Dynamic Phase Transition Behavior and Unusual Hydration Process in Poly(ethylene oxide)-b-Poly(N-vinylcaprolactam) Aqueous Solution

Qiwen Wang, Hui Tang, Peiyi Wu
Department of Macromolecular Science and Laboratory for Advanced Materials, State Key Laboratory of Molecular Engineering of Polymers, Collaborative Innovation Center of Polymers and Polymer Composite Materials, Fudan University, Shanghai 200433, China
Correspondence to: P. Wu (E-mail: peiyiwu@fudan.edu.cn) or H. Tang (E-mail: huitang@fudan.edu.cn)
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ABSTRACT: Dynamic phase transition and self-assembly mechanism of thermosensitive poly(ethylene oxide)-b-poly(N-vinylcaprolactam) (PEO-b-PVCL) copolymer are explored deeply. A gradual dehydration process with predominated hydrophobic interactions among copolymer chains in the phase transition process distinguishes the copolymer from homopolymer. PVCL in the inner zone is restricted and counter-balanced by the PEO segments based on the sequence order of representative groups during the heating-cooling cycles. Remarkably, PEO shell experiences unusual hydration process, which is first discovered. This hydrophilic shell plays as water absorption sponge layer and captures expelled water from PVCL core, accompanied by gradient distribution of water existed in the assembly structures. Peculiarly, pseudo-linear changes of the integral area of free C=O are presented compared with inflection point in the hydrated C=O integral area, which propose that a part of hydrated C=O forms incomplete dehydrated states. During the cooling process, perfect reversibility is observed without obvious hysteresis.

KEYWORDS: dynamic phase transition; FT-IR and phase behavior; incomplete dehydrated states; pseudo-linear changes; self-assembly; thermodynamics; two-dimensional correlation spectroscopy; unusual hydration

INTRODUCTION
With fast development of smart materials in scientific and potential technological applications, 1-3 a lot of significant attention has been paid to stimulus-responsive polymers, which respond in dramatic ways to slight external variations like temperature, pH, light, ionic strength, etc. 4-6 Among them, thermoresponsive polymers, which display lower critical solution temperatures (LCSTs) in physiological ranges, drive appealing blueprints in plenty of fields including functional therapeutic applications, controlled drug delivery systems. 7 Upon heating above their LCSTs, these thermosensitive polymers experience coil-to-globule phase transitions and then return to their original states in the cooling processes, which are meaningful for wide applications.

Among various LCST-typed polymers, poly(N-vinylcaprolactam) (PVCL) with hydrophilic amide groups and hydrophobic carbon backbones has been considered as valuable alternative to poly(N-isopropylacrylamide) (PNIPAM) in biomedical applications. Because in hydrolysis process, PVCL only produces polymeric carboxylic acid without extra toxic small molecules. 8 Since the LCST increased with decrement of polymer chain length and/or solution concentrations in PVCL aqueous solutions, classical Flory-Huggins thermoresponsive behavior has been illustrated. 9 By using IR spectroscopy, changes in hydrogen bonding of the amide groups and detailed information on the hydration states of the alkyl groups during the phase transition of PVCL aqueous solution were observed by Maeda et al. 10 Based on two-dimensional correlation spectroscopy in combination with molecular dynamics simulation, distribution gradient of water molecules in PVCL mesoglobules ranging from hydrophobic core to hydrophilic surface was depicted by Sun and Wu. 11 Due to the absence of self-associated hydrogen bonds and topological constraints, PVCL has a reduced magnitude of conformation changes compared with PNIPAM. 12-13 Recently, Spevacek et al. demonstrated that water molecules which remained hydrogen bonded to the carbonyl groups could serve as intermediaries of interactions between polymer segments in PVCL mesoglobule. Dehydration at temperatures above the phase transition consisted of transformation of structures from hydrogen bonded to two water molecules to structures with single hydrogen bonded water molecule. 14

