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Preparation of micro-scaled multilayer capsules of poly-dimethyl-diallyl-ammonium chloride and sodium cellulose sulfate by layer-by-layer self-assembly technique

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ABSTRACT

Novel homogeneous micro-scaled hollow polyelectrolyte capsules were prepared by using alternate adsorption of poly-dimethyl-diallyl-ammonium chloride (PDMDAAC) and cellulose sulfate sodium (NaCS) on calcium carbonate ($CaCO_3$) template particles. The $CaCO_3$ particles with various shapes were prepared by the reaction of potassium carbonate (K_2CO_3) with calcium chloride ($CaCl_2$) in the presence of carboxylmethyl cellulose (CMC). The results showed that at a CMC concentration of 0.5 g/L, smooth spherical $CaCO_3$ particles were obtained, which were used as core templates. Thereafter four bilayers of PDMDAAC and NaCS polymer film were deposited on the $CaCO_3$ cores. The film buildup was characterized by (i) microelectrophoresis; (ii) scanning electron microscopy (SEM); and (iii) thermogravimetric analysis (TGA). Hollow capsules were then prepared by exposing the coated $CaCO_3$ particles to EDTA-Na. Finally the molecular weight cut-off (MWCO) of the microcapsules was determined by exposing the microcapsules to the *E. coli* cell lysate.

1. Introduction

Poly-dimethyl-diallyl-ammonium chloride–cellulose sulfate sodium (PDMDAAC–NaCS) capsules system, which was an interesting polyelectrolyte capsules system with high mechanical properties and biocompatibility (Groot-Wassink, Dautzenberg, Grunow, & Von Baehr, 1992; Mansfeld, Förster, Schellenberger, & Dautzenberg, 1991), was first reported in the mid-1980s by Dautzenberg, Loth, Fechner, Mehlis, and Pommerening (1985). Over the past decades, this system has been applied to the fields of cell cultures, drug delivery, and the encapsulation of microorganism as bioreactor (Dautzenberg et al., 1999; Pelegrin et al., 1998; Stadlbauer et al., 2006). In the past ten years, our group also have paid much attention on the biocompatibility of PDMDAAC–NaCS system and its application in the fields of bioseparation, cell encapsulation and drug delivery systems (Chen, Yao, Guan, & Lin, 2005; Li & Yao, 2009; Yao, 1998; Zhang, Yao, & Guan, 2005; Zhao, Chen, & Yao, 2006). In general, these PDMDAAC–NaCS capsules were prepared by dropping a solution of NaCS into a solution of PDMDAAC. In fact, their size was almost over 1.5 mm, which was limited for microscope application.

In recent years, micro- and nanometer-sized polyelectrolyte (PE) microcapsules have received considerable attention for their wide potential applications in medicine, biotechnology, catalysis, synthetic chemistry. Recently, voluminous approaches have been developed to design micro- and nano-scaled capsules that enable the encapsulation of various materials (Johnston, Cortez, Angelatos, & Caruso, 2006). The layer-by-layer (LbL) self-assembly technique, by which the microcapsules were produced by alternating deposition of oppositely charged polyelectrolytes onto colloidal templates, followed by removal of the template cores, was first reported by Donath, Sukhorukov, Caruso, Davis, and Möhwald (1998). Since its introduction in 1998, it had been attracted particular interests largely because their properties, such as size, composition, porosity, stability, surface functionality, and colloidal stability, can be broadly manipulated by varying microcapsule wall thickness, composition and introduction of exterior stimuli. Moreover, the preparation is under relatively mild condition. So far, a wide range of components such as artificial polymers (Yap, Quinn, Ng, Cho, & Caruso, 2005), natural polysaccharides (Ye, Wang, Liu, & Tong, 2005) and proteins (Caruso & Schler, 2000) can be used to fabricate capsules. And the core templates could cover from polymers (Caruso, Shi, Caruso, & Susha, 2001), crystals (Anzai & Kobayashi, 2000), and cells (Neu et al., 2001).

