Fabrication and characterization of bifunctional spherical polyelectrolyte brushes

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ABSTRACT
In this work, we prepared two kinds of dual-responsive spherical polyelectrolyte brushes (SPBs) with narrow distribution, of which cores were composed of polystyrene and grafting from copolymers of poly(N-isopropyl acrylamide-co-acrylic acid) (P(NIPAM-AA)) and poly(acrylic acid-co-N,N-diethyl acrylamide) (P(AA-DEA)), respectively. Both kinds of SPBs could response to pH and temperature stimulations by swelling/deswelling shell and electrophoretic mobility (EPM) changes. The low critical solution temperature of SPBs can be fine-tuned in the range of 28–45 °C at pH 3–9. Due to the different reactivity of comonomers (NIPAM and AA or DEA and AA), the acrylic acid (AA) units were distributed at the inner shell of P(AA-DEA) and outer shell of P(NIPAM-AA), respectively, which led to different responses toward pH, temperature, and ionic strength, such as the abnormal ‘salt effect’ of P(AA-DEA) at low ionic strength and lower shell shrinkage ratio (SR) of P(NIPAM-AA). The dye adsorption in SPBs further confirmed the distribution of AA units.

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Introduction
Multi-responsive nanoparticles have received extensive interest due to their smart characteristics, whose size, viscosity, solubility, aggregation, and structure can be modified by physical or chemical stimulations, such as pH, temperature, magnetism, and visible light.[1–5] They are widely applied in many fields, including biomaterials, sensor, drug delivery, and separation.[6–11] The spherical polyelectrolyte brushes (SPBs), prepared by photoemulsion polymerization, are stable colloid systems and sensitive to stimulations through functional polymer chains and modified cores.[4,12–15] SPBs are classified into three kinds: anionic, cationic, and nonionic according to the changes of polymer chains.[14–16] (i) The anionic SPBs, with poly(acrylic acid) (PAA) chains, are swelling at high pH values due to electrostatic repulsion between polymer chains.[14,17] These swollen layers could attract cationic metal ions and in situ generate metal nanoparticles with high catalytic activity, such as Ni.[18] (ii) The cationic SPBs exhibit opposite size variation under pH adjustment which is swelling at acid condition. The cationic SPBs are ideal nanoreactors for immobilization of Au and Pt nanoparticles [19–21] and protein releasing.[22] (iii) The nonionic SPBs are usually grafted with thermo-sensitive chains which are stretched and shrunk with temperature change. For example, the low critical solution temperature (LCST) of SPBs with poly(N-isopropylacrylamide) (PNIPAM) chains is about 32 °C.[15] All three kinds of SPBs could fast respond to the stimulations, and no aggregation is tested during the responding processes. However, the single response limited the applications of SPBs. Many efforts are devoted to the multi-responsive nanoparticles.

The dual-responsive core-shell particles have been studied by many researchers. Huang et al. used atomic force microscopy (AFM) to investigate the morphology and surface adhesion of SPBs with P(NIPAM-co-AA) chains. The increasing surface adhesion to AFM tip upon pH suggested that the AFM was a powerful method of the surface behavior in situ observation of brushes.[23] Tsuji et al. synthesized hairy nanoparticles with AB-type block copolymer of NIPAM and acrylic acid (AA) by living radical graft polymerization. The hairy particles exhibited different response to temperature as the sequence of NIPAM an AA blocks in polymer chains was reversed. [24,25] Their works are focused on the behavior of block copolymer chains toward stimulations, how about the uneven distribution of AA units in copolymer chains? Is their temperature response similar to block copolymer or has distinct properties? If there are some differences, could it be extended to other copolymer? Our work focused on
Fluka), sodium dodecyl sulfate (SDS; J&K), potassium peroxodisulfate (KPS; J&K), 2-(N-morpholino) ethanesulfonic acid (MES; J&K), and sodium chloride (NaCl; J&K) were used without further purification. Styrene (St) and AA were purchased from Sigma-Aldrich, distilled under reduced pressure to remove the inhibitor, and stored in refrigerator at 4 °C before use. The photoinitiator 2-[p-(2-hydroxy-2-methylpropio-phenone)]-ethyleneglycol-methacrylate (HMEM) was synthesized according to our previous publication.

