Comparison of LCST-transitions of homopolymer mixture, diblock and statistical copolymers of NIPAM and VCL in water†

Lei Hou and Peiyi Wu*

The LCST-transitions of linear, well-defined polymers of N-isopropylacrylamide (NIPAM) and N-vinylcaprolactam (VCL), including a homopolymer mixture, diblock and statistical copolymers, in water are explored and compared by applying turbidity and FTIR measurements in combination with twodimensional correlation spectroscopy (2Dcos). Only one transition is observed in all polymer systems, suggesting a dependent aggregation of poly(N-isopropylacrylamide) (PNIPAM) and poly(N-vinylcaprolactam) (PVCL) parts in the phase transition processes. With the help of 2Dcos analysis, it is discovered that the hydrophobic interaction among C–H groups is the driving force for simultaneous collapse of the two distinct thermo-responsive segments. Additionally, the delicate differences within the LCST-transitions thereof have been emphasized, where the phase separation temperatures of the homopolymer mixture and the diblock copolymer are close while that of the statistical copolymer is relatively higher. Moreover, both diblock and statistical copolymers exhibit rather sharp phase transitions while the homopolymer mixture demonstrates a moderately continuous one.

1. Introduction

Over the past decades, considerable efforts have been devoted to the exploration of thermal-responsive polymers because of their great potential in a wide range of applications in tissue engineering scaffolds, drug delivery, biological separation and sensing.1–3 It is generally known that the thermal-responsive polymers possess either a lower critical solution temperature (LCST) or an upper critical solution temperature (UCST) in the solutions during their phase transition processes. Particularly, a large amount of attention has been focused on the polymers that exhibit the LCST-type transitions in aqueous solutions.4 Among them, poly(N-isopropylacrylamide) (PNIPAM) is the most famous example, which undergoes a rapid coil-to-globule transition in the aqueous solution on heating above its LCST (~32 °C).5–7 Another frequently investigated temperature-sensitive polymer is poly(N-vinylcaprolactam) (PVCL).5–10 PVCL is not only thermally responsive but also biocompatible. The hydrophilic amide group linked to the hydrophobic polymer backbone will not be able to produce low molecular-weight amines. Additionally, N-vinylcaprolactam (VCL) has also been reported to be less cytotoxic than N-isopropylacrylamide (NIPAM).11,12

Nowadays, polymers containing more than one thermo-responsive component have also aroused much attention, due to their complex self-assembly behavior and their potential in the development of reversible encapsulation and delivery areas.13 Some of the researches are dedicated to the diblock copolymers which consist of two distinct temperature-sensitive segments. Sato et al.14 have reported the investigation of dehydration and self-association behavior of a novel diblock copolymer composed of poly(2-isopropyl-2-oxazoline) and PNIPAM (PIPOZ-b-PNIPAM) upon heating in water and water–methanol mixtures. It is found out that the phase transitions of two segments that occur depend on each other; even the difference in the transition temperatures of PIPOZ and PNIPAM homopolymers is enhanced by increasing methanol fraction, which may be attributed to the interference of the cosolvency effect of the PNIPAM block and the cosolvency effect of the PIPOZ block. Thermal properties of diblock copolymers PVCL and poly(2-dimethylaminoethyl methacrylate) (PVCL-b-PDMAEMA) in aqueous buffer solutions have been studied by Karesoja et al.15 Those block copolymers displayed one or two separate LCSTs depending on the pH. With increase in temperature, the PVCL block collapsed first and the PDMAEMA chains turned out from the collapsed PVCL globule toward the aqueous phase to provide colloidal stability to the aggregate...
particles. Some of the researches are concentrated on the random copolymers composed of different thermal-responsive units. Cloud points of aqueous solutions of statistic copolymers of NIPAM and \( N \)-isopropylmethacrylamide (NIPAM) have been determined turbidimetrically by Djokpe and Vogt, where a linear dependence of the cloud point on the copolymer content was discovered. Lately, Crespy et al. have described the synthesis of VCL and \( \text{d} \)(ethylene glycol methyl ether methacrylate) copolymers in solution and miniemulsion. It was shown that the cloud point temperature of the random copolymers could be fine-tuned in the temperature range between 26 and 35 °C. Meanwhile, investigations on the phase transition of physically mixed aqueous solutions of two thermosensitive homopolymers have been reported as well. Spevacek et al. studied the heat-induced phase separations in aqueous solutions of poly\((N\)-isopropylmethacrylamide) (PNIPAM)/poly-(vinyl methyl ether) (PVME) and PNIPAM/PNIPAM mixtures by applying \( ^1 \)H NMR spectroscopy. The authors discovered that, in both systems, the phase transition of the component with lower LCST is not affected by the presence of the higher transition component, while the phase transition of the component with higher LCST is affected by the phase separation of the lower transition component in the mixture. More recently, they have investigated the phase transition behavior of the PNIPAM/PVCL mixture in water and found that both transitions of polymeric components were shifted \( \sim 2 \) K toward higher temperatures in comparison with neat polymers, indicating that the existence of the second component hindered the phase transition of the given one either by direct interactions between two segments or indirectly by changing the order and structures of water molecules surrounding the polymer chains. In the work of Chen et al., the aggregation behavior of poly (vinyl methyl ether) (PVME)/poly (2-ethyl-2-oxazoline) (PEOZ) chains in water was probed by elastic light scattering (ELS) spectroscopy, and two onset temperatures of phase transition during heating were identified. However, systematic studies on the comparison of the phase transition behavior of copolymers (both diblock and random copolymers) and mixtures with the homopolymers are rather seldom. Richtering et al. have reported on the thermosensitive properties of a diblock copolymer poly\((N,N\text{-diethylacrylamide})-b\text{-}\text{poly}(N\text{-isopropylacrylamide})\) (PDEAAM-\( b \)-PNIPAM) and compared it to the homopolymers, mixtures thereof, and to the statistical copolymer with the same molecular weight. It was revealed that the macroscopic and microscopic properties of the diblock copolymer resembled more the averaged properties of the homopolymers due to the competition between the block length dependency on the respective cloud point and the effect of connectivity. In contrast, the mixture and the random copolymer exhibited nonideal behavior leading to a synergistic depression of the cloud points.

