

## Synthesis of Thermo-responsive Hyperbranched Polyamidoamine and the Molecular Weight, pH, and Anion Sensitive Thermo-responsive Properties Thereof

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**Abstract:** Hyperbranched polyamidoamine (HPAMAM) polymers, chemically analogous to the commercially available PAMAM dendrimer, were modified with isobutyric anhydride to result in isobutyramide (IBAm) terminated HPAMAMs (HPAMAM-IBAm). The aqueous solutions of HPAMAM-IBAm polymers had the lower critical solution temperature (LCST). The lower molecular-weight HPAMAM-IBAm exhibited higher LCST and the LCST difference was around 18 °C for one pseudo-generation variation. Further, the hyperbranched thermo-responsive polymers exhibited much lower LCSTs than the corresponding dendrimers with similar molecular weight. The LCST of HPAMAM-IBAm was pH sensitive. At pH below 10, the LCST increased significantly upon decreasing the pH, whereas, at pH above 10, the LCST decreased slowly with an increasing pH value. Nine sodium salts were used to measure the anion effect on the LCST of HPAMAM-IBAm. It was found that the LCST could also be modulated up or down in a broad range by simply adding a small amount of different kinds of inorganic anions. The specific ranking of inorganic anions in salting-out HPAMAM4-IBAm polymer was in accordance with the well-known Hofmeister series.

**Keywords:** hyperbranched, lower critical solution temperature, polyamidoamine, thermo-responsive, salt effect.

### Introduction

During the past decade polymers with stimulus-responsive property, such as fast and reversible conformational or phase changes in response to variations in temperature and/or pH, have gained much interest in the field of biology and medicine.<sup>1</sup> One of the most appealing stimulus-responsive species is the thermo-responsive hydrophilic polymer having the lower critical solution temperature (LCST) in aqueous solution. The character of this kind of polymers is that above a specific temperature their solubility in water dramatically decreases.<sup>2</sup>

Specific ion effect on the chemical and physical properties of the synthetic and natural macromolecules are ubiquitous.<sup>3-5</sup> In many cases, the effectiveness of the ions more or less follows the Hofmeister series<sup>6,7</sup> that was first established to rank the salts according to their relative ability to precipitate certain proteins from an aqueous solution (a salting-out effect), versus to raise the solubility of certain proteins in aqueous solution (a salting-in effect). Up to date many insights into the underlying mechanisms behind the Hofmeister series ordering of ion-specific effects have been gained, however, deeper understanding at the molecular level still seems to be elusive.<sup>8-11</sup> One of the prevailing mecha-

nisms for the Hofmeister series ordering is through ion-specific alterations in the hydrogen-bonding network of water.<sup>10-12</sup> The weakly hydrated ions are named as chaotropes (water-structure breakers) that are monovalent ions of low charge density and bind the immediately water molecules less strongly than water binds itself.<sup>10-13</sup> Contrarily, the strongly hydrated ions are categorized as kosmotropes (water-structure makers) that are of high charge density and bind the immediately water molecules more strongly than water binds itself.<sup>10-13</sup> As the mimic of proteins, many synthetic thermo-responsive linear polymers and their hydrogels containing large amount of amide groups have been studied concerning on the salt effects on their phase transition in water.<sup>14-21</sup> Similar to the natural proteins, anions have big influence on their properties and the rank ordering also follows the Hofmeister series.

In 2004 Kono group reported their pioneering work on the thermo-responsive dendrimers whose LCSTs could be tuned in a broad range by varying the molecular weight.<sup>22</sup> Following their pioneering work more kinds of thermo-responsive dendritic polymers were reported.<sup>23-33</sup> However, the influence of salts on the LCSTs of the polymers with dendritic topology was scarcely issued. Only recently, NaCl, KCl, and Na<sub>2</sub>SO<sub>4</sub> have been used to see their effect on the LCST of thermo-responsive hyperbranched polyethylenimine terminated with isobutyramide (IBAm) groups, where the salt ordering also followed the Hofmeister series.<sup>31</sup> Since only two anions

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have been used there, it could not be concluded whether all the inorganic anions would follow the Hofmeister series for the thermoresponsive polymers with dendritic topology. In this contribution IBAm-terminated hyperbranched polyamidoamine (HPAMAM-IBAm) polymers showing LCST property were synthesized. It was found that the inorganic anions could efficiently affect their LCST. All the anions followed the direct Hofmeister series ordering.

