Stable microcapsules assembled stepwise from weak polyelectrolytes followed by thermal crosslinking

Weijun Tong and Changyou Gao*
Department of Polymer Science and Engineering, Zhejiang University, Hangzhou 310027, P. R. China

Received 11 July 2005; Revised 7 September 2005; Accepted 19 October 2005

Stable weak polyelectrolyte (PE) microcapsules were fabricated by applying thermal treatment on poly(styrene sulfonate) (PSS) doped CaCO₃ microparticles coated alternatively with poly(allylamine hydrochloride) (PAH) and sodium poly(acrylic acid) (PAA) in a layer-by-layer (LBL) assembly manner, followed by core dissolution with disodium ethylenediaminetetraacetate. Confocal laser scanning microscopy (CLSM) observations revealed the hollow nature and good dispersion of the resultant capsules. Energy dispersive X-ray spectroscopy (EDX) found that sodium PSS, which was used to modify morphology of the CaCO₃ particles during the preparation process, contributed approximately 37% of the total mass of the capsules. Confocal Raman spectra verified that part of the PSS originating from the cores had been adsorbed or intertwined in the PAH/PAA multilayers, and part had been released into the capsule interiors. Stability of the capsules after thermal treatment was largely improved as evidenced by incubation of the capsules in an alkaline solution with a pH value of 12, or 0.1 M HCl for 3 weeks. By contrast, the untreated PAH/PAA microcapsules completely disappeared after incubation in the same alkaline solution. Spontaneous deposition of rhodamine 6G into the crosslinked PAH/PAA capsules was also observed. It is demonstrated that this simple thermal treatment method can be successfully applied to covalently crosslink the weak PE layers and stabilize the microcapsules. The encapsulated PSS molecules can attract diverse positively charged substances. Thus the structure-stabilized capsules can be good candidates as drug delivery vehicles and microcontainers over a wide pH range. Copyright © 2005 John Wiley & Sons, Ltd.

KEYWORDS: microcapsules; polyelectrolytes; thermal treatment; crosslinking; layer growth

INTRODUCTION

Hollow capsules are of great interest due to their wide applications and fundamental importance. In recent years layer-by-layer (LBL) assembly of oppositely charged polyelectrolytes (PEs) onto removable colloidal particles has been used to create novel nanocapsules and microcapsules. Using this fabrication technique, capsules with well-controlled size and shape, finely tuned capsule wall thickness and variable wall compositions have been produced. Microcapsules with customized physicochemical properties can be obtained by incorporation of one or more functional components such as biomacromolecules, lipids, photoactive dyes, nanoparticles, and multivalent ions onto the capsule walls or into the capsule interiors. The typical hollow capsules through the electrostatic LBL assembly of PEs are mostly composed of at least one strong PE. In recent years, however, great attention has been paid to weak PEs, which has charge that varies depending on the pH of the medium. On planar substrate detailed investigations have been performed with regard to this class of multilayers, exemplified by poly(allylamine hydrochloride) (PAH) and poly(acrylic acid) (PAA). These studies have revealed that the multilayers show many distinct properties from the traditional combinations, i.e. strong PEs. For instance, the environmental pH influences greatly the layer thickness, the interpenetration between PAH and PAA molecular chains through the multilayers, and the absorption ability for various substances. Moreover, it has been reported that pores in the range of hundreds of nanometers on the PAA/PAH multilayers assembled at some pH values have been created as a result of phase separation induced by pH change or salt exposure.

Recently, production of hollow PAA/PAH microcapsules templated on melamine formaldehyde (MF) particles and other cores have been reported. Dissolution of the microcapsules has been demonstrated by exposure to low pH. The pH responsivity and solubility of these novel capsules provide an additional opportunity to tune the permeability, loading and release properties for many further applications.

