Dynamic self-aggregation and disaggregation behavior of thermoresponsive hyperbranched polyethylenimine with peripheral NIPAM groups: an infrared spectroscopic study†

Shengtong Sun,+ Hongna Wang‡ and Peiyi Wu*

Hyperbranched polyethylenimine (HPEI) has been modified with N-isopropylacrylamide (NIPAM) by Michael addition to obtain a series of LCST-type thermoresponsive HPEI–NIPAMs. According to turbidity and calorimetric measurements, the phase transition temperature of HPEI–NIPAM shows negative correlations with both the substitution degree of NIPAM groups and concentration (75 to 38 °C from 0.2 to 10 mg mL\(^{-1}\)). From FT-IR spectra, two kinds of carbonyl groups are found to exist in the amide I region for HPEI–NIPAM, along with the gradual emergence of a rather small band at 1648 cm\(^{-1}\) due to the lower conformational freedom of amide groups compared to linear PNIPAM. The absence of isosbestic points for C–H and C=O groups in the heating-and-cooling cycle also suggests that HPEI–NIPAM has a much more restricted structure. Moreover, two-dimensional correlation infrared spectroscopy was employed to study the thermodynamic self-aggregation and disaggregation behavior of HPEI–NIPAM. A two-step dynamic mechanism was identified during both heating and cooling by distinguishing the sequence order among the hydrogen bond changes, chain collapse and revival as well as water diffusion. Wherein, the formation and breakage of self-associated C=O…D–N hydrogen bonds are found to be always dominating in the phase transition in close relation to the chain aggregation of HPEI–NIPAM unimers and disaggregation of micelles respectively.

1 Introduction

Stimuli-responsive polymers,† especially thermoresponsive ones based on dendritic polymers,‡–† such as dendrimers,§ and hyperbranched polymers,¶ have attracted great interest in recent years. Compared to their linear analogs, dendritic polymers possess many advantages, e.g. a highly branched architecture with a multitude of terminal functional groups, reduced chain entanglement and hydrodynamic volume. Owing to the branched structure and stimuli-responsive properties, thermoresponsive dendritic polymers are of great interest for applications in biomedical fields such as drug release and gene delivery.

To endow dendritic polymers with thermal responsivity, incorporating thermoresponsive chains by block or graft copolymerization is generally considered to be a feasible strategy.

For instance, several groups have introduced poly(N-isopropylacrylamide) (PNIPAM), the most commonly studied thermoresponsive polymer, to develop stimuli-responsive dendritic polymers through grafting from‡–¶ or grafting onto§–‖ methods. Other linear polymers such as PEO,¶ PEG‖ or PDMAMH‖ were also applied to construct thermoresponsive dendritic polymers. Another viable method to induce thermal responsivity is to modify the dendritic polymer by functional groups. For example, Kono’s group has reported a series of researches about thermoresponsive polyamidoamine dendrimers by modifying the terminal groups with alkyl amide groups like isobutyramide‖–— and phenylalanine residues.26 Similar methods to prepare thermoresponsive hyperbranched polymers such as polyglycerol27 and polyethyleneimine,28 were also reported.

Hyperbranched polyethylenimine (HPEI) is a kind of hyperbranched polymer widely used in gene therapy due to its positive charges that can make a complex with DNA and serve as a nanocapsule for guest encapsulation.29–31 To endow the dendritic structure of HPEI with thermal responsivity, several groups have developed different kinds of HPEI-based copolymers by introducing other blocks or functional groups that respond to temperature.14, 28, 32, 33 For HPEI, PNIPAM was also commonly used as the introduced block chain,14, 28, 35–38 which

State Key Laboratory of Molecular Engineering of Polymers, Department of Macromolecular Science, and Laboratory of Advanced Materials, Fudan University, Shanghai 200433, China. E-mail: peiyiwu@fudan.edu.cn
† Electronic supplementary information (ESI) available: Reaction time dependent \(^1\)H NMR spectra and corresponding integral area ratio of NIPAM monomers, calorimetric and DLS measurements, quantitative analysis for \(r(C–H)\) and \(r(C=O)\). See DOI: 10.1039/c2sm27544a
‡ These authors contributed equally to this work.

