Fabrication of complex microcapsules containing poly(allylamine)-g-poly(N-isopropylacrylamide) and their thermal responsivity

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Complex microcapsules containing thermo-responsive poly(allylamine)-g-poly(N-isopropylacrylamide) (PAH-g-PNIPAam) were fabricated by adsorbing one layer of PAH-g-PNIPAam onto CaCO₃ microparticles doped with poly(styrene sulfonate) sodium salt (PSS), followed by dissolution of the CaCO₃ particles. The complex capsules were characterized by confocal microscopy, scanning electron microscopy, transmission electron microscopy and atomic force microscopy. The existence of PAH-g-PNIPAam in the complex microcapsules was confirmed by FTIR spectroscopy, and its thermo-responsive property was studied by ¹H NMR. The capsules showed different morphologies when dried at 25 and 50°C, as well as enhanced stability at 40°C in alkaline solution due to the additional hydrophobic interaction.

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1. Introduction

Hollow microcapsules have been widely used in fields of pharmaceutics, cosmetic, food, textile, adhesive and agricultural industry due to their isolating property, large inner volume and tunable permeability [1–4]. To obtain the microcapsules with versatile structures and properties, new efforts are continuously tried to explore various techniques for fabrication. For example, by the layer-by-layer (LBL) assembly on sacrificial colloidal particles [5–7], multilayer microcapsules with ultrathin wall thickness and tunable wall structures and properties have been fabricated. They have shown potential applications in materials and life science as microreactors, microcontainers, drug-delivery vehicles, protective shells for cells or enzymes, transfection carriers for gene therapy and biosensors, etc. On the other hand, nowadays much attention is paid to smart materials too, which can respond to stimuli such as pH, ionic strength, temperature, light, magnetic field and specific biological substances, etc. [8–10]. For this context, the smart microcapsules in particular thermo-responsive microcapsules are particularly attractive, since their structures and properties can be easily mediated by temperature. This is of great help for many bio-related applications such as controlled drug delivery.

Poly(N-isopropylacrylamide) (PNIPAam) is a typical thermo-sensitive polymer whose lower critical solution temperature (LCST) is around 32–34°C in water solution. Below the LCST, the polymer is soluble while above it the polymer is in a collapsed state thus becomes water insoluble. The phase transition is caused by the dehydration of the polymer chains above the LCST, at which the coiled polymer chains collapse into a globular conformation due to the breakage of hydrogen bonds between the amide groups of PNIPAam and water molecules [11]. Previously we have prepared a grafting copolymer of poly(allylamine)-graft-poly(N-isopropylacrylamide) (PAH-g-PNIPAam), which has an LCST at 34°C, a temperature identical to that of the PNIPAam regardless of their grafting ratios [12–14].

Therefore, incorporation of the thermo-responsive polymers into the multilayers is a typical strategy to obtain the thermo-responsive microcapsules. Indeed, PNIPAam and other thermo-sensitive polymers have been directly assembled into LBL films and capsules by hydrogen bonding [15]. For example, Quinn and Caruso [16] prepared PNIPAam/poly(acrylic acid) (PAA) multilayer thin films. The adsorption temperature of PNIPAam significantly affects both the mass proportion of PNIPAam in the film and the film surface morphology. On the other hand, much attention has been paid to copolymers of PNIPAam and polyelectrolytes such as PSS, PAH, PAA, poly(methacrylic acid) (PMA), poly(ethyleneimine) (PEI) and chitosan. Jaber and Schlenoff [17] reported thermally reversible modulation of ion transport through thermally responsive polyelectrolyte multilayers prepared from charged...
poly(N-isopropylacrylamide) (PNIPAAm) copolymers. Kono et al. [18] fabricated partly crosslinked PMA-PEI complex capsules containing the methacrylic acid-N-isopropylacrylamide copolymers. However, this kind of capsules needs to be crosslinked and only those microcapsules prepared at temperatures higher than that of the LCST have shown the thermal sensitivity. Glinel et al. [19] prepared microcapsules by LBL deposition of oppositely charged diblock copolymers each containing PNIPAAm block onto colloidal particles and subsequent decomposition of the cores. The capsule shrinkage in their size is accompanied by a decrease of the permeability with increasing temperature, which is a result of structural rearrangement in the shells. However, this process is only partially reversible upon cooling.

