Robust Poly(allylamine)-graft-poly(N-isopropylacrylamide) Particles Prepared by Physical Crosslinking with Poly(styrene sulfonate)

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Introduction

Thermosensitive polymers have attracted extensive attention due to their intelligent and reversible responsiveness to environmental stimuli, in particular, temperature variation. In various physical forms such as gels, particles, micelles, and capsules, they have shown intelligent loading and release capabilities for drugs, proteins, nanoparticles, and DNA upon variation of temperature, ionic strength, pH, solvent, and even light, etc.[1] Among the family of thermosensitive polymers, poly(N-isopropylacrylamide) (PNIPAAm) is one of the typical and most widely studied ones, which exhibits a lower critical solution temperature (LCST) in an aqueous solution around 32 °C, below which the polymer is soluble and above which it is in a collapsed phase and water insoluble.[2] The phase transition of PNIPAAm aqueous solution is understood as a result of dehydration of the polymer chains above the LCST, namely, the breakage of hydrogen bonds between amide groups of PNIPAAm and water molecules, thus collapsing the coiled polymer chains into a globular conformation and inducing polymer aggregation at sufficiently high concentration.[3]

Recently, attention has been paid to copolymers of PNIPAAm and charged polymers (polyelectrolytes), typically poly(acrylic acid), poly(methacrylic acid), poly(ethyleneimine), and chitosan.[4] Such copolymers are expected to respond not only to temperature but also to pH and other stimuli such as ionic strength. Also, the phase transition temperature, mechanical strength, and sensitivity can all be modulated by incorporating charged polymers.[4a,5] Another feature is that above the LCST the copolymers become amphiphilic, and thus can easily form

Summary: Robust thermosensitive PAH-g-PNIPAAm/PSS particles were prepared by addition of a poly(allylamine)-graft-poly(N-isopropylacrylamide) particle suspension into poly(styrene sulfonate) solution above the LCST of PAH-g-PNIPAAm. Scanning force microscopy revealed stable and well-separated particles in water at room temperature. The zeta-potential showed a negative surface charge of the particles. Their thermosensitive behavior was demonstrated by dynamic light scattering. The release of rhodamine 6G loaded particles could respond to the incubation temperature.
micelles or particles having thermosensitive cores and pH-sensitive shells.\textsuperscript{[4a,5]} Moreover, when the charged copolymers of PNIPAAm and polyelectrolytes interact with oppositely charged polymers, thermosensitive particles can be formed in the state of a polyelectrolyte complex, some of which exhibit both temperature-responsive and pH-responsive properties by incorporating weakly charged polyelectrolytes.\textsuperscript{[5c,6]}

We have reported previously the synthesis of poly(allylamine)-graft-poly(N-isopropylacrylamide) (PAH-g-PNIPAAm) obtained from the condensation between poly(allylamine) (PAH) and carboxylic end-capped PNIPAAm (PNIPAAm-COOH).\textsuperscript{[7]} These thermosensitive copolymers exhibit an LCST at 34°C regardless of their grafting ratios (50, 29, or 18%), a temperature identical to that of PNIPAAm-COOH oligomers. A temperature cycle reveals completely reversible polymer aggregation and dissolution in pure water above and below the LCST, respectively. On the other hand, the LCST of the copolymers decreases linearly as a function of NaCl concentration ascribed to the "salt out effect."\textsuperscript{[2,3a,8]} For example, it is lowered down to 28°C in 0.5 M NaCl solution. pH variation in a range of 2–11.3, however, does not affect the LCST of the copolymers.\textsuperscript{[7]} A porous sphere model is suggested to depict the structure of the particles formed above the LCST.\textsuperscript{[7]}

Robust thermosensitive particles, micelles, and microspheres have gained great scientific and technological importance because in many applications, it is highly favorable to have a stable structure that is against reversibility.\textsuperscript{[9]} They can be used for various purposes such as protein adsorbent and drug delivery,\textsuperscript{[10]} whose performance is controlled by temperature. However, the hydrophobic force-governed PAH-g-PNIPAAm particles are not stable when the temperature is below the LCST, i.e., the particles will be soluble again. Therefore, crosslinking of the particles would be a straightforward strategy to stabilize their structure, which may endow the particles to preserve their topological structure below the LCST. As the PAH segments can interact with negatively charged polymers to form a stable complex, we make use here of this mechanism to crosslink the PAH-g-PNIPAAm particles (Scheme 1).