However, stabilized capsules are required in many cases so that the capsules can withstand a wide range of pH treatment, solvent etching and shear stress. One of the promising
pathways is covalent crosslinking of the capsule walls, which has been initially established on planar substrates and then extended to curved capsule walls. For example, by incorporation of photosensitive diazoresin (DAR) in a LBL manner, the charge interaction in the multilayers can be converted into covalent bonding via UV irradiation.\textsuperscript{18–20} The crosslinked multilayers exhibit increased stability against solvent etching. Applying this technique on the PE capsules has improved the stability of the capsules against solvent etching and osmotic pressure.\textsuperscript{21} Hydrogen-bonded multilayers composed of polyacrylamide (PAAm) and PAA can be stabilized to neutral pH by thermoinduced or photoinduced crosslinking reactions\textsuperscript{22} or water-soluble carbodiimide chemistry.\textsuperscript{23} Using the carbodiimide chemistry, weak PE capsules\textsuperscript{24,25} and hydrogen-bonded multilayer capsules\textsuperscript{26} are similarly crosslinked, resulting in stable capsules against solvent etching and pH alteration.

For weak PAH/PAA multilayers, another strategy has been used to create a stable structure by formation of a nylon-like structure at elevated temperature.\textsuperscript{27} Neither special PEs nor other chemicals are involved in both the assembly and the crosslinking processes. The crosslinking takes place very easily by simple thermal treatment. Yet the applicability of this method on curved surfaces, in particular for hollow capsule fabrication, is scarcely known up to present. Thus the possibility of this method for the fabrication of stable weak PEs microcapsules is explored in this study. For this purpose, PAA/PAH multilayers are deposited onto inorganic CaCO\textsubscript{3} microspheres by a LBL manner (Scheme 1). The core-shell particles (Scheme 1a) are then treated under an elevated temperature for a given time, leading to a transformation of the ionic pairs into amides (Scheme 1b), which are more stable against harsh environmental conditions such as acid or base treatment. The crosslinked capsule can be thus obtained after removal of the template core (Scheme 1c). The inorganic microspheres are chosen instead of organic ones such as MF and polystyrene because these cores are stable over the thermal treatment and can be removed easily afterwards. MF particles are unable to be decomposed after heat treatment because of the severe crosslinking activated by the elevated temperature.\textsuperscript{28} Moreover, sodium poly(styrene sulfonate) (PSS) molecules, which are used to modify the morphology of the CaCO\textsubscript{3} particles during the preparation process, can be entrapped simultaneously. This can endow the capsules with other properties such as deposition of charged substances.

EXPERIMENTAL SECTION

Materials

PSS ($M_w = 70$ kD), PAH ($M_w = 65$ kD), calcium nitrate tetrahydrate [Ca(NO\textsubscript{3})\textsubscript{2}·4H\textsubscript{2}O], sodium carbonate (Na\textsubscript{2}CO\textsubscript{3}), disodium ethylenediaminetetraacetate dihydrate (Na\textsubscript{-}EDTA), rhodamine 6G (Rd6G) were obtained from Sigma-Aldrich. Sodium PAA ($M_w = 20$ kD) was purchased from Fluka. All chemicals were used as received. FITC-PAH was prepared by labeling PAH with fluorescein isothiocyanate (FITC) according to the literature.\textsuperscript{29} The water used in all the experiments was triple-distilled.

Methods

Calcium carbonate particle preparation

To prepare the CaCO\textsubscript{3} particles, PSS was firstly mixed with 0.025 M calcium nitrate solution under vigorous agitation. To which an equal volume of 0.025 M sodium carbonate solution was rapidly poured at room temperature. The final PSS concentration was 2 mg/ml. After about 10 min, the CaCO\textsubscript{3} particles were collected and washed using membrane filtration apparatus equipped with a cellulose filter having pore size of 0.45 μm. The size of the obtained particles is approximately 8–9 μm. LBL assembly and capsule fabrication

The pH values of both PAA and PAH solutions were adjusted to 6.5 with 0.1 M HCl or NaOH. Adsorption of the PEs (2 mg/ml) onto the CaCO\textsubscript{3} microparticles (~1% w/w in suspension) was conducted in 0.2 M NaCl solution for

![Scheme 1](image)

Scheme 1. Fabrication of covalently crosslinked PAH/PAA microcapsules via a procedure of LBL assembly on PSS doped CaCO\textsubscript{3} cores, thermally induced amide formation and core removal.
10 min followed by three washings with 0.2 M NaCl solution. The excess PEs were removed by centrifugation at 300 g for 5 min. After a desired number of PE layers was assembled, the coated particles were washed in pure water. In the core removal process, the particles were incubated in 0.02 M EDTA (pH 7) solution for 30 min under shaking. The resultant capsules were centrifuged at 1500g for 5 min with three washings in fresh EDTA solution. Finally, the capsules were washed with distilled water three times. As the CaCO₃ particles possess a negatively charged surface due to the existence of PSS, the first layer was always PAH, while the outermost layer in this study was always PAA.

