Dynamic self-aggregation behavior of a PNIPAM-based nonlinear multihydrophilic block copolymer revealed by two-dimensional correlation spectroscopy

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IR spectroscopy in combination with the perturbation correlation moving window (PCMW) technique and 2D correlation spectroscopy (2DCOS) is employed to elucidate the dynamic self-aggregation behavior of a novel miktoarm star PNIPAM-based multihydrophilic block copolymer, poly(N-isopropylacrylamide)–[poly(N-vinylpyrrolidone)-b-poly(acrylic acid)]3 ((PNIPAM)2–(PVP-b-PAA)3).

At pH = 8, (PNIPAM)2–(PVP-b-PAA)2 tends to self-assemble into micelles with PNIPAM in the core and ionized PAA segments in the shell during heating. IR investigation shows that the AA segments exhibit a similar “phase transition” behavior to the PNIPAM segments, which can be ascribed to the indirect influence through the drastic content changes of water molecules along with the hydrophilic-to-hydrophobic transformation of PNIPAM segments. Boltzmann fitting and PCMW easily determine the transition temperature to be ca. 33 °C and the transition temperature range to be 29.5–35 °C. Moreover, 2DCOS discerns a sequential group motion from PNIPAM to PVP and PAA segments. It is concluded that the three polymeric segments have relatively independent phase behavior during the formation of PNIPAM-core micelles, and the chain conformation adjustment induced by hydrophilic-to-hydrophobic transformation of PNIPAM segments should be the driving force of the whole self-aggregation process. The dynamic self-aggregation process we proposed can be further confirmed by dynamic laser scattering (DLS) and zeta potential measurements.

1 Introduction

In recent years, stimuli-responsive double hydrophilic block copolymers (DHBCs)1–3 have received tremendous interest due to their diverse biological and technological applications in drug delivery,4–6 imaging,7 sensing,8 catalysis,9 stabilizing colloids,10 templating crystal growth,11 and so on. These polymers are always amphiphilic molecules composed of two different polymeric segments, which can reversibly switch between micellar and unimer states in water by adjusting different external stimuli, such as pH, temperature, or light irradiation, resulting in various self-assembled nanostructures.12 More recently, plenty of nonlinear and multihydrophilic block copolymers (MHBCs) have also been successfully synthesized, e.g. miktoarm star polymers,10,11 dendritic–linear block copolymers,12 cyclic block copolymers13 and comb-shaped copolymer brushes.14

Among them, temperature sensitivity is one of the most interesting properties in stimuli-responsive water-soluble polymers. As the mostly investigated example, poly(N-isopropylacrylamide) (PNIPAM) was extensively utilized for the construction of thermo-sensitive micelles.15 Take the simple poly(N-isopropylacrylamide)-b-poly(acrylic acid) (PNIPAM-b-PAA) polymer for example, at pH < 4.5 and T < 32 °C, the PAA segments are less soluble than the PNIPAM segments due to the protonation of the carboxylate groups resulting in micelles with PAA as the hydrophobic core and PNIPAM as the hydrophilic shell; however, at pH > 4.5 and T > 32 °C, the PAA segments are much more soluble than the PNIPAM segments resulting in micelles with PNIPAM as the hydrophobic core and PAA as the hydrophilic shell.16 As the temperature increases to above the lower critical solution temperature (LCST), a hydrophilic-to-hydrophobic transformation of PNIPAM segments is believed to account for the formation of colloidal micelles.