For core templates, melamine formaldehyde (MF) resin was used most widely and researched deeply for their advantages such as suitable and controllable size and could be easily removed in

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past years (Gao, Leporatti, Moya, Donath, & Möhwald, 2001; Liu, Gao, Shen, & Möhwald, 2005). However, MF has bad biocompatibility, so it is not the suitable candidate for biocompatible application. In recent years, $CaCO_3$ spherical particles have been intensive used because it was nontoxic and could also be easily removed (De Geest, Vandenbroucke, Guenther, & Sukhorukov, 2006; Petrov, Antipov, & Sukhorukov, 2003; Volodkin, Petrov, Prevot, & Sukhorukov, 2004). In application of $CaCO_3$ as template cores, the particles must have an appreciate morphology and size. Several works reported that the morphology and growth rate of the $CaCO_3$ crystal could be modified in the presence of foreign compounds or additives (Shen et al., 2004; Yua, Zhao, Cheng, & Zhang, 2005; Yue, Jin, Shui, & Xu, 2004). However, the production on a large-scale of homogenous $CaCO_3$ particles with smooth spherical morphology and having uniform size is still a challenge.

In this work, we will prepare homogeneous micrometer-sized hollow polyelectrolyte capsules using alternate adsorption of PDMDAAC and NaCS on $CaCO_3$ template particles. The $CaCO_3$ particles will be formed by the reaction of potassium carbonate (K_2CO_3) with calcium chloride ($CaCl_2$). In the formation process of spherical $CaCO_3$ template cores, the morphology and size of $CaCO_3$ particles will be regulated by adjusting carboxylmethyl cellulose (CMC) concentration. Then four bilayers of PDMDAAC and NaCS polymer film will be deposited on the $CaCO_3$ cores. The film buildup will be characterized by (i) microelectrophoresis; (ii) scanning electron microscopy; and (iii) thermogravimetric analysis. After exposing the coated $CaCO_3$ to EDTA-Na, we will obtain homogeneous hollow microcapsules with a size according to the $CaCO_3$ template particles. This cores dissolution step will be monitored by optical microscopy. The hollow microcapsules were characterized by SEM. Finally, the molecular weight cut-off of the polymer walls of the microcapsules will be determined by a diffusion test with the cell lysate of *E. coli*.

2. Materials and methods

2.1. Materials

NaCS (Mw = 30,000–200,0000 Da, degree of substitution = 0.36–0.51) was prepared by the heterogeneous reaction as described previously in our lab (Yao, 2000). PDMDAAC (Mw = 200,000–350,000 Da, 20 wt%) was purchased from Aldrich Co., USA. CMC was obtained from Sangon Co., China. The double distilled water used in all experiments was purchased from Wahaha Co., China. All other chemicals and reagents were of analytical grade and were used as procured.

2.2. Preparation of $CaCO_3$ microparticles

$CaCO_3$ microparticles were precipitated by pouring the potassium carbonate solution (0.025 M) into calcium chloride (0.025 M) containing varying CMC concentrations with the help of ultrasonication. Then the $CaCO_3$ suspension was incubated for 3 h. During the precipitation and incubation process, the reactive system was kept at 25 °C. The reaction was stopped by a rapid centrifugation and washing step after the $CaCO_3$ particles reached the needed size. Different kinds of micro-particles with varying CMC concentrations were conserved separately and left for the next step.

2.3. Fabrication of multilayer core–shell structure

The polyelectrolyte layers were deposited onto the $CaCO_3$ microparticles by consecutive adsorption of PDMDAAC and NaCS using a centrifugation protocol. In a typical fabrication process, the $CaCO_3$ particles were incubated in 1 ml PDMDAAC solution (1 mg/ml, in 0.5 M NaCl) for 15 min. The suspension was then centrifuged at $1500 \times g$ for 3 min and the supernatant was carefully removed with a pipette. Three washings with 0.5 M NaCl solution at each interval were conducted to remove the undeposited PDMDAAC before the next NaCS adsorption. Following the same adsorption protocol as PDMDAAC, a layer of NaCS was assembled. The adsorption was repeated until 8 layers of polyelectrolytes were assembled with NaCS as the outermost layer.

2.4. Production of multilayer hollow microcapsules

The $CaCO_3$ cores were removed by exposing 1 ml of suspension of polyelectrolyte-coated $CaCO_3$ particles to 10 ml of 0.2 M EDTA-Na solution and allowing 30 min for core dissolution. The hollow microcapsules were then collected by centrifugation at $650 \times g$ for 5 min, exposed to EDTA-Na again, washed an additional three times with 0.5 M NaCl, and finally resuspended and conserved in 0.5 M NaCl solution.