**Thermal crosslinking of the PAH/PAA multilayers on the CaCO₃ particles**

After deposition of four bilayers of PAH/PAA on the CaCO₃ particles, the coated particles were freeze-dried. The dried particles were placed in a glass tube, which was incubated in an oil bath at 180°C for 2 hr under ambient nitrogen. After cooling to room temperature, the particles were re-suspended in water. The cores were then removed by EDTA according to the above-mentioned procedure.

**Confocal laser scanning microscopy (CLSM)**

Confocal images were taken with a Leica confocal scanning system mounted to a Leica Aristoplan and equipped with a 100 × oil immersion objective with a numerical aperture (NA) of 1.4. Images were taken immediately after mixing equal volumes of capsule suspension and FITC-PAH (0.2 mg/ml) or Rd6G (0.02 mg/ml) solution.

**Scanning electron microscopy (SEM) and energy dispersive X-ray (EDX) spectroscopy**

For SEM analysis, samples were prepared by applying a drop of the particle or capsule suspension to a glass slide. After drying overnight, the samples were sputtered with gold and were observed under a Gemini Leo 1550 instrument at an operation voltage of 3 keV. For EDX analysis, an aluminum wafer was employed as a substrate.

**Thermogravimetric analysis (TGA)**

Thermogravimetric performance was examined by a Netzsch TG 209 machine in nitrogen atmosphere at a scanning rate of 20°C/min from room temperature to 800°C.

**Confocal Raman spectroscopy**

Confocal Raman spectra of the CaCO₃ particles and the PE capsules were recorded in water under ambient conditions using a confocal Raman microscope (CRM200, Witec) equipped with a piezo scanner (P-500, Physik Instrument) and high NA microscope objectives (×60, NA = 0.80 or ×100 oil, NA = 1.25, Nikon). In a typical experiment, a circularly polarized laser (CrystaLaser, λ = 532 nm) was focused on the samples with a diffraction limited spot size (~λ/2). An avalanche photodiode detector (APD) was used to record high resolution Raman images.

**Scanning force microscopy (SFM)**

SFM images were obtained with a scanning probe microscope (SPA3800N, Seiko) in dynamic force mode. A drop of capsule suspension was applied to freshly cleaved mica and dried at room temperature.

**RESULTS AND DISCUSSION**

**CaCO₃ microparticles**

Biomimetic synthesis of biominerals such as CaCO₃ crystals in the presence of organic templates and/or additives has been extensively investigated in recent years as reviewed by Co¨lfen. More recently, PEs have been used to control crystallization of the carbonates. Here this strategy is used to fabricate the PSS doped CaCO₃ microparticles. Figure 1a shows that spherical CaCO₃ microparticles with an average diameter of 8–9 μm were obtained through mineralization from Ca(NO₃)₂ and Na₂CO₃ solutions in the

![Figure 1](image-url)
presence of PSS. The homogeneity of both the particle size and the macroscopic shape is very promising for these particles to be used as a template for multilayer deposition.

The magnified SEM image (Fig. 1b), however, reveals that the typical surface texture of the particle is very rough. It is built from smaller, near spherical building blocks with a typical diameter of 50–100 nm. The formation of this structure may arise from a mesoscale self-assembly process in the present case. The PSS-stabilized amorphous nanoparticles act as precursors for the subsequent mesoscale self-assembly and finally form the micron-sized particles. The driving force of formation of the spherical aggregate is likely as it decreases the surface areas and maintains minimum surface energy.32

The existence of PSS in these as-prepared CaCO₃ micro-particles has been identified by confocal Raman spectroscopy.33 Quantification of the entrapped PSS was conducted by TGA (Fig. 2). Three independent weight losses in the TGA curve can be clearly identified. The first weight loss took place in the temperature range 80–300°C, yielding a value of 3.1% which should be the result of evaporation of adsorbed and combined water molecules. The second weight loss occurred in the temperature range 400–550°C, a typically high enough temperature to induce thermal degradation of ordinary carbon polymers. The PSS content in the CaCO₃ can be thus estimated as 5.5%, i.e. the measured weight loss. The last weight loss was determined above 600°C with a very fast rate. This is understood as the result of CaCO₃ degradation. This result has indeed given a hint that the CaCO₃ particles are stable enough at 180°C to allow the next thermal crosslinking of the multilayers.