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Besides specific cosolutes\textsuperscript{10} (like salts, surfactant, and ionic liquids) and cosolvents\textsuperscript{15} which had been applied to manipulate phase transition behavior of PVCL-based systems, copolymerization had also been utilized to regulate phase transition behavior and morphology of thermo-responsive polymers by shifting the hydrophilic/hydrophobic balance. In general, the introduction of hydrophilic comonomers increases the LCSTs whereas hydrophobic comonomers induce an opposite effect.\textsuperscript{16} With cobalt-mediated radical polymerization method, $N$-vinylcaprolactam (VCL) was copolymerized statistically with hydrophilic $N$-vinylamide or hydrophobic vinyl ester monomers to precisely tune LCSTs of the resulting copolymers.\textsuperscript{17}

Through modulated temperature DSC, the LCST behavior and phase separation kinetics of linear PVCL, PVCL-$\text{graft}$-$\text{poly}(\text{ethylene oxide})$, PVCL networks, and poly(ethylene oxide)-grafted PVCL networks were explored. It was found that the introduction of hydrophilic poly(ethylene oxide) grafts onto PVCL lowered the demixing temperature and enhanced the rate of phase separation.\textsuperscript{18} Since double-hydrophilic block copolymers (DHBCs) which comprise two water-soluble blocks of different chemical nature can spontaneously self-assemble \textit{in situ} when one block becomes hydrophobic due to the external stimulus such as pH or temperature variations, self-assembly, and microphase separation behavior of such stimulus-responsive diblock copolymers has attracted a lot of interests nowadays.\textsuperscript{19}

For instance, Hurtgen et al. prepared double hydrophilic poly(vinyl alcohol)-$b$-poly($N$-vinylcaprolactam) (PVOH-$b$-PVCL) copolymers by hydrolyzation of poly(vinyl acetate)-$b$-poly($N$-vinylcaprolactam) (PVAc-$b$-PVCL). When heated at 36 to 42°C, the PVOH-$b$-PVCL got amphiphilic and micron-size aggregates formed which were stabilized in water by PVOH segments.\textsuperscript{20} Recently, the self-aggregation behavior of poly($N$-isopropylacrylamide)-$b$-poly($N$-vinylcaprolactam) (PNIPAM-$b$-PVCL) during the thermal-induced phase transition in water was explored by a combination of FT-IR spectroscopy and correlation analysis. Only one transition point was detected in the heating process of the block copolymer in aqueous solution, indicating the cooperative aggregation of PNIPAM and PVCL segments. Notably, parallel dehydration of PNIPAM and PVCL segments and cooperative aggregation behavior of the LCST transition in the block copolymer had been displayed via two-dimensional correlation analysis.\textsuperscript{21}

Among various hydrophilic additives and comonomers, poly(ethylene oxide) (PEO) has been often represented due to its remarkable properties such as biocompatibility, ability to control the bioactivity of surfaces, and lack of toxicity.\textsuperscript{22} The introduction of hydrophilic PEO in PVCL-based aqueous systems and block copolymer networks had been investigated via two- and three-dimensional phase diagrams. It suggested that the strength of the hydrogen bonds within the PVCL-$\text{H}_2\text{O}$ was weakened by the introduction of PEO which also interacted with water at the same time. According to the DSC investigation of swollen networks, an irregular water distribution was proposed based on detailed phase analysis.\textsuperscript{23} Using PEO with two chloride end group as the initiator, Negru et al. synthesized poly(ethylene oxide)-$b$-poly($N$-vinylcaprolactam)-$b$-poly(ethylene oxide) triblock copolymers via atom transfer radical polymerization method. By increasing the molar ratio of PVCL, the aggregation ($T_{\text{agg}}$) and gelation ($T_{\text{gel}}$) temperatures were decreased.\textsuperscript{24} Prabaharan et al. prepared PEO-$b$-PVCL block copolymer coupled with folic acid as an antibiotic carrier without living polymerization. The block polymer self-assembled into stable micelles in aqueous solutions above 33°C and a slower and more controlled release profile of drug was shown.\textsuperscript{25} Lately, a series of well-defined PEO-$b$-PVCL copolymers from xanthate-capped PEO macro-chain transfer agent (CTA) was prepared by Liu et al. via reversible addition-fragmentation chain transfer (RAFT) polymerization. Through immobilization of the xanthate end group on the surface of gold nanorods, thermodependent optical properties which may be used as smart drug delivery systems were explored deeply.\textsuperscript{26} Nevertheless, although the morphology investigations of PEO-$b$-PVCL aqueous solutions had been discussed occasionally, the association and dissociation process of these stimulus-responsive DHBCs involved the respective contribution of hydrogen bonding and hydrophobic interaction in the heating and cooling processes have seldom been discussed in detail compared with PEO-$b$-PNIPAM copolymers.\textsuperscript{27–29}