The water used in all experiments was purified with Millipore Milli-Q system, and the resistivity was above 18 MΩ cm.

**Synthesis of core–shell nanoparticles**

SPBs were synthesized by photoemulsion polymerization as illustrated in Figure 1, which were similar to the previously report.[12] Please see supporting information for more details.

**Samples preparation**

The ionic strength was adjusted by NaCl solution. The pH was adjusted by 5 mM buffer (MES for pH between 5.0 and 6.5) aqueous. Solution of NaOH (0.1 M) and HCl (0.1 M) was also prepared to adjust pH values. The pH was detected using Crison (GLP 21) pH meter.

**Characterization**

The diameter and electrophoretic mobility (EPM) of SPBs were determined by dynamic light scatting (DLS, NICOMP380) instrument. DLS was performed by Peters ALV 4000 light scattering goniometry with the angle of 90°. The hydrodynamic diameter of nanoparticles can be

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**Figure 1.** Schematic representation of the synthesis of SPBs.
determined by DLS at fully temperature range. UV–vis absorption spectra of samples were recorded on ultraviolet spectrophotometer (UV-spectra, Shimadzu UV 2550).

**Results and discussion**

The synthesis procedure of SPBs included three steps in Figure 1: synthesis of PS cores, photoinitiator coating, and ‘grafting-from’ process of polymer chains. The PS cores were prepared by emulsion polymerization and then polymerized with a thin layer of photoinitiator (HMEM). It should be emphasized that the photoinitiator was added under starved conditions to ensure the well-defined morphology of PS@HMEM. The diameter of PS@HMEM was about 90 nm as shown in Figure S1. Finally, monomers were added into diluted PS@HMEM, and the mixture was exposed to the UV light for 60 min at room temperature. The obtained SPBs were purified for further analysis.

In the experimental section, NIPAM and AA or DEA and AA monomers were mixed and added into the PS@HMEM solution together. Both of them were prone to be polymerized from the surface of PS cores. Which block, NIPAM or AA and DEA or AA would win that chance? The reactivity of comonomers determined the sequence of blocks. According to literatures,[35,36] the sequence of reactivity of comonomers was the following: NIPAM > AA > DEA. In other words, NIPAM had priority to be grafted from the surface of PS cores as competing with AA monomer and then polymerized with AA at the last stage of polymerization. However, there was a kind of random polymerization and small amount of AA was grafted in the inner block. So the AA monomer was supposed to be densely distributed at the outer shell of P(NIPAM-AA) and sparsely copolymerized with NIPAM inside the shell as shown in Figure 2A. As to P( AA-DEA), it was another story. The reactivity of AA was relatively higher than DEA which meant that AA monomer tended to be firstly grafted from PS cores and dispersed inner shell of P(AA-DEA) as shown in Figure 2B, which was opposite to that of P(NIPAM-AA).[37] The different charge distribution caused the SPBs to behave differently toward physical and chemical stimulations.

As increasing the ratio of AA (relative to the mole of NIPAM) from 0 to 5%, the diameter of P(NIPAM-AA) and P(AA-DEA) showed distinct variation in Figure 3. The diameter of P(NIPAM-AA) increased upon AA incorporation. For example, the diameter was about 450 nm as the ratio of AA was 0.5%, which was nearly twice that of without AA addition. The largely increasing dimension of P(NIPAM-AA) was ascribed to the stretch of P(NIPAM-co-AA) chains rather than chain growth, because the addition of AA was too small for long-chain propagation. This phenomenon was different from hairy particles in kawaguchi et al’s work, which AA block was only at the end of P(NIPAM-b-AA) chains.[24] The diameter of this hairy particles was unchangeable with AA addition until the ratio of AA was 3%. This difference was reasonable for the low charge density in the shell of hairy nanoparticles in kawaguchi et al’s work. The AA units were strictly at the end of PNPAM chains of which the length was 300 nm, and the grafting density was referred as about 0.03 nm\(^{-2}\).[25] The charges density was so low that the repulsion was too weak to stretch the P(NIPAM-b-AA) chains. In SPBs systems, though AA units were also dispersed at the outer shell and the grafting density was about 0.03 nm\(^{-2}\), the length of PNPAM chains was only 70 nm and some AA units were located at the inner shell. The charges of SPBs should be much denser and could repel each other to stretch the polymer chains to a large degree.[17]