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undergoes a reversible coil-to-globule phase transition around 32 °C close to physiological temperature. It has been suggested that the hyperbranched structure of HPEI exhibits a certain effect on the phase transition temperature (or lower critical solution temperature – LCST) of PNIPAM blocks,26,35–38 although controversies have arisen since various studies have both reported a slight change26,37 and a significant increase of the LCST values of PNIPAM.35,38 Apart from introducing thermo-responsive polymeric chains, Chen et al. have tried to modify HPEI by using isobutylamide (IBam) groups to obtain thermoresponsive HPEI–IBam.28,39–41 Moreover, introducing NIPAM onto HPEI chains by Michael addition reaction to bring thermal responsivity has also been reported by Fei et al.,42 yet without more details in the phase transition behavior. On the other hand, although these two strategies can both prepare HPEI-based thermo-responsive polymers, they differ greatly in considering the effects of salt, substitution degree and molecular weight on the LCST.43 It is also worth noting that by introducing terminal functional groups to HPEI chains the LCST of the resultant polymers is still strongly dependent on the specific branched structure,24,28,44 which usually results in distinctive phase transition behaviors during temperature variations.45

In the present study, a series of HPEI-based thermo-responsive polymer HPEI–NIPAMs, prepared by Michael addition reaction with NIPAM, were synthesized, and the effects of several factors, such as the substitution degree and concentration, on the LCST-type phase transition behavior of the polymer were carefully investigated. Further study by IR spectroscopy in combination with two investigation methods, perturbation correlation moving window (PCMW) and 2D correlation spectroscopy (2DCOS), will be employed to elucidate more details about the reversible phase transition mechanism. Wherein, PCMW is a technique that cannot only determine the transition point but also monitor the complicated spectral variations along the perturbation direction.46 2DCOS is a mathematical method which could significantly improve the resolution of the 1D spectrum and provide additional information about the sequential molecular motions.47

2 Experimental section

2.1 Materials
Hyperbranched polyethylenimine, HPEI ($M_w = 1800$ g mol$^{-1}$, Alfa Aesar), was dried under vacuum before use. N-Isopropylacrylamide (NIPAM) was purchased from Tokyo Kasei Kogyo Co. and recrystallized from cyclohexane. Benzoylated cellulose membranes (MWCO, 1000 g mol$^{-1}$) were purchased from Shanghai Greenbird Co. Ltd. D$_2$O and CDCl$_3$ were purchased from Cambridge Isotope Laboratories Inc. (D-99.9%).

2.2 Preparation of HPEI–NIPAM and related solutions
The synthesis of HPEI–NIPAM can be simply accomplished by Michael addition, as shown in Fig. 1a according to a previous report.42 In brief, HPEI (1.0 g, 25.4 mmol NH) in 10 mL H$_2$O was added dropwise to a solution of NIPAM (5.7 g, 50.7 mmol) in 30 mL H$_2$O at 60 °C under magnetic stirring. Aliquots (2 mL) were extracted at predetermined time intervals using syringes and quenched immediately with liquid nitrogen. The reaction was terminated after 72 h by cooling. All obtained solutions, including aliquots, were dialyzed against water for two days and then freeze-dried. The substitution degree of NIPAM was ca. 95% calculated from $^1$H NMR.

A set of HPEI–NIPAM aqueous solutions with different concentrations was prepared for turbidity measurements. The concentrations of HPEI–NIPAM for the measurement of dynamic light scattering (DLS) were fixed to 1 and 10 mg mL$^{-1}$. For FT-IR and differential scanning calorimeter (DSC) experiments, the concentration was fixed to 10 wt%. Here, HPEI–NIPAM was dissolved in D$_2$O and the solution was kept at 4 °C for a week before FT-IR experiments to ensure the complete deuteration of all the NH protons.

2.3 Instruments and measurements
$^1$H NMR experiments were carried out on a Mercury plus 400 MHz nuclear magnetic resonance instrument using CDCl$_3$ as the solvent and tetramethylsilane as the internal reference. Calorimetric measurements were performed on a Mettler-Toledo DSC thermal analyzer during heating and cooling. Turbidity measurements were carried out at 500 nm on a Lambda 35 UV-vis spectrometer with deionized water as the reference (100% transmittance). Temperatures were regulated manually with a water-jacketed cell holder at the rate of ca. 0.5 °C min$^{-1}$ with an increment of 1 °C. To ensure thermal equilibrium of the sample cell, each temperature point was held for 2 min before the measurements. DLS was conducted on a Malvern Zetasizer Nano ZS instrument between 28 and 45 °C with an interval of 1 °C and each temperature was maintained for 5 min before the measurements. The sample for FT-IR measurements was prepared by sealing the HPEI–NIPAM solution between two CaF$_2$ tablets. All time-resolved FT-IR spectra were recorded with a resolution of 4 cm$^{-1}$ on a Nicolet Nexus 470 spectrometer by signal-averaging 32 scans. A manual method was performed to control the temperature at a rate of ca. 0.3 °C min$^{-1}$ with an increment of 0.5 °C. The baseline correction process was carried by the Omnic software, ver. 6.1a.