2.5. Determination of molecular weight cut-off (MWCO)

The MWCO of the microcapsules was determined by a diffusion test, in which the cell lysate of *E. coli* was used as the different Mw source. The layer-by-layer PDMDAAC–NaCS microcapsules were prepared as 2.3 and 2.4, and then the hollow capsules were incubated in cell lysate solution and cultured for 12 h at 20 °C and pH 7.0 to make sure that they got mass balance between microcapsules and environment. Then the centrifugation at $650 \times g$ for 10 min was used to collect the capsules, two washings with water were applied to remove the cell lysate out of microcapsules and the protein adsorption onto the microcapsules. Then the capsules were immersed into water for 3 h to release the cell lysate within capsules. The solution sample (Sample 1) in this step was analyzed by SDS-PAGE. At last, all microcapsules were smashed by supersonic generator, and another solution sample (Sample 2) was collected to be analyzed also by SDS-PAGE. Because the components with molecular weight lower than the MWCO in cell lysate would diffuse freely across the capsules to the solution, the largest molecule that can penetrate the membrane can be determined by analyzing the solution outside the capsules by SDS-PAGE (Gel Doc, 2000, Bio-Rad Co., USA).

2.6. Characterization

The $CaCO_3$ microparticles, the core–shell microparticles and the hollow microcapsules were characterized by scanning electron microscopy (SEM, S-4800, HITACHI, Japan). The microelectrophoretic mobility of coated $CaCO_3$ microparticles was measured by Zeta-sizer (Malvern 3000HS, UK). The optical microscope (E200, Nikon, Japan) was used to get images in the core dissolution step. Thermogravimetric analysis (TGA) was conducted by a Pyris 6 thermogravimetric analyzer (Norwalk, CT, Perkin-Elmer, USA) at a heating rate of 20 °C/min from 25 °C to 800 °C. Samples were treated at 100 °C prior to TGA analysis.

3. Results and discussion

3.1. Preparation of spherical $CaCO_3$ particles

$CaCO_3$ crystal growth experiments in the presence of CMC were performed as indicated in Section 2.1. The results showed that CMC could affect observably the morphology and size of $CaCO_3$. At a moderate CMC concentration, $CaCO_3$ particles with smooth spherical morphology were obtained. Fig. 1 shows the SEM micrographs of the dried $CaCO_3$ particles grown in the presence of CMC with various concentrations with 0 g/L in Fig. 1(a), 0.375 g/L in Fig. 1(b), 0.5 g/L in Fig. 1(c). It can be observed in Fig. 1 that $CaCO_3$ particles have almost rhombohedral morphologies and mean size around