Thus, in the present study, self-assembly behavior and information about the molecular motion process including subtle change of hydrogen bonds and sequential order of different characteristic chemical groups of PEO-$b$-PVCL copolymer in aqueous solution were investigated by FT-IR spectroscopy in combination with correlation analysis. Transition points and complicated spectral variations were explored, while detailed and comprehensive hydration behavior was exhibited.

**EXPERIMENTAL**

**Materials**

Hydroxyl-end capped poly(ethylene oxide) (PEO\textsubscript{42}, $M_n = 1900$ g/mol), 4-dimethylaminopyridine (DMAP), 2-bromoisobutyryl bromide, and potassium ethyl xanthogenate were purchased from Aldrich Reagent. $N$-vinylcaprolactam (VCL) was purchased from Alfa Aesar and used after recrystallized. 2,2'-Azobisiso-butynitrile (AIBN) was recrystallized from ethanol. PEO-$b$-PVCL block copolymer was synthesized via reversible addition fragmentation transfer (RAFT) polymerization according to the literature as shown in the Supporting Information.\textsuperscript{26} The two-step synthetic procedure was shown in Supporting Information Scheme S1 and the characterization results of the copolymers were provided in Supporting Information Figure S1. The repeating units of PVCL were calculated according to the integral of signal at 4.20 to 4.53 ppm (CH protons in PVCL main chains) and narrow single peak at 3.71 ppm. (CH\textsubscript{2} protons in PEO main chains). The composition of copolymer was PEO\textsubscript{42}-$b$-PVCL\textsubscript{124} according to \textsuperscript{1}H NMR result. Unless otherwise specified, all other reagents were purchased from commercial suppliers and used as received.
Instruments and Measurements

Turbidity measurements were carried out on a Lambda 35 UV-Vis spectrometer at 500 nm with water as the reference. Temperatures were controlled automatically with water-jacketed cell holder at the rate of 1 °C/min. In order to reach the thermal equilibrium of the sample, each temperature point was stabilized for 120 s before measurement. Calorimetric measurements were tested on a Metter-Toledo differential scanning calorimeter (DSC) thermal analyzer. Hydrated particle radius was measured by dynamic light scattering (DLS) using zetasizer nano instrument (Malvern, England). The heating rate was 0.4 °C/min with an interval of 1.0 °C and the scattering angle was 90°. To ensure equilibrium at each temperature point, the sample cell was held for 10 min before measurements of the diluted sample solutions (1 wt %). Temperature-variable 1H NMR spectra of the copolymer (1 wt %) were recorded on Varian Mercury plus 500 MHz spectrometer with D2O as solvent and internal NMR standard with an increment of 1 °C. Typical conditions were as follow: 90° pulse width 12 µs, acquisition time 4.68 s, four scans. All temperature-variable 1H NMR experiments were made using a 90° excitation pulse and the interval between pulses were 20 s.

Temperature-dependent FT-IR spectra of PEO-b-PVCL copolymer solutions (10 wt %) were recorded by Nicolet Nexus 6700 spectrometer equipped with DTGS detector by signal-averaging 32 scans at a resolution of 2 cm⁻¹. Temperatures were controlled with electronic cell holder at a rate of 0.3 °C/min with an increment of 1 °C. 2D Shige version 1.3 (Shigeki Morita, Kwansei Gakuin University, Japan, 2004-2005) software was used to process data into the contour maps by Origin Program version 8.0. In the contour maps, warm colors (red and yellow) were defined as the positive intensities, while cold colors (blue) indicated negative ones. An appropriate window size (2m + 1 = 11) was chosen to generate spectra with good quality.