2.4 Investigation methods
Perturbation correlation moving window (PCMW). FT-IR spectra collected with an interval of 0.5 °C during heating and cooling (30–45 °C) were used to perform PCMW analysis. Primary data processing was carried out with the method Morita provided and a further correlation calculation was performed using the 2D Shige software, ver. 1.3 (©Shigeki Morita, Kwansei-Gakuin University, Japan, 2004–2005). The final contour maps were plotted with the Origin program, ver. 8.1, with the red color defined as the positive intensities and the green color as the negative ones. An appropriate window size ($2m + 1 = 11$) was chosen to generate PCMW spectra with good quality.
2D correlation spectroscopy (2DCOS). FT-IR spectra chosen from the PCMW analysis were used to perform 2D correlation analysis. 2DCOS was carried out using the same 2D Shige software, ver. 1.3 (©Shigeaki Morita, Kwansei-Gakuin University, Japan, 2004–2005), and was further plotted into the contour maps by the Origin program, ver. 8.1. In the contour maps, the red color was defined as the positive intensities and the green color as the negative ones.

3 Results and discussion

3.1 Preparation and characterization of HPEI–NIPAM

Commercially available HPEI contains primary, secondary and tertiary amines with molar ratios of 37 : 35 : 28, as calculated by inverted gate 13C NMR spectra. The feed ratio of NIPAM to NH in HPEI is 2 : 1, thus nearly all primary and secondary amines would participate in the Michael addition when the reaction time is long enough. Aliquots were extracted from the reactive solution a h or 12 h to monitor the reaction. As the reaction proceeds, the 1H NMR spectra (Fig. S1) and relative integral area ratio (Table S1) show a gradually reduced content of NIPAM monomer protons compared to the final HPEI–NIPAM protons. Corresponding calorimetric measurements reveal a negative relation between the transition temperature (T_p) and the reaction time (Fig. S2a and b). As the reaction took place, more NIPAM monomers react with HPEI. Obviously, the increase of the substitution degree makes HPEI–NIPAM aqueous solutions undergo phase transitions at much lower temperatures. The phenomenon where LCST decreases with the increase of the substitution degree has also been reported previously. After 72 h, the reaction completely finished and HPEI–NIPAM with a high substitution degree was obtained. From the 1H NMR spectra in Fig. 1b, the presence of the resonances at 4.0 ppm (–CH(CH3)2, d), 2.3 ppm (–CH2CO, c) and 1.13 ppm (–CH(CH3)2, e) strongly indicates the incorporation of NIPAM moieties. In addition, Fig. 2 shows the FT-IR spectrum of HPEI–NIPAM compared to that of HPEI, where the emergence of rss(CH3) at 2968 cm⁻¹, amide I around 1644 cm⁻¹ and amide II around 1552 cm⁻¹, also implies the successful introduction of NIPAM moieties onto HPEI chains.

3.2 Turbidity

For most LCST-type polymers, solution concentrations can have more or less effects on their transition temperatures. Generally, according to previous reports, with increasing concentrations, the T_p increases or decreases depending on the types of polymers. For example, for poly(2-methoxyethyl vinyl ether) (PMOVE), poly(vinyl methyl ether) (PVME) and poly(N-vinylcaprolactam) (PVCL) (higher than 5 wt%), T_p exhibits positive correlations with concentration. But for poly(ethylene glycol acrylamide) (PEPA) and PNIPAM, the effects of concentration on T_p suggest negative correlations.

Here, we perform turbidity measurements to determine T_p and try to find out the relationship between concentrations and T_p. Purified HPEI–NIPAM was used to prepare polymer solutions of various concentrations ranging from 0.2 to 10 mg mL⁻¹. The turbidity results during a heating-and-cooling cycle are shown in Fig. 3. For each concentration, T_p was taken as the middle point of the transmittance versus temperature curves. With concentrations ranging from 0.2 to 10 mg mL⁻¹, T_p decreases from 75 to 38 °C as shown in Fig. 3b. The negative correlation is similar to PEPA and PNIPAM, and more obvious at low concentrations but less significant when concentrations are above 5 mg mL⁻¹. Moreover, the phase transition of HPEI–NIPAM is completely reversible with a very small hysteresis, for example at 10 mg mL⁻¹ the hysteresis is about 0.7 °C, which can

![Fig. 1](image1.jpg) (a) Synthesis of HPEI–NIPAM by Michael addition reaction. (b) 1H NMR spectra of HPEI–PNIPAM and HPEI in CDCl3.

