorbed on the oil–water interface of every droplet. The number of $CaCO_3$ microparticles N adsorbed on one droplet of water phase can be calculated from following equation (Duan et al., 2005):

$$N = \frac{W_P}{\rho V_w} \times \left(\frac{D}{d}\right)^3 \quad (1)$$

where W_P is the weight of $CaCO_3$ microparticles (g), ρ the $CaCO_3$ microparticle density (1.1 g/mL) (Wang et al., 2006), V_w the volume of the disperse phase (water). Then, the packing density f, i.e., the surface area ratio for one colloidosome bead covered by $CaCO_3$ microparticles, is

$$f = \frac{Nd^2}{4D^2} = \frac{D}{4\rho d} \times \frac{W_P}{V_w} \quad (2)$$

According to Eq. (2), f is 0.60, 0.78 and 0.99 for the colloidosomes prepared at $W_p/V_w = 0.03$, 0.04 and 0.05 g/mL, respectively. If the $CaCO_3$ microparticles are closely covered on the colloidosome surface, the maximum two-dimensional f value will be 0.82 (Visscher and Bolsterli, 1972), lower than 0.99 at $W_p/V_w = 0.05$ g/mL. Therefore, we observed $CaCO_3$ microparticle aggregation on the surface of colloidosome when $W_p/V_w = 0.05$ g/mL (Fig. 3c).

3.1.3. Volume fraction of water

Fig. 4 represents microscopical photos of the colloidosomes produced at $\Phi_w = 0.2$ (a, d, g), 0.3 (b, e, g), and 0.5 (c, f, i) with W_p/V_w fixed at 0.04 g/mL. This corresponds to increase the $CaCO_3$ microparticle concentration in sunflower oil with increasing V_w to keep W_p/V_w constant. When $\Phi_w = 0.2$, the colloidosome was stable with spherical shape (Fig. 4a). There was almost no coalescence in the emulsion. When $\Phi_w = 0.3$, some colloidosomes coalesced, but they were stable with spherical shape (Fig. 4b). Increasing Φ_w to 0.5, we found deformation of some colloidosomes to non-spherical shape and even broken for some colloidosomes (Fig. 4c). At the same time, we found that macroscopic gel on the inner wall of the container when $\Phi_w = 0.5$, which was not included in colloidosome beads. The increase of Φ_w up to 0.5 resulted in excessive water solution of alginate in addition to the W/O emulsion colloid, which was also