A number of methods have been developed to study the self-aggregation behavior of DHBCs or MHBCs, such as turbidity,17 NMR,18 IR,19–21 Raman,22 light scattering,23,24 calorimetry,25 viscometry26 and transmission electron microscopy.27 However, only a few previous studies were concerned with a dynamic...
description of chain conformation transformations and relevant complicated interactions at the molecular level.\textsuperscript{19,20,24} As we know, interactions between polymeric segments and water molecules as well as intra- and inter-polymer interactions\textsuperscript{18,29} may all be involved in the dynamic self-aggregation behavior of MHBCs. Thus, to discern the driving force of phase separation and the dominating interactions in different hydration stages is particularly helpful for our understanding of the formation of micelles and molecular design. In this paper, IR spectroscopy, as well as the perturbation correlation moving window (PCMW) technique\textsuperscript{60,31} and 2D correlation spectroscopy (2DCOS)\textsuperscript{12,31}, is employed to elucidate the dynamic self-aggregation behavior of a novel miktoarm star PNIPAM-based MHBC ((PNIPAM)\textsubscript{2}–(PVP-b-PAA)\textsubscript{2}, as shown in Scheme 1). This novel nonlinear MHBC consisting of pH-responsive PAA segments, thermo-responsive PNIPAM segments, and hydrophilic, non-charged and biocompatible PVP segments tends to self-assemble into three different types of micellar aggregates and unimers dependent on different pH and temperature conditions.\textsuperscript{34} Herein, we mainly focus on the thermodynamic self-aggregation behavior of (PNIPAM)\textsubscript{2}–(PVP-b-PAA)\textsubscript{2} from the unimers to PNIPAM-core micelles at pH = 8. In this condition, the PAA segments are partially ionized for good solubility in water. Dynamic laser scattering (DLS) and zeta potential measurements were also used to confirm our 2DCOS results.

2 Experimental section

2.1 Materials

(PNIPAM)\textsubscript{2}–(PVP-b-PAA)\textsubscript{2} was synthesized via a combination of single-electron transfer living radical polymerization and reversible addition–fragmentation chain transfer (RAFT) polymerization techniques.\textsuperscript{34} The number average molecular weight of (PNIPAM)\textsubscript{2}–(PVP-b-PAA)\textsubscript{2}, \(M_n = 2.9 \times 10^4\) and polydispersity index \(M_d/M_n = 1.22\) were determined by GPC measurements with monodisperse polystyrene as the standard and DMF as the eluent phase at 30°C. \(\text{D}_2\text{O}\) were purchased from Cambridge Isotope Laboratories Inc. (D-99.9\%). For calorimetric measurements, (PNIPAM)\textsubscript{2}–(PVP-b-PAA)\textsubscript{2} solution in \(\text{D}_2\text{O}\) at pH = 8 was placed at 4°C for a week before FT-IR measurements to ensure complete dissolution and deuteration.

2.2 Instruments and measurements

The sample of (PNIPAM)\textsubscript{2}–(PVP-b-PAA)\textsubscript{2} solution (10 wt% in \(\text{D}_2\text{O}\)) for FT-IR measurements was prepared by sealing it between two CaF\textsubscript{2} tablets. All time-resolved FT-IR spectra at different temperatures were recorded on a Nicolet Nexus 470 spectrometer with a resolution of 4 cm\textsuperscript{-1}, and 32 scans were available for an acceptable signal-to-noise ratio. Temperatures were manually controlled with an electronic cell holder at rates of ca. 0.3°C min\textsuperscript{-1} with an increment of 0.5°C. Raw spectra were baseline-corrected by the software Omnic, ver. 6.1a.

Calorimetric measurements of (PNIPAM)\textsubscript{2}–(PVP-b-PAA)\textsubscript{2} in \(\text{D}_2\text{O}\) were performed on a Mettler-Toledo differential scanning calorimeter (DSC) thermal analyzer with varying scanning rates and concentrations from 5 to 60°C. The temperature-dependent average hydrodynamic radius (\(R_h\)) and zeta potential measurements of (PNIPAM)\textsubscript{2}–(PVP-b-PAA)\textsubscript{2} in \(\text{H}_2\text{O}\) (1.0 mg mL\textsuperscript{-1}, pH = 8) were performed on a DLS-zetasizer nanosystem (Malvern) with increments of 2°C from 26 to 50°C.