![Fig. 2](image2.jpg) FT-IR spectral comparison between HPEI–NIPAM and HPEI.
aqueous solutions with the concentration of 1 and 10 mg mL\(^{-1}\) may be caused by the structural disruption at too high a temperature (90 °C).

Particularly, it is worth noting that the \(T_p\) variation here is nearly 40 °C for HPEI–NIPAM from 0.2 to 10 mg mL\(^{-1}\). However, for PNIPAM aqueous solutions, the variation is fairly small in a rather wide concentration range.\(^{26,21}\) Even for PNIPAM, stereoregularity strongly affects the concentration–transition temperature relationship. The \(T_p\) variation was found to be more obvious in isotactic-rich PNIPAM than atactic PNIPAM, which was attributed to dominant intermolecular interactions over intramolecular interactions.\(^{28}\) Other LCST-type polymers, such as PVCL,\(^{54}\) and HPEI–IBAm\(^{49}\) without intramolecular self-association, also show an obvious concentration dependence of the phase transition behaviors. Thus we consider that, although the connection between NIPAM moieties and HPEI is random, due to the hyperbranched architecture the phase transition of HPEI–NIPAM should be dominated by the interchain aggregation. It is reasonable that a low concentration would cause less interchain aggregation resulting in the apparent increase of \(T_p\). This can also be proved by DSC measurements. As reported,\(^{29}\) a polymer with the demixing temperature strongly dependent on concentration has a broader DSC peak, as shown in Fig. S2c and d.\(^{†}\) Additionally, as shown in Fig. S2d,\(^{†}\) a higher scanning rate leads to a higher \(T_p\), which has an analogous effect to decreasing the concentration, suggesting the existence of strong interchain aggregation.

We also performed DLS measurements of HPEI–NIPAM aqueous solutions with the concentration of 1 and 10 mg mL\(^{-1}\) during heating, as shown in Fig. S3.\(^{†}\) With increasing temperature, the hydrodynamic radius (\(R_h\)) of HPEI–NIPAM hardly changes until the temperature is above \(T_p\), corresponding to the occurrence of the phase separation or the formation of micelles according to turbidity measurements. Note that due to multiple scattering, the \(R_h\) value may not reflect the true size of the micelles formed by HPEI–NIPAM. The \(R_h\) remains unchanged before the phase transition (~3 nm), which is ascribed to be the size of the HPEI–NIPAM unimer. It reveals that there is no obvious pre-contraction or intrachain association of HPEI–NIPAM unimers before phase transition, which also suggests the dominating role of interchain aggregation during the phase transition of HPEI–NIPAM.

3.3 Conventional IR analysis

To further understand the hysteresis and phase transition behavior, temperature-dependent FT-IR spectra of 10 wt% HPEI–NIPAM in D\(_2\)O during a heating-and-cooling cycle from 30 to 45 °C with an interval of 0.5 °C were collected, as presented in Fig. 4. Here, D\(_2\)O was selected as the solvent to eliminate the overlap of the \(\delta(OH)\) band of water around 1640 cm\(^{-1}\) with the amide I band, as well as the wavenumber interference of \(\nu(OH)\) of H\(_2\)O around 3300 cm\(^{-1}\) with \(\nu(CH)\) of HPEI–NIPAM.

The band regions of \(\nu(CH)\) at 2900–2800 cm\(^{-1}\) and \(\nu(C=O)\) at 1665–1580 cm\(^{-1}\) show obvious changes and reflect nearly all the group motions of HPEI–NIPAM during the phase transition process, and are selected for further analysis. During heating, all the C–H stretching bands shift to a lower frequency, while C=O exhibits a binary spectral intensity change. During cooling, the case is just opposite to the heating process.

