Exploring the influence of the poly(4-vinyl pyridine) segment on the solution properties and thermal phase behaviours of oligo(ethylene glycol) methacrylate-based block copolymers: the different aggregation processes with various morphologies†

Yalan Dai and Peiyi Wu*

The assembly properties, thermal phase behavior and microdynamics of well-defined P(MEO2MA-co-OEGMA)-b-P4VP, (poly(2-(2-methoxyethoxy)ethylmethacrylate)-co-poly(oligo(ethylene glycol) methacrylate))-b-poly(4-vinyl pyridine), in aqueous solution during heating are investigated in detail by dynamic light scattering (DLS), turbidity measurements, temperature-variable $^1$H NMR and FTIR spectroscopy in combination with two-dimensional correlation spectroscopy (2Dcos) and the perturbation correlation moving window (PCMW) technique. It is observed that the chain length of the relatively hydrophobic P4VP segment strongly affects the temperature-induced phase transition behavior of the block copolymers: the copolymers with shorter P4VP$_{10}$ segments exhibit an abrupt phase transition process, while the copolymer with longer P4VP$_{19}$ blocks presents a relatively gradual transition behavior. Moreover, the two systems with different P4VP segment lengths have different morphologies in aqueous solution: a single-chain globule for shorter P4VP$_{10}$ systems and a core–shell micelle consisting of a relatively hydrophobic P4VP core and a hydrophilic POEGMA-based shell for the longer P4VP$_{19}$ system. Analysis of spectral results clearly illustrates that the dehydration of the C–H groups at the linkages between backbones and pendant chains predominates the sharp phase transition of P(MEO$_2$MA-co-OEGMA)-b-P4VP$_{10}$, while the dehydration of hydrophobic C–H groups on the side chains in P(MEO$_2$MA-co-OEGMA)-b-P4VP$_{19}$ leads to the continuous increase of the hydrodynamic diameter ($D_h$) upon heating.

1. Introduction

Over the past few decades, temperature-responsive polymers have been the focus of considerable interest from both fundamental and applied perspectives, with applications in controlled drug delivery, bio-separation and sensing, protein purification, tissue engineering scaffolds, etc.1–4 As the most extensively exploited thermosensitive polymer, poly(N-isopropylacrylamide) (PNIPAM) is regarded as a classic polymer exhibiting a lower critical solution temperature (LCST) in water with a thermally reversible coil-to-globule phase transition.5 However, it is found that PNIPAM has several inherent defects such as obvious hysteresis, releasing toxic amines during hydrolysis and strong adhesion to proteins by collaborative hydrogen bonding interactions.6 Recently, an emerging class of thermoresponsive polymers that may compete with or even surpass PNIPAM are polymers bearing oligo ethylene glycol (OEG) side chains, such as poly(oligo(ethylene glycol) methacrylate) (POEGMA), which combine the biocompatibility of polyethylene glycol (PEG) with a controllable and versatile LCST behavior.7,8 The LCST value of OEGMA-based polymers can be fine-tuned in the range of physiological temperature by simple controllable random copolymerization of OEGMA monomers with different initial comonomer ratios and oligo(ethylene glycol) side chain lengths.9–12 Owing to its excellent features including tunable LCST, good biocompatibility, antifouling properties below LCST, and nearly complete reversibility of thermal transition, POEGMA has been widely used in the design of functional polymers with complex topology structure, hydrogels, polymer brushes, nano-hybrid materials, bioactive surfaces, etc.13–17
Recently, the synthesis and self-assembly of well-defined stimuli-responsive block copolymers, especially double hydrophilic block copolymers (DHBCs), consisting of two different hydrophilic blocks, have attracted considerable interest because of their potential applications in the fields of drug delivery, nanotechnology, biological vectors, etc. As is well known, upon subtle adjustment of the environmental conditions such as pH, light intensity, temperature or ionic strength, these block copolymers can self-assemble into a variety of nanostructures, such as micelles. Moreover, DHBCs will exhibit a specific ‘schizophrenic’ character when both blocks are environment-sensitive, i.e., the AB block copolymer can form different micelles with the A-core or the B-core in response to external stimuli.