2.3 Investigation methods

Perturbation correlation moving window (PCMW) analysis. FT-IR spectra collected in increments of 0.5°C during heating were used to perform PCMW analysis. Primary data processing was carried out with the method Morita provided and further correlation calculations were performed using the software 2D Shige, ver. 1.3 (© Shigeki Morita, Kwansei-Gakuin University, Japan, 2004–2005). The final contour maps were plotted by Origin program, ver. 8.1, with warm colors (red and yellow) defined as positive intensities and cool colors (blue) as negative ones. An appropriate window size (2m + 1 = 11) was chosen to generate the PCMW spectra with good quality.

2D correlation spectroscopy (2DCOS). FT-IR spectra chosen from the PCMW analysis were used to perform 2D correlation analysis. 2D correlation analysis was carried out using the same software 2D Shige ver. 1.3 (© Shigeki Morita, Kwansei-Gakuin University, Japan, 2004–2005), and was further plotted into the contour maps by Origin program ver. 8.1. In the contour maps, warm colors (red and yellow) are defined as positive intensities, while cool colors (blue) as negative ones.

3 Results and discussion

3.1 Calorimetric measurements

We performed the calorimetric measurements to examine the concentration and scanning rate effects on the aggregation behavior of (PNIPAM)\textsubscript{2}–(PVP-b-PAA)\textsubscript{2} in \(\text{D}_2\text{O}\), as shown in Fig. 1. As reported for PNIPAM aqueous solutions, a faster scanning rate usually leads to a higher transition temperature and more asymmetric DSC curves during heating as a result of the competition between intrachain contraction and interchain association.\textsuperscript{35} That is, a slow heating rate allows the linear PNIPAM chains to collapse and aggregate simultaneously, while a faster heating rate leads to more intrachain contraction and less interchain association. Decreasing the concentration has an analogous effect to decreasing the scanning rate by causing more intrachain contraction.\textsuperscript{36} It is notable that despite one

Scheme 1 Chemical structure of (PNIPAM)\textsubscript{2}–(PVP-b-PAA)\textsubscript{2} and its corresponding schematic illustration. Where \(m = 47, n = 38, r = 45\).
end-constrained conformation of PNIPAM segments, (PNIPAM)$_2$-(PVP-$b$-PAA)$_2$ exhibits similar changes to linear PNIPAM chains with variation in scanning rate and concentration. This suggests that the PNIPAM segments may have relatively independent phase behavior during the aggregation process of miktoarm multihydrophilic block (PNIPAM)$_2$–(PVP-$b$-PAA)$_2$ chains.

### 3.2 Conventional IR analysis

To further examine its dynamic aggregation behavior, temperature-dependent FT-IR spectra of (PNIPAM)$_2$–(PVP-$b$-PAA)$_2$ in D$_2$O (10 wt%, pH = 8) were collected during heating between 26 and 40 °C, as shown in Fig. 2. It should be noted that we used D$_2$O instead of H$_2$O as the solvent here in order to eliminate the overlap of $\delta$(O–H) band of H$_2$O around 1640 cm$^{-1}$ with the $\nu$(C=O) bands as well as the broad $\nu$(O–H) band of H$_2$O around 3300 cm$^{-1}$ with the $\nu$(C–H) bands of (PNIPAM)$_2$–(PVP-$b$-PAA)$_2$. For the thermo-responsive PNIPAM segments, the transition temperature in D$_2$O is ca. 0.7 °C higher than that in H$_2$O. Thus, the deuterium isotope effect can be considered to cause no obvious changes on the phase separation of (PNIPAM)$_2$–(PVP-$b$-PAA)$_2$.