Fabrication and characterizations of the thermal crosslinked capsules

The biggest problem in assembly of weak PE combinations onto colloidal particles is the severe aggregation, raised mainly by low charge density of the PEs. Here a pH value of 6.5 was chosen for multilayer deposition, because at this condition a more flat and more highly ionically crosslinked multilayer film on the CaCO₃ particles can be assembled,34 and the severe aggregation can be avoided.

The CaCO₃ cores were removed using EDTA. This is a very promising way especially when pH sensitive weak PEs are utilized as the layer components in the present case. The hollow nature of the thermally treated microcapsules was directly confirmed by CLSM in aqueous solution and by SFM in the dry state (Fig. 3). For visualization under CLSM, an additional FITC-PAH layer was adsorbed on the capsules surfaces. Figure 3(a) shows that the capsules could still be well suspended and dispersed in water. The capsules resembled almost the macroscopic shape and size of their CaCO₃ template. From the SFM image of one typical four-bilayer PAH/PAA capsule shown in Fig. 3(b), one can find that the thickness of the double walls is as large as 174 nm, meaning an “apparent” layer thickness of 11 nm. This is 20 times thicker than that of PAA/PAH capsules templated on MF particles and assembled under similar conditions.17 Since the untreated PAA/PAH capsules have the similar thickness, the influence of thermal treatment can be ruled out. The influence of the core remaining can also be ruled out because EDX analysis detected only 1.5 wt% Ca²⁺ in the
capsules, a neglectable value compared with the wall thickness. Capsules with thicker walls have also been reported previously. For example, Sukhorokov and coworkers have found a huge increase of the thickness of PSS/PAH capsule walls templated on ultra rough and porous CaCO₃ templates. They attribute this thickness increase to the PE complex formation in the pores. This could be one reason in the present case since the surface of the template CaCO₃ particles is also very rough (Fig. 1b).

Another reason should be the entrapment of PSS. Actually, confocal Raman spectra of thermally crosslinked (PAH/PAA)₄ microcapsules recorded strong Raman shifts at 1128 and 1600 cm⁻¹ at positions on both the capsule wall and interior (Fig. 4), demonstrating the existence of the PSS molecules inside the hollow capsules. Since no PSS is used in the assembly process, these results would mean that part of the PSS originated from the cores has been absorbed or intertwined in the PAH/PAA multilayers, and part has been released into the capsule interiors. EDX measurements found that the sulfur content in the capsules was ~6 wt%, which corresponds to about 37% mass contributed by PSS. Moreover, from a simple calculation one can know that about 8.6% PSS originated from the CaCO₃ cores was preserved within the capsules (for a detailed calculation process see ref. 33). This would mean that more than 90% of PSS has been released through the pores or defects on the capsule walls. Therefore, the PSS amount itself is not high enough to cause the huge increase of the capsule wall thickness measured by
SFM. Yet incorporation of interior materials may have caused incomplete collapse of the capsules. Together with the rough surface of the particles, the huge wall thickness can be thus explained.

Interestingly, no obvious capsule swelling has been observed even though PSS had been entrapped. This is distinct from previous observations, where obvious capsule swelling occurs when PSS or dextran sulfate is encapsulated in the PE capsules.\textsuperscript{36,37} Capsule swelling or not is dependent on both the state of the PE inside and the strength of the capsule wall. Only free PE can produce enough osmotic tension onto the capsule walls to induce swelling assuming the pressure exceeds the critical point. While the PE in an associated or complex state can hardly contribute to the osmotic pressure.\textsuperscript{38} Since no apparent capsule swelling occurred either for the untreated PAA/PAH capsules, one can thus conclude that most of the PSS should be in an associated or complex state (for example, adhering onto the capsule walls, or forming a complex with Ca\textsuperscript{2+}) other than a highly free state. Actually, EDX detected approximately 1.5 wt\% Ca\textsuperscript{2+} in the thermally treated capsules. Of course these Ca\textsuperscript{2+} ions may also associate (even more strongly) with the free carboxylate groups besides the sulfonate groups. Anyway, capsules with this feature could be used as micro-containers to load drugs spontaneously, and micro-reactors to perform chemical reactions in their restricted volume.\textsuperscript{36}