Several systems, such as poly(4-vinylbenzoic acid)-block-poly((2-diethylamino)ethyl methacrylate) (PVBA-b-PDEA), poly(methacrylic acid)-block-poly((2-diethylamino)ethyl methacrylate) (PMAA-b-PDEA), poly(N-isopropylacrylamide)-block-poly(3-[N-(3-methacrylamidopropyl)-N,N-dimethylammonio propyl]sulfonate) (PNIPAM-b-PSSP), and poly(N-isopropylacrylamide)-block-poly(4-vinyl pyridine) (PNIPAM-b-P4VP) have been investigated. As one of the commonly used biomaterials in biomedical applications, such as drug release systems, gene therapy studies and antimicrobial polymeric systems, poly(4-vinyl pyridine) (P4VP) is soluble in water when the pH is below 4.7 and becomes insoluble at higher pH with adjustable hydrophilicity. In particular, the hydrophilicity and solution behavior of poly(vinyl pyridine) can be easily modulated by changing the structure of the quaternizing agent. Zhu et al. used the atom transfer radical polymerization (ATRP) method to prepare the PNIPAM-b-P4VP block copolymer which could assemble into micelles with the P4VP core when the temperature is below its LCST and the pH is above 4.7, and a reverse micelle could be formed under modulating external conditions. The pH- and thermo-induced micellization behavior of the PNIPAM-co-b-P2VP block copolymer using reversible addition-fragmentation chain transfer polymerization (RAFT) in dilute aqueous solution was investigated by Shi et al. Similarly, a reversible transition between P2VP-core and PNIPAM-core micelles was observed through an intermediate unimer state in aqueous solution. From the viewpoint of application, Dincer and his coworkers studied the di-block copolymers of 4VP and OEGMA as potential drug delivery systems and demonstrated that the copolymer chains formed micelles at pH values higher than 5, whereas unimeric polymers were observed at pH below 5 due to the repulsion of positively charged P4VP blocks. However, to the best of our knowledge, most of the research studies have only focused on the formation of various micelle morphologies in a DHBC system upon distinct changes in external stimuli, and the variation of the microstructure and the thermal microdynamics of thermoresponsive blocks, such as POEGMA, influenced by the introduction of the P4VP segment have been rarely studied.

Herein, a series of well-defined P(MEO2MA-co-OEGMA)-b-P4VP block copolymers, especially with a relatively higher molar fraction of hydrophilic OEGMA-based polymer blocks, are synthesized via the RAFT technique. Different from previous research studies that focused only on light scattering techniques and fluorescence measurements, temperature-variable 1H NMR and temperature-resolved IR spectroscopy in combination with two-dimensional correlation spectroscopy (2Dcos) and the perturbation correlation moving window (PCMW) technique are applied here to illustrate the microdynamical phase separation mechanism of P(MEO2MA-co-OEGMA)-b-P4VP with different 4VP block lengths in aqueous solutions upon thermal perturbation. In particular, the influence of the relatively hydrophobic P4VP segment on the solution properties of P(MEO2MA-co-OEGMA)-b-P4VP will be discussed. Detailed information on the sequential order corresponding to chain conformation changes during phase transition could be extracted.

2. Experimental section

2.1 Synthesis of P(MEO2MA-co-OEGMA)-b-P4VP block copolymers

P(MEO2MA-co-OEGMA)-b-P4VP block copolymers were synthesized according to previous report. Materials and the typical synthesis process are provided in the ESI. The molecular structures of copolymer samples used in the investigation are shown in Scheme 1 and Table 1. Details of NMR spectra and GPC traces of all the copolymers are provided in Fig. S1 (ESI†).

2.2 Instruments and measurements

1H NMR spectra were recorded on a Varian Mercury plus 400 M spectrometer with CDCl3 as solvent. A gel permeation chromatograph (GPC) instrument with a G1362A refractive index detector was used to identify the weight-average molecular weights (Mw), number-average molecular weights (Mn), and the polydispersity index (PDI, Mw/Mn). N,N-Dimethylformamide (DMF) was used as the solvent.

![Scheme 1 Chemical structure of P(MEO2MA-co-OEGMA)-b-P4VP, wherein m ≈ 3 and n ≈ 28.](image)

<table>
<thead>
<tr>
<th>Table 1 Characterization results and phase transition temperature (Tp) of the copolymers</th>
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<tbody>
<tr>
<td>Copolymer</td>
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<tr>
<td>P(MEO2MA38-co-OEGMA13)</td>
</tr>
<tr>
<td>P(MEO2MA38-co-OEGMA13)-b-P4VP1</td>
</tr>
<tr>
<td>P(MEO2MA38-co-OEGMA13)-b-P4VP10</td>
</tr>
<tr>
<td>P(MEO2MA38-co-OEGMA13)-b-P4VP15</td>
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a Calculated using 1H NMR data. b Measured by GPC in DMF (PMMA as calibration). c Determined by turbidity measurements at 0.1 wt% copolymer concentration.
the eluent at a flow rate of 1.0 mL min\(^{-1}\) and monodispersed poly(methyl methacrylate) (PMMA) was used as the standard for the calibration curve. The temperature-variable average \(D_h\) of P(MEO\(_2\)MA-co-OEGMA)-b-P4VP in H\(_2\)O (0.1 wt\%) was measured on a dynamic light scattering (DLS)-Zetasizer nanosystem (Malvern) (scattering angle: 90°) with an increment of 1 °C. Transmittance measurements were performed at 550 nm using a Lambda 35 UV-vis spectrometer with a water-jacketed cell holder at the rate of 1 °C min\(^{-1}\). It was held for 2 min at each temperature point before measurement to ensure thermal equilibrium of the sample cell. Temperature-variable \(^1\)H NMR spectra of copolymers in D\(_2\)O solutions (10 wt\%) were recorded on a Bruker AV (500 MHz) spectrometer.

