

Note

Preparation and Characterization of Thermoresponsive Hyperbranched Polyethylenimine with Plenty of Reactive Primary Amine Groups*

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Abstract Certain amount of primary amine (NH_2) groups of hyperbranched polyethylenimine (HPEI) was first protected by Boc groups. Subsequently, the residual reactive amine groups were reacted with isobutyric anhydride to introduce isobutyramide (IBAm) groups to HPEI. Finally, Boc groups were deprotected to result in HPEI-IBAm- NH_2 with 18% of primary amine terminals on the periphery and 80% of IBAm terminal groups (abbreviated as HPEI-IBAm_{0.80}- NH_2). ^1H -NMR characterization proved the successful preparation of the product in each step. Compared with its spatial isomer HPEI-IBAm_{0.80} without primary amine groups, ^1H -NMR spectra verified that more IBAm groups were located in the interior of HPEI-IBAm_{0.80}- NH_2 . The further modification of HPEI-IBAm_{0.80}- NH_2 and HPEI-IBAm_{0.80} with *p*-nitrobenzaldehyde demonstrated that HPEI-IBAm_{0.80}- NH_2 was more reactive than HPEI-IBAm_{0.80} due to its possession of primary amines. Turbidimetry measurements showed that HPEI-IBAm_{0.80}- NH_2 was thermoresponsive in water. In the pH range of 9.5–10 its cloud point temperature (T_{cp}) was constant, and it increased obviously upon decreasing the pH below 9.5. The thermoresponsive HPEI-IBAm_{0.8} exhibited the similar trend, but the pH threshold to achieve the constant T_{cp} was around 8.5. Moreover, HPEI-IBAm_{0.8}- NH_2 showed higher T_{cp} and broader phase transition than HPEI-IBAm_{0.8}. The mechanism leading to the different thermoresponsive properties between HPEI-IBAm_{0.8}- NH_2 and its spatial isomer HPEI-IBAm_{0.8} was discussed.

Keywords: Hyperbranched; Phase transition; Polyethylenimine; Spatial isomer; Thermoresponsive polymers.

INTRODUCTION

During the past decade, polymers with stimuli-responsive properties, such as fast and reversible conformational or phase changes in response to variations in temperature and/or pH, have attracted much interest^[1]. An appealing stimuli-responsive species is thermoresponsive polymers with a lower critical solution temperature (LCST) in aqueous solutions, which show a large decrease in solubility in water above a specific temperature^[2–4].

In 2004 Kono's group reported their pioneering work on the thermoresponsive dendrimers whose LCST could be tuned in a broad range by varying the molecular weight^[5]. Following their pioneering work, more kinds of thermoresponsive dendritic polymers were reported^[6–18]. It was found that the thermoresponsive dendritic polymers as a new member of the family of thermoresponsive polymers exhibited the obvious difference in properties compared with the traditional thermoresponsive linear polymers^[5, 18–20]. For instance, the phase transition temperature of the compact hyperbranched polymers exhibited higher sensitivity to ions than that of the traditional thermoresponsive linear polymers^[18, 19].

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Hyperbranched polyethylenimine (HPEI) is a well-known functional polymer and its application fields are very wide^[21–23]. Endowing HPEI with a further thermoresponsive property will definitely expand its application^[24–27]. It is well-known that adjusting the contents of hydrophilic/hydrophobic units in thermoresponsive polymers is an effective method to modulate the phase transition temperature. Until now, certain hydrophobic units have been reported by us to successfully render the hydrophilic HPEI thermoresponsive property through partial modification of its reactive amino groups^[15–18]. Unlike dendrimers, the degree of branchings of hyperbranched polymers is usually less than 100%, that is, hyperbranched polymers consist of terminal, linear and dendritic structures, and the functional end-groups coming from the terminal and linear structure are located not only in the periphery of the spheroid-like materials, but also in the inner parts. Due to the steric hindrance effect, it can be expected that the modification reaction for the end-groups of hyperbranched polymers starts preferentially from the periphery to the interior. The residual interior functional groups are therefore less reactive and more difficult for the further manipulation. In this contribution we attempted to transform the less-reactive interior amino groups of HPEI into the isobutyramide (IBAm) groups that are mainly responsible for the thermoresponsive property of HPEI, while many reactive primary amine groups mainly located in the periphery were left. Moreover, we found that modulating the spatial distribution of IBAm units in thermoresponsive hyperbranched polymer was also a good method to alter the phase transition temperature.