At a temperature above the LCST, the PAH-g-PNIPAAm will accumulate into particles with porous structure [Scheme 1(a)]. When the particle suspension is added dropwise into a poly(styrene sulfonate) (PSS) solution with the same temperature, the PSS molecules may cover the surfaces and penetrate into the interiors of the particles to combine with the positively charged PAH segments. Here the PSS molecules function as crosslinkers. As a result, the structure of the particles will be stabilized by electrostatic crosslinking [Scheme 1(b)]. The stabilized particles should still preserve their swelling behavior below the LCST of PAH-g-PNIPAAm because of the hydration effect, but they have the ability to keep their topological structure [Scheme 1(c)]. The internal volume of the polymer network is temperature tunable, which would provide different permeability for a given substance at different temperatures. Dynamic light scattering (DLS) and scanning force microscopy (SFM) are employed to demonstrate the structure and thermosensitivity of the resultant particles. By using rhodamine 6G (Rd6G) as a probe, the release property of the particles at different temperatures is also compared.

**Experimental Part**

**Preparation of the PAH-g-PNIPAAm/PSS Particles**

PAH-g-PNIPAAm copolymers, with a PNIPAAm grafting degree of 29% (denoted as PAH-g-PNIPAAm29), were

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**Scheme 1.** Schematic illustration to show the formation of robust PAH-g-PNIPAAm particle by incorporation of PSS. (a) PAH-g-PNIPAAm particle with porous structure formed above the LCST. Positively charged components cover the surface and interfaces of the particle. (b) Introduction of PSS will crosslink the particle by electrostatic force between the PAH segments and the PSS molecules. (c) The particle is reversibly deswelling and swelling in response to temperature.
synthesized by grafting carboxylic end-capped PNIPAAm (PNIPAAm-COOH, $M_w$ 2 800) onto PAH ($M_w$ 65 000, Aldrich) chains as reported previously. Previous study has demonstrated that the copolymers can form porous particles above the LCST (34 °C in water or 28 °C in 0.5 m NaCl). In a typical procedure to prepare the PSS ($M_w$ 70 000, Aldrich) crosslinked PAH-g-PNIPAAm particles, 0.5 of mL PAH-g-PNIPAAm/0.5 m NaCl solution (3 mg · mL$^{-1}$) and 1 mL of PSS/0.5 m NaCl solution (2 mg · mL$^{-1}$) were incubated in a water bath at 35 °C for 10 min, respectively. Then the PAH-g-PNIPAAm solution was added dropwise into the PSS solution under shaking at 35 °C. 10 min later, the solution was centrifuged at 200 rpm to remove larger polyelectrolyte complex. The particles in the supernatant were subjected to further studies.

Characterizations

A drop of particle suspension at 22 (room temperature) or 40 °C was applied onto newly cleaved mica with the same temperature. After drying at 22 or 40 °C, the samples were subjected to SFM characterization. Images were recorded in air using a Nanoscope III Multimode SFM (Digital Instrument Inc., Santa Barbara, CA).

The mass weighted hydrodynamic radius ($R_h$) as a function of temperature was measured by DLS on a Malvern High Performance Particle Sizer (HPPS 500). The machine is equipped with a He-Ne laser with a maximum output power of 3.0 mW at 633 nm and a detector of Avalanche photodiode (QE > 50% at 633 nm). Correlator has a minimum sample time of 125 ns and a maximum number of data channels of 560. The effective hydrodynamic diameter, that can be detected, ranges from 0.6 to 6 000 nm. Measurements of the autocorrelation functions were performed using a Signal processor of Non-Invasive Back-Scatter technology. Data points were collected from 3 to 125. DLS results were analyzed by a fit of a regularized mode to give a mass weighted size distribution using a Laplace inversion routine. The grid points were set at 75. At each temperature the samples were equilibrated at least for 10 min before measurement. All data were averaged from three to five measurements and expressed as mean ± standard deviation.

Zeta-potentials were tested at 25, 37, and 40 °C on a Malvern Zetasizer 4. All data were averaged from ten measurements.

Temperature-Tuned Release

Temperature-tuned release of the particles was checked by using R6G as a probe. 0.25 mL of R6G solution (1 mg · mL$^{-1}$) was mixed with 2 mL of particle solution. After the mixture was shaken for 1 h at room temperature, the solution was centrifuged at 8 000 rpm to remove the unloaded R6G. Then the R6G-loaded particles were re-dispersed in 1 mL of water. Two release protocols were selected to compare the release behavior at different temperatures. In protocol 1, 0.5 mL of R6G-loaded particle suspension was incubated at 5 °C and then at 50 °C under agitation, each for 2 h. In protocol 2, the incubation sequence was reversed, i.e., first at 50 °C then at 5 °C. The released R6G in each hour was quantified by measuring the absorbance of the supernatant by a Microplate Reader (Bio-Rad Model 550) at 570 nm. After each measurement, equal volume of water was supplied.