## Experimental

**Materials.** Isobutyric anhydride (98%) was purchased from Alfa Aesar and used without further purification. Benzoylated cellulose membrane (MWCO 1,000 g/mol) was purchased from Sigma. Triethylamine (A.R., TEA) was dried over  $\text{CaH}_2$  and distilled before use. Methyl acrylate (A.R.) was purchased from Tianjin University Kewei Chemical Company and used directly. All the inorganic salts (spectra pure grade) for the LCST measurement were purchased from Tianjin Guangfu Fine Chemical Research Institute and used directly. De-ionized water was distilled before use. Tris(2-aminoethyl)amine was purchased from Aldrich and was distilled under reduced pressure before use. Tris(2-di(methylacrylate)aminoethyl)amine was synthesized according to the method of Dvornic.<sup>34</sup> Pseudo generation 4 HPAMAM (HPAMAM4,  $M_w=1.36\times 10^4$ , PDI=1.4) and pseudo generation 5 HPAMAM (HPAMAM5,  $M_w=3.03\times 10^4$ , PDI=2.2) were synthesized according to the literatures.<sup>34-36</sup>

**Methods.**  $^1\text{H}$  NMR spectra were recorded on a Varian INOVA 500 MHz spectrometer. The chemical shifts are given in parts per million (ppm). 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 is  $0.5\text{ }^\circ\text{C}/2\text{ min}$ . The cloud point was defined as the temperature corresponding to the initial break points in the resulting transmittance versus temperature curve. FTIR spectra were recorded on a Nicolet 5DXC FTIR spectrometer. The measurement was done using KBr pellets, and the scanning range was  $4000\text{--}400\text{ cm}^{-1}$ .

**Syntheses of IBAm Terminated HPAMAMs.** The synthesis of IBAm terminated HPAMAM is exemplified for HPAMAM4-IBAm: Under nitrogen atmosphere, isobutyric anhydride (1.523 g, 9.624 mmol) was added dropwise to the mixture of HPAMAM4 (1.637 g, 8.020 mmol of primary amines) and triethyl amine (1.071 g, 10.59 mmol) in 20 mL of chloroform at  $0\text{ }^\circ\text{C}$  with vigorous stirring. Subsequently, the reaction mixture was kept and carried out at room temperature for 24 h. Finally, the reaction temperature was raised to  $65\text{ }^\circ\text{C}$  for 2 h to finalize the reaction. After cooling down to room temperature, the produced salt was filtered off. Volatile in the filtrate was removed under vacuum and the residue was dissolved in 20 mL of methanol. One gram of potassium carbonate was added to the solution and the mixture was stirred at room temperature for 4 h. After

filtration, the solution was concentrated to *ca.* 10 mL and then purified by dialysis against methanol using a benzoylated cellulose membrane (MWCO 1,000 g/mol) for 2 days in order to remove low molecular weight impurities. Finally, the methanol solvent was removed under vacuum, and the product was dried in vacuum for 24h.  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ ):  $\delta=0.91\text{--}0.92$  ( $(\text{CH}_3)_2\text{CHCON-}$ );  $2.15\text{--}3.60$  ( $(\text{CH}_3)_2\text{CHCON-}$ , ethylene protons of HPAMAM moieties);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta=1.10$  ( $(\text{CH}_3)_2\text{CHCON-}$ );  $2.15\text{--}3.75$  ( $(\text{CH}_3)_2\text{CHCON-}$ , ethylene protons of HPAMAM moieties).

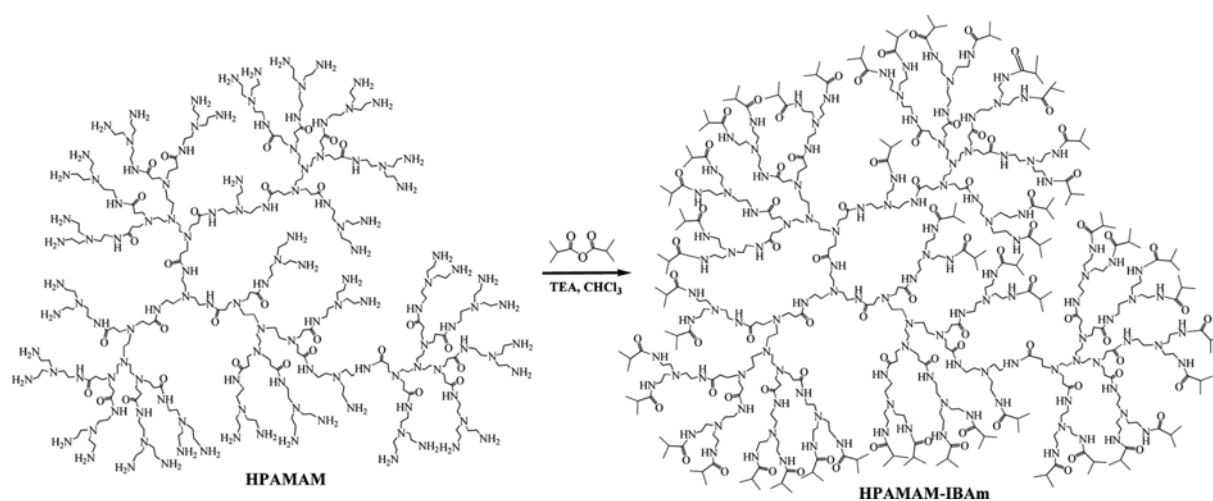
**Adjusting the pH of the Aqueous Solution of HPAMAM4-IBAm.** The pH value of HPAMAM4-IBAm in double-distilled de-ionized water (20 g/L) was measured to be 9.09. Aqueous solutions of HCl (0.1 M) and NaOH (1 M) were used to lower and elevate the pH of this polymer solution, respectively.