Herein, two spectral regions are mainly focused upon: C–H related stretching vibrations (3030–2845 cm$^{-1}$) involving all C–H groups of (PNIPAM)$_2$–(PVP-$b$-PAA)$_2$, C=O and COO$^-$ related stretching vibrations (1750–1530 cm$^{-1}$) consisting of the COOD groups of the PAA segments around 1710 cm$^{-1}$, amide I groups of the PNIPAM and PVP segments around 1626 cm$^{-1}$ and COO$^-$ groups of the ionized PAA segments around 1570 cm$^{-1}$. Therefore, the changes of the $\nu$(C–H) bands can be explained by a gradual strengthening of the hydrophobic interaction of polymer chains, which should mainly arise from the PNIPAM segments.
In addition, during heating the amide I groups of (PNIPAM)$_2$–(PVP-b-PAA)$_2$ show a binary spectral intensity change, also very similar to the spectral variation of pure PNIPAM in D$_2$O, indicating that in (PNIPAM)$_2$–(PVP-b-PAA)$_2$, despite being constrained in the miktoarm architectures, the phase separation behavior of PNIPAM segments is not obviously influenced. The ν(C=O) band can be roughly considered to be the combination of two bands at 1626 and 1651 cm$^{-1}$, which can be assigned to C=O stretching vibrations in C=O⋯D.O and C=O⋯D–N hydrogen bonds, respectively. Thus the binary change of amide I in the PNIPAM segments during heating can be explained by the transformation of the hydrogen bonds of C=O from being with water to being self-associated groups. Interestingly, ionized and nonionized COOD groups in the PAA segments exhibit the opposite intensity changes, strongly indicating that during heating the COOD groups are gradually ionized into COO$^-$ groups.

To quantitatively describe the frequency shift of the ν(C–H) bands, the content changes (reflected by integral areas) of amide I groups in the PNIPAM segments, as well as the changes of COOD and COO$^-$ group content in the PAA segments, we plotted their temperature-dependent tendencies in Fig. 3. It is surprising that the changes in COOD and COO$^-$ group content in the PAA segments both show an S or anti-S shaped variation, which is only characteristic for phase transition polymers such as PNIPAM. It is also totally different from PNIPAM-co-AA random copolymers, in which the COOD of the AA moieties exhibits a gradual intensity increase due to the formation of stable hydrogen bonds between the AA moieties and the NIPAM moieties. It is presumed that in (PNIPAM)$_2$–(PVP-b-PAA)$_2$ the PNIPAM segments do not form massive hydrogen bonds with the PAA segments due to the block architecture. Considering that the gradual ionization of COOD groups in the PAA segments are remarkably influenced by the phase separation of the PNIPAM segments, we ascribe this phenomenon to the drastic change in the content of water molecules surrounding the (PNIPAM)$_2$–(PVP-b-PAA)$_2$ unimers along with the hydrophilic-to-hydrophobic transformation of the PNIPAM segments during heating. This is also the first report of the indirect influence between thermo-responsive polymer segments and pH-responsive polymer segments through changes in the content of water molecules in the DHBC and MHBC systems.

For an accurate determination of the transition temperature of (PNIPAM)$_2$–(PVP-b-PAA)$_2$, Boltzmann fitting was employed for all the points in Fig. 3. The corresponding equation is as follows:

$$y = \frac{A_1 - A_2}{1 + e^{(x-x_0)/\Delta x}} + A_2$$

In eqn (1), $A_1$ is the minimum value of the function; $A_2$ is the maximum value of the function; $x_0$ is the value on the x axis corresponding to the inflection of the curve, which also equals to the transition temperature; and $\Delta x$ is the domain where this value lies. Boltzmann fitted results have all been presented in Fig. 3, which are closely approximate to that obtained from DSC ($\approx$32.4 °C, 10 °C min$^{-1}$). Determined from the transition temperatures during heating in Fig. 3, we can roughly conclude that in (PNIPAM)$_2$–(PVP-b-PAA)$_2$, the PNIPAM segments have an earlier response than the PAA segments, while for the PNIPAM segments separately, the CH-related groups respond slightly earlier than the CO-related groups, which is consistent with the observations of pure PNIPAM in D$_2$O.

