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Fabrication of pH-Responsive Microcapsules by Precipitation Polymerization on Calcium Carbonate Templates

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ABSTRACT: A simple and fast method was developed to fabricate pH-responsive microcapsules. After the copolymerization of *N*-isopropylacrylamide (NIPAAm), methacrylic acid (MAA), and a crosslinker, poly(ethylene glycol) diacrylate, at 65°C in the presence of sacrificial $CaCO_3$ microparticles, hollow microcapsules were acquired by template removal. A higher temperature and the presence of MAA/NIPAAm were found to be critical factors for the successful fabrication of the microcapsules. The entire fabrication step was simple, fast, and easy to scale up. The microcapsules were characterized by Fourier transform infrared spectroscopy, scanning electron microscopy, and transmission electron microscopy. The pH responsiveness of the microcapsules was confirmed by confocal laser scanning microscopy and ζ-potential analysis. Together with the fastness and simplicity of the fabrication process, this kind of pH-responsive microcapsule holds potential for drug- and gene-delivery systems. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 129: 3601–3605, 2013

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INTRODUCTION

Microcapsules have been applied in many fields, including the pharmaceutical, cosmetic, textile, catalysis, and agricultural industries.[1,2] Considerable scientific interests have been devoted to stimuli-responsive hollow microcapsules because of their unique features in tunable encapsulation/release behavior.[3] Stimuli-responsive microcapsules have found applications in many fields, including microreactors, microsensors, controlled release, and enzyme immobilization systems.[4–6] Stimuli-responsive polymers can respond to either external or internal stimuli (e.g., ionic strength, pH, light, electric, magnetic, redox, temperature[7,8]) by exhibiting distinct property changes, such as changes in the permeability, mechanics, or shape.[9,10] For example, poly(methacrylic acid) (PMAA) exhibits pH-dependent swelling/collapse behavior because of the ionization/deionization of the carboxyl groups.[11] At low pH (<5.5), the PMAA chain collapses because of the deionization of the carboxylic groups. At high pH values, the PMAA chain shows stretched conformations as a result of the repelling force of the ionized COO^- groups.[12] The pH value is the stimulus that can be easily applied and controlled both *in vitro* and *in vivo*.[13] Recent biomedical applications have aroused the interest of microcapsules that could respond to the pH value.[14] For example, some tumor sites show increases in local temperature[15] and decreases in the extracellular pH value[16] compared with normal tissues, so microcapsules that respond to pH[17–19] will have better controlled release effects.[18]

Many articles have been published on the fabrication of pH-responsive microcapsules. Several methods are usually used to fabricate pH-responsive microcapsules; these include coacervation, layer-by-layer, and grafting/precipitation polymerization onto templates. Stable microcapsules can be prepared under mild conditions without the use of organic solvents or surfactants by the coacervation method; however, the size and shell thickness of the capsules are difficult to control.[20] The layer-by-layer technique has been widely used in the construction of pH-responsive microcapsules.[21,22] This provides a strategy for rationally designing film properties through the precise control of the composition, number of layers, and thickness of the films at a molecular level.[23] However, it is both time consuming and labor intensive. Grafting/precipitation polymerization on sacrificial templates is another technique for fabricating pH-responsive microcapsules because of its simplicity, robustness, and the use of well-characterized monomer/polymers.[24] However, with

this technique, there is still a need to pretreat the templates with anchoring reagents before polymerization.[25]