On the contrary, the diameter of P(AA-DEA) decreased from 258 to 172 nm as the ratio of AA increased from 0 to 5%. It was rare that the diameter of nanoparticles decreased with monomers increasing [12] except cross-linkers which could contract the polymer chains by formation of physical or chemical bondings.[15] So the inner shell distributed AA units probably acted as cross-linkers that formed numbers of strong physical cross-links to prevent the polymer chains
were swollen again in the cooling process. The coil-to-globule process of P(NIPAM) was completed in 3 °C in the heating process, which was much sharper than 6 °C of P(DEA).[39] The slight hysteresis of globule-to-coil transition (cooling process) of P(NIPAM) appeared as comparing with the coil-to-globule transition (heating process), while the coil-to-globule-to-coil processes were superposed of P(DEA) at full temperature range.[28] The differences in response rate and coil-to-globule-to-coil transition were due to distinct chemical structure of NIPAM and DEA. The lack of amide hydrogen of DEA could only act as hydrogen accepter rather than both hydrogen accepter and donator of secondary amide of NIPAM, so it could not form intramolecular hydrogen bonds which could promote the contraction of polymer chains. So the volume transition of P(DEA) was relatively continuous comparing with abrupt volume change of P(NIPAM). These intramolecular hydrogen bonds of P(NIPAM) also resulted from swelling.[38] These cross-links were also responsible for the unique ionic strength response of P(AA-DEA) in the next section.

**Effect of ionic strength on SPBs**

Figure 4 shows the diameter of P(NIPAM-AA) and P(AA-DEA) as a function of ionic strength. As the ionic strength increased from 0.01 to 100 mM, the diameter of P(NIPAM) and P(DEA) kept constant indicating the insensitivity to ionic strength if without AA incorporation. As for P(NIPAM-AA), the diameter largely decreased with ionic strength due to the ‘salt screening effect’ and was close to that of P(NIPAM) as ionic strength was 100 mM in Figure 4A.[14] For the case with P(AA-DEA) system, the diameter of P(AA-DEA) highly increased at low ionic strength and then decreased gradually with ionic strength in Figure 4B. This abnormal salt effect at low ionic strength was probably due to partial replacement of protons within the shell by salt ions, which consequently caused the dissociation of carboxylic groups and osmotic pressure increasing that stretched polymer chains.[14] Another reason was that the cross-links between monomers were releasing as salt ions effectively competed with DEA and BIS, which extended the polymer layers. However, further increasing ionic strength could induce the increase in osmotic pressure in solution and caused shrinkage of the shell.

**LCST of P(NIPAM) and P(DEA)**

As we know, thermo-sensitive particles have coil-to-globule transition above LCST due to the disruption of hydrogen bonds between polymer chains and water molecules. Figure 5 shows the temperature-dependent diameter of P(NIPAM) and P(DEA) within the heating and cooling processes. Both P(NIPAM) and P(DEA) had obvious shrinkage at 32 °C and 28 °C, respectively, and were swollen again in the cooling process. The coil-to-globule process of P(NIPAM) was completed in 3 °C in the heating process, which was much sharper than 6 °C of P(DEA).[39] The slight hysteresis of globule-to-coil transition (cooling process) of P(NIPAM) appeared as comparing with the coil-to-globule transition (heating process), while the coil-to-globule-to-coil processes were superposed of P(DEA) at full temperature range.[28] The differences in response rate and coil-to-globule-to-coil transition were due to distinct chemical structure of NIPAM and DEA. The lack of amide hydrogen of DEA could only act as hydrogen accepter rather than both hydrogen accepter and donator of secondary amide of NIPAM, so it could not form intramolecular hydrogen bonds which could promote the contraction of polymer chains. So the volume transition of P(DEA) was relatively continuous comparing with abrupt volume change of P(NIPAM). These intramolecular hydrogen bonds of P(NIPAM) also resulted

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**Figure 4.** Diameter of P(NIPAM-AA) (A) and P(AA-DEA) (B) with the increase in ionic strength.