The stability and spontaneous deposition property of the crosslinked capsules

The PAH/PAA complex is intrinsically unstable at lower or higher pH because of the fully decharging of the PAA or PAH molecular chains, respectively. Interestingly, the microcapsules fabricated in this case are much more stable compared with those in a previous study.\textsuperscript{17} For example, even incubated in 0.1 M HCl solution for about 30 min most of the capsules could still keep their macroscopic contours, although they were severely swollen and the walls had become buckled (Fig. 5a). Undoubtedly, the involved PSS should be responsible for the improved stability of the capsules, since PSS molecules are still negatively charged at low pH and they can form a stable complex with PAH.\textsuperscript{39} However, the capsules are dissolved so fast at pH 12 that it is hard to record the dissolution process.\textsuperscript{40} At a relative lower pH such as 11, the dissolution speed of the capsules was comparatively slow. For example, after incubation for 10 min some of the capsules were dissolved to form flocules, while some were still stable to keep their macroscopic contours (Fig. 5b). However, longer incubation yielded complete dissolution as well. By contrast, thermally treated PAH/PAA capsules have shown a dramatic improvement in their stability against concentrated acid or base solutions. For instance, no visible change in capsule morphology has been found after exposure to a solution with a pH value of 12 for 3 weeks (Fig. 5c). Similar results were observed at pH 1. A previous study has demonstrated that the –COO\textsuperscript{−}/NH\textsubscript{3}\textsuperscript{+} ionic bonds in the PAA/PAH multilayers can be undoubtedly converted into the covalent amide bonds by thermal treatment under the same condition.\textsuperscript{27} These newly-formed covalent bonds endow the capsules with improved stability. Thus one can conclude that the thermal treatment is a simple but effective way to improve the capsule stability against harsh stimuli such as extremely low or high pH.

The PAA/PAH capsules with entrapped PSS show readily the spontaneous deposition of some water-soluble substances into the capsule interiors driven by charge interaction. For example, when the capsule suspension was mixed with Rd6G solution, a very strong fluorescence was recorded from the capsule interiors than that from the bulk (Fig. 6a), demonstrating the spontaneous deposition of the positively charged dyes. After thermally crosslinked, this spontaneous deposition property was completely reserved (Fig. 6b). It has to mention that the capsules without strong fluorescence inside are most possibly ruptured during the core removal process, allowing the encapsulated PSS to escape and preventing the dye complexion.

CONCLUSIONS

The successful fabrication of stable microcapsules assembled from weak PEs on PSS doped CaCO\textsubscript{3} microparticles has been demonstrated. Thermal treatment transformed the –COO\textsuperscript{−}/NH\textsubscript{3}\textsuperscript{+} ionic bonds to the covalent amide bonds. These
covalently crosslinked microcapsules could still be suspended and dispersed in water, with improved stability over a wide pH range. Another feature of the microcapsules is that a part of the PSS molecules were simultaneously entrapped inside the microcapsules. Positively charged dyes can be spontaneously deposited inside. Stable capsules over a wide pH range. Another feature of the microcapsules suspended and dispersed in water, with improved stability covalently crosslinked microcapsules could still be

Acknowledgements
The authors would like to thank Professor J. C. Shen and H. Möhwald for their continuous support and stimulating discussions. R. Pitschke and W. F. Dong are greatly acknowledged for their assistance in SEM and Raman spectra measurements, respectively. The authors would also like to thank the Max-Planck Society for a visiting scientist grant. H. Möhwald for their continuous support and stimulating discussions. R. Pitschke and W. F. Dong are greatly acknowledged for their assistance in SEM and Raman spectra measurements, respectively. The authors would also like to thank the Max-Planck Society for a visiting scientist grant.

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