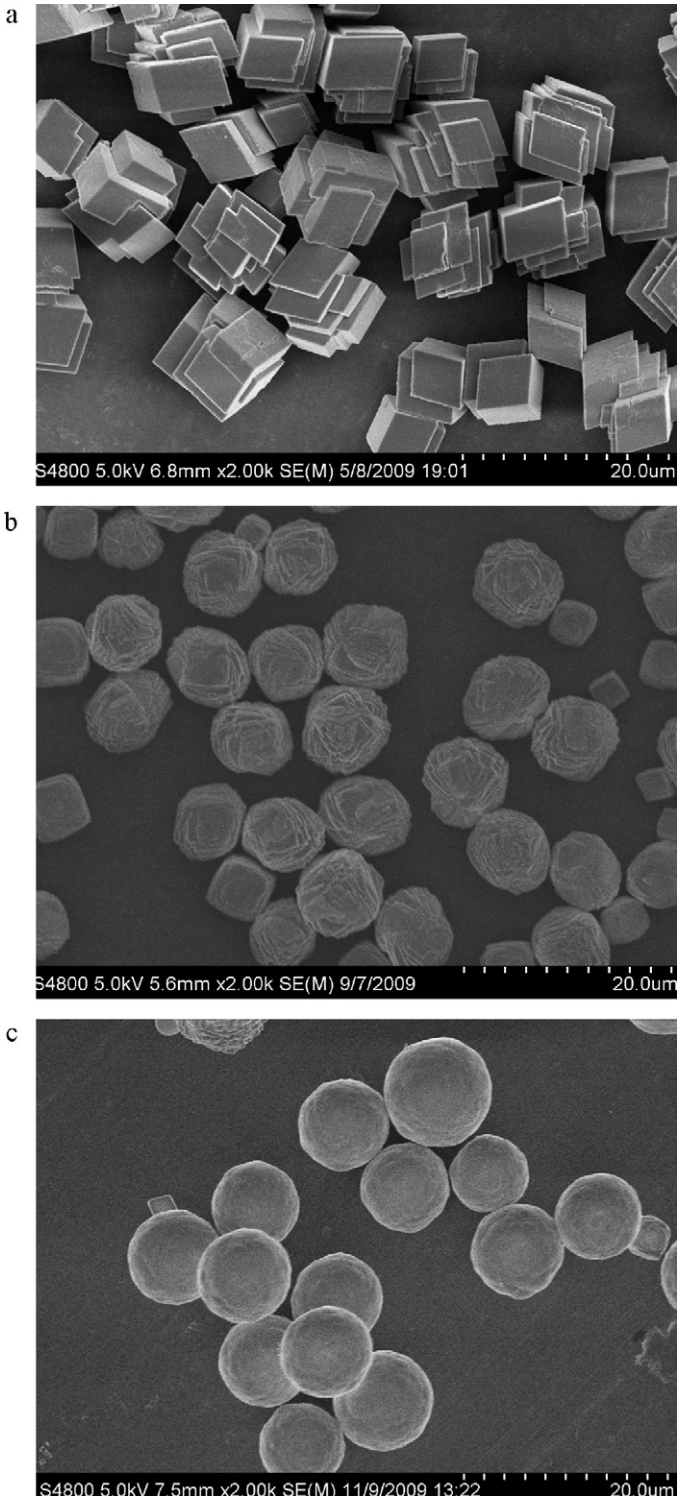

Fig. 1. CMC concentration effects on the morphology and size polydispersity of the $CaCO_3$ particles. CMC concentration: (a) 0 g/L; (b) 0.375 g/L; (c) 0.5 g/L.

6 μm in the absence of CMC (Fig. 1(a)). By adding 0.375 g/L CMC, $CaCO_3$ particles having rough spherical morphology and the uniform size around 6 μm were obtained (Fig. 1(b)). At 0.5 g/L CMC concentration, the shape of $CaCO_3$ particles changed to smooth spherical and its average size was 9.5 μm (Fig. 1(c)). The results showed that the concentration of polyelectrolytes had strong influence on the $CaCO_3$ crystal growth, which was similar to many other manuscripts (Shen et al., 2004; Yua et al., 2005; Yue et al., 2004).

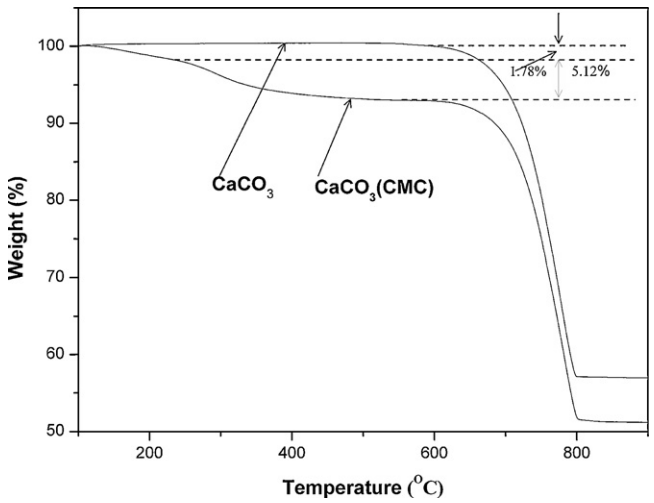

Fig. 2. Thermogravimetric analysis of $CaCO_3$ and $CaCO_3$ (CMC) microparticles.

After the spherical $CaCO_3$ particles were prepared, the CMC was quantified by thermogravimetry analysis (Fig. 2). Compared with the pure $CaCO_3$ particles in the temperature range 100–520 °C, a considerable large amount (7.0%) of mass was lost for the $CaCO_3$ (CMC) particles. The weight loss of 1.78% in the temperature range 100–240 °C should represent the evaporation of water, where as the weight loss of 5.12% in the temperature range 240–520 °C corresponds to decomposition of CMC. The sharpest decreases in weight loss were extrapolated to be 700 °C and 690 °C for pure $CaCO_3$ and $CaCO_3$ (CMC) particles, respectively, indicating that the decomposition temperature of $CaCO_3$ was hardly influenced by incorporation of the CMC.

3.2. Fabrication of multilayer core–shell structure

The fabrication of multi-layered PDMDAAC/NaCS films on $CaCO_3$ microparticles via LBL deposition was illustrated in Fig. 3(a). The structures of NaCS and PDMDAAC were also depicted. The microelectrophoretic measurements were utilized to follow adsorption of layers on the $CaCO_3$ templates. Fig. 3(b) shows the ζ-potential of $CaCO_3$ microparticles coated with PDMDAAC/NaCS multilayers as a function of layer number. Because of the existence of CMC, the ζ-potential of $CaCO_3$ cores at pH 7.0 was −37.3 mV (Fig. 3(b)). After deposition of a layer of PDMDAAC, the ζ-potential exhibited a positive value, +11.3 mV. Then the ζ-potential value changed to −23.5 mV after deposition of NaCS. Regular surface charge reversal was observed with adsorption of each polyelectrolyte layer, indicating the successful deposition of PDMDAAC/NaCS on the microparticles.