EXPERIMENTAL

Nomenclature

HPEI-IBAm_x represents the traditional HPEI terminated with plenty of IBAm groups, in which all the outer amino groups are replaced by IBAm groups, and *x* means the degree of substitution (DS) relative to the total reactive primary and secondary amines of HPEI. HPEI-IBAm-NH₂ represents the new HPEI terminated with plenty of IBAm and primary amine (NH₂) groups. Boc-HPEI represents Boc protected HPEI. Boc-HPEI-IBAm represents Boc protected HPEI-IBAm.

Materials

Hyperbranched polyethylenimine (Aldrich, $M_n = 1.0 \times 10^4$ g/mol, $M_w/M_n = 2.5$) was dried under vacuum prior to use. Isobutyric anhydride (97%), trifluoroacetic acid (99%), *p*-nitrobenzaldehyde (99%) and Boc anhydride (97%) were purchased from Alfa Aesar and used without further purification. Benzoylated cellulose tubing (MWCO 1200) was purchased from Sigma and used directly. Triethyl amine (A.R., TEA) was dried over CaH₂ and distilled before use. De-ionized water was double-distilled before use. The traditional HPEI-IBAm was prepared as that reported previously through the one-step amidation reaction between HPEI and isobutyric anhydride^[19].

Procedure for Synthesis of Boc_{0.18}-HPEI

Under nitrogen atmosphere, Boc anhydride (1.48 g, 6.78 mmol) in 10 mL of anhydrous chloroform was added dropwise to the solution of HPEI (2.00 g, 34.0 mmol of terminal groups) in 25 mL of anhydrous chloroform at 0 °C with vigorous stirring. Subsequently, the reaction mixture was stirred at 35 °C for 9 h. The chloroform was removed under vacuum. The residue was purified by dialysis against methanol using a benzoylated cellulose membrane (MWCO 1200 g/mol) for 3 days. Finally, the methanol solvent was removed under vacuum, and the product was dried in vacuum for 24 h.

Procedure for Synthesis of Boc_{0.18}-HPEI-IBAm_{0.80}

Under nitrogen atmosphere, isobutyric anhydride (4.54 g, 28.7 mmol) was added dropwise to the mixture of Boc_{0.18}-HPEI (1.89 g) and triethyl amine (4.35 g, 43.0 mmol) in 20 mL of chloroform at room temperature with vigorous stirring. Subsequently, the reaction mixture was kept at room temperature for 24 h. Finally, the reaction temperature was raised to 72 °C for 2 h to finalize the reaction. The chloroform was removed under vacuum and the residue was dissolved in 35 mL of methanol. 3 g of potassium carbonate was added to the solution and the

mixture was stirred at room temperature for 4 h. After centrifugation, the solution was concentrated to around 10 mL and then purified by dialysis against methanol using a benzoylated cellulose membrane (MWCO 1200 g/mol) for 3 days. Finally, the methanol solvent was removed under vacuum, and the product was dried in vacuum for 24 h.

Procedure for Synthesis of HPEI-IBAm_{0.80}-NH₂

Under nitrogen atmosphere, trifluoroacetic acid (8.17 g) in 10 mL of anhydrous chloroform was added dropwise to the solution of Boc_{0.18}-HPEI-IBAm_{0.80} (2.69 g) in 25 mL of anhydrous chloroform at room temperature with vigorous stirring. Subsequently, the reaction mixture was stirred at room temperature for 12 h. The chloroform was removed under vacuum and the residue was dissolved in 35 mL of methanol. Three grams of potassium carbonate was added to the solution and the mixture was stirred at room temperature for 4 h. After centrifugation, the solution was concentrated to *ca.* 10 mL and then purified by dialysis against methanol using a benzoylated cellulose membrane (MWCO 1200 g/mol) for 3 days. Finally, the methanol solvent was removed under vacuum, and the product was dried in vacuum for 24 h.