RESULTS AND DISCUSSION

Turbidity Measurements

Turbidity measurements of the copolymer in aqueous solutions were carried out to determine the LCSTs of copolymer under different concentrations, as performed in Supporting Information Figure S2. Narrow transmittance changes with increment of temperatures under various concentrations were observed. The heating and cooling curves were identical and no obvious hysteresis could be observed. Kareoja et al. reported similar good resilience of mesoporous silica particles grafted with PEO-b-PVCL copolymers in turbidity measurement and they attributed significant stability of hydrophilic PEO block to the main reason.30 Taken the initial turn points in the transmittance versus temperature curves as the cloud points, the phase transition temperature varied from 40 °C to 36 °C with increment of concentration from 0.1 wt % to 10 wt %. This relationship between concentration and LCST of copolymer was similar to the PVCL homopolymer. With temperature increment, the hydrophilicity of PVCL decreased and inter-chain collapsing occurred to form larger and denser aggregates with higher concentration, which lead to the lower LCST. According to previous study, Laukkanen et al. indicated that Tc, were 31.8 and 35.7 °C for PVCL-330 and PVCL-30 homopolymers in H2O (0.1 wt %), respectively. In contrast, the cloud point of copolymer increased to some degree due to the stabilization effect of hydrophilic PEO segments.

Calorimetric Measurements

Typical DSC curves of copolymer aqueous solutions during the heating and cooling cycles with different concentrations were presented in Figure 1. Although good resilience in macroscopic turbidity measurements, perceptible hysteresis could be detected in microscopic enthalpy DSC measurements. With increment of the concentration from 1 wt % to 10 wt %, the hysteresis decreased from 7.3 to 3.7 °C, which was accordance with previous studies of PVCL systems.31 Liu et al. illustrated that PEO chains on the surface of PVCL aggregations exhibited rapid chain motions and had large excluded volume, resulting in extraordinary persistent colloidal stability of PEOylated particles under hydrophilic or hydrophobic conditions.16 Notably, the LCSTs measured by DSC decreased with the increment of concentration. It could be figured out that both heating and cooling curves were broad with more than 10 °C temperature intervals, indicating gradual dehydration process of copolymer. Meanwhile, asymmetric curves with sharp increment of the heat capacity below LCST and gradual decrement of the heat capacity above LCST could be noted. Laukkanen et al. investigated thermoresponsive behavior of PVCL homopolymers with molecular weight ranging from 2.1 × 10⁶ to 1.5 × 10⁶ g/mol and presented similar asymmetric DSC curves. Previous mechanism explorations about PVCL aqueous solutions put forward that asymmetric DSC curve responded with discontinuous changes of dehydration process of PVCL.11,12
Dynamic Light Scattering Measurements (DLS)

Former study reported that hydrodynamic radiiues were about 80 to 90 nm for both of PVCL-1300 and PVCL-330 homopolymers at 50 °C. And the apparent mean $R_h$ were 24, 15 and 4 nm for PVCL-1300, PVCL-330, and PVCL-30 at 20 °C, respectively. Notably, the sizes of the homopolymers were far larger than the size of a single chain in water. It was considered that the particles resulted from the aggregation of several collapsed polymer chains. To analyze self-assembly behaviors of the block copolymer together with information about the hydrated sizes, DLS investigation was performed as shown in Figure 2. Dramatic increment in $R_h$ within narrow temperature range was detected. Obviously, thermoinduced self-assembly structures were formed above 36 °C due to the dehydration of PVCL segments. When the temperature was increased to 38 °C, PVCL collapsed tensely and progressive decrement of the $R_h$ was observed which could be attributed to the shrinkage of PVCL cores by removal of water inside. Above 46 °C, hydration radius kept stable at 236 nm, indicating assembly structures formed completely.