SEM was employed to observe the morphology and size of the $CaCO_3$ particle before and after LbL self-assembly. Fig. 4 displays SEM images of $CaCO_3$ particles and $CaCO_3$ particles coated with eight polyelectrolyte layers. It showed obviously that the polymer layers could be found on the PDMDAAC/NaCS covered particles' surface to make it rougher (Fig. 4(a)) than the uncovered ones (Fig. 4(b)). As shown in the images, $CaCO_3$ particles before and after LbL self-assembly have almost the same size. The results showed that the coated $CaCO_3$ particles were stable when stored in an aqueous medium, reflecting the stability of the adsorbed layers.

TGA was conducted to analyze the $CaCO_3$ microparticles and core–shell microparticles. It can be observed in Fig. 5 that the weight loss in the temperature range of 240–520 °C corresponding to polymers was 5.12% and 7.99% for $CaCO_3$ and $CaCO_3/(PDMDAAC/NaCS)_4$, respectively, indicating that the mass of deposited polyelectrolytes was 2.87%.

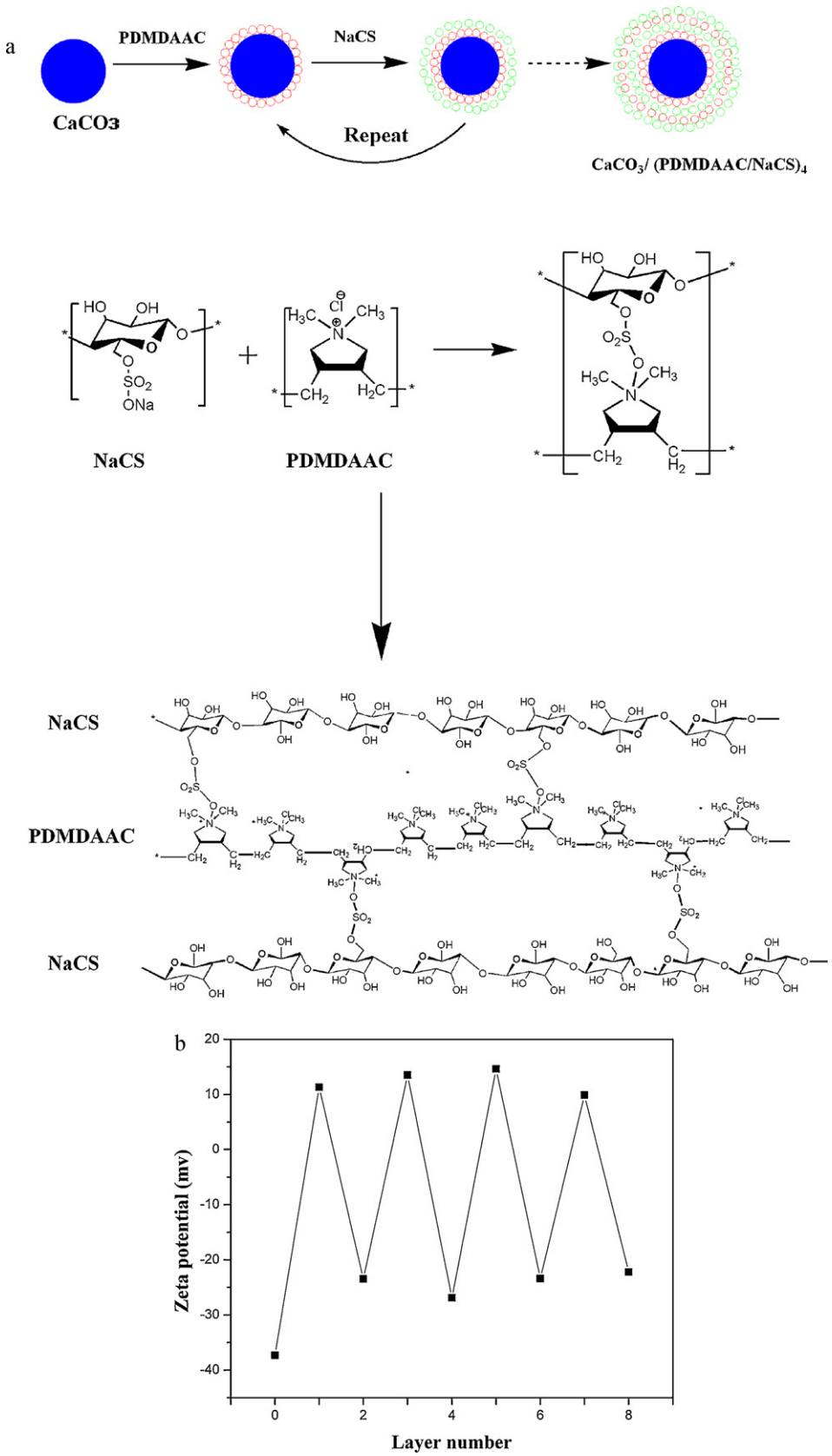

Fig. 3. Fabrication of multilayer core–shell structure. (a) Schematic diagram illustrating the fabrication of multi-layered PDMDAAC/NaCS films on $CaCO_3$ microparticles via LBL deposition. (b) ζ-Potential of $CaCO_3$ microparticles coated with PDMDAAC/NaCS multilayers as a function of layer number.

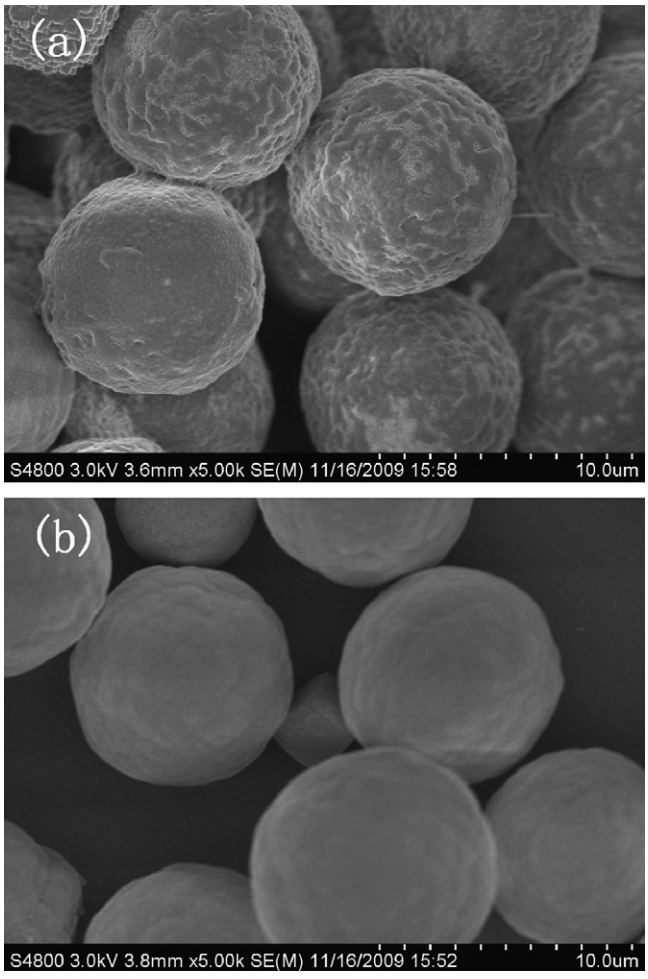

Fig. 4. The SEM images of (a) $CaCO_3$ microparticles and (b) $CaCO_3$/$(PDMDAAC/NaCS)_4$.

3.3. Production of multilayer hollow microcapsules

After the core–shell microparticles were obtained, the dissolution of core templates and formation of hollow microcapsules were conducted. $CaCO_3$ could be normally dissolved by EDTA-Na.

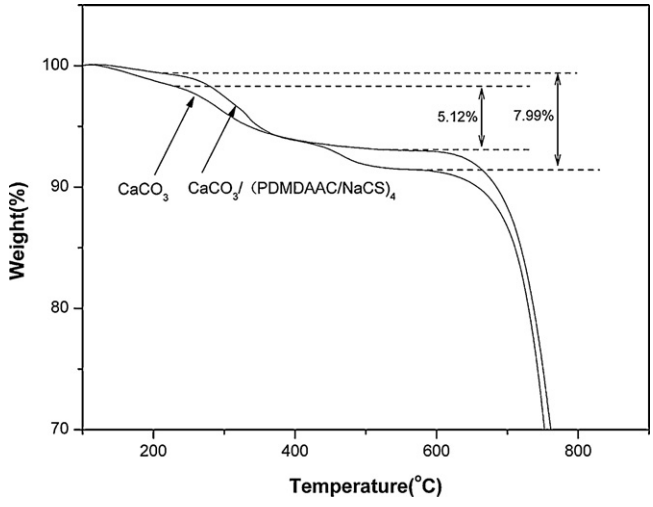

Fig. 5. Thermogravimetric analysis of $CaCO_3$ and $CaCO_3$/$(PDMDAAC/NaCS)_4$.