The samples of P(MEO\(_2\)MA-co-OEGMA)-b-P4VP solutions (10 wt\%) in D\(_2\)O for FT-IR measurements were prepared, and sealed between two ZnS tablets. All the temperature-dependent FTIR spectra were recorded on a Nicolet Nexus 6700 spectrometer equipped with a deuterated triglycine sulfate (DTGS) detector. And it was collected by signal-averaging 32 scans at a 4 cm\(^{-1}\) resolution to obtain an appropriate signal-to-noise ratio. Temperature was controlled with an electronic cell holder at a rate of ca. 0.3 °C min\(^{-1}\) with a programmed increment of 1 °C. Baseline correction was carried out using OMINIC 8.0 software.

Perturbation correlation moving window (PCMW) and two-dimensional correlation analysis (2Dcos) analysis were performed with the collected FTIR spectra with an interval of 1 °C during heating. Primary data processing was carried out and further correlation was calculated using software 2D Shige, ver. 1.3 (Shigeaki Morita, Kwansei-Gakuin University, Japan, 2004–2005) with an appropriate window size \(2m + 1 = 11\). Moreover, it was plotted using Origin program ver. 8.0 for the final contour maps, with warm colors (red and yellow) defined as positive intensities and cool color (blue) regions as negative ones.

### 3. Results and discussion

#### 3.1 Turbidity measurements

Herein, UV/vis transmittance measurements of 0.1 wt% copolymer aqueous solution as a function of temperature were performed, as shown in Fig. 1. The phase transition temperature \(T_p\) is taken as the initial temperature point where the transmittance drops below 50%. It can be found that the P(MEO\(_2\)MA-co-OEGMA)-b-P4VP copolymers, with the introduction of P4VP segments, show lower phase transition temperatures compared with the P(MEO\(_2\)MA-co-OEGMA) precursor, and the LCST of the copolymer aqueous solution shifts to a lower temperature upon increase of the P4VP block length. This phenomenon indicates that the poly(4-vinyl pyridine) segment in aqueous solution is regarded as a hydrophobic segment, and the transition temperature decreases with the higher degree of chain collapse and a lower degree of hydration of the OEGMA-based segments due to the assembled structures of these double hydrophilic block copolymers upon heating. Moreover, obviously, the transition temperature range of P(MEO\(_2\)MA-co-OEGMA)-b-P4VP is much broader than P(MEO\(_2\)MA-co-OEGMA)-b-P4VP and P(MEO\(_2\)MA-co-OEGMA)-b-P4VP, illustrating that the phase transition of the copolymer with longer P4VP blocks occurs more slowly than the shorter P4VP block copolymers. It may be attributed to the different hydrophilic-hydrophobic properties by introducing P4VP blocks of different lengths and the various micelle morphologies existing in aqueous solution. The difference will be discussed in detail later.

#### 3.2 DLS measurements

To further ascertain the results shown in the turbidity curves and investigate the LCST-type transition of P(MEO\(_2\)MA-co-OEGMA)-b-P4VP from a micro aspect, DLS measurements were carried out with 0.1 wt% copolymer aqueous solutions, as presented in Fig. 2. The \(D_h\) value of P(MEO\(_2\)MA-co-OEGMA)-b-P4VP chain aggregates can be determined and the aggregation behavior of the copolymers can be monitored under variation of solution temperature. As expected, upon heating, the \(D_h\) sizes of all the copolymers undergo three stages: the relative equilibrium stage, then, the increase, and the final slight decrease. However, the \(D_h\) size exhibits an abrupt increase at the cloud points, 33 and 32 °C, respectively, indicating the aggregation of copolymer chains for P(MEO\(_2\)MA-co-OEGMA)-b-P4VP and P(MEO\(_2\)MA-co-OEGMA)-b-P4VP, in contrast, a continuous increase occurs for the longer P4VP block system, P(MEO\(_2\)MA-co-OEGMA)-b-P4VP, which parallels well with the turbidity curves. It indicates that the increased length of...
hydrophilic P4VP blocks broadens the transition temperature region and results in a lower LCST of P(MEO2MA-co-OEGMA)-b-P4VP19. Since there might exist hydrogen bond associated with pyridine and water molecules as well as weak pyridine–pyridine interaction,36-40 and the hydration of the copolymers in aqueous solution may be destabilized, it is inferred that the specific interaction of P4VP and water may impact the initial interaction pattern between the POEGMA chain segment and water molecules, altering the thermal phase behavior consequently. In addition, P(MEO2MA-co-OEGMA)-b-P4VP and P(MEO2MA-co-OEGMA)-b-P4VP19 show a hydrodynamic diameter of 5–10 nm in water at 5 °C, and a higher $D_h$ (~600 nm) at 55 °C. While, a relatively narrow $D_h$ (~200 nm) at initial temperature and a final $D_h$ (~300 nm) at high temperature belong to P(MEO2MA-co-OEGMA)-b-P4VP19.