Results and Discussion

Morphology of the PAH-g-PNIPAAm/PSS Particles

It has been found that the PAH-g-PNIPAAm copolymers exhibit the LCST at 28 °C in 0.5 m NaCl. Crosslinking the particles at 35 °C will then endow the particles with enough stability to withstand a hydration force below the LCST, thus maintaining their topological structure. SFM characterization of the particles dried at 22 °C (room temperature) indeed revealed that the as-prepared sample contained well-separated particles [Figure 1(a) and (b)]. The average radius was 36 nm (minimum 5.5 nm, maximum 83 nm).

When the particle suspension was dried at 40 °C, a few particles with an average radius of 43 nm and a larger number of particles with an average radius of 12 nm were obtained [Figure 1(c) and (d)]. Moreover, few particles with radius above a 100 nm could also be found (image not shown). These values match well with that of their corresponding precursor particles, whose radii were measured as 35–40 nm and a few nanometers by SFM. The slight enlargement of the particles can be regarded as a sign of PSS adsorption. This has been actually demonstrated by zeta-potential measurement. At 25, 37, and 40 °C, almost the same zeta-potential values of −29.8 ± 0.4 mV, −30.4 ± 0.9 mV, and −30.9 ± 0.3 mV were determined, respectively. Since PAH-g-PNIPAAm is positively charged, the negative value must be exclusively attributed to the involvement of PSS. Moreover, the quite constant value as a function of temperature implies that the particle structure is rather robust regardless of its swelling or deswelling state.

Thermosensitivity of the PAH-g-PNIPAAm/PSS Particles

The SFM results have provided an indirect proof that the size of the PAH-g-PNIPAAm/PSS particles can respond to thermal stimuli. To elucidate the thermosensitivity of the PAH-g-PNIPAAm/PSS particles, DLS was employed to check the variation of hydrodynamic radius ($R_h$) as a function of temperature. Figure 2 compares the distributions of $R_h$ at temperatures of 32, 37, and 50 °C, respectively. When the temperature was ≤32 °C, two independent peaks centered at 81 nm (wt.-2%, calculated from the $R_h$ distribution curve) and 429 nm (wt.-98%) were detected [Figure 2(a)]. For simplicity, we denote the two kinds of particles as Particle 1 and Particle 2, respectively. After the temperature was elevated to 37 °C, a volume change occurred [Figure 2(b)]. In this case, three kinds of particles were detected with $R_h$ of 31 nm (wt.-1.5%), 250 nm (wt.-35%), and 1 500 nm (wt.-63.5%), respectively. Due to the deswelling of the thermosensitive PNIPAAm segments,
it is reasonable that the radii of the original Particle 1 and Particle 2 are decreased from 81 and 429 nm to 31 and 250 nm, respectively. The newly emerging particles with $R_h$ of 1500 nm (denoted as Particle 3) should be the result of particle accumulation at an elevated temperature. From the weight change of each kind of particles, one knows that this accumulation should occur mainly for Particle 2. A simple calculation also reveals that a particle with a radius of 1500 nm (Particle 3) contains $\geq 200$ particles with a radius of 250 nm (Particle 2). This can explain why these large particles with a higher weight ratio were rarely observed in the SFM image, since the number of Particle 2 was approximately 200 times larger than that of Particle 3. When the temperature was elevated to 50 °C, as a result of particle accumulation Particle 1 completely disappeared [Figure 2(c)]. The radii of Particle 2 and Particle 3 decreased to 152 (wt.-26.5%) and 904 nm (wt.-73.5%), respectively.

To further check the thermosensitivity of the particles, the particle radius was plotted during a temperature cycle. For clarity, in this section we consider only Particle 1 and Particle 2 as shown in Figure 3. For both Particle 1 and Particle 2, a temperature increase caused a sharp decrease of the particle volume between 32 and 33 °C. By contrary, a temperature decrease led to swelling of the particle again. Figure 3 shows that the volume change with very fast response due to deswelling-swelling is completely reversible during a temperature cycle. This is important because it reveals that the particles can provide temperature-tunable internal volume while maintain their topological structure. It is worth noting that the accumulation temperature was 33 °C, at which Particle 3 could be detected.