### 3.3 Perturbation correlation moving window analysis

PCMW is a newly developed technique, whose basic principles date back to conventional moving window analysis proposed by Thomas et al., which were later improved in 2006 Morita et al. to give this technique much wider applicability through introducing the perturbation variable into the correlation equation. Except for its original ability in determining transition points as conventional moving window did, PCMW can additionally monitor complicated spectral variations along the perturbation direction.

Fig. 4 presents PCMW synchronous and asynchronous spectra as well as the corresponding transition temperature region plot of (PNIPAM)$_2$–(PVP-b-PAA)$_2$ in D$_2$O (10 wt%) at pH = 8 during heating between 26 and 40 °C. PCMW synchronous spectra are very helpful to find transition points. Thus we can easily determine the transition temperature of (PNIPAM)$_2$–(PVP-b-PAA)$_2$ to be ca. 33 °C, in good conformity with the Boltzmann fitting results. Determined from different transition temperatures of different groups in PCMW...
synchronous spectra in Fig. 4, we can draw the same conclusion that the PNIPAM segments have an earlier response than the PAA segments. For the PNIPAM segments, the CH-related groups are nearly synchronized with the CO-related ones, perhaps owing to their one end-constrained conformation compared to end-free PNIPAM chains.

In addition to determining transition points, PCMW can also monitor the spectral variations along temperature perturbation combining the signs of synchronous and asynchronous spectra by the following rules: positive synchronous correlation represents an increasing spectral intensity, while negative one represents it decreasing; positive asynchronous correlation can be observed for a convex spectral intensity variation while a negative one can be observed for a concave variation. Based on this point, we can ascertain that during heating both the CH and CO related bands of (PNIPAM)$_2$-(PVP-b-PAA)$_2$ show S-shaped or anti-S-shaped spectral changes, consistent with the above conventional IR analysis.

The transition temperature region can also be determined by the peaks in the asynchronous spectra which are all turning points of the sigmoid curves. Thus we can conclude that (PNIPAM)$_2$-(PVP-b-PAA)$_2$ experiences a phase transition in D$_2$O between 29.5 and 35 °C during heating. This served as an important basis for the segmental mode of the following 2DCOS analysis.

### 3.4 Two-dimensional correlation analysis

2DCOS is a mathematical method whose basic principles were first proposed by Noda in 1986. Up to now, 2DCOS has been widely used to study 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 external variables, additional useful information about molecular motions or conformational changes can be extracted which cannot be obtained straight from conventional 1D spectra.

On the basis of the phase transition evolving regions obtained from PCMW, we chose to perform 2DCOS analysis on all the spectra between 29.5 and 35 °C, as shown in Fig. 5. 2D synchronous spectra reflect simultaneous changes between two given wavenumbers. The bands at 2972, 2927, 2872, 1647,
1570 cm\(^{-1}\) all have positive cross-peaks, indicating that their spectral intensities had similar responses to temperature perturbation—that is, they all increase during heating, as determined from the raw spectra. On the other hand, the bands at 2991, 1686 and 1626 cm\(^{-1}\) have spectral intensities that decrease during heating.

2D asynchronous spectra can significantly enhance the spectral resolution. In Fig. 5, many subtle bands such as the bands at 2947, 2860 and 1657 cm\(^{-1}\) attributed to the \(v_{as}(\text{CH}_2)\), \(v_{as}(\text{CH}_3)\) and \(\nu(C=O)\) of the PVP segments, which are heavily overlapped by the PNIPAM segments, can be identified. These newly found bands corresponding to subtle group conformations would facilitate further 2DCOS analysis for discerning sequential group motions. For the convenience of discussion, all the bands found in the asynchronous spectra and their corresponding assignments have been presented in Table 1.