To find out the changes of \(\nu(CH)\) and \(\nu(C=O)\) during temperature variation, FT-IR spectra of HPEI–NIPAM in D\(_2\)O at 30, 35, 40 and 45 °C were chosen to make the comparison with the original neat sample, as presented in Fig. 4c and d. Since neat HPEI–NIPAM cannot form hydrophobic hydration and hydrogen bonding with water, we can ascribe the C–H groups to be those free of water and C=O groups to be free and self-associated ones. After the addition of D\(_2\)O, all the \(\nu(CH)\) peaks shift to higher wavenumbers, suggesting a hydration interaction with neighboring water molecules. With increasing temperature, all the bands dehydrate and exhibit a red shift. It should be noted that even at 45 °C after phase transition, the peaks of \(\nu(CH)\) in solution are still slightly higher than those of the neat sample, which indicates a weak hydration of the C–H groups. For \(\nu(C=O)\) bands, with water surrounding the polymer, C=O groups tend to form hydrogen bonds with D\(_2\)O and give rise to a band at a lower wavenumber around 1619 cm\(^{-1}\). During heating, a new band located at 1648 cm\(^{-1}\) emerges, which is close to the peak of the self-associated C=O groups in the neat sample. It can be inferred that after phase transition,
there are at least two types of C=O groups at 1619 and 1648 cm\(^{-1}\), respectively. Furthermore, from the quantitative analysis for \(\nu(C-H)\) and \(\nu(C=O)\) during heating and cooling (Fig. S4†), there is a small hysteresis in accordance with turbidity and calorimetric measurements. An incomplete return for both \(\nu_{as}(\text{CH}_2)\) and \(\nu(C=O)\) can be explained since HPEI–NIPAM has a relatively restricted structure with less conformational freedom, resulting in a slow hydration process during cooling.\(^{43}\)

It should be noted that amide I (C=O groups at 1664–1580 cm\(^{-1}\)) contains at least two types of C=O groups at 1619 and 1648 cm\(^{-1}\). By second-derivative analysis, the bands in the region of \(\nu(C=O)\) can be distinguished as presented in Fig. 5. The second derivative curves clearly demonstrate that there are two dominant bands in the amide I region. According to previous studies,\(^{60-62}\) 1619 cm\(^{-1}\) and 1648 cm\(^{-1}\) can be ascribed to C=O···D\(_2\)O and C=O···D–N hydrogen bonds, respectively. During heating, there is a binary change in which the band at 1619 cm\(^{-1}\) decreases as the band at 1648 cm\(^{-1}\) gradually increases. The variation is in the opposite direction in the cooling process. It is considered that as temperature rises the hydrogen bonds between C=O and D\(_2\)O transform into C=O···D–N ones and conversely during cooling.

Although HPEI–IBAm,\(^{45}\) HPEI–NIPAM and PNIPAM\(^{60,62}\) are very similar polymers with the same thermoresponsive moieties, they present obvious differences with respect to amide I during phase transition, as shown in Fig. 6. In terms of the number of bands, HPEI–NIPAM and PNIPAM show only two bands while there are three bands in HPEI–IBAm. As previously reported, the three bands of HPEI–IBAm at 1600, 1625 and

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**Fig. 4** Temperature-dependent FT-IR spectra of 10 wt% HPEI–NIPAM in D\(_2\)O (30–45 °C) during (a) heating and (b) cooling with an interval of 0.5 °C. Spectral comparison of 10 wt% HPEI–NIPAM in D\(_2\)O at several temperatures during heating with neat HPEI–NIPAM for (c) \(\nu(C-H)\) and (d) \(\nu(C=O)\).

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**Fig. 5** FT-IR and corresponding second derivative spectra of 10 wt% HPEI–NIPAM in D\(_2\)O at several temperatures during heating and cooling.
1648 cm\(^{-1}\) are attributed to the singly, doubly hydrogen bonded C=O groups with water and the C=O–D–N hydrogen bonds, respectively.\(^4\) In light of the molecular structures of these three polymers, the C=O groups of HPEI–IBAm contain two kinds of amide groups, secondary and tertiary amides with the molar ratio of 37 : 35. However, only secondary amides can afford protons to form hydrogen bonds with the C=O groups. So partial tertiary amide groups are exposed directly to water and form strong hydrogen bonds (C=O–D\(_2\)O) with the band at 1600 cm\(^{-1}\) without the competition from the N–D–OD\(_2\) or C=O–D–N hydrogen bonds. However, for HPEI–NIPAM and PNI-PAM, only secondary amides exist and both N–D and C=O parts can form hydrogen bonds with water or between themselves, leading to fewer C=O–D\(_2\)O hydrogen bonds due to fierce competition.

Furthermore, during heating, all the three polymers exhibit binary changes. C=O groups at lower wavenumbers gradually transform to those at higher wavenumbers; that is, hydrogen bonds between C=O and D\(_2\)O decrease with increasing C=O–D–N hydrogen bonds. Although HPEI–NIPAM contains the same thermoresponsive NIPAM moieties as linear PNIPAM, there are much fewer C=O–D–N hydrogen bonds formed after the phase transition, as shown in Fig. 6. Moreover, the band becomes a little broader. As HPEI–NIPAM is a dendritic polymer with a branched structure, NIPAM moieties in the periphery have much less conformational freedom compared to those in linear polymers. Thus, one can imagine that after the phase transition, amide groups of HPEI–NIPAM can only form hydrogen bonds with neighboring ones instead of undergoing large conformation changes.