For clarity, the $D_h$ number-average size distributions of these block copolymers are portrayed under the same conditions in Fig. 3. Herein, P(MEO2MA-co-OEGMA)-b-P4VP and P(MEO2MA-co-OEGMA)-b-P4VP19 are chosen as representatives to study the thermal transition behavior of POEGMA-based copolymers with shorter and longer P4VP block lengths. At low temperature, it is interesting to note that unimers ($D_h$ ~5–10 nm) are observed in P(MEO2MA-co-OEGMA)-b-P4VP10, demonstrating that the system has a single-chain structure, probably a loose single-chain globule with P4VP segments located at the inside.13,41-44 While P(MEO2MA-co-OEGMA)-b-P4VP19, shows a homogeneous $D_h$ size around 200 nm at the starting temperature since P4VP is a pH-sensitive polymer which is soluble in water (when the pH value is below 4.7) and becomes insoluble when the pH is high.31 At low temperature, a core–shell micelle is expected to be formed in aqueous solution with the relatively hydrophobic core of P4VP blocks and the hydrophilic shell of POEGMA-based blocks.26 With the $D_h$ size increasing with temperature, the thermo-responsive POEGMA-based shell probably undergoes assembly by the hydrophobic interaction with the water molecule, forming some small aggregates. When it is above the transition temperature, the $D_h$ shows a slight decrease with the temperature increasing persistently, which may be due to the further collapse of the aggregates by the removal of more water molecules and the formation of more compact and regular structures. The same phenomenon is also observed in PNIPAM-b-poly(ionic liquid).45 Notably, at high temperature, the aggregates of copolymers with shorter P4VP blocks have a larger structure ($D_h$ sizes are larger) as a result of a higher aggregation number and weaker hydrophobic interaction, in contrast, the aggregates with long P4VP19 blocks are formed with a smaller size and aggregation number, which can be ascribed to the stronger hydrophobic interactions derived from the longer P4VP19 segment chains.

3.3 Temperature-variable $^1$H NMR analysis

To quantitatively describe the phase transition degree of P(MEO2MA-co-OEGMA)-b-P4VP10 and P(MEO2MA-co-OEGMA)-b-P4VP19, temperature-variable $^1$H NMR measurements were performed, as shown in Fig. 4(a) and (b), respectively. Almost all the proton types in P(MEO2MA-co-OEGMA)-b-P4VP copolymers can be observed in $^1$H NMR spectra in D$_2$O with corresponding assignments in Fig. 4(a). Compared with the sharp and intense proton signals in CDCl$_3$ in Fig. S1(b) (ESI†), it is noted that in D$_2$O, the intensities of the signals from the pyridine ring ($H_1$, $H_6$), the backbone ($H_4$, $H_5$), and protons close to the backbone ($H_2$) are considerably weakened and broadened, revealing that the hydration of the copolymers in D$_2$O is not uniform with more water molecules around the hydrophilic side chains and less water molecules surrounding the hydrophobic pyridine rings and the backbones.44

Obviously, in Fig. 4, almost all the protons shift toward a lower field along with a drastic intensity decrease upon heating, similar to the case of PNIPAM and POEGMA.46-48 Furthermore, after the phase transition, the signals of protons corresponding to pyridine rings ($H_1$, $H_6$), the backbone of P(MEO2MA-co-OEGMA)-b-P4VP($H_4$, $H_5$), and protons in the proximity of the backbone ($H_2$) are hardly detectable, and only the proton signals from the hydrophilic side chains in the surface of aggregates can be detected. It is helpful in understanding the microdynamic responses of P4VP segments and different parts of POEGMA-based chains during the phase transition process by quantitatively analyzing the varying pattern of specific peaks. Hence, the integral area changes of $H_1$, $H_2$, $H_3$, $H_4$ were quantitatively analyzed to figure out the difference phase behaviors between P(MEO2MA-co-OEGMA)-b-P4VP10 and P(MEO2MA-co-OEGMA)-b-P4VP19. Note that the integral areas are calculated with the HDO peak as the reference.

As presented in Fig. 5, it is obvious that the integral areas of all the protons decrease upon heating, indicating that the protons are wrapped after phase transition. Notably, the integral area shifts of all the peaks in P(MEO2MA-co-OEGMA)-b-P4VP10 solution are more drastic than those in P(MEO2MA-co-OEGMA)-b-P4VP19, which demonstrates that the changes in the hydration degree in P(MEO2MA-co-OEGMA)-b-P4VP19 is larger than that in P(MEO2MA-co-OEGMA)-b-P4VP10 solution. Compared with P(MEO2MA-co-OEGMA)-b-P4VP10, it is inferred that the weak hydration degree in P(MEO2MA-co-OEGMA)-b-P4VP19 solution at initial temperature is due to the more dehydrated environment caused by the core–shell micelle structures formed in aqueous solution. Additionally, $H_1$, $H_2$, $H_3$, $H_4$, $H_5$, and $H_6$ in P(MEO2MA-co-OEGMA)-b-P4VP10 as well as $H_5$ and $H_6$ in P(MEO2MA-co-OEGMA)-b-P4VP19 exhibit “anti-S” shaped variations, while the integral area shifts of $H_4$, $H_5$, and $H_6$ in P(MEO2MA-co-OEGMA)-b-P4VP19 are inconspicuous and display approximately linear changes. From the macroscopic aspect, it is consistent with the drastic phase transition with P(MEO2MA-co-OEGMA)-b-P4VP10.
3.4 Conventional IR analysis