Modification of HPEI-IBAm_{0.80}-NH₂ with *p*-Nitrobenzaldehyde

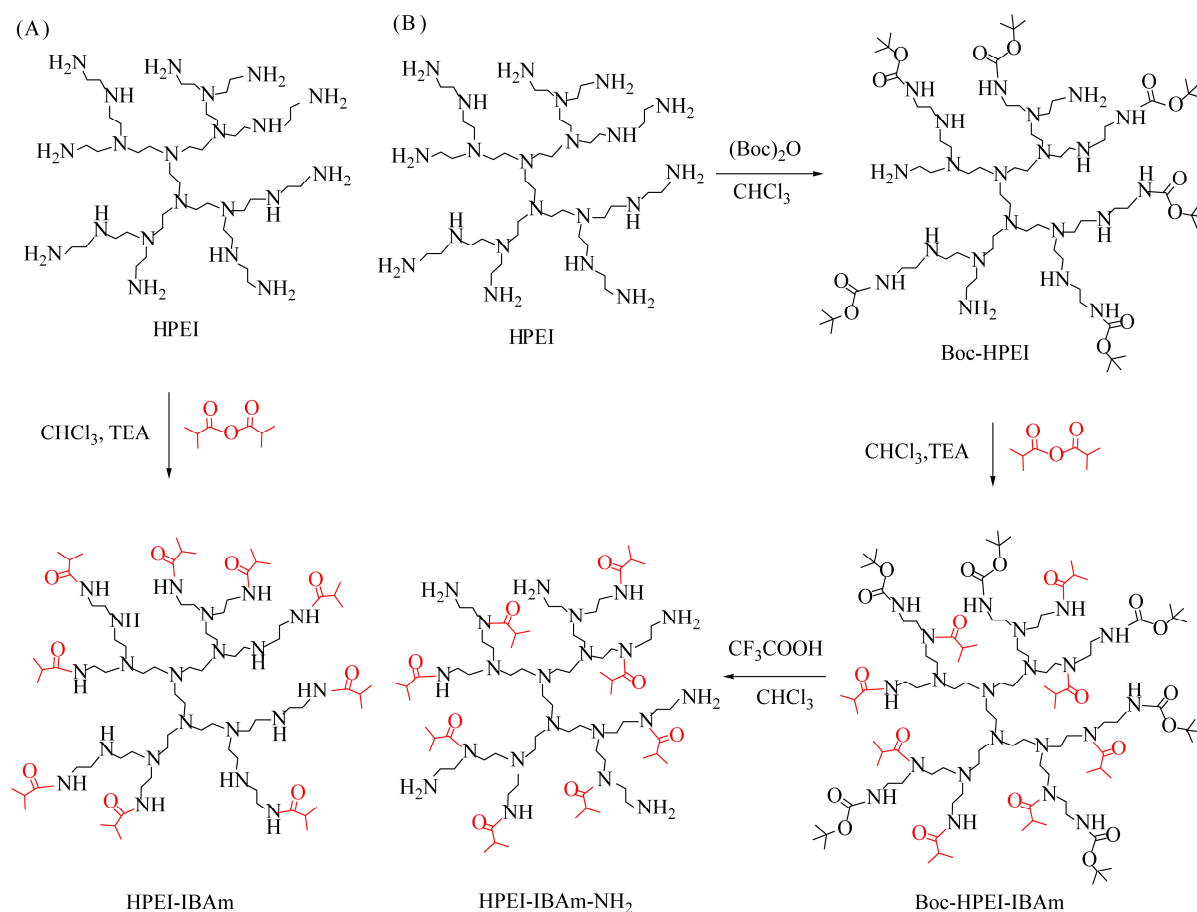
Under nitrogen atmosphere, *p*-nitrobenzaldehyde (78.6 mg) in 15 mL of anhydrous chloroform was added dropwise to the solution of HPEI-IBAm_{0.80}-NH₂ (0.760 g) in 15 mL of anhydrous chloroform at room temperature with vigorous stirring over a period of 2 h. Subsequently, the reaction temperature was raised to 65 °C for 3 h. Finally, the reaction temperature was raised to 85 °C for another 3 h, and the water byproduct was removed through the Dean-Stark.