**Preparation of the Aqueous Salt Solution of HPAMAM4-IBAm.** Different amount of salt solid was added to the 3 mL of aqueous solution of HPAMAM4-IBAm (20 g/L). The mixture was shaken gently. After the solid salt dissolved completely, the clear solution was used for the cloud point measurement.

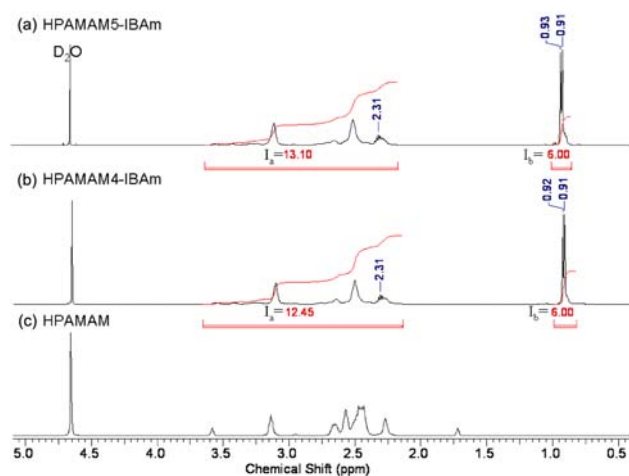
## Results and Discussion

Hyperbranched polyamidoamine (HPAMAM) polymers with  $M_w$  similar to the commercially available generation 4 and 5 PAMAM dendrimers, namely HPAMAM4 and HPAMAM5, were synthesized according to the literatures.<sup>34-36</sup> HPAMAM contains primary amine, tertiary amine and secondary amide functional groups. The syntheses of hyperbranched polymers having IBAm end groups were carried out through the amidation reaction of HPAMAMs with isobutyric anhydride according to Scheme I. The resultant polymers were characterized by  $^1\text{H}$  NMR (Figure 1(a) and (b)). Compared with the  $^1\text{H}$  NMR spectrum of HPAMAM precursor (Figure 1(c)), the new doublet peak at about 0.9 ppm in Figure 1(a) assigned as the methyl protons of IBAm moieties is the strong evidence of the successful attachment of IBAm groups to the HPAMAMs. The multiple peaks of methine protons of IBAm moieties can be also seen clearly at around 2.31 ppm in Figure 1(a) and (b) although the peaks are overlapped with the HPAMAM scaffold. All the other signals between 2.15 and 3.60 ppm are assigned as the ethylene protons of the HPAMAM scaffold.

HPAMAM reacted with isobutyric anhydride through its terminal primary amine groups. The degree of substitution relative to the primary amines can be calculated according to the corresponding  $^1\text{H}$  NMR spectra (Figure 1(a) and (b)). The integration ( $I_b$ ) of six protons from the two methyl groups of IBAm divided by 6 ( $I_b/6$ ) represents the relative amount of IBAm moieties. The integration ( $I_a$ ) between 2.15-3.60 ppm includes four protons from the ethylene units of HPAMAM scaffold and one methine proton from IBAm



**Scheme I.** Synthesis of hyperbranched polyamidoamine terminated with isobutyric amide groups.



**Figure 1.** Typical  $^1\text{H}$  NMR spectra of (a) isobutyric amide (IBAm) terminated HPAMAM5 (HPAMAM5-IBAm); (b) isobutyric amide (IBAm) terminated HPAMAM4 (HPAMAM4-IBAm); (c) HPAMAM precursor (HPAMAM4).