Temperature-Tuned Release

Researches show that graft copolymers can be made into robust microspheres by crosslinking. After loading a certain drug by physical adsorption, the microspheres exhibit a drug release property that is thermosensitive.[11] For the PAH-g-PNIPAAm/PSS particles, the response of the particle volume to thermal stimuli has readily provided the capability for the particles to release loaded substance with temperature-tunable behavior. To demonstrate the different release performance at swelling and deswelling
states, two protocols were selected and the results are summarized in Table 1. For both release protocols, the hourly released amount of Rd6G decreased as a function of time, and the total released amount was also very similar. When comparing their release process, however, differences can be found between protocol 1 and protocol 2. During the first 2 h, the release amount was inversely proportional to the environmental temperature, i.e., larger amount (Abs 0.0795) was released for protocol 1 at 5°C while smaller amount (Abs 0.056) for protocol 2 at 50°C. In the next 2 h, the incubation temperatures were exchanged either from 5 to 50°C (protocol 1) or from

Figure 2. Typical DLS spectra to show the hydrodynamic radius ($R_h$) and radius distribution of PAH-g-PNIPAAm/PSS particles at (a) 32°C, (b) 37°C, and (c) 50°C.

Figure 3. Hydrodynamic radius as a function of temperature during a temperature increase and decrease cycle. (a) Particle 1 and (b) Particle 2.
50 to 5°C (protocol 2). Again the release amount was inversely proportional to the environmental temperature. Interestingly, in protocol 2 there was only a slight decrease of the release amount in the third hour (Abs 0.022) from that in the second hour (Abs 0.0225). On the contrary, when the process was done oppositely, the release amount dropped dramatically (from Abs 0.03 to Abs 0.014). These results have demonstrated that at a temperature above the LCST of PNIPAAm, the polymer networks within the particles become more compact as a result of macroscopic collapse. Therefore, the release will proceed at a relatively slow rate. At lower temperature (below the LCST of PNIPAAm) the polymer network within the particles should be looser because of the swelling, which can then result in a faster release rate. This can explain the result in protocol 2 when the temperature was decreased from 50 to 5°C, since at 5°C the entrapped Rd6G could be more easily released as a result of particle swelling.

**Conclusion**

A facile strategy has been established to prepare stable thermosensitive particles from a copolymer of PAH-g-PNIPAAm. Here, instead of covalent crosslinking or other interactions, electrostatic force binds the positively charged PAH segments and the added negatively charged PSS. For this to occur, PAH-g-PNIPAAm particles are formed at a temperature above the LCST (28°C in 0.5 M NaCl). Then the particle suspension is dropped into a PSS solution with the same temperature. By crosslinking the PAH segments with PSS, particles stable at room temperature have thus been fabricated. Since the precursor PAH-g-PNIPAAm particles have a porous structure, the PSS molecules should thus penetrate into the particles and cover the particle surfaces, as partly evidenced by the negative zeta-potential of the resultant particles. SFM characterization observes well-separated particles dried at both room temperature and 40°C. DLS measures particles with hydrodynamic radii of several tens of nanometers and several hundred nanometers, which are largely reduced when the temperature is above the LCST of PNIPAAm. A temperature cycle causes completely reversible particle deswelling and swelling. A faster release rate below the LCST of PNIPAAm is recorded than that above the LCST. In conclusion, by crosslinking with PSS, stable PAH-g-PNIPAAm particles have been obtained with preserved thermosensitive properties, which might find some applications in drug delivery and other fields in the future.

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**Table 1. Release amount of Rd6G from the PAH-g-PNIPAAm/PSS particles.**

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Time (temperature)</th>
<th>Hourly release (Abs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protocol 1</td>
<td>1 h (5°C)</td>
<td>0.0495</td>
</tr>
<tr>
<td></td>
<td>2 h (5°C)</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>3 h (5°C)</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td>4 h (5°C)</td>
<td>0.006</td>
</tr>
<tr>
<td>Protocol 2</td>
<td>1 h (50°C)</td>
<td>0.0335</td>
</tr>
<tr>
<td></td>
<td>2 h (50°C)</td>
<td>0.0225</td>
</tr>
<tr>
<td></td>
<td>3 h (5°C)</td>
<td>0.022</td>
</tr>
<tr>
<td></td>
<td>4 h (5°C)</td>
<td>0.015</td>
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