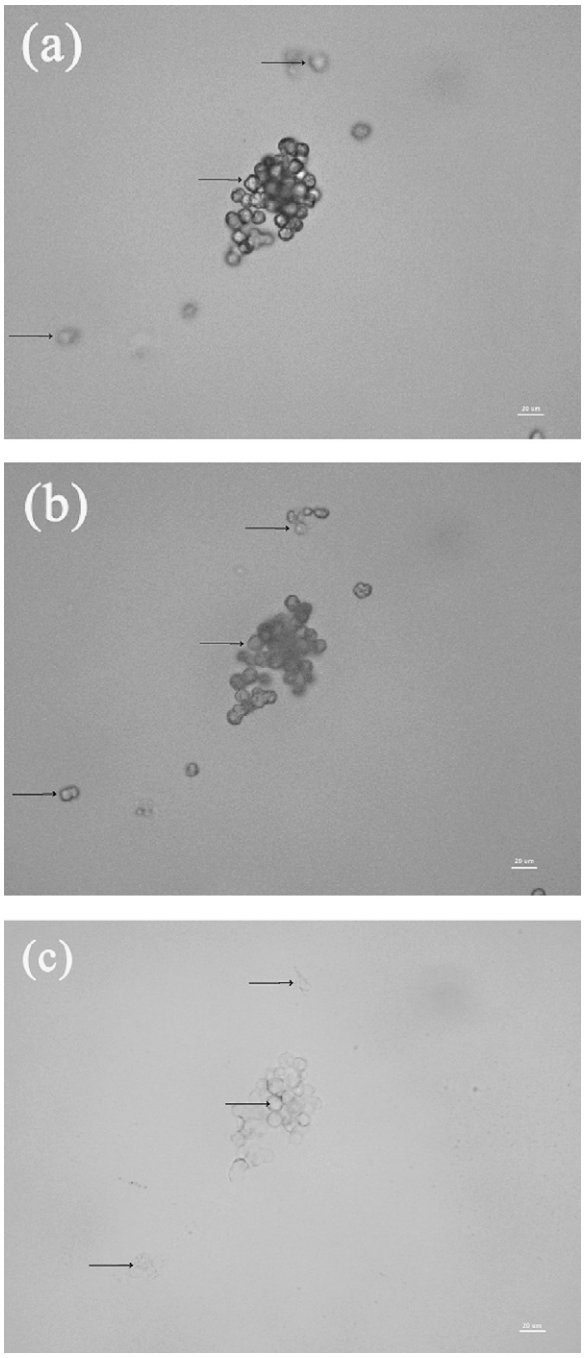

Fig. 6. Core dissolution step characterized by an optical microscope. (a) Before the cores were dissolved; (b) in the process of core dissolution; (c) after the cores were dissolved.

The optical microscope was used to get images in the core dissolution step. The results were shown in Fig. 6(a)–(c) in which the arrow represented the same location in the microscope. $CaCO_3$ particles appeared as dark areas as a result of their impermeability of light. When EDTA-Na was added in the system, the capsules' colors gradually changed from opaque black to nearly colorless and transparent, exhibiting that the cores had been dissolved. The capsules were intact after dissolution of cores besides a little swelling, owing to higher osmotic pressure within them in this step. This result demonstrated that one major advantage of the LbL process was the ability to easily control the microcapsule size by their templates.

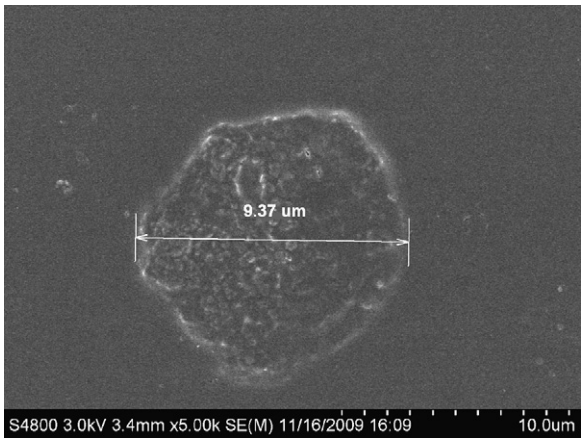

Fig. 7. The SEM images of $(PDMDAAC/NaCS)_4$ hollow microcapsule.