Except for enhancing spectral resolution, 2DCOS can also discern the specific order taking place under external perturbation. An asynchronous cross-peak can develop only if the intensities of two spectral features change out of phase with each other (i.e., delayed or accelerated if time is the external variable). The determining rule can be summarized as Noda’s rule—that is, if the asynchronous spectra have the same sign, the change at \(v_1\) may occur prior to that of \(v_2\), and vice versa. A simplified method for determination of sequence order has ever been described before.\(^4\)

In short, multiplicity was performed on the two signs of each cross-peak in the synchronous and asynchronous spectra, the final results of which are presented in Table 2. For each sign of the cross-peaks in Table 2, according to Noda’s rule, if the sign is positive (+), the larger wavenumber or the bottom wavenumber will respond to the external perturbation earlier than the smaller wavenumber or the left wavenumber. Similarly, if the sign is negative (−), the left wavenumber will respond earlier than the bottom one. If the sign is zero (or blank), we cannot make an exact judgement. Thus we can easily deduce the final specific order for the heating process of \((\text{PNIPAM})_2–(\text{PVP-b-PAA})_2\) in \(\text{D}_2\text{O}\) at pH = 8 (− means prior to or earlier than): 2991 cm\(^{-1}\) → 1647 cm\(^{-1}\) → 1626 cm\(^{-1}\) → 1657 cm\(^{-1}\) → 2972 cm\(^{-1}\) → 2872 cm\(^{-1}\) → 1614 cm\(^{-1}\) → 2927 cm\(^{-1}\) → 1570 cm\(^{-1}\) → 1686 cm\(^{-1}\) → 2860 cm\(^{-1}\) → 2883 cm\(^{-1}\) → 2947 cm\(^{-1}\) → 1714 cm\(^{-1}\).

Table 1 Tentative band assignments of (PNIPAM)\(_2–(PVP-b-PAA)_2\) in \(\text{D}_2\text{O}\) at pH = 8 according to the 2DCOS results\(^3,39-41\)

<table>
<thead>
<tr>
<th>Frequency/cm(^{-1})</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>2991</td>
<td>(v_{as}(\text{CH}_3)) (Hydrated) (PNIPAM)</td>
</tr>
<tr>
<td>2972</td>
<td>(v_{as}(\text{CH}_3)) (Dehydrated) (PNIPAM)</td>
</tr>
<tr>
<td>2947</td>
<td>(v_{as}(\text{CH}_2)) (PVP)</td>
</tr>
<tr>
<td>2927</td>
<td>(v_{as}(\text{CH}_2)) (Dehydrated) (PNIPAM)</td>
</tr>
<tr>
<td>2883</td>
<td>(v_{as}(\text{CH}_2)) (Hydrated) (PNIPAM)</td>
</tr>
<tr>
<td>2872</td>
<td>(v_{as}(\text{CH}_2)) (PNIPAM)</td>
</tr>
<tr>
<td>2860</td>
<td>(v_{as}(\text{CH}_2)) (PVP)</td>
</tr>
<tr>
<td>1714</td>
<td>(\nu(C=O)–D-O(A_A)) (PAA)</td>
</tr>
<tr>
<td>1686</td>
<td>(\nu(C=O)–D-O(D)) (PAA)</td>
</tr>
<tr>
<td>1657</td>
<td>(\nu(C=O)–D-O(D)) (PVP)</td>
</tr>
<tr>
<td>1647</td>
<td>(\nu(C=O)–D-N)) (PNIPAM)</td>
</tr>
<tr>
<td>1626</td>
<td>(\nu(C=O)–D-O(D)) (PNIPAM)</td>
</tr>
<tr>
<td>1614</td>
<td>(\nu(C=O)–2D-O(D)) (PNIPAM)</td>
</tr>
<tr>
<td>1570</td>
<td>(\nu(\text{COO}^+)) (PAA)</td>
</tr>
</tbody>
</table>