The aim of this work is to fabricate complex microcapsules containing PNIPAAm and study their thermal responsivity. We have previously reported a facile route to fabricate complex polyelectrolyte microcapsules via in situ coacervation [20,21]. In this work this in situ coacervation method is applied to fabricate the thermo-responsive microcapsules by utilizing PAH-g-PNIPAAm as one of the polyelectrolytes. In this protocol, CaCO₃ particles doped with PSS are used as the template, onto which only one single layer of PAH-g-PNIPAAm is adsorbed. The capsule walls are formed during the core dissolution, in which the doped PSS is released and instantaneously complexed with the adsorbed PAH-g-PNIPAAm to form the shells eventually. The chemical structure and morphology of the complex hollow microcapsules are studied by Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and transmission electron microscopy (TEM) and scanning force microscopy (SFM). The capsules show better stability at 40°C than at 20°C in alkaline solution. To the best of our knowledge, this is the first report on the effect of temperature on the morphology and stability in alkaline solution of the microcapsules containing PNIPAAm.

2. Experiment

2.1. Materials and methods

Sodium poly(styrene sulfonate) (PSS, MW ~ 70 kDa), poly(allylamine hydrochloride) (PAH, MW ~ 70 kDa), rhodamine 6G (Rd6G), fluorescein sodium (FL) were obtained from Sigma–Aldrich. Calcium nitrate tetrahydrate (Ca(NO₃)₂·4H₂O), sodium carbonate (Na₂CO₃), disodium ethylene diamine tetraacetate dihydrate (EDTA) were obtained from Shanghai Chemical Reagent Company, China. All chemicals were used as-received. The water used in all experiments was triple distilled.

PAH-g-PNIPAAm copolymers were synthesized by grafting carboxylic end-capped PNIPAAm (PNIPAAm–COOH, Mw 2500) onto PAH (Mw 65 kDa, Aldrich) chains as described previously [12]. The
The grafting degree of PNIPAAm was measured as 33% by elemental analysis.

PSS-doped CaCO₃ microparticles were prepared as described previously [20]. Briefly, 200 mg PSS was dissolved in 200 mL 0.05 M calcium nitrate solution, into which an equal volume of 0.05 M sodium carbonate solution was rapidly poured at room temperature under ultrasonication. 10 s later, the mixture was stored undisturbed for 1 h and the precipitated CaCO₃ particles were collected by centrifugation and washed 3 times with water.

The PSS-doped CaCO₃ microparticles (~0.5% (w/v) in suspension) were then incubated in 2 mg/mL PAH-g-PNIPAAm/0.5 M NaCl solution for 30 min under shaking. The particles were separated from the solution by centrifugation at 500 x g for 5 min, followed by three washings with water. To obtain hollow capsules, the PAH-g-PNIPAAm coated microparticles were treated with 0.02 M EDTA solution for 30 min under gentle shaking. The capsules were separated by centrifugation at 1500 x g for 5 min, followed by three washings in fresh EDTA solution. Finally, the capsules were washed with water for 3 times.

Confocal laser scanning microscopy (CLSM) was employed to detect the size variation of the capsules in alkaline solution (pH 11, 11.5 and 12) at 20 and 40 °C. The size variation of the capsules was described by \((D - D_0)/D_0 \times 100\%\), where \(D\) and \(D_0\) are the average diameters measured at the specific pH value and pH 7 and 20 °C, respectively.

### 2.2. Instrumentation

\(^1H\) NMR spectra were recorded by a Bruker 300 MHz spectrometer employing D₂O as solvent. FTIR spectra were acquired on a Bruker FTIR spectrometer. A drop of capsule suspension was dropped onto a glass slide and air-dried. After sputtering with gold, the samples were observed by a scanning electron microscope (SIRION-100 FEI). Copper grids sputtered with carbon films were used to support the microcapsules for transmission electron microscopy (TEM) study. 10 µL of the microcapsule suspension was dropped on the copper grid and air-dried. The measurement was carried on a JEOL JEM-200CX TEM. A drop of sample suspension was applied to freshly cleaved mica and air-dried for scanning force microscopy (SFM) study. The images were acquired by a Seiko Instruments scanning force microscope (SFM, SPI3800N Probe Station and SPA400 SPM Unit) in air at room temperature using a tapping mode. CLSM images were taken with a Bio-Rad Radiance 2100 confocal laser scanning microscope, equipped with a 100 x oil immersion objective with a numerical aperture of 1.4. Rh6G was used to stain the microcapsules. The \(\zeta\)-potentials of the CaCO₃ microparticles and the microcapsules were measured using a Zetasizer Nano instrument Nano Z. Each value was averaged from five parallel measurements.

### 3. Results and discussion

By adsorption of one layer of PAH-g-PNIPAAm on the PSS-doped CaCO₃ microparticles and subsequent core removal, PSS/PAH-g-PNIPAAm microcapsules were successfully acquired as shown in Fig. 1. The capsules dispersed very well in water and no microscopically detectable breakages of the capsule walls were observed in both the CLSM (Fig. 1(a)) and transmission (Fig. 1(b)) images. The hollow nature of the resulted microcapsules was identified by SEM (Fig. 1(c)) and TEM (Fig. 1(d)) measurements, in which folds and creases on the capsules could be similarly observed as that of the PSS/PAH capsules [20].