3.4. Characterization of hollow microcapsules

After removal of the $CaCO_3$ cores, the hollow microcapsules were prepared. The SEM was employed to identify the size and morphology of capsules. Fig. 7 indicated that the micro-capsules' surfaces were collapsed because they were completely dried before testing. The hollow microcapsule was almost the same size as the origin core template.

MWCO of membrane was defined as the lowest Mw of the substance that was not able to permeate through the membrane of microcapsules. Cell lysate of *E. coli*, which contained a group of proteins with Mws from 1 kDa to over 100 kDa, was used to characterize the MWCO by a diffusion test. In this test, the MWCO into the microcapsules (MWCO-in) and the MWCO out of the microcapsules (MWCO-out) were measured. As a result of the different environment inside/outside the microcapsules, the mass transfer behavior would be affected. As described in Section 2.5, Sample 1 represented MWCO-out, and Sample 2 was the MWCO-in. The SDS-PAGE analysis (Fig. 8) of Sample 1 and Sample 2 are shown in Fig. 8. It proved that the MWCO-in and MWCO-out were almost the same, and both of them were larger than 50 kDa which was large enough that these microcapsules could be used to encapsulate ordinary protein drugs as a potential drug delivery system.

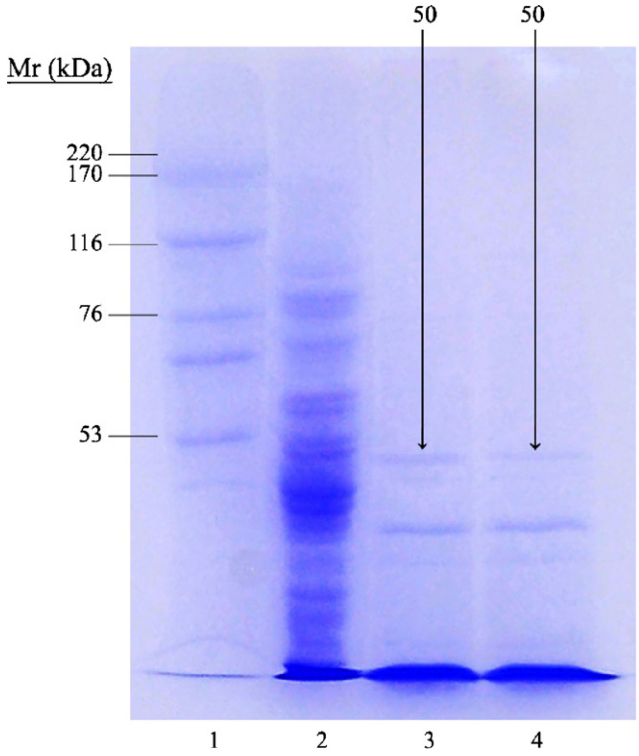

Fig. 8. SDS-PAGE of the proteins that permeated through the microcapsules. Line 1: Marker; Line 2: original proteins from cell lycate; line 3: protein could permeate out of the capsule; line 4: 3 h later, protein within the capsule.

4. Conclusions

In present work, novel homogeneous micro-sized hollow PDMDAAC-NaCS microcapsules were prepared by LbL technique. $CaCO_3$ particles with various shapes were obtained by the reaction of K_2CO_3 with $CaCl_2$ in the presence of CMC. At a CMC concentration of 0.5 g/L, smooth spherical $CaCO_3$ particles with an average size of 9.5 μm were obtained. Thereafter four bilayers of PDMDAAC and NaCS polymer film were deposited on the $CaCO_3$ cores. The film buildup was characterized by (i) microelectrophoresis; (ii) scanning electron microscopy (SEM); and (iii) thermogravimetric analysis (TGA). After exposing the coated $CaCO_3$ particles to EDTA-Na, hollow particles with a size of 9.37 μm according to the core templates were obtained, which was characterized by optical microscopy and SEM. The MWCO of membrane of PDMDAAC-NaCS microcapsule was larger than 50 kDa as determined by diffusion tests, which was relatively large enough for many materials to permeate through the membrane of this microcapsule. These properties would bring a bright perspective for application of this capsule system in the field of biotechnology, and biochemical engineering.

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