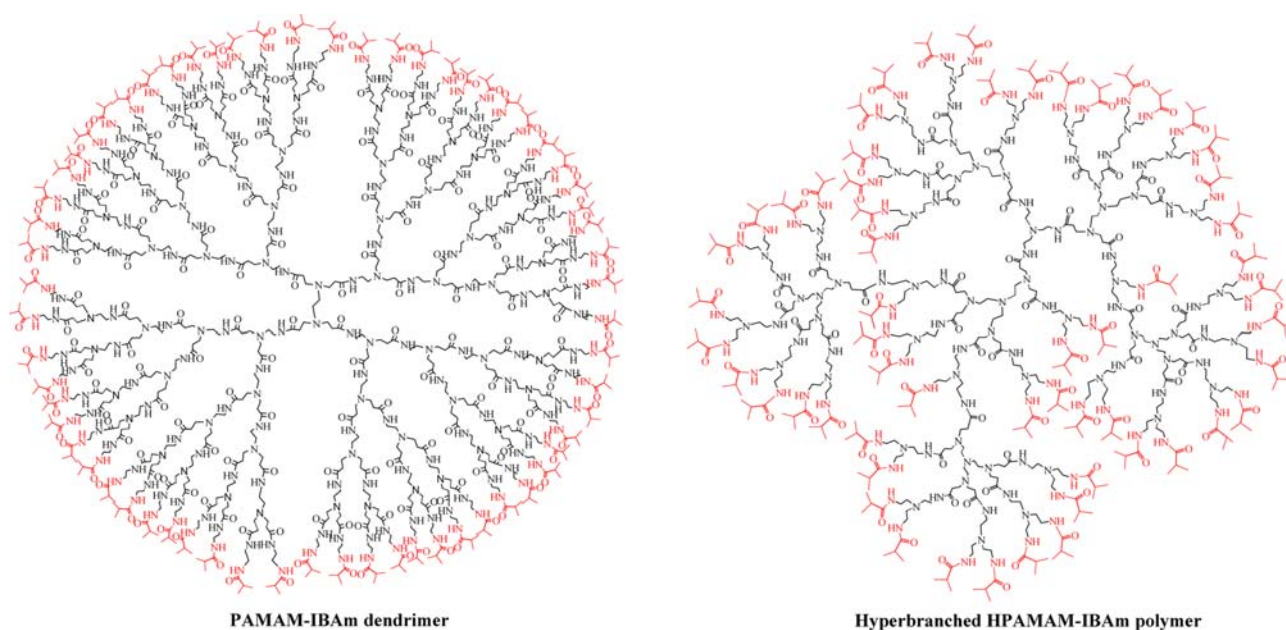
moiety, therefore the real integration of ethylene units of HPAMAM scaffold is  $I_a$  minus  $I_b/6$ . According to the HPAMAM structure shown in Scheme I it is clear that each ethylene unit corresponds for one nitrogen atom, thus, the relative nitrogen amount of HPAMAM-IBAm can be deduced to be the integration of the ethylene protons divided by 4. The percentages of the primary amines relative to the total nitrogens in HPAMAM4 and HPAMAM5 are 37% and 38%, respectively. Therefore, the degree of substitutions can be calculated according to eq. (1) and (2) for HPAMAM4-IBAm and HPAMAM5-IBAm, respectively.

$$\begin{aligned} \text{Degree of substitution (HPAMAM4-IBAm)} \\ = (I_b/6)/[(I_a - I_b/6)/4 \times 0.37] \end{aligned} \quad (1)$$

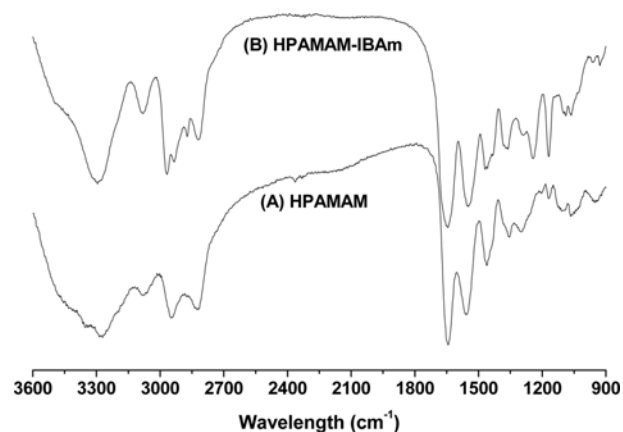
$$\begin{aligned} \text{Degree of substitution (HPAMAM5-IBAm)} \\ = (I_b/6)/[(I_a - I_b/6)/4 \times 0.38] \end{aligned} \quad (2)$$