Characterization

¹H-NMR spectra were recorded at 25 °C on a Varian INOVA spectrometer, operated at 500 MHz. Light transmittance of the polymer solution was measured on a temperature-controlled Purkinje General (China) T6 UV/Vis Spectrophotometer at 660 nm, and the heating rate was 0.5 K/2 min. The cloud-point temperature (*T*_{cp}) was taken from the intersection of the maximal slope tangent and the initial horizontal tangent in the resulting transmittance versus temperature curve. Sharpness of the phase transition ($\Delta T_{1/2}$) is defined as half the values of temperature difference of final break point with initial break point in the transmittance versus temperature curves. The temperature error is ± 0.2 °C.

RESULTS AND DISCUSSION

Commercially available HPEI with $M_n = 1.0 \times 10^4$ g/mol contains primary, secondary and tertiary amine functional groups. Comparing the integrals of carbons adjacent to primary, secondary and tertiary amine groups in the inverted gate ¹³C-NMR spectra^[28], the ratio of primary, secondary and tertiary amines was found to be 33:40:27. In the previous publication, thermoresponsive HPEIs with different amount of IBAm groups (normal HPEI-IBAm) were prepared through the one-step amidation reaction between the primary/secondary amines and isobutyric anhydride (Scheme 1A)^[18, 19]. For the HPEI-IBAm polymers with DS of IBAm less than 100%, it can be expected that the outer primary amines are more reactive toward isobutyric anhydride due to steric hindrance effect, thus only the inner less reactive secondary amines are left in HPEI-IBAm after the modification reaction, which has been also verified by the previous 2D ¹H-¹H NOESY NMR characterization^[29]. Since primary amine is more reactive and its reaction types are more versatile, it is more meaningful for certain primary amines to be left in the thermoresponsive HPEI. Targeted by such aim, three-step modification reactions were adopted (Scheme 1B). First, certain amount of primary amines of HPEI were reacted with Boc anhydride, resulting in Boc protected HPEI (Boc-HPEI). Subsequently, the residual inner amines in Boc-HPEI were reacted with isobutyric anhydride to result in Boc-HPEI-IBAm. Finally the protecting Boc groups were deprotected by trifluoroacetic acid, leading to the targeted HPEI-IBAm-NH₂.



Scheme 1 Synthetic procedures for HPEI-IBAm and HPEI-IBAm-NH₂

The product in each step was characterized by ¹H-NMR (Fig. 1). It is known that the ethylene protons of neat HPEI appear in the range of $\delta = 2\text{--}3$ ^[15]. The new broad singlet at around $\delta = 1.4$ and the new broad multiple signals between $\delta = 3.0$ and $\delta = 3.9$ in Fig. 1(A) can be assigned as the methyl protons of Boc units and the methylene protons of HPEI moiety directly connected with Boc units, respectively, pointing out the successful preparation of Boc-HPEI. Compared with Fig. 1(A), a new broad singlet appears at around $\delta = 1.1$ in Fig. 1(B), and it can be assigned as the methyl protons of IBAm moieties. Moreover, the intensity of the broad multiple signals between $\delta = 3.0$ and $\delta = 3.9$ becomes stronger, indicating that more amide groups are formed due to the successful attachment of IBAm units to HPEI. The signal of methine proton of IBAm moiety is overlapped with that of HPEI scaffold since its chemical shift is also in the range of $\delta = 2\text{--}3$ ^[29]. All these evidences prove that Boc-HPEI-IBAm is successfully prepared. In Fig. 1(C) the signal at around $\delta = 1.4$ disappears totally, while the other signals from the IBAm and HPEI moieties are still there, demonstrating that Boc groups are effectively deprotected and HPEI-IBAm-NH₂ is formed. From the integrals of the signals coming from Boc, IBAm and HPEI moieties in the ¹H-NMR spectra (Figs. 1A–1C), the average DS values can be calculated. The DS value of Boc unit in the obtained Boc-HPEI is around 18%, while the DS value of IBAm unit in Boc-HPEI-IBAm is around 80%. Thus, it can be deduced that the terminal groups of the resulting HPEI-IBAm-NH₂ contain around 80% of IBAm groups, 18% of primary amines and 2% of secondary amines. For comparison, the traditional HPEI-IBAm with the same DS value of IBAm unit was prepared, and its ¹H-NMR spectrum is shown in Fig. 1(D). Figure 1(C) exhibits a minor difference with Fig. 1(D) in the intensity of the signal at around $\delta = 2.9$. It is known that the signals of methine protons of IBAm moieties at the periphery of HPEI-IBAm appear at

around $\delta = 2.4$, whereas those in the interior occur at around $\delta = 2.9$ ^[29]. Thus, the higher intensity of the signal at $\delta = 2.9$ in Fig. 1(C) implies that HPEI-IBAm_{0.80}-NH₂ has more IBAm groups located in the interior than its spatial isomer HPEI-IBAm_{0.80}. This further supports that there are certain amount of primary amines in HPEI-IBAm_{0.80}-NH₂.