From the above analysis, it reveals that during the phase transition, both C–H and C=O groups of HPEI–NIPAM experience an interesting variation in terms of band shift, shape and integral areas. Here, as presented in Fig. 7, isosbestic points were
Table 1 Existences of isosbestic points during heating and cooling for C–H and C–O groups of HPEI–NIPAM, HPEI–IBAm, PNIPAM aqueous solutions and the PNIPAM hydrogel

<table>
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<tr>
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<th>Heating</th>
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<td>C–H</td>
<td>C–O</td>
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<tr>
<td>HPEI–NIPAM</td>
<td>No</td>
<td>No</td>
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<tr>
<td>HPEI–IBAm</td>
<td>Yes</td>
<td>No</td>
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<td>PNIPAM solution</td>
<td>Yes</td>
<td>Yes</td>
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<td>PNIPAM hydrogel</td>
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employed to determine whether these transitions are quantitatively converted. For clarity, we chose only six spectra with the same temperature interval. No isosbestic points are observed either for ν(CH) or ν(C=O) during the heating and cooling cycle. In other words, C–H and C–O groups undergo variations with intermediates in both the chain aggregation and disaggregation processes. However, for comparison (Table 1), HPEI–IBAm exhibits isosbestic points for C–H groups during heating and C=O groups during cooling; PNIPAM hydrogel exhibits isosbestic points in the cooling process for both C–H and C–O groups; PNIPAM aqueous solution shows isosbestic points in both heating and cooling processes. It should be noted that HPEI–IBAm, HPEI–NIPAM and the PNIPAM hydrogel all have a restricted structure compared to linear PNIPAM. It indicates that a more restricted structure with a lower conformational freedom of groups would result in fewer isosbestic points. As NIPAM monomers can react with both primary and secondary amines of HPEI, the density of terminal groups in HPEI–NIPAM is higher than that in HPEI–IBAm. It in turn leads to a much lower conformational freedom, which may account for the absence of isosbestic points during both heating and cooling for HPEI–NIPAM.

3.5 Two-dimensional correlation analysis

2DCOS is a mathematical method whose basic principles were first proposed by Noda in 1986. Up to the present, 2DCOS has been widely used to study the spectral variations of different chemical species under various external perturbations (e.g. temperature, pressure, concentration, time, electromagnetic, etc.). Due to the different response of different species to an external variable, additional useful information about molecular motions or conformational changes can be extracted which cannot be obtained straight from the conventional 1D spectrum.

According to PCMW analysis, spectra from 33 to 41.5 °C were chosen for the 2DCOS analysis. 2D synchronous and asynchronous spectra of 10 wt% HPEI–NIPAM in D$_2$O are illustrated in Fig. 9. From the 2D synchronous spectrum, we can derive the simultaneous changes between two related wavenumbers. The bands at 2987, 2881, 2858 and 1619 cm$^{-1}$ all show positive cross-peaks, which indicate similar changes during temperature variation. In combination with 1D spectra, it can be inferred that these bands all decrease during heating and increase during cooling. The bands at 2964, 2927 and 1648 cm$^{-1}$ experience a phase transition in the opposite direction. In addition, the 2D asynchronous spectrum can enhance the resolution significantly. For instance, the bands at 2933 cm$^{-1}$ attributed to ν$_a$(hydrated CH$_2$) and the three splitting bands of ν(C=O···D$_2$O) at 1625, 1619 and 1591 cm$^{-1}$ can be identified. For clarity, all the bands observed from 2DCOS spectra are listed in Table 2 together with the tentative assignments.

Additionally, from the 2DCOS spectra, we can deduce the sequence order of all the C–H and C=O groups’ response to temperature changes. The judging rule of the sequence can be summarized as Noda’s rule – that is, if the cross peaks ($r_{12}$, $r_{21}$, and assume $r_1 > r_2$) in synchronous and asynchronous spectra have the same sign, the change at $r_1$ may occur prior to $r_2$, and vice versa.

(1) The heating process. Derived from the 2DCOS spectra, we can deduce the sequence order for the groups during heating as follows: 1648 > 2933 > 2927 > 1619 > 2987, 2881 > 2858 > 2838 > 1591 cm$^{-1}$ (> means prior to or earlier than).