Isobutyric anhydride with 1.2 time excess to the primary amines were used to react with HPAMAM4 and HPAMAM5, resulting in two polymers, HPAMAM4-IBAm and HPAMAM5-IBAm, with degree of substitutions being only about 92% and 89%, respectively, both less than 100%. This phenomenon was also found for the modification of hyperbranched polyethylenimine, where the employment of isobutyric anhydride always tended to be less than 100% substitution.<sup>31</sup> The less degree of substitution than expected may be ascribed to the steric hindrance effect since some of the terminal amino groups are embedded in the interior of the hyperbranched polymer structure, unlike PAMAM dendrimer whose terminal groups are only located in the periphery of the spheroid-like materials (Figure 2).<sup>37</sup> Whereas, the more reactive isobutyryl chloride is not adopted here since it has been found that one primary amine may react with two molecules of the very reactive isobutyryl chloride,<sup>31,38</sup> which will complicate the structure of the obtained polymers.

The resulting polymers and their HPAMAM precursors were also characterized by the FTIR (Figure 3). The strong absorptions between  $2800$  and  $3000\text{ cm}^{-1}$  are characteristic for the C-H stretching bond frequency of alkane groups. As for HPAMAM, the peaks in the C-H stretching absorption region derived from its ethylene groups are doublet. Whereas multiple peaks occur in the C-H stretching absorption region for IBAm terminated HPAMAMs, which can be ascribed to the introduction of methyl and methine groups originating from IBAm moieties. The N-H stretching bond frequency of primary amine is a broad band in the  $3300\text{ cm}^{-1}$  region, whereas, that of secondary amide is a broad band in the  $3300\text{ cm}^{-1}$  region superimposed with one or more sharp spikes. Due to the almost equally co-existence of primary amine and secondary amide groups in HPAMAM, the shape of the N-H stretching bond in the  $3300\text{ cm}^{-1}$  region is a broad band superimposed with small spikes. The attachment of IBAm groups to HPAMAM reduces the number of



**Figure 2.** Comparison of PAMAM-IBAm dendrimer with hyperbranched HPAMAM-IBAm polymer.

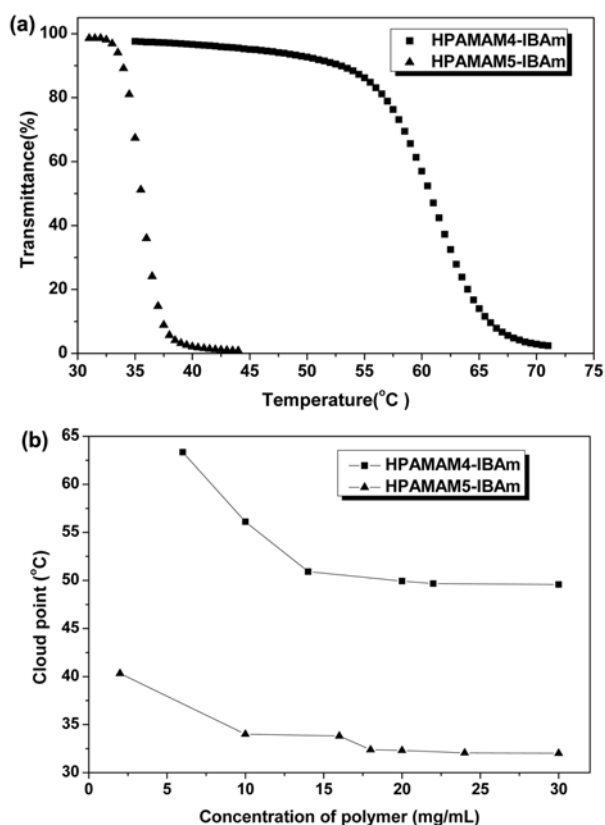


**Figure 3.** Comparison of the FTIR spectra of (A) HPAMAM and (B) HPAMAM-IBAm.

N-H bonds of primary amines, whereas increases the number of N-H bonds of secondary amide groups. Thus, the band in the  $3300\text{ cm}^{-1}$  region for HPAMAM-IBAm is less broad and the spike becomes much bigger.