In the FTIR spectrum (Fig. 2) of PSS/PAH microcapsules, the aromatic ring stretch absorbance was found at ∼1600, 1510, and 1450 cm⁻¹. The NH₂ scissor bend was found at ∼1613 cm⁻¹, which overlapped with the symmetric aromatic ring stretch at ∼1600 cm⁻¹. For the PSS/PAH-g-PNIPAAm microcapsules, the peak at 1651 cm⁻¹ (amide I, C=O stretching) overlapped with the NH₂ scissor bend peak and benzene symmetric ring stretch peak at ∼1613 cm⁻¹, and the peak at 1541 cm⁻¹ (amide II, N–H bending) overlapped with another aromatic ring stretch absorbance at 1510 cm⁻¹ found in the PSS/PAH microcapsules too. The antisymmetric bend of –CH₃ at 1458 cm⁻¹ overlapped with the ‘side way’ ring stretch absorbance of PSS at about 1450 cm⁻¹, and two splitted methyl umbrella bends were found at 1387 and 1367 cm⁻¹ which are the characteristic peaks of –C(CH₃)₂. The existence of amide and isopropyl groups confirms the successful incorporation of PAH-g-PNIPAAm into the complex microcapsules.
AFM characterization revealed that the double walls have a thickness of $36 \pm 6$ nm, corresponding to a wall thickness of $\sim 18$ nm, which is almost the same as that of the PSS/PAH complex capsules [20,21]. The microcapsules obtained here are apparently thicker than the same number of regular bilayer PAH/PSS (3–4 nm), which is one of the advantages of the in situ coacervated method. By this way, the capsules are formed dynamically along with the dissolution of the CaCO$_3$ particles and release of the PSS. The rough surface and pores of the CaCO$_3$ particles adsorbs a large amount of PAH-g-PNIPAAm, which combines with the released PSS to form the thicker capsule shells during the core dissolution.

Elemental analysis found that the molar ratio of PSS:PAH-g-PNIPAAm in the microcapsules (1.1) was smaller than that of the PSS:PAH microcapsules (1.3), revealing that the amount of PSS was decreased while the amount of PAH-g-PNIPAAm was increased. This is well consistent with the variation of the chemical structures of the polycations. In the PAH-g-PNIPAAm molecules, around 1/3 of the ionizable amine groups were substituted by the PNIPAAm chains, thus the ionic density of the polymers was significantly decreased. Moreover, the pendent PNIPAAm chains can block the nearby amine groups from forming electrostatic binding with PSS. Consequently, more amount of PAH-g-PNIPAAm is required to compensate with the same amount of PSS.

Figs. 3 and 4 compared the results of AFM characterization of PSS/PAH-g-PNIPAAm complex capsules dried under 25 and 50°C. Although the capsule size and wall thickness did not change obviously, the surface morphology turned out to be quite different. The capsules dried at 25°C (Fig. 3) show rougher surfaces with a root-mean-square roughness (RMS) of $\sim 5.8$ nm, while those dried at 50°C (Fig. 4) was smoother (RMS $\sim 3.2$ nm). Grains on those capsules dried at 25°C were found to be 115 nm in diameter and 17 nm in height, respectively. Interstitials between the grains were clearly detected. In contrast, much larger domain size (238 nm) was found for the capsules dried at 50°C with a height of 11 nm, and deep invagination between the grains were not observed. A comparable study revealed that the PSS/PAH complex capsules fabricated by this protocol had a RMS of $\sim 5.8$ nm, with a domain size of the order of ca.100–200 nm regardless of the drying temperature.

Early investigation have found that besides the influence of capsule swelling during core removal, the lateral organization of the multilayers and their roughness depend mainly on polyelectrolyte aggregation and subsequent segregation of these complexes caused by evaporation of water [22]. Since there is little difference on surface texture of the PSS/PAH complex capsules dried at different temperatures, the surface texture difference for the PSS/PAH-g-PNIPAAm complex capsules dried at different temperatures can be only attributed to the existence of PNIPAAm. It is known that the PNIPAAm chains become hydrophobic at higher temperature. The hydrophobic interaction between the PNIPAAm chains can enhance the interaction between the polymer chains and thereby lead to the homogeneity of the capsules. Besides, dehydration of the polymer chains above the LCST will largely reduce water content in the capsule walls. This may contribute to the diminished segregation of the previously well-separated polyelectrolyte domains, resulting in smoother surface texture but larger grain size.