Not considering the differences of vibration modes of the C–H and C=O groups, we have CH\(_3\) (PNIPAM) → C=O (PNIPAM) → C=O (PVP) → CH\(_2\) (PNIPAM) → COO\(^-\) (PAA) → CH\(_2\) (PVP) → COOD (PAA). Obviously, during the heating of \((\text{PNIPAM})_2–(\text{PVP-b-PAA}_2)\), the PNIPAM segments respond first, followed by PVP segments and PAA segments sequentially. This order is in good conformity with the linking sequence of \((\text{PNIPAM})_2–(\text{PVP-b-PAA}_2)\). It seems that the three polymeric segments have relatively independent phase behavior during the formation of the PNIPAM-core micelles. Except for the structural linking, the interplay among them is mainly achieved through the changes in the content of water molecules surrounding the \((\text{PNIPAM})_2–(\text{PVP-b-PAA}_2)\) unimers. In other words, it is the conformation adjustment induced by the hydrophilic-to-hydrophobic transformation of the PNIPAM segments that drives the formation of the PNIPAM-core micelles. Therefore, in combination with above conventional IR and PCMW analysis, the dynamic self-aggregation behavior of \((\text{PNIPAM})_2–(\text{PVP-b-PAA}_2)\) can be described as follows: at temperatures below the LCST, both PNIPAM, PVP, PAA segments are hydrophilic and soluble in water resulting in a four-arm unimer with relatively extended conformation; as the temperature increases to above the LCST, the two PNIPAM arms collapse and transform to hydrophobic chains resulting in an amphiphilic macromolecule. However, the unimers in this conformation are not stable and tend to aggregate into micelles with the PNIPAM segments in the core and the more ionized PAA in the shell, which finally leads to the micellization of \((\text{PNIPAM})_2–(\text{PVP-b-PAA}_2)\).

Along with the drastic decrease in the number of water molecules induced by the hydrophilic-to-hydrophobic transformation of the PNIPAM segments, the PAA segments are indirectly influenced and exhibit similar “phase transition” behavior. A schematic illustration of this dynamic self-aggregation process has been presented in Fig. 6.

3.5 DLS and zeta potential analysis

DLS and zeta potential are particularly useful methods for investigating the dynamic aggregation behavior of various charged or noncharged water-soluble polymers.\(^6,49-51\) Herein, the concentration of \((\text{PNIPAM})_2–(\text{PVP-b-PAA})_2\) in water was fixed at 0.2 or 1.0 mg mL\(^{-1}\) to make the polymer chain behave as a unimer. From Fig. 7 we can see that the \(R_h\) of \((\text{PNIPAM})_2–(\text{PVP-b-PAA})_2\) exhibits phase transition curve profiles at ca. 41 and 38 °C at 0.2 and 1.0 mg mL\(^{-1}\) respectively, which are both a little higher than that obtained from the IR measurements (10 wt%) due to the concentration effect. The \(R_h\) value of \((\text{PNIPAM})_2–(\text{PVP-b-PAA})_2\) at 0.2 mg mL\(^{-1}\) (〜9.0 nm) is a little higher than that at 1.0 mg mL\(^{-1}\) (〜6.0 nm) before the phase transition, probably owing to pH fluctuation. Take the sample at 1.0 mg mL\(^{-1}\) for example, a slow decrease in \(R_h\) occurs before the transition from 7.5 nm at 26 °C to 4.9 nm at 36 °C, indicating that the \((\text{PNIPAM})_2–(\text{PVP-b-PAA})_2\) unimer has a gradual hydrophilic-to-hydrophobic conformation adjustment due to the intrachain contraction of PNIPAM segments, in agreement with our previous 2DCOS analysis results. Then \(R_h\) drastically increases to 200 nm above the LCST, corresponding to the unimers’ aggregation into micelles with PNIPAM segments in the core and ionized PAA in the shell. The \(R_h\) of...
At 0.2 mg mL\(^{-1}\)/C\(_0\), the change before and after the phase transition is similar. However, it is worth noting that the final size of the PNIPAM-core micelles at 0.2 mg mL\(^{-1}\)/C\(_0\) is only 1/3 of that at 1.0 mg mL\(^{-1}\)/C\(_0\). This reveals that by increasing the concentration, more (PNIPAM)\(_2\)–(PVP-b-PAA)\(_2\) unimers would participate in the formation of PNIPAM-core micelles, resulting in a much larger micelle size. Similar results have also been obtained in relation to the phase transition behavior of hyperbranched poly(ethylenimine) with iso-butryamide residues.