Despite the similarities in the phase transition behavior of PNIPAM and PVCL, differences for both polymers are obvious. For instance, the LCST transition is rather sharp and rapid for PNIPAM, whereas PVCL exhibits a relatively broad and continuous change. The cloud point of PVCL could range from 30 to 50 °C, strongly dependent on the molecular weight and concentration, while that of PNIPAM is somewhat stable. Moreover, PNIPAM displays conon solvency (insolubility in a mixture of good solvents), which is hardly detectable for PVCL. Previously, we had explored the self-aggregation behavior of the diblock copolymer PNIPAM-\( b \)-PVCL during the heat-induced phase transition in water, in which only one transition point was observed, indicating that the two distinct temperature-sensitive segments behaved cooperatively. It is generally considered that the monomer pattern along the polymer chain plays a vital part in its final chemical and physical properties. Under such a circumstance, we are interested on the influence of NIPAM and the VCL pattern along the polymer chain on the eventual LCST-behavior. In addition, the mixing of two thermally responsive polymers demonstrates a facile method to develop novel “smart” materials. As a result, how one given component is affected by the appearance of the other one is of great significance in practical applications. Moreover, by comparing the phase transitions of the homopolymer mixture, diblock and statistical copolymers of NIPAM and VCL in water, we will be able to figure out what role each thermoresponsive component possesses in different polymer structures and better understand the inherent nature of thermosensitive behavior.

Thus, in this work, we utilize turbidity measurements to examine the transition points and features of homopolymers of PNIPAM and PVCL, the mixture thereof, diblock and statistical copolymers of NIPAM and VCL firstly, which may provide a macroscopic vision of the phase transition processes. Then, Fourier transform infrared spectroscopy (FTIR), proved to be a quite useful tool to probe variations in the hydration states or hydrogen bonds of individual chemical groups, is employed to describe the microscopic changes with regard to the LCST-transitions of different investigated systems and uncover the underlying diverse molecular interactions thereinto. Furthermore, the literature lacks integrated explorations of the phase transition behavior of the homopolymer mixture and the statistical copolymer of PNIPAM and VCL, which will be emphasized in our work. Therefore, two-dimensional correlation spectroscopy (2Dcos) analysis is applied hereby to illustrate the dynamic dehydration behavior of the homopolymer mixture and the statistical copolymer during the heating process. In this way, additional information on the specific order taking place related to chain conformation changes during the transition could be extracted, which is very helpful for better understanding of the evolution mechanisms.

2. Experimental

2.1 Materials

\( N \)-Isopropylacrylamide (NIPAM) was obtained from Aladdin reagent Co. and recrystallized from cyclohexane. \( N \)-Vinylcaprolactam (VCL) was purchased from Alfa Aesar Co. and purified by passing through a short alumina column prior to use. \( 2,2' \)-Azobisiso-butronitrile (AIBN, Aladdin reagent Co.) was recrystallized from ethanol. Methanol was distilled under vacuum after drying with calcium hydride for 12 h. \( D_2^0 \) was purchased from Cambridge Isotope Laboratories Inc. (D-99.9%). The reversible addition–fragmentation transfer (RAFT)
agent, O-ethyl-S-(1-phenylethyl)dithiocarbonate (CTA), was prepared according to the literature procedure.30–32

2.2 Polymer synthesis

All the polymers studied in this work were synthesized via RAFT polymerization according to ref. 33. The chemical structures and reactions are presented in Fig. 1.

A typical procedure for the preparation of the statistical copolymer (PNIPAM-st-PVCL) was as follows. NIPAM (0.56 g, 5 mmol), VCL (1.13 g, 10 mmol), AIBN (0.0032 g, 0.02 mmol) and CTA (0.0236 g, 0.1 mmol) were placed in a 25 ml flask and dissolved in 5 g of methanol. After three freeze-evacuate-thaw cycles, the reaction mixture was sealed under vacuum and the polymerization was conducted at 60 °C for 24 h. The reaction was then quenched by rapid cooling in liquid nitrogen and exposure to air. After purifying by three times precipitation with diethyl ether, the product was finally vacuum-dried for 24 h. The detailed synthesis procedures of other polymers are shown in the ESI.†

2.3 Instruments and measurements

The number average molecular weight ($M_n$) and polydispersity index (PDI = $M_w$/$M_n$) of PNIPAM, PVCL, diblock and statistical copolymers of NIPAM and VCL were determined by GPC measurements with monodisperse poly(methylmethacrylate) (PMMA) as a standard and DMF as an eluent phase at 35 °C. $M_n$ NMR spectra of the investigated polymers were recorded on a Varian Mercury plus (400 MHz) spectrometer using CDCl$_3$ as the solvent. Turbidity measurements were performed on a Lamda 35 UV-vis spectrometer at 500 nm with deionized water as a reference (100% transmittance). Temperatures were manually regulated with a water-jacketed cell holder at the rate of ca. 0.4 °C min with an increment of 1 °C. Each temperature point was held for 2 min before measurements to ensure the thermal equilibrium of the sample cell. The samples for turbidity measurements were highly diluted in water (0.2 mg ml$^{-1}$) to prevent aggregation.