Temperature-resolved FT-IR measurements of P(MEO₂MA-co-OEGMA)-b-P4VP₁₀ and P(MEO₂MA-co-OEGMA)-b-P4VP₁₉ in D₂O (10 wt%) are measured upon heating to elucidate their dynamic mechanism of the thermoresponsive behavior, as shown in Fig. 6. Taking account of the slight effect on the thermoresponsive phase behavior of the OEGMA-based polymer by the deuterium isotope effect,⁶ H₂O was replaced by D₂O to eliminate the overlap of the δ(O–H) band of H₂O around 1640 cm⁻¹ with ν(C=O) of POEGMA-based blocks as well as the broad δ(O–H) band of H₂O around 3300 cm⁻¹ with the C–H stretching region.⁴⁷,⁴⁸ Herein, the C–H stretching region (3050–2810 cm⁻¹), the C=O stretching region (1755–1680 cm⁻¹), the C=C stretching region in pyridine (1618–1589 cm⁻¹) and the C–O–C stretching region (1118–1053 cm⁻¹) are specifically focused on.

In Fig. 6, we can find that all the C–H stretching bands of the two copolymers shift to lower wavenumbers during the heating process. Since water clathrates exist around the hydrophobic moieties of water-soluble polymers in a well-ordered structure, a higher number of water molecules surrounding C–H groups would result in higher vibrational frequency.⁴⁹,⁵⁰ The red shift of the C–H band confirms that the aliphatic groups in the copolymers suffer from dehydration process upon heating. Besides, obviously, the C–H groups of P(MEO₂MA-co-OEGMA)-b-P4VP₁₉ have an relatively abrupt decrement of absorbance compared with the gradual decrease of P(MEO₂MA-co-OEGMA)-b-P4VP₁₀, suggesting that the dehydration of C–H groups in P(MEO₂MA-co-OEGMA)-b-P4VP₁₉ probably play a vital role in the whole dehydration process during heating. As for C=O and C–O–C groups, both the copolymers exhibit a blue shift, demonstrating the dehydration of hydrophobic ester linkages and the breakage of hydrogen bonds between ether oxygen groups and water.⁶ However, it should be mentioned that the...
blue shifts of P(MEO2MA-co-OEGMA)-b-P4VP10 are much more notable than P(MEO2MA-co-OEGMA)-b-P4VP19, which can be ascribed to the different dehydration degrees of C=O and C–O–C groups, and we will analyze them quantitatively in detail later. Similar to the C–H group, a relatively hydrophobic moiety of water soluble copolymers, the red shift changes of C=C groups in the pyridine ring of these two copolymers are observed during heating in the present study. Accordingly, it is inferred that the relatively hydrophobic pyridine rings in the inner of the loose single chain globules or micelle structure, leading to the less flexible movement of dehydrated groups. In a sense, by comparison of the shifts of C–H and C=O groups below and above phase transition temperatures, it seems that the dehydration degrees of C=O in P(MEO2MA-co-OEGMA)-b-P4VP10 and the C–H group in P(MEO2MA-co-OEGMA)-b-P4VP19 copolymer are more obvious than the others, which will be deeply investigated in the following FTIR study.

Additionally, we quantitatively examined the temperature-dependent frequency shifts of the three bands around 2832, 1720, and 1600 cm⁻¹, corresponding to ν(CH₂)(–OCH2CH2O–), ν(C=O) and ν(C=C) in pyridine, to obtain more detailed information on the influence on the hydration states of different chemical groups during the phase transition, as presented in Fig. 8. Obviously, the frequency shifting tendency of the three bands all exhibits an asymmetric sigmoid curve with a sharp increase. The phenomena may result from the formation of the single-chain globules or micelle structure, leading to the less flexible movement of dehydrated groups. In a sense, by comparison of the shifts of C–H and C=O groups below and above phase transition temperatures, it seems that the dehydration degrees of C=O in P(MEO2MA-co-OEGMA)-b-P4VP10 and the C–H group in P(MEO2MA-co-OEGMA)-b-P4VP19 copolymer are more obvious than the others, which will be deeply investigated in the following FTIR study.

Fig. 6 Temperature-dependent FTIR spectra of P(MEO2MA-co-OEGMA)-b-P4VP10 (20–50 °C) and P(MEO2MA-co-OEGMA)-b-P4VP19 (10–40 °C) in D₂O (10 wt%) during heating with an interval of 1 °C in the regions 3050–2810, 1755–1680, 1618–1589, and 1118–1053 cm⁻¹.