The zeta potential mainly reflects the charge changes of the PAA segments in (PNIPAM)\(_2\)–(PVP-b-PAA)\(_2\) unimers and micelles. Only the case with a concentration of 1.0 mg mL\(^{-1}\) is shown in Fig. 7. The zeta potential curve shows an anti-S shaped profile, in accordance with our previous analysis on the “phase transition” behavior of the PAA segments. It is also notable that the zeta potential related to the PAA segments has a “transition temperature” (ca. 35 °C) lower than that observed from the \(R_\text{h}\) measurements related to the unimer-to-micelle transition. This reveals that the zeta potential of the (PNIPAM)\(_2\)–(PVP-b-PAA)\(_2\) unimer shows a drastic decrease just before the growth in size indicating that the ionization of the PAA segments is indirectly influenced by the hydrophilic-to-hydrophobic transformation of the PNIPAM segments.

Conclusions

In this paper, FT-IR spectroscopy, in combination with the perturbation correlation moving window (PCMW) technique and 2D correlation spectroscopy (2DCOS), is used to elucidate the dynamic self-aggregation behavior of a novel miktoarm multihydrophilic block copolymer, (PNIPAM)\(_2\)–(PVP-b-PAA)\(_2\). Upon heating at pH = 8, (PNIPAM)\(_2\)–(PVP-b-PAA)\(_2\) tends to self-assemble from unimers to form PNIPAM-core micelles. In the unimers, the PAA segments are partially ionized. Interestingly, both ionized and nonionized PAA segments exhibit a similar “phase transition” behavior to the PNIPAM segments, which can be ascribed to the indirect influence through changes in the content of water molecules induced by hydrophilic-to-hydrophobic transformation of the PNIPAM segments. Boltzmann fitting and PCMW analysis easily confirm the transition temperature to be ca. 33 °C and the transition temperature range

### Table 2: The final results of multiplication on the signs of each cross-peak in synchronous and asynchronous spectra during heating

<table>
<thead>
<tr>
<th>Cross-peak</th>
<th>Synchronous</th>
<th>Asynchronous</th>
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<tbody>
<tr>
<td>1570</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1614</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1626</td>
<td>+</td>
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Fig. 6: Schematic illustration of dynamic self-aggregation behavior of (PNIPAM)\(_2\)–(PVP-b-PAA)\(_2\) in D\(_2\)O at pH = 8 during heating. Note that the number of unimers participating in the formation of micelles is only representative, and actually, the number would increase with increasing concentration.

Fig. 7: DLS (0.2 and 1.0 mg mL\(^{-1}\)) and zeta potential (1.0 mg mL\(^{-1}\)) measurements of (PNIPAM)\(_2\)–(PVP-b-PAA)\(_2\) in H\(_2\)O at pH = 8 during heating with increments of 2 °C from 26 to 50 °C. Scanning rate: 0.3 °C min\(^{-1}\).
to be 29.5–35 °C. By discerning the sequence of different group motions, 2DCOS results reveal that the PNIPAM segments respond first, following by the PVP segments and the PAA segments sequentially, and it is the conformation adjustment induced by the hydrophilic-to-hydrophobic transformation of the PNIPAM segments that drives the formation of the PNIPAM-core micelles. These results have also been confirmed by DLS and zeta potential measurements.

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References