For clarity, distribution of the hydrodynamic radiiues at three different temperature points 25, 38, and 50 °C were shown in Figure 2(b) to represent different hydration states in the heating process. At lower temperature (25 °C), bimodal distribution of the diagram was observed which indicated that a fraction of larger aggregations existed (~100 nm) although water was a good solvent for both PEO and PVCL. In comparison, PVCL homopolymer (25,000 g/mol) was also tested by DLS, presented in Supporting Information Figure S3. There was no similar distribution of the same size and its $R_h$ was about 17 nm at 25 °C, which was a little larger than single chains but much smaller than that of copolymer. Similar large associations had previously been reported in PEO-b-PNIPAM system by several groups, and they ascribed the presence of these large species below LCST to the formation of loose associates owing to the incompatibility of PEO and PNIPAM blocks.

Kermagoret et al. reported the existence of aggregates ($R_h$ ~100 nm) in PVCL-b-poly(N-vinylcaprolactam-stat-N-vinylpyrrolidone) copolymers and they attributed the large associated objects under LCST to the loose aggregates. Thus, although several adjacent chains of homopolymer tended to associate (<30 nm) under LCST, in copolymer system, much larger aggregations (~100 nm) could be formed which consisted with more chains compared with homopolymer. When temperature rose to 38 °C, the average sizes of copolymer reached several hundred nanometers, indicating that PVCL segments had volume phase transition and the copolymer began to aggregate and assembled into core-shell structures. With further rising temperature to 50 °C, the hydrodynamic radius of copolymer decreased by extruding internal water molecules with denser core structures.

Temperature-Variable $^1$H NMR Measurements

Temperature-variable $^1$H NMR measurements of PEO-b-PVCL in D$_2$O were carried out to quantitatively describe the phase transition degree, as shown in Figure 3(a). For normalization, HDO peak (4.8 ppm) was used as internal NMR standard. Several temperature points stood for different hydration conditions. It was obvious that the peaks corresponding to PEO-b-PVCL copolymer did not have significant changes in chemical shifts with rising temperature. In contrast, the protons of PVCL segments experienced drastic intensity decrement with increment of temperature, which was similar to the case of PVCL homopolymer; PNIPAM, and poly(oligo(ethylene glycol) methacrylate) (POEGMA) thermoresponsive polymers. Moreover, temperature-dependent integral area of different protons were depicted in Figure 3(b) The integral area of $H_c$ on the caprolactam rings decreased dramatically compared with other protons in the copolymer, suggesting that the pendant chains twisted inside of the cores and lost interactions with water molecules during the heating process.

For more meticulous presentation of the phase transition process, phase separation fraction $p$ was employed to characterize the degrees of phase transition, and $p$ was defined as:
where $I$ and $I_o$ were the normalized integrated intensities of a selected resonant peak at a specified temperature and at 25 °C, respectively.\textsuperscript{14,36} Temperature dependences of the phase separation fraction $p$ for different proton types of PEO-b-PVCL in D$_2$O were presented in Figure 3(c). A phase separation degree of about 0.8 after the phase transition at 46 °C was showed, indicating that the PVCL blocks underwent dehydration process with compacted inner core and decrement of water molecules inside. Taking the middle point of the curves as the phase transition temperature ($T_p$), the $T_p$ was determined to be about 40 °C with wide transition temperature regions. Similar $^1$H NMR measurement of PVCL-D$_2$O system showed that the transition regions was approximate 7 °C broad.\textsuperscript{14} Swanson referred that the conformation transition of PVCL was not as sharp or intense as that observed for PNIPAM, and it underwent a thermally induced conformational transition from relatively open coil to compact globular structure based on fluorescence investigations.\textsuperscript{12} Moreover, the phase separation degree of copolymer (0.8) above LCST was lower than that obtained from PVCL-D$_2$O system (0.9),\textsuperscript{14} indicating that the dehydration process was insufficient compared with PVCL-D$_2$O system. Diversely, the phase separation fraction of $H_a$ underwent a very slight decrement change and its $p$ value was negative ($< -0.06$). Since hydrophilic PEO did not have thermosensitive properties in this temperature range and the increment of $p$ value illustrated the phase separation typically, we speculated that C-H main chains of PEO segment did not have any dehydration phase transition process even if bonded with PVCL and its C-H states almost maintained steady with a very slight hydration process, compared with massive dehydration process of PVCL blocks. This unusual phenomenon had also been observed and verified by later FT-IR analysis.