**Figure 5.** The diameter of P(NIPAM) (filled circle heating process, unfilled circle cooling process) and P(DEA) (filled triangle heating process, unfilled triangle cooling process) and with temperature, I = 5 mM. The inset image was the diameter of P(NIPAM) and P(DEA) around LCST.
in a small hysteresis in heating and cooling process that retarded the swelling of shell as temperature decreases.[40] Both P(NIPAM) and P(DEA) were stable and monodisperse during heating and cooling process and no aggregation was tested at full temperature (inset of Figure 5).

AA effect on LCST and diameter of SPBs

There are various ways to tune the LCST of PNIPAM and PDEA.[41–45] Adding comonomer to alter the hydrophilic/hydrophobic balance of polymers is one of the effective ways.[46] In this work, we incorporated AA monomer into thermo-sensitive polymer chains to adjust the hydrophilic/hydrophobic balance of PNIPAM and PDEA chains. Herein, we introduced shell shrinkage ratio (SR) as one of parameters to evaluate the thermo-sensitivity of SPBs. $SR = \frac{V_{shell}}{V_{shell}'}$ (swollen state)/$V_{shell}'$ (collapsed state), $V_{shell}$ was the shell volume of nanoparticle.

Figure 6 shows the temperature effect on the diameter of P(NIPAM-AA)-0.5, 1, 5% at different pH values. The LCST of P(NIPAM-AA) was well-tuned in a wide range as the ratio of AA increased from 0.5 to 5%. For example, the P(NIPAM-AA)-0.5% had volume transition in the range of 29–43 °C as pH increased from 3 to 9 in Figure 6A and in the range of 27–48 °C of P(NIPAM-AA)-1% in Figure 6B. The dissociated AA units increased the hydrophilic of P(NIPAM-co-AA) chains that enhanced the LCST of P(NIPAM-AA) at high pH values, but the P(NIPAM-AA) became dull to temperature change as the ratio of AA was more than 1%. For example, the diameter of P(NIPAM-AA)-5% was almost constant with temperature increasing at pH 9 in Figure 6C. The affinity between water molecules and P(NIPAM-co-AA) chains enhanced with AA addition, but too strong would deprive the thermo-sensitivity of P(NIPAM-AA). 5% addition of AA could seriously retard the shrinkage of P(NIPAM-AA) above pH 7, suggesting the importance of AA addition for retaining the thermo-sensitivity of P(NIPAM-AA). So the P(NIPAM-AA) exhibited both pH and temperature sensitivity at full pH range as the ratio of AA was below 1%. It should be noted that the two-stage volume transition appeared in the P(NIPAM-AA)-0.5% at pH 5 which was discussed later.

Figure 6. The diameter of P(NIPAM-AA) nanoparticle with temperature (A) P(NIPAM-AA)-0.5% (B) P(NIPAM-AA)-1% (C) P(NIPAM-AA)-5%. It was measured at (■) pH 3, (○) pH 5, (▲) pH 7 (▽) pH 9, $i = 5$ mM.

Figure 7. The diameter of P(NIPAM-AA) with temperature at pH (A) 3, (B) 5, (C) 7, $i = 5$ mM. (■) P(NIPAM), (○) P(NIPAM-AA)-0.25%, (▲) P(NIPAM-AA)-0.5%, (▽) P(NIPAM-AA)-1% (◆) P(NIPAM-AA)-5%. The inset Figure in (A) was the enlarged version of A.
The preparation of P(AA-DEA) was in a similar method as P(NIPAM-AA). Due to the inner shell distribution of AA units, the P(AA-DEA) exhibited some differences of pH and temperature responses with P(NIPAM-AA).