In the FTIR measurements of polymers solutions, the concentration of different samples was fixed at 10 wt%. Particularly, in the homopolymer mixture, the weight ratio of PNIPAM : PVCL was 1 : 1. Hereby, the solutions were sealed between two pieces of CaF$_2$ tablets, which had no absorbance in the middle infrared bands. All the temperature-dependent FTIR spectra were recorded by applying a Nicolet Nexus 6700 FTIR spectrometer equipped with a DTGS detector. 32 scans with a resolution of 4 cm$^{-1}$ were accumulated to achieve an acceptable signal-to-noise ratio. Temperatures were under programmed control with an electronic cell holder at a heating rate of ca.1 °C per 3 min with an increment of 0.5 °C (accuracy: 0.1 °C). The neat films of the studied polymers for FTIR tests were prepared by drop casting on the CaF$_2$ tablets from their CH$_2$Cl$_2$ solutions. The baseline correction was performed by the software of OMINIC 6.1a.

2.4 Investigation method

2.4.1 Two-dimensional correlation spectroscopy (2Dcos).

The temperature-resolved FTIR spectra recorded at an increment of 0.5 °C in specific wavenumber ranges were selected to conduct 2Dcos analysis. 2Dcos was carried out by utilizing the software of 2D Shige ver. 1.3 (Shigeaki Morita, Kwansei Gakuin University, Japan, 2004–2005) and then plotted into contour maps with Origin Program ver. 8.0. In the contour maps, red colors are defined as positive intensities, while the green colors are defined as negative ones.

3. Results and discussion

3.1 Polymer preparation and characterization

The diblock and statistical copolymers of NIPAM and VCL, as well as their homopolymers are synthesized via RAFT polymerization. And the corresponding molecular characterizations are summarized in Table S1.† Hereby, all the studied polymers are of almost the same molecular weight, but different segmental arrangements. Moreover, to better control the variations, the diblock and statistical copolymers, together with the homopolymer mixture are prepared with similar compositions (mole ratio of NIPAM to VCL is around 1/0.8), which can be determined from the $^1$H NMR spectra (Fig. S1†). The chemical compositions can be also confirmed from the FTIR spectra of those polymers, where C–H stretching bands of the diblock and statistical copolymers and the homopolymer mixture are nearly in the same shape (Fig. 2).

3.2 Turbidity measurements

To illustrate the thermoresponsive properties of the homopolymer mixture, diblock and statistical copolymers, turbidity
measurements are first employed to provide a direct view of the solubility changes of their dilute aqueous solutions upon heating. For the sake of comparison, aqueous solutions of pure PNIPAM and PVCL, prepared by RAFT polymerization, are also analyzed under the same conditions. The resulting cloud point curves of those studied polymers at the concentration of 0.2 mg ml\(^{-1}\) in the heating process are displayed in Fig. 3. Herein, the cloud point is taken as the initial turn point in the transmittance versus temperature curves. Notably, it is shown that the cloud point temperature (\(T_{cp}\)) of PVCL is remarkably higher than that of PNIPAM, which could be attributed to the effect of dilution since the transition point of PVCL is highly concentration dependent.\(^{22}\) To some extent, the enhanced difference of \(T_{cp}\)s of PNIPAM and PVCL homopolymer aqueous solutions with dilution will help us to better understand the phase transition behavior of the homopolymer mixture, diblock and statistical copolymers. Under such conditions, several observations can be made by comparing the turbidity behavior of the samples. It is clearly demonstrated that there exists only one LCST-transition in all the cloud point curves, indicating that the dehydration of PNIPAM and PVCL segments does not occur independently in the investigated systems. Moreover, the \(T_{cp}\)s of the homopolymer mixture and the diblock copolymer are close, and they are both slightly higher than that of pure PNIPAM, which might be attributed to the increased hydrophilicity of the polymer component with the incorporation of the PVCL part. Diblock copolymers are usually expected to form the micelle structures in selective solvent. However, such a phenomenon has not been observed here in the PNIPAM-b-PVCL system, which could be explained by the hydrophobic interactions of PNIPAM and PVCL segments.\(^{26}\) To be specific, after heating the solution above the cloud point of PNIPAM but below that of PVCL, PVCL segments preferentially move towards the collapsed PNIPAM segments, attracted by the hydrophobic interactions, rather than dissolve and spread into water. In this way, the PNIPAM and PVCL segments in the diblock copolymer exhibit cooperative aggregation during the phase transition process. Unlike the sharp decrease in transmittance of the diblock copolymer system at the cloud point, the homopolymer mixture exhibits a more continuous transition, behaving in a similar way to pure PVCL. This reveals that the dehydration process of polymers occurs differently in the homopolymer mixture and the diblock copolymer systems, which will be further discussed later. As for the statistical copolymer, it displays a relatively abrupt LCST-transition with the cloud point lying between that of pure PNIPAM and PVCL. Besides, the \(T_{cp}\) of the statistical copolymer is obviously higher than that of the homopolymer mixture and the diblock copolymer, which could be ascribed to that copolymerization of the VCL unit with PNIPAM would not only interrupt the interactions between NIPAM units but also increase the hydrophilicity of the whole polymer chains. Similar results can be as well confirmed by the dynamic light scattering (DLS) curves (Fig. S2†), where the hydrodynamic diameter (\(D_h\)) displays an abrupt increase at the \(T_{cp}\) which locates at almost the same temperature as that found in the turbidity curves for each polymer system. In addition, it is found out that both unimers and associates exist in the mixture, diblock and statistical copolymer solutions below the cloud points (Fig. S3†), suggesting the possibility of the formation of micelle structures in those thermal-responsive systems, which might be derived from the slight interactions (hydrogen bonding or hydrophobic interaction) among PNIPAM or PVCL units. Nevertheless, it is should be noted that the scattering intensity of large particles is far stronger than that of small ones in DLS measurements. Thus, it is presumed that mainly unimers exist in the solutions below the cloud points, and the amount of associates could be neglected.