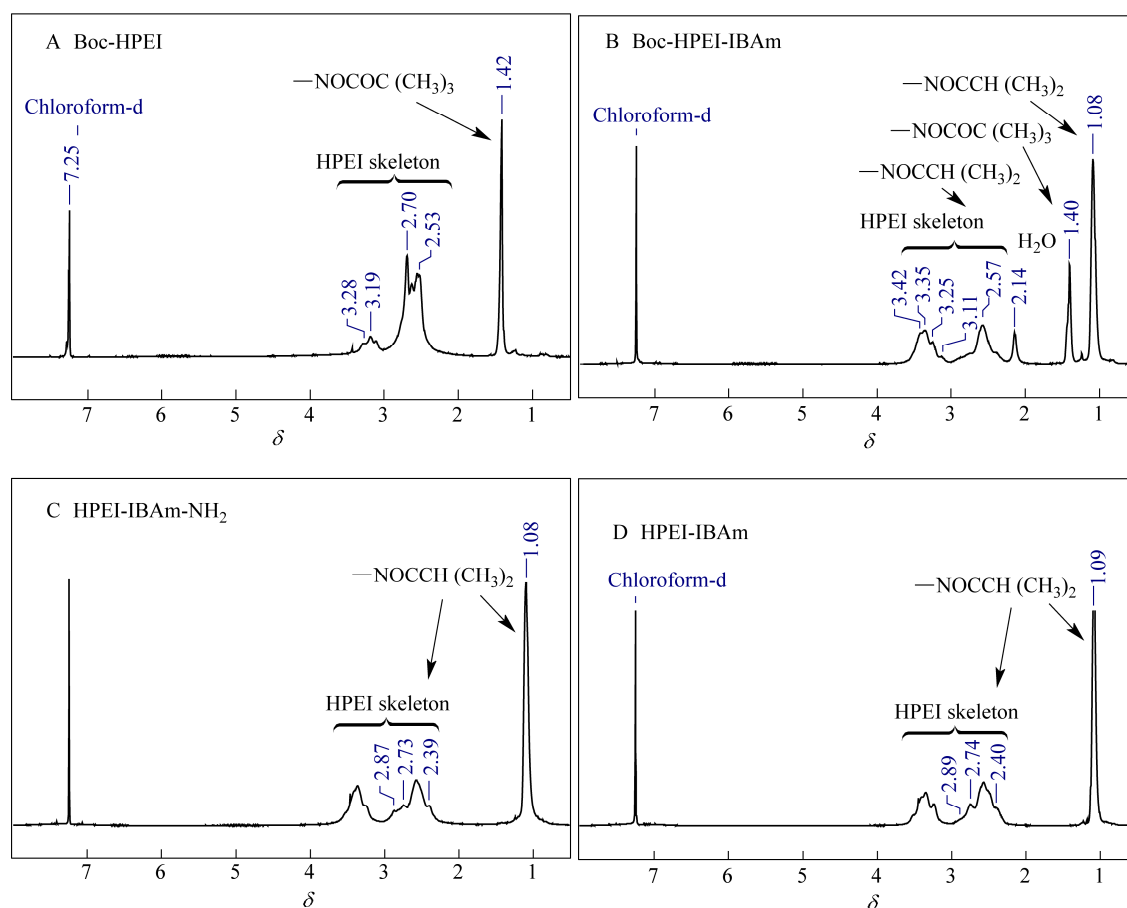


Fig. 1 Typical ¹H-NMR spectra of (A) Boc-HPEI, (B) Boc-HPEI-IBAm, (C) HPEI-IBAm-NH₂ and (D) HPEI-IBAm in CDCl₃