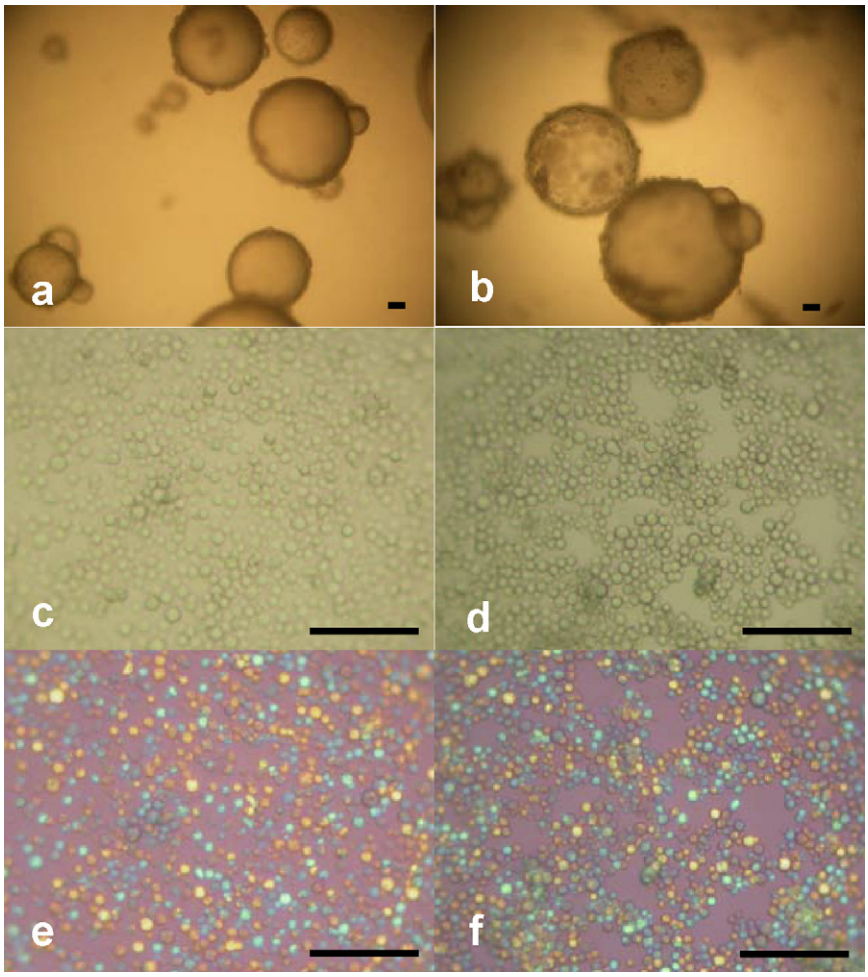

Fig. 5. Optical micrographs of the colloidosomes prepared with 0.5 M (a, c, e) and 1.0 M NaCl (b, d, f) in alginate aqueous phase; c and f are the photos under crossed polarizer and analyzer of b and e, respectively. Black bars are 50 μm.

gelated with the GDL hydrolysis. We can see from Fig. 4d to i, that all shells of the colloidosomes at these Φ_w values are almost closely covered. This also suggests that the packing density f would be independent of Φ_w.

3.1.4. NaCl concentration

We also investigated the effect of NaCl concentration of the water phase on the appearance of colloidosomes at $W_p/V_w = 0.04$ g/mL and $\Phi_w = 0.2$. The results are illustrated in Fig. 5. With adding 0.5 and 1.0 M of NaCl into the aqueous alginate solution, the colloidosome beads were still spherical but easily coalesced (Fig. 5a and b). With an increase in NaCl concentration, the $CaCO_3$ microparticles aggregated and the uncovered area on the gel core surface increased as seen from Fig. 5c to f. It is well known that addition of electrolytes into colloids will suppress the thickness of the double-layer and cause aggregation when beyond the critical concentration of coagulation (Tcholakova et al., 2005). Reynaert et al. found that adding NaCl caused the aggregation of sulfonated polystyrene particles at decane–water interface, which reduced the coverage of the particle on the oil–water interface (Reynaert et al., 2006). Golemanov et al. (2006) also reported that addition of NaCl induced the aggregation of sulfonated polystyrene particles at the hexadecane–water interface.

3.1.5. Appearance of colloidosome shell

SEM and CLSM photos of the colloidosome are displayed in Fig. 6a and b, respectively. Though the alginate gel core of colloidosomes was destroyed during drying process for SEM observation, the $CaCO_3$ microparticles could be recognized with round shape from Fig. 6a. The shell closely covered by the $CaCO_3$ microparticles was visualized with RITC-labeled $CaCO_3$ microparticles using a CLSM as shown in Fig. 6b. This photo confirms that the $CaCO_3$ microparticles were adsorbed on the surface of the alginate gel core and no $CaCO_3$ microparticles appear in the alginate gel core. This means that the colloidosome beads with $CaCO_3$ microparticle shells and alginate gel cores have been successfully fabricated by templating water-in-oil emulsion and in situ gelaton.