### 3.3 Conventional IR analysis

To further elucidate and compare the phase transition behavior of those polymer systems from the molecular level, temperature-dependent FTIR spectra of the homopolymer mixture, diblock and statistical copolymers in D\(_2\)O (10 wt%) were
obtained, as shown in Fig. 4. It should be noticed that D$_2$O, rather than H$_2$O, is selected as the solvent here in order to eliminate the overlap of the δ(OH) relating to H$_2$O around 1640 cm$^{-1}$ with the amide I region of PNIPAM and PVCL, as well as reduce the influence of broad v(OH) of H$_2$O on v(CH). As have been reported, the transition temperatures of both PNIPAM and PVCL are only slightly affected by the isotopic substitutions.$^{23,24}$ Thus, it is suitable to use D$_2$O instead of H$_2$O for this study.

Herein, we will mainly focus on the C–H stretching vibration region (3020–2835 cm$^{-1}$) and the C=O stretching vibration region (1680–1560 cm$^{-1}$), so that we are able to trace nearly all the group motions in both PNIPAM and PVCL parts. By carefully inspecting the temperature-resolved FTIR spectra, two main changes can be observed upon heating in these three systems: all the bands related to C–H stretching vibration exhibit an evident red shift while the Amide I regions present an apparent bidirectional spectral intensity variation. It is considered that water clathrates, where water molecules have a well-ordered structure, exist around the hydrophobic moieties (such as C–H groups) of water-soluble polymers.$^{35}$ The higher the number of water molecules surrounding C–H groups is, the higher the vibrational wavenumber is.$^{36}$ From this point of view, the changes of the v(CH) bands could be explained by the dehydration of C–H groups with increased temperature. Referring to the v(C=O) bands, they can all be roughly divided into two parts: the higher wavenumber moiety and the lower one. It is clearly demonstrated in the spectra that the intensity of the higher wavenumber moiety increases while the lower one decreases in the heating process, indicating that there exists a transformation among different states of carbonyl groups in the phase transition of those LCST-type polymers. Additionally, it is easy to find that the homopolymer mixture and diblock copolymer share a relatively similar peak shape and variation of the v(C=O) region, which differs largely from that of the statistical copolymer. This phenomenon, to some extent, parallels the results of $T_{c_p}$s in the turbidity curves.

For better understanding of those discoveries within the C=O groups, the second-derivative analysis of the amide I region is carried out, as presented in Fig. 5. It is believed that the sharpened minima in the second-derivative curves can be related to the maxima in the original absorption spectra. For the homopolymer mixture and diblock copolymer systems, the second-derivative curves look the same and four peaks, located at around 1650, 1625, 1610 and 1590 cm$^{-1}$ can be identified. On the basis of previous reports,$^{7,25,26}$ those four peaks could be distributed to hydrogen bonds of C=O···D–N (PNIPAM), C=O···D–O–D (PNIPAM), C=O···D–O–D (PVCL) and C=O···2O–D–D (PVCL), respectively. With regard to the statistical copolymer, the situation is somewhat different. As shown in the figure, only three distinct bands, namely 1650, 1625 and 1590 cm$^{-1}$, are recognized. The band, 1610 cm$^{-1}$, which appears in the homopolymer mixture and diblock copolymer systems, is missing here. Through deeper examination, we should find that the missed band in the statistical copolymer system is compensated by the increased intensity of band at 1590 cm$^{-1}$. Meanwhile, it is also the origin of the above observation where the temperature-dependent FTIR spectra of the statistical copolymer in the v(C=O) region express differently. By taking the corresponding assignments of those two bands into consideration, it can be deduced that the C=O groups of PVCL segments in the statistical copolymer aqueous solution system are more hydrated than that in the homopolymer mixture and diblock copolymer systems. In spite of the dissimilarities among those three systems, the responses of the C=O groups during the phase transition upon heating all follow the analogous change: interactions between carbonyl groups (of both PNIPAM and PVCL parts) and water molecules gradually break with the formation of intra-/inter-molecular hydrogen bond C=O···D–N (PNIPAM part) and free or relatively free C=O (PVCL part). Apart from above observations, we could as well figure out that the direct hydrogen bonding interaction of C=O in PVCL parts and D–N in PNIPAM parts is not that preferential in the three systems since no obvious bands related to C=O (PVCL)···D–N (PNIPAM) have been detected in the second-derivative spectra of the amide I region. Such a situation is quite different from that observed in the PNIPAM/PDEAAM systems,$^{22,37}$ where intra-/inter-molecular hydrogen bonding between the hydrogen-bond donor (PNIPAM) and the hydrogen-bond acceptor (PDEAAM) holds priority in the homopolymer mixture and the statistical copolymer. In some sense, this inspection might explain the difference of the LCSTs of the homopolymer mixture and the statistical copolymer between PNIPAM/PVCL and PNIPAM/PDEAAM systems.
As has been discussed in our previous report,26 we are able to monitor the dehydration behavior of PNIPAM and PVCL parts separately by simply tracking the frequency variations of $\nu_{as}(\text{CH}_3)$ and $\nu_{s}(\text{CH}_2)$, respectively. Therefore, quantitative analysis of the temperature-dependent frequency shifts of these C–H stretching bands is carried out, which are plotted in Fig. 6. For the convenience of comparison, the heat-induced changes of $\nu_{as}(\text{CH}_3)$ in pure PNIPAM and $\nu_{s}(\text{CH}_2)$ in pure PVCL are also displayed hereby. It is observed that both C–H bands exhibit decrease in wavenumber during the phase transition process in all investigated systems, revealing the decreased hydrophobic hydration of C–H groups upon heating. However, we can also find that different systems demonstrate different change behavior. For example, the dehydration behavior of C–H groups in the LCST-transition of pure PNIPAM is rather sharp while that in pure PVCL is relatively continuous, which is in consistence with the previous literature.$^{7,23}$ indicating that PNIPAM presents a sharp transition while PVCL presents a continuous one. Under such a circumstance, we may wonder how the transition behavior of PNIPAM and PVCL parts will be in the homopolymer mixture, diblock or statistical copolymers.