In this article, we demonstrate a simple and fast approach for the fabrication of pH-responsive microcapsules by precipitation polymerization on sacrificial $CaCO_3$ microparticles without template functionalization. The copolymerization of *N*-isopropylacrylamide (NIPAAm), methacrylic acid (MAA), and poly (ethylene glycol) diacrylate (PEGDA) were carried out in the presence of spherical $CaCO_3$ particles as sacrificial templates.[26] MAA was chosen in this system as both a coupling reagent of the $CaCO_3$ templates (because of the strong interaction between the calcium cations and carboxyl groups) and as a monomer for pH-responsive polymers. NIPAAm was applied in the system to increase the adsorption/precipitation of polymers on the template.[27–29] Poly(*N*-isopropylacrylamide) (PNIPAAm), which undergoes a volume phase transition at its lower critical solution temperature (LCST; $\sim$ 32°C),[30,31] had good adsorption properties above the LCST and a negligible adsorption capacity below the LCST.[32] PEGDA was applied as a crosslinker, which ensured the formulation of continuous microcapsule shells. Microcapsules with pH responsiveness were obtained after template removal. The entire process (including template preparation, polymerization, and template removal) lasted less than 5 h and was easy to scale up. Together with the fastness and simplicity of the fabrication process, these kinds of pH-responsive microcapsules may find applications in controlled drug- and gene-delivery systems.[33,34]

EXPERIMENTAL

Materials

NIPAAm, MAA, and PEGDA (number-average molecular weight = 258) were purchased from Sigma-Aldrich (Shanghai, China). Na_2CO_3, $CaCl_2$, $K_2S_2O_8$, and ethylenediaminetetraacetic acid were purchased from Sinopharm (Shanghai, China). All of the reagents were used without further purification.

Fabrication Methods

We fabricated the $CaCO_3$ microparticles by pouring a 0.33*M* Na_2CO_3 solution into a 0.33*M* $CaCl_2$ solution under vigorous stirring. The precipitates were centrifuged, washed twice with deionized water, and used directly as templates.[35]

NIPAAm, MAA, and PEGDA at a weight ratio of 64 : 16 : 20 were dissolved in water to make a 5% solution; 1 g of $CaCO_3$ was then added to 20 mL of such solutions. After N_2 bubbling for 20 min, the polymerization were initiated by the addition of 0.1 g of $K_2S_2O_8$ into the solution, and the temperature was increased to 65°C. The polymerization proceeded for 4 h under mild stirring. The acquired complex particles were washed three times by centrifugation at 1000 rpm for 4 min and then resuspended in water. The complex particles were subsequently treated with a large amount of 0.1*M* ethylenediaminetetraacetic acid for 10 min, centrifuged at 5000 rpm for 10 min, and then washed three times to obtain hollow microcapsules.

Characterization

The morphologies of the $CaCO_3$ microspheres, complex microparticles, and hollow microcapsules were characterized by a SIRION-100 FEI electron microscope (FEI, Netherlands). Fourier transform infrared (FTIR) spectra were recorded on a VETOR 22 spectrophotometer (Bruker, Germany). The ζ potentials of the microcapsules were measured by a Zetasizer nanoinstrument Nano Z equipment (Malvern, England). The fluorescent images were captured with a Zeiss LSM 510 confocal laser scanning microscopy (CLSM) instrument (Zeiss, Germany). Optical images of the microcapsules at different pH values and temperatures were recorded. The diameters of the microcapsules were determined by the measurement of at least 200 microcapsules.

RESULTS AND DISCUSSION

Preparation and Characterization of the Microcapsules

Figure 1(a) shows the scanning electron microscopy (SEM) image of the $CaCO_3$ microparticles, which shows the regular spherical shape with diameters of 3–4 μm. Figure 1(b) shows the $CaCO_3$/copolymer complex after copolymerization. We observed that the surface of the composite particles became rougher than the bare $CaCO_3$ microparticles; this implied that the copolymer had precipitated onto the templates. Figure 1(c,d) shows the SEM and transmission electron microscopy (TEM) images of the hollow microcapsules dried at pH 7.4. Clear folds and creases on the collapsed microcapsules could be seen on both images; this confirmed the successful deposition of the copolymers on the templates. The collapse of the microcapsules was the consequence of core removal and the evaporation of water from the interior. Interestingly, if the microcapsules were dried under acidic conditions, the morphology of the microcapsules was quite different from those dried under neutral conditions. The microcapsules dried at pH 4.0 [Figure 1(e,f)] showed a pancakelike form; no folds/creases could be found on the surfaces of the capsules. The morphological changes under different drying conditions were believed to be the pH responsiveness of the microcapsules; this is discussed in detail later.