The structure variation of PAH-g-PNIPAAm at 25 and 50°C was verified by $^1$H NMR (Fig. 5). At 25°C, PAH-g-PNIPAAm was fully solvated and signals associated with isopropyl groups (signals ‘a’ and ‘b’) were clearly visible. Other peaks at $\sim 1.4$ and 1.8 ppm are
assigned to –CH_2 and –CH groups on the backbone of both PAH and PNIPAAm, and the peaks between 2.8 and 3.2 ppm are assigned to –CH_2 of PAH adjacent to –NH_2 and –NH groups. An increase of the solution temperature to 50 °C resulted in complete disappearance of the signal ‘b’ and substantive weakening of signal ‘a’. The overall higher chemical shifts of all the remaining resonance peaks at 50 °C should be the result of alteration of the chemical environment. It is known that Above the LCST, the PNIPAAm chain adopts a compact and collapsed conformation, which makes it difficult for the hydrophobic isopropyl group to interact with water molecules and thereby accounts for the chemical shift of the peaks. On the other hand, existence of the hydrophilic PAH component inside the complex may increase the water content than that of the pure PNIPAAm, which can explain the incomplete disappearance of the signal ‘a’ in Fig. 5. The PNIPAAm signal was regained when the temperature dropped to 25 °C, illustrating the reversible thermal responsivity.

Stability of the microcapsules in alkaline conditions was then studied (Fig. 6). To better understand the contribution of the PNIPAAm, firstly the PSS/PAH complex capsules (Fig. 6(a)) were compared. At 20 °C, the average capsule diameter was only increased about 5% when the pH value was increased from 7 to 11, but rapidly increased 11% at pH 11.5. When the pH value reached to 12, however, the capsule size was decreased ~9%. When the pH value is above the pK_a of PAH (8.4) [23], significant deprotonation of PAH shall occur. Since PSS is a strong polyelectrolyte, the remaining negative charges on the capsule walls cause the polyelectrolytes to increase their mutual distance by electrostatic repulsion [24], which causes the capsule swelling. When the pH value is higher than 11.5, the amino groups of PAH are deprotonated almost completely [25], leading to slow release of PSS from the capsule walls by same charge repulsion. This was confirmed by UV–vis measurement, in which PSS was detected in the supernatant of the microcapsules treated in pH 12 solution. When the pH value is further increased, the quick disassembly of the polyelectrolyte complex cause the fast dissolution of the PAH/PSS polyelectrolyte capsules [26]. The PSS/PAAm capsules showed a little diameter decrease in pH 7 solution (~<1%) when the temperature was increased from 20 to 40 °C. Previous study has showed that higher temperature facilitates the transition towards a more coiled arrangement of the individual polyelectrolyte molecules in multilayers and can accelerate the transient breakage of the electrostatic bonds. At pH 11, there was no significant difference between the extent of diameter variation at 20 °C (5%) and 40 °C (2%). But at pH 11.5, while the capsules were still swollen (11%) at 20 °C, they began to shrink at 40 °C (~5%); at pH 12, the extent of shrinking was much more prominent at 40 °C (~58%) than that at 20 °C (~9%). These results manifest that higher temperature deteriorates the stability of the microcapsules.

As to PSS/PAH-g-PNIPAAm capsules (Fig. 6(b)), the extent of diameter variation at pH 11, 11.5, 12 were 10%, 10% and ~53%, respectively at 20 °C, revealing that the PSS/PAH-g-PNIPAAm capsules have poorer stability in alkaline solution, especially at pH 12. This is attributed to the lower ionic density of the PAH-g-PNIPAAm and thereby the weaker interactions between the PSS/PAH-g-PNIPAAm pairs. When the temperature was increased to 40 °C, no detectable diameter change occurred in pH 7 solution. Interestingly, at pH 11, 11.5 and 12, the extent of diameter variation became 2%, 2% and ~26%, respectively, manifesting an enhanced base stability at higher temperature. Incorporation of the PNIPAAm pendent chains can provide additional hydrophobic interaction above the LCST, which may maintain the macroscopic topology of the capsules even the electrostatic interaction at higher pH has been significantly weakened or destroyed. In this case, the PSS chains should be kinetically trapped within the enhanced crosslinked network through entanglements, which can either block the capsule swelling induced by electrostatic repulsion or prevent quick leakage of PSS from the capsules.

4. Conclusion

In summary, complex capsules containing thermo-responsive PAH-g-PNIPAAm were successfully fabricated by in situ polyelectrolyte coacervation on PSS-doped CaCO_3 particles. The thermo-responsive property of PAH-g-PNIPAAm was studied by ^1H NMR, the incorporation of PAH-g-PNIPAAm into the complex microcapsules was confirmed by FTIR spectroscopy. This kind of capsules showed different morphologies when dried at 25 and 40 °C and enhanced stability at 40 °C than that at 20 °C in alkaline solution. With the advantages of time/materials saving and thermal sensitivity, this kind of microcapsules may find some applications in drug delivery and other biotechnology fields.

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References