In the phase transition of the homopolymer mixture, the sudden frequency decrease of $\nu_{as}(\text{CH}_3)$ and $\nu_{s}(\text{CH}_2)$ begins at almost the same temperature, which, to some extent, confirms the results of turbidity curves and suggests that there should exist some kind of interaction between PNIPAM and PVCL during the LCST-transition of the homopolymer mixture system. Then, by examining the variation behavior of the C–H groups in the homopolymer mixture more carefully, we can discover that the changes with regard to PNIPAM and PVCL parts show obvious inconformity, where PNIPAM exhibits an abrupt change while PVCL exhibits a gradual one around the LCST. In a sense, this detection clearly implies that PNIPAM and PVCL parts resemble the properties of their own homopolymers in the phase transition of the homopolymer mixture system apart from those interactions. In contrast, the changes of PNIPAM and PVCL parts are identical, namely, both sharp, in the LCST-transition of the diblock copolymer aqueous solution.

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**Fig. 5** Second-derivative spectra in the C=O stretching region of 10 wt% mixture, diblock and statistical copolymers in D$_2$O during the heating process.

**Fig. 6** Temperature-dependent frequency shifts of $\nu_{as}(\text{CH}_3)$ and $\nu_{s}(\text{CH}_2)$ corresponding to PNIPAM (black), mixture (red), diblock (blue), statistical copolymer (green) and PVCL (pink) in D$_2$O during the heating process.
upon heating. Then, we are able to conclude that the chemical bond between PNIPAM and PVCL within the diblock copolymer chain plays an important role in the cooperative dehydration of both segments. In this way, the difference in the phase transition between the homopolymer mixture and the diblock copolymer can be emphasized, though their LCSTs stay close. Moreover, this microscopic difference should be also responsible for the dissimilarity of the optical change in the LCST-transition of the homopolymer mixture and diblock copolymer systems, where the transmittance loss of the former is markedly slower than that of the latter during the heating process (Fig. 3). Concerning the statistical copolymer, it is noted that both the transition temperature and the transition behavior of the PNIPAM and PVCL segments act in unison, hinting that PNIPAM and PVCL segments herein work collaboratively during the phase separation process. Furthermore, the dehydration procedure of the statistical copolymer chains consists of two steps: a somewhat steep dehydration near the LCST and a relatively continuous change thereafter, which approximates to the situation of the diblock copolymer and could be originated from the combined LCST-transition features of pure PNIPAM and PVCL.\textsuperscript{26} Even though, when focusing on the details, we can apparently find that the statistical copolymer exhibits a rather broader LCST-transition than the diblock copolymer. Considering that the sharp phase transition of pure PNIPAM is rooted from the formation of inter/intra-molecular hydrogen bonds C\textsuperscript{=O} \cdots H\textsuperscript{-N}, we might attribute this finding to the fact that the incorporation of VCL units into PNIPAM chains would interrupt the construction of hydrogen bonding structure C\textsuperscript{=O} \cdots H\textsuperscript{-N} among PNIPAM segments, further making the LCST-transition less precipitous. What’s more, it is clearly demonstrated in the curves that the initial wavenumber of \(v_{\text{as}}(\text{CH}_3)\) is lower and \(v_{\text{s}}(\text{CH}_2)\) is higher in the homopolymer mixture, diblock and statistical copolymer systems than that in the corresponding pure PNIPAM and PVCL ones, respectively, indicating that water molecules have been redistributed along the polymer chains when PNIPAM and PVCL segments are brought into one system. To be more specific, water molecules bind preferentially onto PVCL sites, while that around PNIPAM ones are reduced in the composite system. This could be explained by the fact that VCL units are more hydrophilic than NIPAM ones. Additionally, the situation is especially remarkable in the statistical copolymer system, which can be also verified in the above second-derivative analysis of the amide I region. And we might ascribe it to the enhanced competition of hydration between PNIPAM and PVCL segments caused by the monomer pattern along the statistical copolymer chains.