It is well known that primary amine can react with aldehyde to form imine, whereas secondary amine cannot. Thus, *p*-nitrobenzaldehyde was used to react with HPEI-IBAm_{0.80}-NH₂ and its isomer HPEI-IBAm_{0.80} without adding any catalyst. The final mixtures were directly analyzed by ¹H-NMR (Fig. 2). It is clear that *p*-nitrobenzaldehyde can be anchored onto both HPEI-IBAm_{0.80}-NH₂ and HPEI-IBAm_{0.80}. With the integral of methyl protons of IBAm groups (around $\delta = 1.1$) as the standard, it can be known that HPEI-IBAm_{0.80}-NH₂ links more *p*-nitrobenzaldehyde units than HPEI-IBAm_{0.80}, indicating HPEI-IBAm_{0.80}-NH₂ is more reactive than HPEI-IBAm_{0.80}. The ortho-position protons (around $\delta = 8.1$) of *p*-nitrobenzaldehyde is more sensitive to the variation of its ketal group. When ketal group is transformed into imine unit, conjugation situation does not change, but electron-withdrawing effect becomes a little weaker. Therefore, the chemical shift of the ortho-position protons moves upfield a little from *ca.* 8.1 to 7.9 for HPEI-IBAm_{0.80}-NH₂ linked with *p*-nitrobenzaldehyde units. With respect to HPEI-IBAm_{0.80} linked with *p*-nitrobenzaldehyde units, the chemical shift of the ortho-position protons moves upfield to 7.6, which can be attributed to the destruction of long conjugation due to the reaction between ketal group and secondary amine. The above results demonstrate that HPEI-IBAm_{0.80}-NH₂ still bears many reactive primary amines, whereas its isomer HPEI-IBAm_{0.80} does not.

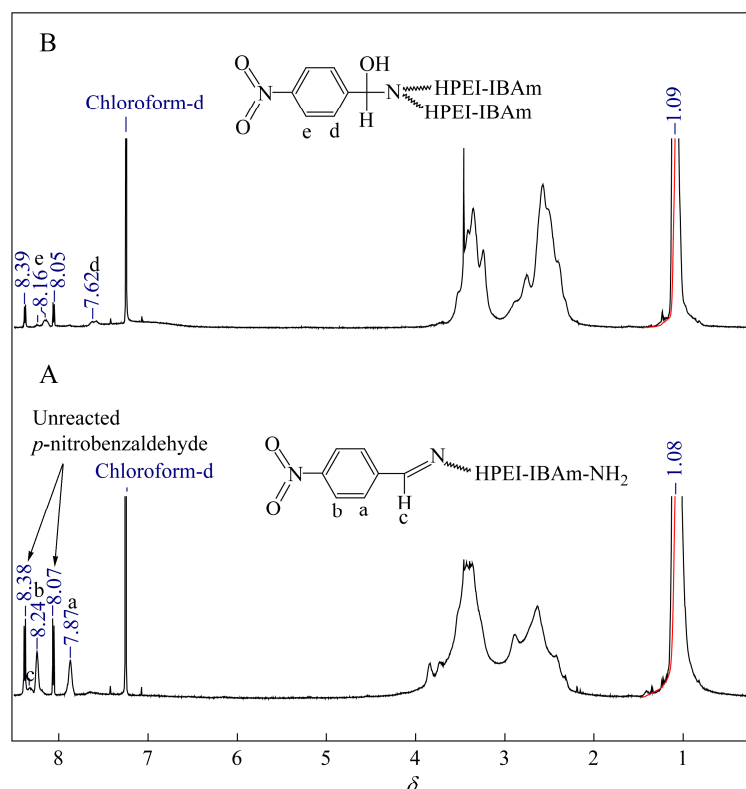


Fig. 2 ^1H -NMR spectra of (A) HPEI-IBAm_{0.80}-NH₂ reacted with *p*-nitrobenzaldehyde and (B) HPEI-IBAm_{0.80} reacted with *p*-nitrobenzaldehyde