The chemical composition of the copolymer microcapsules was investigated by FTIR spectroscopy (Figure 2). The band at 3305 cm^{-1} could be attributed to the amide group in PNIPAAm and the carboxyl group in PMAA. The peak at 1729 cm^{-1} corresponded to the characteristic stretching vibrations of the ester group of PEGDA and the carboxyl group of PMAA. The peaks at 1649 and 1546 cm^{-1} were ascribed to amide I (viz., carbonyl stretching) and amide II (viz., N—H bending) vibrations. The peaks at 1454, 1389, and 1367 cm^{-1} were assigned to the C—H symmetric and asymmetric stretching vibrations of the isopropyl group. The peak at 1173 cm^{-1} was attributed to the C—O—C bond vibrations of the ether groups. The spectrum further confirmed the incorporation of PNIPAAm/PMAA/PEGDA in the microcapsule shell.

Two factors were critical to the successful fabrication of the microcapsules. One was the incorporation of MAA into the copolymerization system. Our results show that no microcapsules could be formed without MAA during copolymerization. It is well known that the carboxyl group in MAA can chelate with Ca^{2+} of $CaCO_3$ with a way of two carboxyl groups interacting with one calcium cation. The association constant of MAA to free calcium was reported to be 37,000 M^{-1}.[36] Therefore, MAA might act as the surface modifier of the $CaCO_3$ templates before polymerization because of the strong interaction