The phase transition behavior of the homopolymer mixture, diblock and statistical copolymers in their aqueous solutions, as well as the inherent distinction among them, has been basically pictured until now. For clarity, the comparison of the phase transition temperatures and behavior of the PNIPAM, PVCL, PNIPAM/PVCL mixture, diblock and statistical copolymers are summarized in Table 1. While, a more concrete description with regard to the group motions from the molecular level still remains unclear. Thereupon, 2Dcos analysis of FTIR spectra is performed. Herein, we will mainly focus on the homopolymer mixture and statistical copolymer systems, due to the lack of exploration.

### 3.4 Two-dimensional correlation spectroscopy (2Dcos)

2Dcos is a mathematical method, and its basic principles were firstly proposed by Noda.\textsuperscript{38,39} Until now, it has been intensively utilized to interpret spectroscopic fluctuations of various chemical species under different types of external perturbations (e.g., temperature, time, pressure, concentration, electromagnetic, etc.).\textsuperscript{40–42} By spreading the original peaks along a second dimension, those complex or overlapped features, not readily visible in the conventional analysis, can be sorted out and thus, an improved spectral resolution is obtained. Furthermore, additional information on molecular motions or conformational changes can be achieved in 2Dcos for different responses of different species to external variables, which will help to understand the perturbation-induced change from an intuitionistic view.

#### 3.4.1 2Dcos analysis of the homopolymer mixture system

To acquire the 2Dcos maps of the homopolymer mixture system, all the FTIR spectra during heating from 25 to 45 °C with an increment of 0.5 °C have been employed and the corresponding synchronous and asynchronous contour maps are

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<td>Statistical copolymer</td>
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* The transition temperatures herein are determined as the initial turn point in turbidity curves and temperature-dependent frequency shifts of \(v_{\text{as}}(\text{CH}_3)\) and \(v_{\text{s}}(\text{CH}_2)\).*
shown in Fig. 7. A 2D synchronous map can provide information of simultaneous variations between two given wavenumbers. For instance, the bands at 2991 and 1587 cm$^{-1}$ hold positive cross-peaks in the synchronous map, reflecting a similar response of spectral intensities to the increasing temperature of those two bands. After reading the raw data for reference, we will find that the intensities of the two bands both decrease upon heating. Meanwhile, the 2D asynchronous map can significantly enhance the resolution of the original spectra. In the C–H stretching region, hydrated and dehydrated states of C–H groups, which have been superimposed in the original spectra, are recognized. Moreover, several subtle bands related to C=O groups appear in the 2D asynchronous map, revealing the diversity in the hydrogen bonding structure with regard to those C=O groups. For the convenience of discussion, all the bands detected in the 2D asynchronous map and their corresponding assignments are listed in Table 2.

In addition to enhancing spectral resolution, 2Dcos can be also used to discern the specific sequence occurring along external perturbations. A cross-peak in the 2D asynchronous map can develop only if the intensities of two spectral features change out of phase with each other (i.e., accelerated or delayed when the external variable is time). The determining rule can be outlined as Noda’s rule$^{43}$—namely, if the cross-peaks ($v_1$, $v_2$, assuming $v_1 > v_2$) in the synchronous and asynchronous map have the same sign, the change at $v_1$ may take place prior to or earlier than that of $v_2$, and vice versa. A simplified method for the determination of the sequence order has been reported previously.$^{44}$ And herein, the final sequence order for the heat-induced phase transition of the homopolymer mixture in D$_2$O is described as follows: 2953 → 1628 → 2856 → 2914 → 2929 → 291 → 2972 → 1647 → 1660 → 1587 → 1606 cm$^{-1}$ or $v_{as}$(CH$_2$) (hydrated) (PNIPAM and PVCL segments) → $v$(C=O···D–O–D) (PNIPAM segments) → $v_{as}$(CH$_2$) (dehydrated) (PNIPAM and PVCL segments) → $v_{as}$(CH$_2$) (dehydrating) (PNIPAM and PVCL segments) → $v_{as}$(CH$_3$) (hydrated) (PNIPAM segments) → $v_{as}$(CH$_3$) (dehydrated) (PNIPAM segments) → $v$(C=O···D–O–D) (PNIPAM segments) → $v$(free C=O) (PNIPAM segments) → $v$(C=O···D–O–D) (PVCL segments) → $v$(C=O···D–O–D) (PVCL segments).

Deduced from the overall sequence, it is noticeably demonstrated that almost all the hydrophobic C–H groups hold an earlier response to temperature rising than the C=O groups, indicating that the hydrophobic interaction among C–H groups should be the driving force for the LCST-transition of the homopolymer mixture. Accordingly, we may ascribe such an interaction to be the root of simultaneous dehydration of PNIPAM and PVCL parts. Considering the bands of C=O groups separately, we can discover that the changes of PNIPAM parts take place prior to that of PVCL ones. It is generally known that