Figure 3(A) shows the temperature-dependent light transmittance of the aqueous solution of HPEI-IBAm_{0.80}-NH₂ at different pH, and obvious phase transition can be observed in the pH range of 7–10, indicating that HPEI-IBAm_{0.80}-NH₂ is also a thermoresponsive polymer. For comparison, the temperature-dependent light transmittance of the aqueous solution of HPEI-IBAm_{0.80} at different pH was also measured and plotted in Fig. 3(B). From Figs. 3(A) and 3(B), the T_{cp} values are read off and plotted in Fig. 3(C). In the pH range of 9.5–10 the T_{cp} of HPEI-IBAm_{0.80}-NH₂ is nearly constant, but below pH 9.5 decreasing the pH raises the T_{cp} obviously. As for HPEI-IBAm_{0.80}, its T_{cp} does not change in a wider pH range, 8.5–10. Below pH 8.5, further reducing the pH also raises the T_{cp} obviously. Comparing the T_{cp} values of HPEI-IBAm_{0.80}-NH₂ and HPEI-IBAm_{0.80} at the same pH, it is clear that the T_{cp} of HPEI-IBAm_{0.80}-NH₂ is obviously higher than that of HPEI-IBAm_{0.80}. The compositions of HPEI-IBAm_{0.80}-NH₂ and HPEI-IBAm_{0.80} are the same, and their main difference lies in the spatial distribution of IBAm groups on HPEI skeleton. Therefore, it can be deduced that varying the spatial distribution of thermoresponsive units in hyperbranched polymers is also an effective method to alter the phase transition temperature. The sharpness of the phase transition of HPEI-IBAm_{0.80}-NH₂ and HPEI-IBAm_{0.80} is also compared (Fig. 3D). The phase transition of HPEI-IBAm_{0.80}-NH₂ is broader than that of HPEI-IBAm_{0.80}. The difference of their $\Delta T_{1/2}$ values is smaller in the high pH range where the T_{cp} is stable. In the lower pH range where T_{cp} changes obviously the $\Delta T_{1/2}$ difference becomes bigger.

The above pH responses of HPEI-IBAm_{0.80}-NH₂ and HPEI-IBAm_{0.80} are interpreted as follows: As the acidity of an aqueous solution of the polymers increases, more amino groups of HPEI-IBAm_{0.80}-NH₂ and HPEI-IBAm_{0.80} are protonated to form more polar N⁺ groups. The significant increase in polarity increases T_{cp} since it is well-known that increasing the hydrophobicity of thermoresponsive polymers decreases the phase transition temperature, whereas increasing the hydrophilicity increases it^[30]. Increasing basicity of the polymer solutions gradually turns the more polar protonated N⁺ groups into less polar amines, decreasing T_{cp} . When all

the polar protonated N^+ groups are transformed back to amines, the polarity of the polymer does not vary, and thus its T_{cp} does not change in a certain pH range. The pH threshold to achieve the constant T_{cp} is around 9.5 and 8.5 for HPEI-IBAm_{0.80}-NH₂ and HPEI-IBAm_{0.80}, respectively, which can be attributed to the different pKa values of primary amine (pKa 9.64) and secondary amine (pKa 8.59) groups of HPEI^[31]. Such phenomenon further verifies that many primary amine groups are left in the obtained HPEI-IBAm_{0.80}-NH₂. Moreover, HPEI-IBAm_{0.80}-NH₂ shows higher phase transition temperature and broader phase transition than HPEI-IBAm_{0.80} at the same pH. This can be explained as follows. The phase transition behavior of polymers in solution measured by the turbidity method reflects the aggregation extent of polymers at different temperatures. The aggregation of hyperbranched polymers is mainly due to the temperature-induced increase of the hydrophobic-hydrophobic interaction among the thermoresponsive units of different macromolecules^[17, 19]. Therefore, more thermoresponsive units on the periphery favor the inter-macromolecular hydrophobic-hydrophobic interaction. This leads to the more efficient aggregation of polymers, embodied as the lower phase transition temperature and narrower phase transition. HPEI-IBAm_{0.80} has more IBAm groups on the periphery of HPEI skeleton than HPEI-IBAm_{0.80}-NH₂, thus, it shows lower T_{cp} and narrower phase transition than HPEI-IBAm_{0.80}-NH₂.