All the polymers were qualitatively analyzed to see whether they had thermoresponsive property in aqueous media by dipping the vial containing the polymer solutions into the thermostated water bath. Over the experimental temperature range, the solutions of unmodified HPAMAMs were always transparent, indicating that HPAMAMs themselves were not thermoresponsive in aqueous media. However, the solutions of all the obtained hyperbranched polymers with IBAm groups became turbid after being heated to certain temperature, and became transparent again when the solutions were cooled down, which pointed out that the introduction of

IBAm groups to HPAMAMs rendered them thermoresponsive. The cloud point temperature ( $T_{cp}$ ) of the solutions of the obtained polymers was determined by a temperature-controlled UV-vis spectrometer at 660 nm. Figure 4(a) shows the respective temperature dependence of the light transmittance of aqueous solutions of HPAMAM4-IBAm and HPAMAM5-IBAm. Compared with those of their dendrimer analogues,<sup>22</sup> their phase transition ranges are relatively broad due to their broad molecular weight distribution. The  $T_{cp}$  is defined as the temperature corresponding to the initial break point of the curve in Figure 4(a). Figure 4(b) shows the influence of the polymer concentration on the  $T_{cp}$ . It is clear that  $T_{cp}$  decreases upon raising the polymer concentration at the beginning, but levels off when the polymer concentration reaches around 20 mg/mL. The lowest  $T_{cp}$  value at certain polymer concentration is regarded as the LCST of the polymers. From Figure 4(b) the LCSTs of HPAMAM4-IBAm and HPAMAM5-IBAm can be read off to be about 50 and 32 °C, respectively. The LCST difference of HPAMAM-IBAm is around 18 °C for one pseudo-generation variation. It was known that if the primary structure of thermoresponsive linear polymer was not changed, its LCST could be altered only insignificantly by changing its molecular weight. For example, the LCST of (hydroxypropyl)cellulose dropped *ca.* 4 °C as the molecular weight was raised from  $10^5$  to  $10^6$ ; The LCST difference of poly(*N*-isopropylacrylamide) (PNIPAM) with the average molecular weight between 5,400 and 160,000 was only *ca.* 2 °C.<sup>14</sup> Thus, it can be deduced that the compact and spheroidal topology of hyperbranched polymers are essential for the significant molecular-weight dependent LCST. Furthermore, the higher LCST exhibited by the lower molecular-weight hyperbranched polymer might



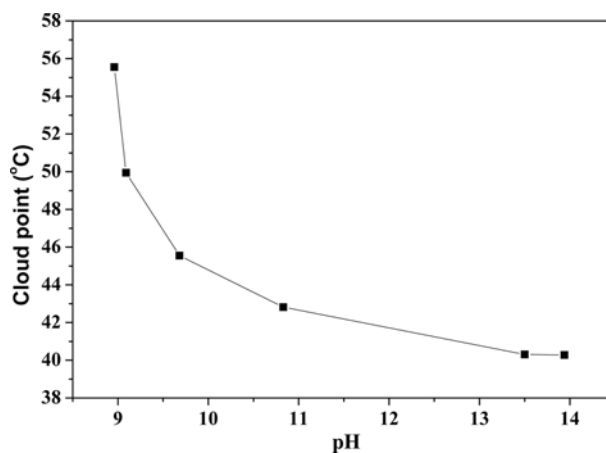
**Figure 4.** The aqueous solutions of (■) HPAMAM4-IBAm and (▲) HPAMAM5-IBAm: (a) the typical curves of light transmittance versus temperature; (b) influence of polymer concentration on the cloud points.

be ascribed to the lower density of its hydrophobic terminal IBAm groups just like dendrimers,<sup>37</sup> resulting that the dehydration of the terminal IBAm groups occurred at higher temperature.<sup>22</sup>

From Figure 4(b) it can be known further that the LCSTs of HPAMAM-IBAm polymers are much lower than the respective 62 and 45 °C of their corresponding PAMAM-IBAm dendrimers,<sup>22</sup> implying that the thermoresponsive properties of hyperbranched polymers have obvious difference from those of their perfect-structured dendrimer analogues. Comparing the structures of PAMAM-IBAm dendrimer with its hyperbranched analogue (Figure 2), it is clear that they have significant topological difference in the location of functional end-groups. The functional IBAm groups are only concentrated in the periphery of the spheroid-like dendrimers to form a relatively hydrophobic shell around the relatively hydrophilic PAMAM core, and the interface of core and shell is distinct. As for the thermoresponsive hyperbranched polymers, their IBAm groups are distributed not only in the periphery, but also in the inner part. Therefore it can be deduced that the peripheral IBAm groups of the hyperbranched polymers are less dense than those of the dendrimers with the similar molecular weight; and the