### Table 2

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<thead>
<tr>
<th>Wavenumber (cm$^{-1}$)</th>
<th>Tentative assignments</th>
</tr>
</thead>
<tbody>
<tr>
<td>2991</td>
<td>$v_{as}$(hydrated CH$_2$) (PNIPAM segments)</td>
</tr>
<tr>
<td>2972</td>
<td>$v_{as}$(dehydrated CH$_3$) (PNIPAM segments)</td>
</tr>
<tr>
<td>2953</td>
<td>$v_{as}$(hydrated CH$_2$) (PNIPAM and PVCL segments)</td>
</tr>
<tr>
<td>2929</td>
<td>$v_{as}$(dehydrating CH$_2$) (PNIPAM and PVCL segments)</td>
</tr>
<tr>
<td>2914</td>
<td>$v_{as}$(dehydrated CH$_2$) (PNIPAM and PVCL segments)</td>
</tr>
<tr>
<td>2856</td>
<td>$v$(CH$_2$) (PVCL segments)</td>
</tr>
<tr>
<td>1660</td>
<td>$v$(free C=O) (PNIPAM segments)</td>
</tr>
<tr>
<td>1647</td>
<td>$v$(C=O···D–O–D) (PNIPAM segments)</td>
</tr>
<tr>
<td>1628</td>
<td>$v$(C=O···D–O–D) (PNIPAM segments)</td>
</tr>
<tr>
<td>1606</td>
<td>$v$(C=O···D–O–D) (PVCL segments)</td>
</tr>
<tr>
<td>1587</td>
<td>$v$(C=O···D–O–D) (PVCL segments)</td>
</tr>
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</table>
the sharp LCST-transition of pure PNIPAM comes from the transformation of the hydrogen bonding structure C==O⋯H–O–H to C==O⋯H–N. In this case, the earlier response of C==O groups in PNIPAM herein is also responsible for its abrupt change in the homopolymer mixture system, as have been observed in Fig. 6. Thereupon, in combination with above conventional FTIR analysis, the chain collapse mechanism of PNIPAM and PVCL in the homopolymer mixture in D₂O during the heating process could be proposed. At temperatures lower than LCST, both PNIPAM and PVCL chains are hydrophilic and well soluble in water, with C–H and C==O groups associating with water molecules. As temperature increases, connections between polymer chains and water molecules gradually break, leading to the strengthened hydrophobic interactions among both polymer chains. As soon as the LCST is reached upon heating, those hydrophobic C–H groups in both PNIPAM and PVCL parts start to aggregate abruptly, further resulting in the parallel collapse of both kinds of polymer chains. Due to the lack of chemical bonds between PNIPAM and PVCL chains, the cooperative dehydration among PNIPAM and PVCL chains is not that significant. Hence, PNIPAM chains exhibit a sharp and exhaustive collapse while PVCL chains display a rather continuous and slow one.

3.4.2 2Dcos analysis of the statistical copolymer system. To further gain the details of group motions in the LCST-transition of the statistical copolymer aqueous solution, 2Dcos analysis is also applied with all the FTIR spectra on heating between 25 and 45 °C with an interval of 0.5 °C, and the obtained synchronous and asynchronous maps are presented in Fig. 8. Thanks to the enhanced spectra resolution of 2Dcos, many subtle bands with regard to various states of C–H and C==O groups have been identified. Herein, it should be noted that though the original peak shape of C==O stretching region differs much in the homopolymer mixture and diblock copolymer systems, the differentiated bands related to C==O groups achieved by the 2D asynchronous map remain almost the same, inferring that the species of C==O groups do not vary between the two systems. This phenomenon, in some sense, confirms our above discussion that the distinctive shape of the C==O stretching band of the statistical copolymer system is not derived from the formation of new hydrogen bonding structures but the transformation among different ones. For clarity, Table 3 presents all the bands recognized from 2D asynchronous maps and their tentative assignments.

Similarly, the specific change sequence of different chemical groups taking place under heating of the statistical copolymer in D₂O can be discerned. According to Noda’s rule, the final sequence is deduced: 2979 → 2939 → 2860 → 1628 → 2970 → 1649 → 2918 → 2852 → 1661 → 1589 → 1599

<table>
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<th>Tentative assignments</th>
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</thead>
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<tr>
<td>2979</td>
<td>ν asym (hydrated CH₃) (PNIPAM segments)</td>
</tr>
<tr>
<td>2970</td>
<td>ν asym (dehydrated CH₃) (PNIPAM segments)</td>
</tr>
<tr>
<td>2939</td>
<td>ν asym (hydrated CH₂) (PNIPAM and PVCL segments)</td>
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<tr>
<td>2918</td>
<td>ν asym (dehydrated CH₂) (PNIPAM and PVCL segments)</td>
</tr>
<tr>
<td>2860</td>
<td>ν asym (hydrated CH₄) (PVCL segments)</td>
</tr>
<tr>
<td>2852</td>
<td>ν asym (dehydrated CH₄) (PVCL segments)</td>
</tr>
<tr>
<td>1661</td>
<td>ν (free C==O) (PNIPAM segments)</td>
</tr>
<tr>
<td>1649</td>
<td>ν (C==O⋯D–N) (PNIPAM segments)</td>
</tr>
<tr>
<td>1628</td>
<td>ν (C==O⋯D–O–D) (PNIPAM segments)</td>
</tr>
<tr>
<td>1599</td>
<td>ν (C==O⋯D–O–D) (PVCL segments)</td>
</tr>
<tr>
<td>1589</td>
<td>ν (C==O⋯2D–O–D) (PVCL segments)</td>
</tr>
</tbody>
</table>