The $CaCO_3$ microparticles in the shell can be removed by dissolution in HCl. Fig. 7a depicts the colloidosome beads after removing the $CaCO_3$ microparticles, which testifies the in situ gelation of the alginate core. The surface of the alginate core was flocculent and not smooth when the $CaCO_3$ microparticles

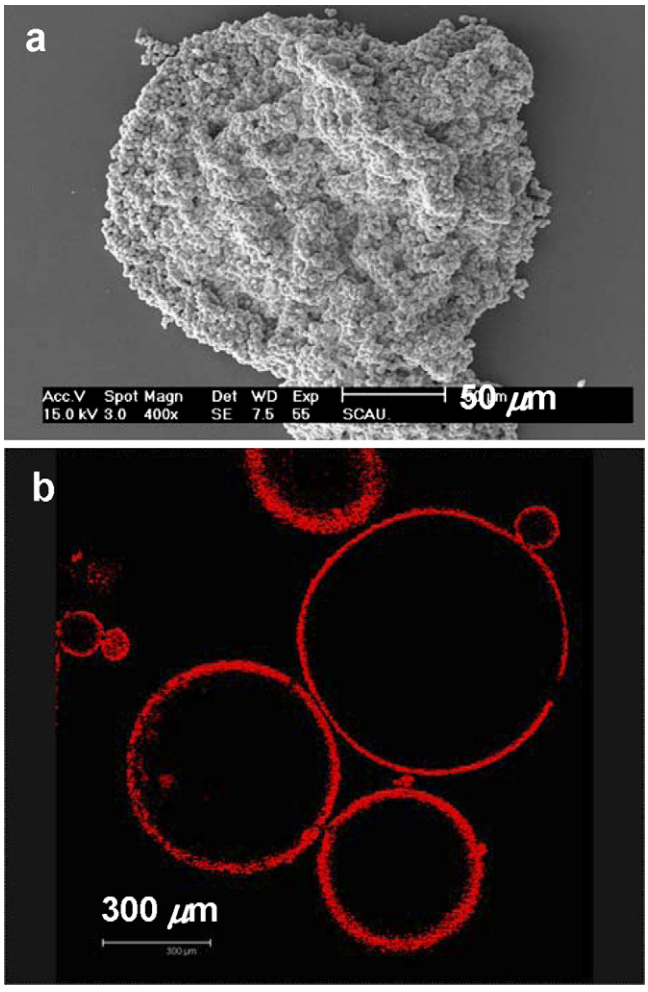

Fig. 6. SEM (a) and CLSM photos (b) of the colloidosome prepared at $W_p/V_w = 0.04$ g/mL, $\Phi_w = 0.2$ and without NaCl.

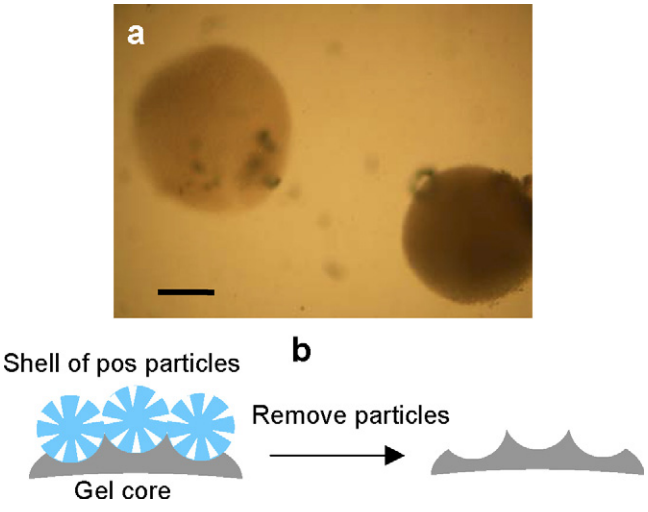

Fig. 7. (a) Optical micrograph of alginate gel cores after removing $CaCO_3$ microparticles in shells of the colloidosome; (b) schematic illustration for the formation of flocculent surface. Black bar is 200 μm.

were dissolved. The possible explanation for this is schematically represented in Fig. 7b. This was the appearance of alginate gel formed in the interstices between the $CaCO_3$ microparticles in the shell. On the other hand, alginate solution was soaked into the pores of $CaCO_3$ microparticles during emulsion process due to capillarity and gelated by calcium cations, which reinforced the interaction between the $CaCO_3$ microparticles and alginate gel core. This made the $CaCO_3$ microparticles stick firmly on the surface of the alginate gel core to form colloidosomes, standing for the transfer from sunflower oil to water by centrifuge.