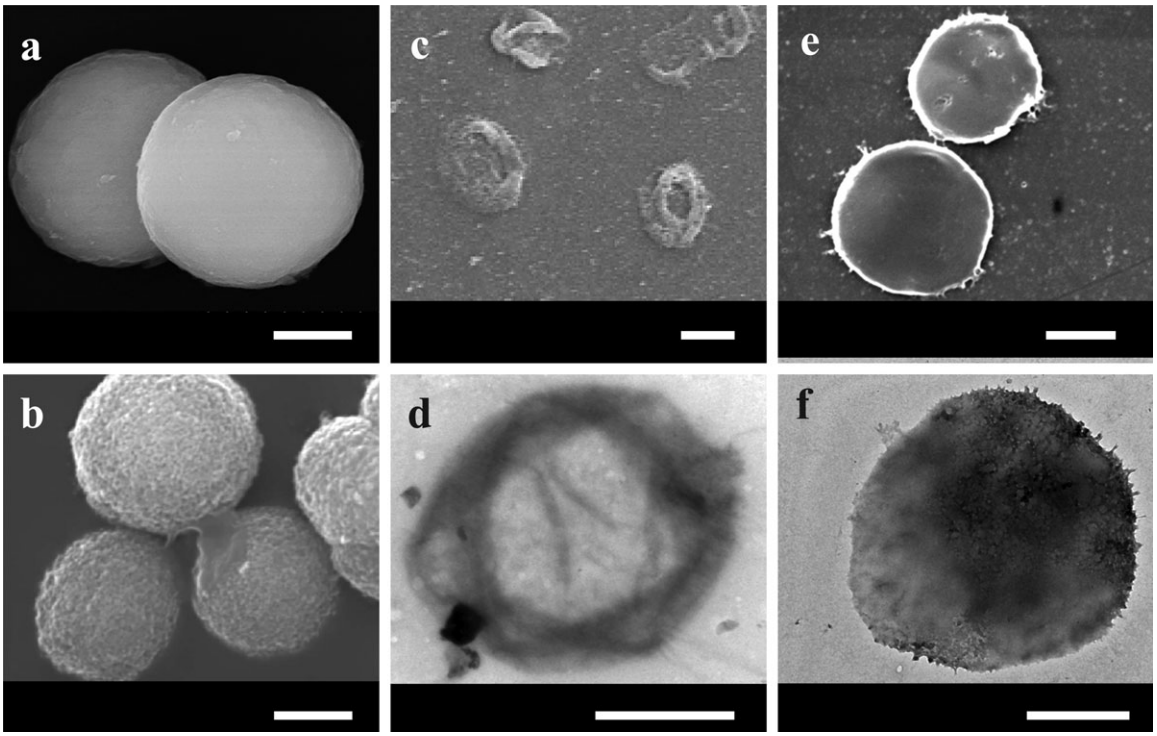

Figure 1. SEM images of (a) $CaCO_3$ and (b) the $CaCO_3$/copolymer complex. SEM images of the copolymer microcapsules dried under pH values of (c) 7.4 and (e) 4.0. TEM images of the copolymer microcapsules dried under pH values of (d) 7.4 and (f) 4.0. The scale bar represents 2 μm.

between calcium cations and carboxyl groups.[37] It will functionalize the interface with double bonds and provide anchoring sites for the successful deposition of the copolymers. The other decisive factor for the successful fabrication of microcapsule was temperature. Few microcapsules were found after core removal when copolymerization occurred at temperatures lower than 30°C. The reason might have been that the newly formed PNIPAAm-rich copolymers precipitated at higher temperatures because of the intramolecular hydrogen bond of the PNIPAAm chains. The precipitated polymers deposited on the interfaces to decrease their surface energy,[38] which made the formation of continuous polymer shells on the template surfaces easy.

pH Responsiveness of the Microcapsules

The diameter and ζ potential of the microcapsules as a function of the pH value are shown in Figure 3(a). It shows that at low pH values (<4.0), the diameter of the microcapsules was relatively small under acidic conditions (2.8 μm at pH 2.0), but it increased very quickly with increasing pH value. The diameter at pH 7.4 (6.1 μm) was nearly twice that at pH 4.0 [3.2 μm; Figure 3(b)]. The diameter showed little change at higher pH values (>7.4). The pH responsiveness of the microcapsules was attributed to the incorporation of PMAA in the capsule shell. The pK_a of PMAA was reported to be 5.5.[12] Our ζ-potential analysis also showed that at low pH values, the surface charge of the microcapsules was practically neutral (0.5 mV at pH 2.0), but it decreased very fast from pH 4.0 (−1.5 mV) to 7.4 (−19 mV). This confirmed that the COOH groups were ionized when the pH value was higher than the pK_a (~ 5.5). The electrostatic repulsion among ionized carboxylate group led to the conformational changes of PMAA chains and the consequent swelling of the microcapsules.

The pH responsiveness of the microcapsules could also be seen from morphological differences among the capsules dried at different pH values, as mentioned previously [Figure 1(c–f)]. The capsules dried under acidic conditions had much fewer folds/creases than those dried in basic environments. The reasons might have been twofold. One was the electrostatic-repulsion-induced swelling of the capsules at high pH values, which resulted in the increasing of the diameter and the thinning of the capsule wall. Another possible reason was the hydrogen

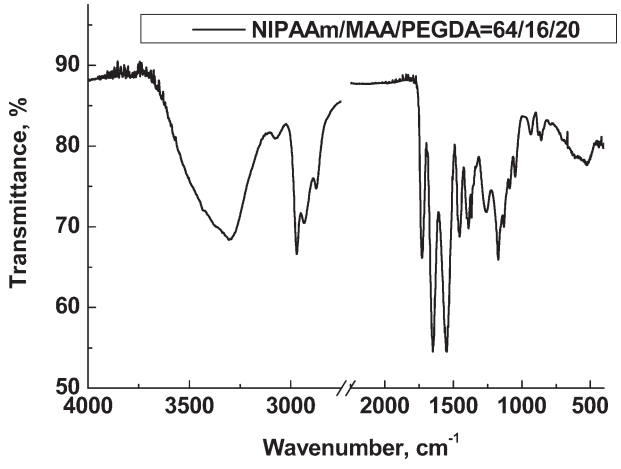

Figure 2. FTIR spectrum of the NIPAAM/MAA/PEGDA copolymer microcapsules.