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Fig. 8 2D synchronous and asynchronous spectra of 10 wt% statistical copolymer of NIPAM and VCL in D₂O obtained from all spectra between 25 and 45 °C during heating (red colors are defined as positive intensities, while the green colors are defined as negative ones).
cm$^{-1}$ or $\nu_{as}(CH_3)$ (hydrated) (PNIPAM segments) $\rightarrow \nu_{as}(CH_3)$ (hydrated) (PNIPAM and PVCL segments) $\rightarrow \nu_{s}(CH_2)$ (hydrated) (PVCL segments) $\rightarrow \nu(C=O-D-O-D)$ (PNIPAM segments) $\rightarrow \nu(C=O-D-N)$ (PNIPAM segments) $\rightarrow \nu_{as}(CH_3)$ (dehydrated) (PNIPAM and PVCL segments) $\rightarrow \nu_{s}(CH_2)$ (dehydrated) (PVCL segments) $\rightarrow \nu($free C=O$)$ (PNIPAM segments) $\rightarrow \nu(C=O-2D-O-D)$ (PVCL segments) $\rightarrow \nu(C=O-2D-O-D)$ (PVCL segments).

Without considering the differences in stretching modes, we have C–H $\rightarrow$ C=O, which behaves in the same way as the homopolymer mixture demonstrates, indicating that the LCST-transition of statistical copolymer is also driven by hydrophobic interactions among those C–H groups. Nevertheless, unlike the obviously predominant rule of the dehydration of C–H groups in the homopolymer mixture system, the changes of C=O and C–H take place alternately herein, revealing that C=O and C–H groups work cooperatively in the phase transition process of the statistical copolymer aqueous solution. Through careful examination of the sequence order, it was figured out that both C–H and C=O groups in PNIPAM segments, respectively, exhibit an earlier response than those in PVCL segments. As has been discussed before, due to the close connection of PNIPAM and PVCL segments along the statistical copolymer chains, the competition of hydration between them is enhanced. Since PVCL segments are more hydrophilic, water molecules will distribute preferentially around PVCL segments. Under this circumstance, hydrophobic interactions in PNIPAM segments are intensified, which would further bring about their quicker response in the LCST-transition. On the basis of above discussion, the comprehensive self-aggregation behavior of the statistical copolymer of NIPAM and VCL in water can be obtained. The copolymer chains are well soluble in water through hydrophobic hydration of C–H groups and hydrogen bonding of hydrophilic C=O groups with water molecules at lower temperatures. Due to the competitive hydration between PNIPAM and PVCL segments, there exists a heterogeneous distribution of water molecules, where PVCL segments are surrounded by more water molecules. After heating the solution above LCST, the whole polymer chains begin to collapse, driven by enhanced hydrophobic interactions among C–H groups with increase in temperature. Particularly, PNIPAM segments present a relatively earlier response to heating by virtue of their less hydrated states. With the help of the cooperative dehydration of C–H and C=O groups in both segments and the formation of inter/intra-molecular hydrogen bonds C=O-H-N among PNIPAM segments, the statistical copolymer exhibits an abrupt LCST-transition in water upon heating. Moreover, on account of the continuous dehydration of PVCL segments, further collapse of the statistical copolymer chain is observed at the later stage of the LCST-transition. For limpidity of demonstration, the illustration and comparison of the LCST-transition processes of the homopolymer mixture, diblock and statistical copolymers of NIPAM and VCL in water upon heating are displayed in Fig. 9.

The description of the LCST-transition of diblock copolymer in water is according to ref. 26.

Fig. 9  Schematic illustration and comparison of the LCST-transition processes of the homopolymer mixture, diblock and statistical copolymers of NIPAM and VCL in water.

4. Conclusion

In this work, we mainly employed turbidity and FTIR measurements, as well as 2D correlation spectroscopy (2Dcos) analysis to explore the LCST-transitions of the homopolymer mixture of PNIPAM and PVCL, diblock and statistical copolymers of NIPAM and VCL aqueous solutions in comparison with pure PNIPAM and PVCL. Only one transition point is detected for all of the three investigated polymer systems, revealing that PNIPAM and PVCL segments do not behave independently in the LCST-transitions. The cloud point temperatures ($T_{cep}$) and macroscopic features of the transitions are determined from the turbidity curves, where the homopolymer mixture and the diblock copolymer display close $T_{cep}$ while the statistical copolymer presents a relatively higher one. Besides, both diblock and statistical copolymers exhibit rather sharp phase transitions while the homopolymer mixture demonstrates a moderately continuous one. Such observations can be then confirmed and explained by the quantitative analysis of temperature-dependent FTIR spectra on the frequency shifts of $\nu_{as}(CH_3)$ and $\nu_{s}(CH_2)$, which reflects the dehydration behavior of PNIPAM and PVCL segments from the molecular level upon heating, respectively. By comparing with pure homopolymers, it is discovered that water distributions are slightly changed along the polymer chains in the composite polymer systems, in which PNIPAM segments become less hydrated while PVCL segments become more hydrated. This situation is especially obvious in the statistical copolymer system, and it could be derived from the enhanced competition of hydration between PNIPAM and PVCL segments under close connection. In addition, it is displayed in both 2Dcos analyses of the homopolymer mixture and statistical copolymer systems that C–H groups, as the hydrophobic part, exhibit an earlier response than C=O groups to temperature rising, which is also in accordance with our previous finding in the diblock copolymer system, indicating that the hydrophobic interaction is the driving force and predominates the LCST-transitions thereof.
Acknowledgements

We are very grateful for the financial support of the National Natural Science Foundation of China (NSFC) [no. 21274030].

Notes and references