To consider C=O related vibrations separately, it can be described as 1648 > 1619 > 1591 cm$^{-1}$. There are more than two bands here, which is in agreement with what we discussed in the isosbestic points where HPEI–NIPAM undergoes transition...
gradually with intermediates. Upon heating, the C=O⋅⋅⋅D–N hydrogen bonds between neighboring HPEI–NIPAM unimers gradually form and water molecules are expelled out of the polymer, which in turn leads to the breakage of the C=O⋅⋅⋅D2O hydrogen bonds.

As for C–H groups, according to the assignments in Table 2, the sequence order can be described as: r-as(hydrated CH2) > r-as(dehydrated CH3) > r-as(hydrated CH3), r-s(CH3) > r-s(dehydrated CH3). Without considering the differences in stretching modes, the sequence can be described as CH2 > CH3. It reveals that the branches of HPEI–NIPAM respond earlier than the terminal groups. Only considering the stretching modes of the CH3 groups in the branches, it is interesting to find that the asymmetric stretching modes respond earlier than the symmetric stretching modes. As reported,68 the direction of the asymmetric stretching vibration is parallel to the polymer chain axis while that of the symmetric stretching vibration is vertical to the polymer chain axis. Thus, it can be deduced that HPEI–NIPAM tends to collapse before water molecules are expelled out of the system.

Considering all the groups, it reveals that C=O groups respond earlier than C–H groups. The phase transition of HPEI–NIPAM during heating is driven by the formation of self-associated C=O⋅⋅⋅D–N hydrogen bonds followed by the collapse of the branched chains.

(2) The cooling process. Similarly, the sequence order for all the groups during cooling is described as: 1648 > 2838 > 2858 > 2881 > 2964 > 2927 cm⁻¹. For C=O groups separately, the sequence order will be 1648 > 1625 > 1619 > 1591 > 1580 cm⁻¹. It indicates that with decreasing temperature, the breakage of the self-associated C=O⋅⋅⋅D–N hydrogen bonds still has the earliest response followed by the formation of C=O⋅⋅⋅D2O hydrogen bonds.

As for the C–H groups, it will be r-as(dehydrated CH3) > r-s(hydrated CH3) > r-s(CH3) > r-as(hydrated CH3) > r-as(dehydrated CH3). Without considering the stretching modes, we have CH2 > CH3, very similar to the heating process, indicating that the branches of HPEI–NIPAM respond earlier than the thermoresponsive terminal groups during cooling. In addition, it indicates an opposite change to the heating process, that it is the symmetric stretching modes respond prior to the asymmetric modes if we only consider the stretching modes of CH2 groups in the branches. Therefore, we can conclude that during cooling, water molecules diffuse into the polymer system first to allow the revival or swelling of the branched chains.

For all the groups during cooling, we have C=O > CH, also consistent with the heating process. It reveals that upon cooling, the dehydration process of HPEI–NIPAM is driven by the breakage of the self-associated C=O⋅⋅⋅D–N hydrogen bonds, suggesting the dominating role of interchain aggregation and disaggregation in the reversible phase transition of HPEI–NIPAM, in accordance with our turbidity, DSC and DLS observations.

Combining the above sequence order analysis for both the heating and cooling processes, we can draw an integrated profile of the dynamic self-aggregation and disaggregation behavior of HPEI–NIPAM during the whole phase transition, as illustrated in Fig. 10. For convenience, only two particles are shown here for clarity. Below LCST, HPEI–NIPAM unimers dissolve in water with water molecules surrounding the polymer. C–H groups are hydrated and amide I groups, according to the assignments in Table 2, 2878 | 2885

CH3) > r-as(dehydrated CH3). Without considering the stretching modes, we have CH2 > CH3, very similar to the heating process, indicating that the branches of HPEI–NIPAM respond earlier than the thermoresponsive terminal groups during cooling. In addition, it indicates an opposite change to the heating process, that it is the symmetric stretching modes respond prior to the asymmetric modes if we only consider the stretching modes of CH2 groups in the branches. Therefore, we can conclude that during cooling, water molecules diffuse into the polymer system first to allow the revival or swelling of the branched chains.

For all the groups during cooling, we have C=O > CH, also consistent with the heating process. It reveals that upon cooling, the dehydration process of HPEI–NIPAM is driven by the breakage of the self-associated C=O⋅⋅⋅D–N hydrogen bonds, suggesting the dominating role of interchain aggregation and disaggregation in the reversible phase transition of HPEI–NIPAM, in accordance with our turbidity, DSC and DLS observations.