Figure 8A–C displayed the temperature effect on diameter of P(AA-DEA)-0.5, 1, 5%. The AA incorporation could adjust the LCST of P(AA-DEA) in a wide range as well as P(NIPAM-AA). The LCST of P(AA-DEA)-0.5% was in the range of 27–34 °C and 26–45 °C of P(AA-DEA)-1% at pH 3–9. The P(AA-DEA)-1% had the two-stage volume transition at pH 7 and 9 which would be discussed later. Figure 8A, B, C illustrated the diameter of P(AA-DEA) versus temperature at different pH values. Due to the pKa of P(AA-DEA) was above 5,[44] the LCST decreased with increment of AA at pH 3 and 5. For example, the LCST of P(AA-DEA)-5% decreased to 25 °C at pH 3. Above pH 5, the LCST could be enhanced with AA incorporation in Figure 8C. Both P(AA-DEA)-1% and P(AA-DEA)-2% appeared as two-stage volume transition at pH 7. For example, the LCST of P(AA-DEA)-1% was 34 and 43 °C, respectively. The two-stage volume transition had been observed in hairy particles with block copolymer chains by Tsuji et al.[47] In the P(AA-DEA) system, the AA units were densely located at the inner shell that highly increased the LCST of the second stage. Different from Tsuji’s work, the LCST of the first stage slightly increased for sparse AA distribution in the outer shell, the character

![Figure 8](image-url)
nanoparticles if the shell SR was above 30%. The SR of P(NIPAM-AA) and P(AA-DEA) at different pH values was illustrated in Figure 10. The SR of both P(NIPAM) and P(DEA) could reach 95% at full pH range indicating the complete collapse of shell and high thermo-sensitivity. As the ratio of AA increased, the SR of P(NIPAM-AA) and P(AA-DEA) largely decreased if the pH was above pKa as shown in Figure 10A. As the ratio of AA increased from 0.25 to 5%, the SR of P(NIPAM-AA) decreased from 94 to 18% at pH 7 and from 86 to 0.6% at pH 9. The low SR of P(NIPAM-AA)-5% implied the bluntness toward temperature stimulation above pH 7.

As for P(AA-DEA), all the SR was above 30% except that of P(AA-DEA)-5% at pH 9 in Figure 10B. With the same ratio of AA, the SR of P(NIPAM-AA) was smaller than that of P(AA-DEA), which meant the AA units exhibited larger effect on P(NIPAM-AA). For example, the SR of P(AA-DEA)-0.5% was about 85% while only 66% of P(NIPAM-AA)-0.5% at pH 9. It was ascribed to the outer shell AA distribution of P(NIPAM-AA) which had more mobility to prevent polymer layers from shrinkage than inner shell distribution of P(AA-DEA).[35]

Cationic dye adsorption of SPBs

Cationic Basic Violet 14 dye was introduced to be adsorbed in the shell of P(NIPAM-AA)-0.5% and P(AA-DEA)-0.5% with temperature increment in Figure 11. There are three factors determined the dye adsorption, including electrostatic interaction, volume of layers, and hydrophobicity. The electrostatic interaction between
due to more hydrophobicity of DEA. It should be noted that the P(NIPAM-AA)-0.5% reached the platform at 43 °C which was earlier than 45 °C that of platform of EPM. The asynchronous change of EPM and adsorption was caused by collapsed layers which limited the capacity of dye adsorption.

**Conclusions**

In this work, pH- and thermo-sensitive SPBs (P(NIPAM-AA) and P(AA-DEA)) with narrow distribution were synthesized by photoemulsion polymerization. Due to different activity of comonomers (AA and NIPAM or AA and DEA), the AA units were densely distributed in the inner shell of P(AA-DEA) and outer shell of P(NIPAM-AA). This distinct distribution of AA units led to different responses to ionic strength, temperature, and pH stimulations. The LCST of both P(NIPAM-AA) and P(AA-DEA) could be tuned in the wide range by the ratio of AA and pH values. Besides, the temperature-dependent dye adsorption was further proved the charges distribution of P(NIPAM-AA) and P(AA-DEA). This work provided valuable suggestions for synthesis and application of dual-responsive SPBs with asymmetrical charges distribution. This simple synthetic method of bifunctional nanoparticles could be extended to other wisdom copolymer synthesis and applied in the catalytic environment and biomaterial engineering.

**Supporting information**

All experimental details and diameter of SPBs with temperature increment at different ionic strength were shown in supporting information.

**Disclosure statement**

No potential conflict of interest was reported by the authors.
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