Fig. 7 Spectral comparison of P(MEO2MA-co-OEGMA)-b-P4VP10 and P(MEO2MA-co-OEGMA)-b-P4VP19 in D₂O (10 wt%) at low and high temperatures and neat film samples.
in the inner of the relatively loose P(MEO₂MA-co-OEGMA)-b-P₄VP₁₀ single-chain globule, accompanied by the adjustment of conformation and the formation of aggregates.

3.5 Perturbation correlation moving window (PCMW)

In consideration of the asymmetric sigmoid curves displayed in Fig. 8, it is difficult for us to accurately determine the specific transition points of the corresponding groups due to the gradual changes during heating. Therefore, PCMW technique is employed to ascertain the transition temperatures as well as transition temperature regions regarding various chemical groups in the two copolymers with different P₄VP segment lengths. Further introduction of PCMW technique is covered in the ESI.†

Herein, the synchronous and asynchronous spectral maps of the PCMW are drawn for deeper investigation of the phase transitions of P(MEO₂MA-co-OEGMA)-b-P₄VP₁₀ (20–50 °C) and P(MEO₂MA-co-OEGMA)-b-P₄VP₁₀ (10–40 °C) in D₂O during heating, as shown in Fig. S2 (ESI†). For clarity, all the points read from PCMW synchronous and asynchronous spectra are plotted in Fig. 9. In P(MEO₂MA-co-OEGMA)-b-P₄VP₁₀, the phase transition temperature of C—O groups is determined to be 30 °C, which is lower than those of C—H and C—C groups (around 33 °C), indicating that C—O groups have an earlier response than the other three groups. Additionally, the transition temperature range of C—H, C—C in pyridine and C—O-C groups in P(MEO₂MA-co-OEGMA)-b-P₄VP₁₀ is determined to be between 29 and 35 °C, while C—O groups experience the transition process between 27 and 33 °C. The relatively narrow transition temperature regions suggest that the C—H, C—C in pyridine and C—O-C groups of P(MEO₂MA-co-OEGMA)-b-P₄VP₁₀ have cooperative responses to the increasing temperature under the driving of C—O groups. As for P(MEO₂MA-co-OEGMA)-b-P₄VP₁₀, there are relatively wide transition temperature ranges almost from 17 to 26 °C of different groups, presenting a slow dehydration process. These observations accord well with turbidity and DLS results that P(MEO₂MA-co-OEGMA)-b-P₄VP₁₀ presents a sharp phase transition behavior while P(MEO₂MA-co-OEGMA)-b-P₄VP₁₀ reveals a relatively gradual one. In fact, incorporating P₄VP moieties into linear POEGMA leads to the formation of micelles and more interaction between water and the copolymer, and a longer P₄VP length may impact the hydrophilic–hydrophobic balance and the hydrophobic aggregation of POEGMA. Furthermore, it is noteworthy that in P(MEO₂MA-co-OEGMA)-b-P₄VP₁₀, the C—H groups respond earlier than C—O and the other groups, which differ from P(MEO₂MA-co-OEGMA)-b-P₄VP₁₀. It is inferred that the higher P₄VP moieties in the block copolymer significantly impact the microscopic dynamic process of dehydration and the response of C—H groups may play a crucial role in the continuous phase transition behavior of the longer P₄VP₁₀ block system.

3.6 Two-dimensional correlation spectroscopy (2Dcos)

2Dcos analysis of P(MEO₂MA-co-OEGMA)-b-P₄VP₁₀ (20–50 °C) and P(MEO₂MA-co-OEGMA)-b-P₄VP₁₀ (10–40 °C) spectra during heating with an internal temperature of 1 °C is employed to elucidate the contraction of the POEGMA-based block and the microscopic phase transition mechanism influenced by the relatively hydrophobic P₄VP blocks of different lengths, as presented in Fig. 10 and 11. The generated synchronous spectra which reflect simultaneous changes between two wavenumbers and asynchronous spectra greatly enhancing the spectral resolution of the two systems are shown. All the peaks observed in asynchronous spectra as well as their tentative assignments are listed in Table 2. Many additionally observed bands related to subtle group conformations are obtained, and they could provide more detailed information and significantly assist in figuring out the mechanism of the complex phase transition process. Besides, 2Dcos can also discern the specific order taking place under external perturbation. According to Noda’s rule (in the ESI†), the resultant sequence orders of all groups in the two copolymers can be inferred directly and more detailed information can be figured out in the following discussion.