3.2. In vitro release

Brilliant blue (BB) as a drug model was loaded into the colloidosome beads by dissolved in the alginate aqueous solution before gelation (1 g/L). The release behavior of BB from the colloidosomes at pH 7.4 was investigated with fluorescence spectra. The release profile is shown in Fig. 8 compared with that from alginate gel spheres. BB-loaded alginate gel spheres were prepared in the way similar to that for the BB-loaded colloidosome preparation except that no $CaCO_3$ microparticles were used as the stabilizer. BB released fast from alginate gel spheres with the half release time for 50% BB of 50 min and the total release time of 600 min. BB release from the colloidosomes was much slower than that from the alginate gel spheres. Only about 54% BB was released during 600 min. The reason appears to be the function of the porous $CaCO_3$ microparticles, which adsorb some of BB dissolved in the alginate gel cores into the nanopores through the capillary force and block the diffusion of BB from the alginate gel core to the release medium. Consequently, the $CaCO_3$ microparticles play an important role in sustained release of BB from the colloidosome beads.

The cumulative release data were analyzed according to Eq. (3) (Ritger and Peppas, 1987):

$$M_t/M_\infty = kt^n \quad (3)$$

here M_t/M_∞ is the fractional drug released at time t. M_t is the mass of solute released at time t, M_∞ the total mass of solute that

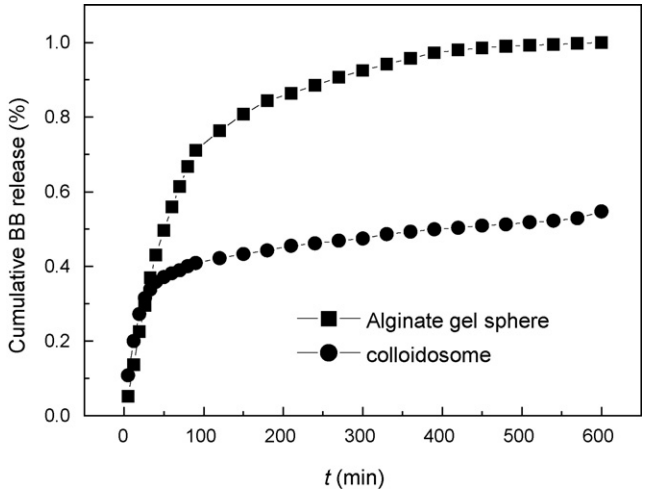

Fig. 8. Release profiles of BB from alginate gel spheres and colloidosomes at pH 7.4.

was released from the gel beads. n is the diffusional exponent. The variables k and n are constants, which can be related to diffusion coefficients and the specific transport mechanism. In the drug release studies that were carried out, since the gels were still releasing the drug beyond the time period that the experiment was conducted in, the value of M_∞ is not known. So the amount of drug was normalized over the total amount of drug initially loaded instead of the total amount of drug that was released. So, M_∞ was substituted by M_0, the amount of drug that was loaded into the gel beads (Ankareddi and Brazel, 2007). Fitting the curves in Fig. 8 with the above equations, n is 0.32 and 0.19 for pure alginate gel spheres and colloidosome beads, respectively. Both release systems are non-Fickian diffusion.

4. Conclusions

Colloidosome beads with porous $CaCO_3$ microparticle shell and alginate gel core were prepared by self-assembly of colloid $CaCO_3$ particles at the interface of water-in-sunflower oil emulsion and subsequently in situ gelation of aqueous alginate droplets caused by Ca^{2+} from the $CaCO_3$ particles, which was released due to lowering pH with slow hydrolysis of GDL. The $CaCO_3$ microparticles in shells were trapped and supported by the gel cores and difficult to be lost from the colloidosome beads. The packing density of $CaCO_3$ microparticles of the shell increased with the increase in W_p/V_w and decreased with addition of NaCl. The BB release from the colloidosome beads was slowed down because of the formation of the shell of $CaCO_3$ microparticles. The colloidosome has potential applications as the delivery vehicle for drugs, cosmetics, food additives and living cells.

Acknowledgments

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