existence of the internal IBAm groups renders the core of hyperbranched polymer to be more hydrophobic than that of the dendrimer. As far as dendrimers are concerned, the higher the density of peripheral IBAm groups is, the stronger the interaction between the IBAm groups is. This results in the efficient dehydration at the relatively low-temperature region. Thus, the less dense peripheral IBAm groups are supposed to enhance the LCST of the thermoresponsive hyperbranched polymers. However, the existence of the internal IBAm groups renders the core of hyperbranched polymers relatively hydrophobic, thus lowering their LCSTs. From the experimental results it can be known that the existence of the relatively hydrophobic IBAm groups in the inner part of the hyperbranched polymers is the main factor to decide their LCST variation trend. Moreover, unlike the perfect-structured thermoresponsive dendrimers the LCSTs of the obtained thermoresponsive hyperbranched polymers can be controlled in the physiological range, rendering them to be potentially applied in the bio-related field.

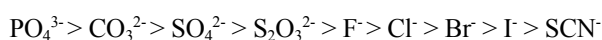
Figure 5 shows the pH effect on the  $T_{cp}$  of the aqueous solution of HPAMAM4-IBAm polymer (20 g/L). It is clear that below pH=10 the  $T_{cp}$  of HPAMAM-IBAm can be elevated efficiently upon lowering the pH of the solution. Above pH=10 the  $T_{cp}$  of HPAMAM-IBAm decreases with the increase of the pH value of the solution, however, the  $T_{cp}$  variation is not so pronounced as that at pH below 10. The obtained HPAMAM-IBAm has large amount of tertiary amines and some residual primary amines. The Protonation of these amino groups leads to the expansion of the hyperbranched polymer due to the charge-charge repulsion, which tends to reduce the density of the terminal IBAm groups and increases the  $T_{cp}$ .<sup>22</sup> Furthermore the protonation of these amino groups also increases the hydrophilicity of the polymer, resulting in the higher  $T_{cp}$ . The pH of the solution of 20 g/L HPAMAM4-IBAm polymer in double-distilled de-ionized water was measured to be 9.09. It has been known that in the amine-



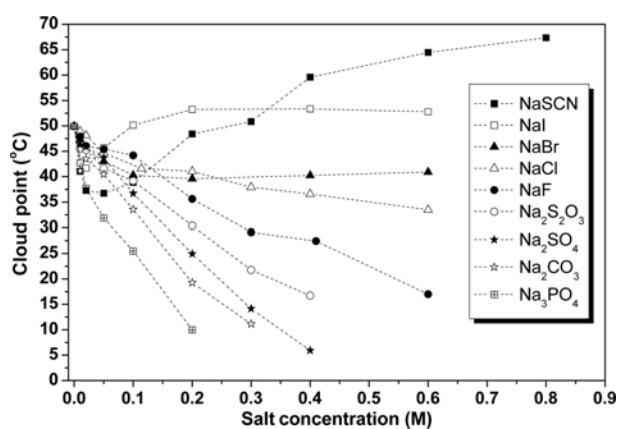
**Figure 5.** Typical influence of pH on the cloud point of HPAMAM4-IBAm in de-ionized water (polymer concentrations are fixed at 20 mg·mL<sup>-1</sup>).

terminated PAMAM dendrimer the protonation of the primary amine groups occurs between pH 7 and 10, whereas the protonation of the tertiary amino groups takes place between pH 3 and 7.8.<sup>39-41</sup> Therefore, in the double-distilled de-ionized water the residual primary amine groups of HPAMAM4-IBAm polymer are partially protonated, whereas the tertiary amines are intact. The high pH-sensitivity of the  $T_{cp}$  of HPAMAM-IBAm at pH below 10 can be ascribed to the different degree of protonation of the primary amine groups. At pH above 10 both primary and tertiary amines are in the original state, however, the  $T_{cp}$  of HPAMAM-IBAm can still be influenced by the variation of pH even though the sensitivity becomes weak. This might be due to the existence of the OH<sup>-</sup> anion since it is well-known that inorganic anions usually have influence on the properties of synthetic and natural macromolecules.<sup>16</sup>

It has been known that the effects of cations on the properties of synthetic and natural macromolecules are usually not so significant as the anions.<sup>16</sup> Therefore, in this paper we mainly studied the anion effect. Figure 6 shows the influence of nine sodium salts on the  $T_{cp}$  of HPAMAM4-IBAm polymer. It's clear that the  $T_{cp}$  of HPAMAM4-IBAm can be modulated to different extent by the addition of different inorganic anions. In the moderate to high salt concentration region, the specific ranking of inorganic anions in salting-out HPAMAM4-IBAm polymer is as follows:



This sequence is in accordance with the well-known Hofmeister series for the biopolymers and synthetic water-soluble polymers.<sup>42,43</sup> From this ordering it can be known that the  $T_{cp}$  of HPAMAM4-IBAm polymer can be lowered efficiently by the inorganic anions with high charge number. Among the inorganic anions with the same charge number, the one with the highest charge density has the strongest ability to salt-out the polymer, such as F<sup>-</sup> and CO<sub>3</sub><sup>2-</sup>. The different effect of these anions on the  $T_{cp}$  of HPAMAM4-



**Figure 6.** Influence of different salts on the cloud point of HPAMAM4-IBAm in de-ionized water (polymer concentration is fixed at 20 mg·mL<sup>-1</sup>).

IBAm can be ascribed to two factors. One is their ability to form hydrogen bonds with the hydration water wrapped on the hydrophobic units and the polar groups of the polymer chains; the other is their ability to directly interact with the polar amide groups that have a kind of resonance providing the amide nitrogen with a partial positive charge and the carbonyl with a partial negative charge.<sup>16</sup> The first factor helps to salt-out the polymers from water since it will weaken the interaction between hydration water and the polymer chains.<sup>16</sup> Thus, the interaction of hydrophobic groups of polymers is strengthened, leading that polymers are demixed with water at lower temperature. The second factor helps to salt-in the polymers since it will enhance the hydrophilicity of the polymers, resulting in the higher  $T_{cp}$ .<sup>16</sup> Therefore, from Figure 6 it can be deduced that at medium to high salt concentration the second factor will be dominant in the system with I<sup>-</sup> or SCN<sup>-</sup>, whereas the first factor will dominate in the system with the other anions. Why is it so? It might be explained by the different hydration ability of the anions exemplified by the different physical data of anion-water interaction, such as Jones-Dole viscosity  $B$ -coefficients,<sup>44</sup> hydration of entropy,<sup>16</sup> Gibbs free energy of hydration,<sup>45,46</sup> *etc.* For instance, the viscosity  $B$ -coefficients for PO<sub>4</sub><sup>3-</sup>, SO<sub>4</sub><sup>2-</sup>, F<sup>-</sup>, Cl<sup>-</sup>, Br<sup>-</sup>, I<sup>-</sup>, and SCN<sup>-</sup> are 0.590, 0.208, 0.10, -0.007, -0.032, -0.068, and -0.103 (mol<sup>-1</sup>), respectively. The higher the value of viscosity  $B$ -coefficient is, the stronger the hydration ability of the anion is. The kosmotropic anions have positive or less negative viscosity  $B$ -coefficient values, whereas the chaotropic anions show large negative values. At one aspect, the stronger hydration ability of kosmotropic anions makes them effectively reduce the hydration shell from the polymers. At another aspect the stronger hydration ability of kosmotropic anions also makes them consume much energy to dehydrate the hydration shell around themselves before the direct interaction with polymer. Thus, the first salting-out factor is dominant in the system with kosmotropic anions. As for the chaotropic anions, their weak hydration abilities not only render them to have less influence on the hydration shell around the polymers, but also make them consume less energy to dehydrate their hydration shell before the direct interaction with the polar groups of the polymer. Thus, the chaotropic anions have more chance to directly interact with the polar amide groups of the polymers, resulting in the higher  $T_{cp}$ .

## Conclusions

Thermoresponsive hyperbranched polymers have been synthesized successfully by transforming the reactive primary amine groups of HPAMAMs into IBAm groups. The obtained HPAMAM-IBAm polymers showed LCST in aqueous media. The lower molecular weight HPAMAM-IBAm exhibited higher LCST and the LCST difference was around 18 °C for one pseudo-generation variation. The hyperbranched

thermo-responsive polymers exhibited much lower LCSTs than the corresponding dendrimers with the similar molecular weight. The  $T_{cp}$  of HPAMAM-IBAm was pH-sensitive, which could be enhanced significantly upon lowering the pH to certain point. The  $T_{cp}$  of HPAMAM-IBAm could also be elevated or lowered intentionally in a broad range by simply adding a small amount of different kinds of inorganic anions. At the moderate to high salt concentration the specific ranking of inorganic anions in salting-out HPAMAM4-IBAm polymer is in accordance with the well-known Hofmeister series, that is  $\text{PO}_4^{3-} > \text{CO}_3^{2-} > \text{SO}_4^{2-} > \text{S}_2\text{O}_3^{2-} > \text{F}^- > \text{Cl}^- > \text{Br}^- > \text{I}^- > \text{SCN}^-$ .

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