Analysis of 2Dcos for P(MEO₂MA-co-OEGMA)-b-P₄VP₁₀ with shorter P₄VP blocks. According to the 2Dcos spectra, using a simplified method for the determination of sequence order described before, the final sequence order of different
chemical groups in the P(MEO2MA-co-OEGMA)-b-P4VP10 sample during heating is displayed as follows (→ means prior to or earlier than): 1727 → 1099 → 1079 → 1710 → 1598 → 2838 → 3002 → 2952 → 2927 → 2939 → 2987 → 2894 → 2879 → 1608 → 2825 cm⁻¹ or ν(C=O) (dehydrated) → ν(C–O–C) (dehydrated) → ν(C=O) (hydrating) → ν(C=C in pyridine) (dehydrated) → νas(CH3)(–OCH3) (hydrated) → νas(CH3)(–OCH2CH2O–) (hydrated) → νas(CH2)(–OCH2CH2O–) (hydrated) → νas(CH2) (backbone) → νas(CH3)(–OCH2CH2O–) (dehydrated) → νas(CH3)(–OCH3) (dehydrated) → νas(CH2) (backbone).

Without considering the differences in stretching modes of the chemical groups in the copolymer with shorter P4VP blocks, the specific order can be summarized as follows: C–O → C=O–C → C=C in pyridine → –OCH2CH2O→ –OCH3 → CH2 (backbone).

The dehydration of hydrophobic ester groups responds to the temperature perturbation at the earliest during the heating
Table 2 Tentative band assignments of P(MEO2MA-co-OEGMA)-b-P4VP10 and P(MEO2MA-co-OEGMA)-b-P4VP19 in D2O according to 2Dcos results.\textsuperscript{52–54}

<table>
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<th>Wavenumber (cm(^{-1}))</th>
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<td>3082/3000</td>
<td>(\nu_C(CH_3))−(\nu_C(CH_2)) (hydrated)</td>
</tr>
<tr>
<td>2994/2987</td>
<td>(\nu_C(CH_3)) (dehydrated)</td>
</tr>
<tr>
<td>2954/2952</td>
<td>(\nu_C(CH_2))−(\nu_C(CH_3)) (hydrated)</td>
</tr>
<tr>
<td>2939/2937</td>
<td>(\nu_C(CH_3))−(\nu_C(CH_2)) (dehydrated)</td>
</tr>
<tr>
<td>2927/2923</td>
<td>(\nu_C(CH_2)) (backbone)</td>
</tr>
<tr>
<td>2894/2890</td>
<td>(\nu_C(CH_2))−(\nu_C(CH_3)) (hydrated)</td>
</tr>
<tr>
<td>2879/2877</td>
<td>(\nu_C(CH_2))−(\nu_C(CH_3)) (dehydrated)</td>
</tr>
<tr>
<td>2838/2836</td>
<td>(\nu_C(CH_2))−(\nu_C(CH_2))−(\nu_C(CH_3)) (hydrated)</td>
</tr>
<tr>
<td>2827/2825</td>
<td>(\nu_C(CH_2))−(\nu_C(CH_2))−(\nu_C(CH_3)) (dehydrated)</td>
</tr>
<tr>
<td>1727/1729</td>
<td>(\nu(C=O)) (dehydrated)</td>
</tr>
<tr>
<td>1716/1710</td>
<td>(\nu(C=O)) (hydrating)</td>
</tr>
<tr>
<td>1704</td>
<td>(\nu(C=O)) (hydrated)</td>
</tr>
<tr>
<td>1606</td>
<td>(\nu(C=O)) in pyridine (hydrated)</td>
</tr>
<tr>
<td>1598</td>
<td>(\nu(C=O)) in pyridine (dehydrated)</td>
</tr>
<tr>
<td>1106/1099</td>
<td>(\nu(C=O-C=O)) (dehydrated)</td>
</tr>
<tr>
<td>1079/1064</td>
<td>(\nu(C=O-C=O)) (hydrated)</td>
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</table>

process in the block system, which is in good agreement with the results obtained from PCMW. Obviously, different from the POEGMA based linear polymer system in which the dehydration of C–H groups on the side chains has an early response during the transition process,\textsuperscript{52} the sensitivity of C–H groups to temperature seems to be considerably weakened. In other words, the driving force for phase transition of the P(MEO2MA-co-OEGMA)-b-P4VP\textsubscript{10} sample is the damage of hydrogen bonds with C–O groups and water. Among the four stretching regions, it is reasonable to believe that C–O groups at the linkages between backbones and pendant chains play a vital part in the self-aggregation transition of the single chain globules formed in aqueous solution. Unexpectedly, the dehydrated C–O and C–O–C respond prior to the hydrated ones in the sequence. This unusual phenomenon indicates that C–O and C–O–C groups work cooperatively to make the polymer chains collapse under the driving of C–H groups and gradually expel water molecules out of the system.

Moreover, upon a careful observation of C–O regions, compared to P(MEO2MA-co-OEGMA)-b-P4VP\textsubscript{10} above in which only two bands are observed, 1710 and 1727 cm\(^{-1}\) corresponding to the (relatively) hydrated C–O and the (relatively) dehydrated C–O, there are three subtle bands, 1704 cm\(^{-1}\), 1716 cm\(^{-1}\), 1729 cm\(^{-1}\), emerging in the asynchronous spectra of the carbonyl groups in the longer P4VP\textsubscript{19} block system. It indicates the existence of diverse dehydrating states of C–O during the phase transition. And it is inferred that the closer to the P4VP core, the more dehydrated state belongs to C–O groups. Actually, the various dehydrating states of C–O groups have also been observed in the POEGMA-based hydrogel system.\textsuperscript{52} Thus, it reveals that the water molecules are gradually squeezed out around C–O groups, resulting in the existence of a distribution gradient of water molecules in P(MEO2MA-co-OEGMA)-b-P4VP\textsubscript{19}, which parallels well with the continuous transition of C–O groups in Fig. 8.