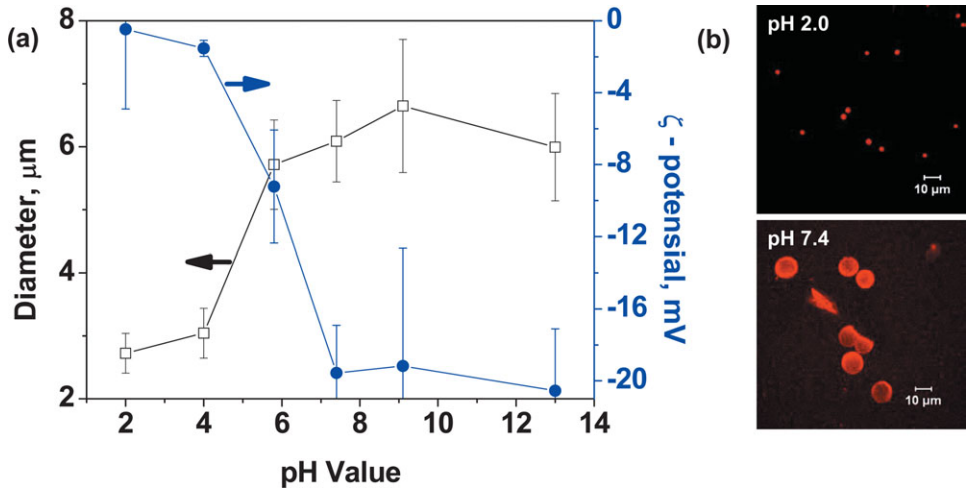

Figure 3. (a) Diameter and ζ potential values of the microcapsules as a function of the pH value and (b) CLSM images of the microcapsules at pH values of 2.0 and 7.4 at room temperature. The microcapsules were labeled with dextran-TRITC (2000 kDa). The microcapsules were first mixed with a 1 mg/mL dextran-tetraethyl rhodamine isothiocyanate (TRITC), (2000 kDa) solution for 10 min and then underwent centrifugation/washing three times to remove unadsorbed dextran-TRITC before imaging. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

bonding between the carboxylic acid and amide groups[39] of the PMAA/PNIPAAm[40] pairs and between the carboxylic acid and ether groups[41] of the PMAA/poly(ethylene glycol) pairs. These strong interactions could only exist under acidic conditions, which indicated that the capsules might have been stiffer[42] in the acid environment than in the basic environment. This could also explain the morphology differences between the samples dried under different conditions. However, the diameter differences of the microcapsules at different pH values were not so significant after drying (Figure 1). One possible reason was that the pH value might have changed after HCl evaporation for the acidic solutions (pH 4.0); this would have caused insignificant diameter differences after drying.

The diameters of the microcapsules at different temperatures were observed by optical microscopy. With an increase in the temperature from room temperature to 40°C, the diameter of the microcapsules decreased from 3.2 ± 0.15 to 2.3 ± 0.1 μm. The thermoresponsiveness of the PMMA/PNIPAAm copolymer microcapsules was not significant compared with its pH responsiveness. A possible reason was that the PNIPAAm in this system might have been trapped into a 3D crosslinking network after copolymerization with the crosslinker, whose ability to dehydrate upon heating might have been hampered compared with the free or grafted PNIPAAm chain.[43]

CONCLUSIONS

In summary, we have presented a simple and rapid route for the preparation of pH-responsive microcapsules by precipitation polymerization. The microcapsules were fabricated by the precipitation of a NIPAAm/MAA/PEGDA copolymer on $CaCO_3$ template microparticles followed by core removal. A higher temperature and the presence of MAA were found to be critical factors for the successful fabrication of the microcapsules. The pH responsiveness was validated by optical microscopy and -CLSM. This technique has potential applications in drug- and gene-delivery systems.

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