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HPEI–NIPAM chains. Then, water molecules are expelled out of the polymer system. For the cooling process, along with the disaggregation of HPEI–NIPAM micelles, water molecules diffuse into the polymer system first to break the self-associated C=O⋯D–N hydrogen bonds. Then, the C–H groups tend to hydrate with water gradually, which consequentially promotes the revival or swelling of the branched chains. During both heating and cooling the formation and breakage of the self-associated C=O⋯D–N hydrogen bonds always dominate the phase transition of HPEI–NPAM in close relation to the chain aggregation and disaggregation.

Obviously, this mechanism for the phase transition of HPEI–NIPAM is largely different from that of linear PNIPAM, which shows a two-step dehydration of the CH3 groups first, followed by the main-chain diffusion and aggregation along with the hydrogen bond transition. The dynamic sequence in the cooling process of linear PNIPAM is just the opposite.60 However, due to the branched architecture and the absence of intrachain self-association, the interchain aggregation and disaggregation induced by the transition between self-associated C=O⋯D–N hydrogen bonds and C=O⋯D2O ones always dominates the heating and cooling processes.

Table 2  Tentative band assignments of HPEI–NIPAM in D2O according to 2DCOS results

<table>
<thead>
<tr>
<th>Frequency/cm⁻¹</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>2987</td>
<td>ν₁ as(hydrated CH₃)</td>
</tr>
<tr>
<td>2964</td>
<td>ν₁ as(dehydrated CH₃)</td>
</tr>
<tr>
<td>2933</td>
<td>ν₁ ad(hydrated CH₂)</td>
</tr>
<tr>
<td>2927</td>
<td>ν₁ ad(dehydrated CH₂)</td>
</tr>
<tr>
<td>2881</td>
<td>ν₁(CH₃)</td>
</tr>
<tr>
<td>2858</td>
<td>ν₁(hydrated CH₂)</td>
</tr>
<tr>
<td>2838</td>
<td>ν₁(dehydrated CH₂)</td>
</tr>
<tr>
<td>1648</td>
<td>ν(C=O⋯D–N)</td>
</tr>
<tr>
<td>1625, 1619, 1591</td>
<td>ν(C=O⋯D₂O)</td>
</tr>
</tbody>
</table>

Fig. 9  2D synchronous and asynchronous spectra of 10 wt% HPEI–NIPAM in D₂O from all the spectra between 33 and 41.5 °C during heating (left) and cooling (right). Red colors are defined as positive intensities, while green colors as negative ones.
Conclusions

Thermoresponsive HPEI–NIPAM was synthesized by Michael addition reaction between NIPAM monomers and HPEI. A series of HPEI–NIPAM samples with different reaction times were obtained. The LCST values are found to decrease with the increase of the substitution degree and concentration, and the concentration effect is much more obvious at relatively low concentrations than at high concentrations with an abnormal large variation range of ca. 40 °C determined by turbidity measurements.

Conventional FT-IR of 10 wt% HPEI–NIPAM in D2O has been performed to further understand the phase transition behavior. Compared to the neat sample it reveals that, for HPEI–NIPAM in D2O, C–H groups hydrate with water and C=O···D2O hydrogen bonds are formed. Especially, there are two dominant bands in the amide I region. Although with only secondary amide groups like PNIPAM, HPEI–NIPAM presents a rather small emergence at 1648 cm⁻¹ after phase transition due to the reduced conformation freedom of the peripheral NIPAM groups. The disappearance of the isosbestic points indicates that the phase transition of HPEI–NIPAM occurs gradually with intermediates. Moreover, PCMW was employed to determine the phase transition temperature, which was ca. 36.5 °C during heating and ca. 36 °C during cooling, and the transition temperature range, which was 33–41.5 °C.

Further study on the groups’ interactions and phase transition mechanism was carried out by 2DCOS analysis. The identification of the sequence order finally promotes an integrated profile of the dynamic self-aggregation and disaggregation behavior of HPEI–NIPAM during both heating and cooling. That is, during heating, self-associated C=O···D–N hydrogen bonds between amide groups are firstly formed resulting in the collapse of the HPEI–NIPAM chains. Then, water molecules are expelled out of the polymer system. Whereas, for the cooling process, water molecules diffuse into the polymer system first to break the self-associated C=O···D–N hydrogen bonds. Then, C–H groups tend to hydrate with water gradually, which consequentially promotes the revival or swelling of the branched chains. We expect that our study of a thermoresponsive hyperbranched polymer and its comparison to similar polymers will provide useful information for understanding the relationship between structure and their responsive properties.

Acknowledgements

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