Based on the above analysis, we can clearly understand the phase transition dynamic mechanisms of P(MEO2MA-co-OEGMA)-b-P4VP\textsubscript{10} and P(MEO2MA-co-OEGMA)-b-P4VP\textsubscript{19} aqueous solutions upon heating. For a more intuitive demonstration of the dynamic mechanisms, a schematic illustration is depicted in Scheme 2.

In general, for the P(MEO2MA-co-OEGMA)-b-P4VP\textsubscript{10} solution, below LCST, a relatively loose single-chain globule with relatively hydrophobic P4VP blocks on the inside is formed, and sufficiently hydrated in the aqueous solvent. When the temperature increases above LCST, compared with the hydrophobic alkyl chains that are connected to the side chains of POEGMA blocks, the hydrophobic ester carbonyls at the linkage between the backbone and the pendant chains mainly provide the hydrophobic interactions, which are the driving force for the LCST-type phase transition. At the same time, the conformational change happens and the single-chain globules start to merge together and further collapse under the increasing hydrophobic interaction upon heating. As a result,
a core–shell assembly structure with relatively hydrophobic P4VP segments wrapped in the inside is formed subsequently. With continuous heating, the pendant ether segments start to dehydrate and collapse toward the backbone simultaneously, and the D2O molecules around the pyridine ring inside the aggregates should be squeezed out, then the hydration of hydrophilic side chains on the POEGMA-based shell is damaged continuously until the whole phase transition assembly process is finished.

In the case of P(MEO2MA-co-OEGMA)-b-P4VP19 solution, at temperatures below LCST, it exhibits a more compact micelle in the solution due to the increased length of P4VP blocks, leading to enhanced hydrophobic interactions near the core. When the temperature increases above the LCST, the dehydration of the hydrophobic side chain of POEGMA-based blocks is carried out, which plays a dominant role during the whole process of dehydration. And accompanying the aggregation of side chain and collapse of the micelle shell, the much less water around the pyridine rings in the core of the micelles is expelled out subsequently. Then with the help of cooperative dehydration among the C–O and C–O–C groups and the resulting adjustment of the polymer chains in the system, water molecules are ultimately squeezed out of the surrounding environment step by step.

4. Conclusion

In this work, P(MEO2MA-co-OEGMA)-b-P4VP block copolymers are synthesized via the RAFT method. Turbidity, DLS, 1H NMR as well as FTIR measurements in combination with 2Dcos methods are used to investigate the thermal phase transition behavior and the assembly properties of P(MEO2MA-co-OEGMA)-b-P4VP solutions with different P4VP segment lengths at the molecular level. As shown by turbidity measurements, the transition temperature of P(MEO2MA-co-OEGMA)-b-P4VP decreases with the increase of the P4VP block length. Specifically, the copolymers with shorter P4VP7/10 blocks exhibit a relatively narrow phase transition temperature range and a higher LCST, while the copolymer with longer P4VP19 segments displays a broad transition region and a lower LCST during heating. Additionally, the DLS measurements suggest the noteworthy distinctions of hydrodynamic diameters and morphologies between the shorter and longer P4VP block systems. The copolymers with shorter P4VP7/10 blocks possess a single-chain structure (single-chain globule) in solution while the copolymer with longer P4VP19 blocks is regarded as a micelle consisting of a relatively hydrophobic P4VP core and a hydrophilic shell of the POEGMA block in aqueous solution. The results are further confirmed by temperature-variable 1H NMR analysis. And the temperature-dependent frequency shifts of C–H, C–O, and C–C in pyridine and C–O–C groups in conventional FT-IR analysis indicate that the gradient distribution of water molecules exists in the copolymer solutions ranging from relatively hydrophobic pyridine rings to hydrophilic POEGMA chains. On the basis of the 2D correlation analysis, we attribute the gradual dehydration of C–O groups at the linkages between backbones and pendant chains to the dominant effect on the sharp phase transition behavior of P(MEO2MA-co-OEGMA)-b-P4VP10. While the hydrophobic C–H groups on the side chains of POEGMA-based blocks, due to the relatively more dehydrated environment resulting from the increased P4VP length and the formation of micelles with a hydrophilic POEGMA-based shell, are the driving forces for the continuous phase transition and play a significant role during the whole dehydration process of P(MEO2MA-co-OEGMA)-b-P4VP19.

Acknowledgements

We gratefully acknowledge the financial support from the National Science Foundation of China (NSFC) (No. 21274030 and 51473038).
Notes and references