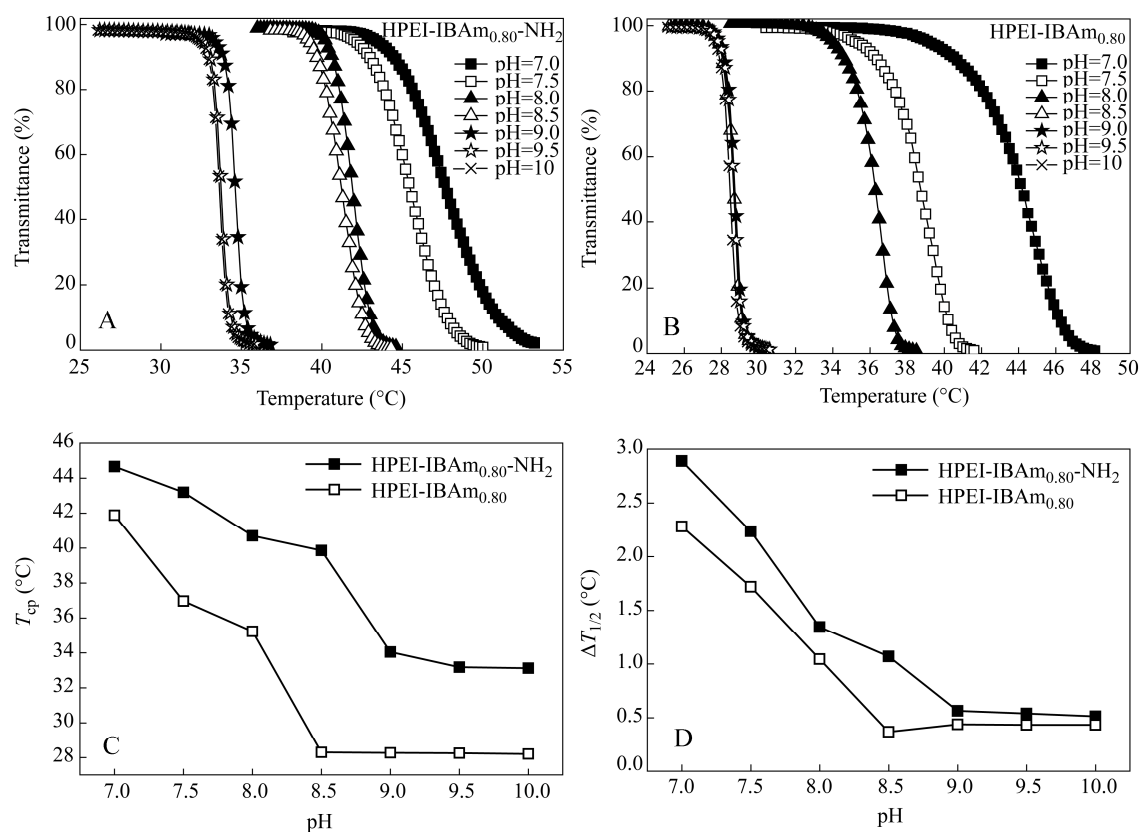


Fig. 3 Typical influence of temperature on the light transmittance of (A) HPEI-IBAm_{0.80}-NH₂ and (B) HPEI-IBAm_{0.80} at different pH; Influence of pH on (C) T_{cp} and (D) $\Delta T_{1/2}$ of HPEI-IBAm_{0.80}-NH₂ and HPEI-IBAm_{0.80} (Concentration of polymer is 20 mg/mL.)

CONCLUSIONS

HPEI-IBAm-NH₂ with many primary amine groups on the periphery can be prepared through three-step reactions, including (1) Boc protection on the primary amines of HPEI, (2) subsequently, amidation reaction between the residual reactive amines and isobutyric anhydride and (3) finally, Boc deprotection. HPEI-IBAm_{0.8}-NH₂ is more reactive for the further modification than its spatial isomer HPEI-IBAm_{0.8} without primary amine

groups. HPEI-IBAm_{0.8}-NH₂ in water is thermoresponsive. In the pH range of 9.5–10 the T_{cp} of HPEI-IBAm_{0.8}-NH₂ is constant, and it increases obviously upon decreasing the pH below 9.5. Compared with HPEI-IBAm_{0.8}, HPEI-IBAm_{0.8}-NH₂ shows higher T_{cp} and broader phase transition, indicating that varying the spatial distribution of thermoresponsive units in hyperbranched polymers is also an effective method to alter the phase transition temperature.

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