**Conventional FT-IR Analysis**

In order to explore the PEO-b-PVCL copolymer assembly behavior and microscopic molecular motions, FT-IR technique in combination with correlation analysis which had been widely used to analyze spectra variation of thermosensitive systems under external temperature was applied here to supervise the transition process.\textsuperscript{11,21,37,38} It should be noted that we utilized D$_2$O as the solvent to eliminate the overlap of $\delta$(O-H) band with $\nu$(C=O) as well as overlap of the broad $\nu$(O-H) band with $\nu$(C-H) band.\textsuperscript{39} Temperature-dependent FT-IR spectra of PEO-b-PVCL in D$_2$O during the heating and cooling cycles were presented in Figure 4. Three wavenumber regions C=H (3000–2837 cm$^{-1}$), C=O (1645–1546 cm$^{-1}$), and C-O-C (1100–1056 cm$^{-1}$) were applied as the most representative chemical groups for detailed characteristic structures analysis. The band of $\nu$(C=H) at 3000 to 2837 cm$^{-1}$ shifted to lower wavenumbers with rising temperature, as shown in Figure 4(a). Referring to the previous studies,\textsuperscript{11,21} the red shift of C-H stretching band was caused by the interaction variations between hydrophobic polymers and water molecules in systems. Dissolved polymers in well-ordered structure would cause water clathrates near their hydrophobic moieties and lead to higher vibrational frequency.\textsuperscript{60,61} Thus, it was concluded that C-H group in PEO-b-PVCL copolymers underwent dehydration process during the heating process which was mainly generated by the PVCL segments. In C=O region [Fig. 4(b)], bidirectional spectral intensity changes of C=O bands were observed in which the intensity of 1611 cm$^{-1}$ band became weaker while a new band at 1635 cm$^{-1}$ appeared with the increment of temperature. These two bands were roughly assigned as the $\nu$(hydrated C=O) and free C=O bonds, respectively. Interestingly, C-O-C region had an abnormal red shift to lower frequency with rising temperature in Figure 4(c). Previous studies on POEGMA-based polymers had revealed that the C-O-C shifted to higher frequency during the heating, suggesting the abruption of hydrogen bonds between ether oxygen groups and water molecules. Similar FT-IR spectroscopy investigation revealed that the dehydration of hydrophilic oxyethylene side chain groups took place before the dehydration of hydrophilic carbonyl groups and backbones, and the
conformation changes of side chain groups along with dehydration process played key role in the formation of micelles as the starting point.35,42 However, in the present study, the ether groups which located at the main chains and blocked with thermo-responsive PVCL segments presented particular red shift. Integrated with previous temperature-variable 1H NMR result, it could summarize that ether bonds in PEO-b-PVCL system experienced exceptional mild dehydration process and special conformation changes with increment of temperature. As for successive cooling process shown in Figure 4(d–f), C@O groups underwent converse hydration process while C@H reformed hydrogen bonds with water.

To quantitatively describe the phase transition process during the heating process, the frequency shifts of C@H, C@O–C groups and integral area of C@O were plotted in Figure 5. The transition range was wide which reconfirmed that dehydration of C=H was a gradual and persistent process. Taking the middle point of curve as the phase transition temperature (T_p), the T_p was determined to be 38 °C. The frequency of the CH2 stretching bands shifted to lower wavenumbers and showed nearly unchanged both before and after the phase transition (typical S-shaped curve). In contrast, a typical anti-S-shaped curve with sharp shift below LCST and gradual shift above LCST had been identified in PVCL homopolymer. Considering the topological constraints of caprolactam rings compared with PNIPAM, distribution gradient of water existed with increment of temperature which resulted in the persistent dehydrating process in PVCL mesoglobules.11,12 Lebedev et al. demonstrated specific hydration and coil-globule transition of PVCL based on SANS results. The coexistence of globules and disordered chains was illustrated.13 Herein, the S-shaped curve together with less degree of phase separation fraction in 1H NMR of copolymer compared with PVCL homopolymer implied that PEO chains might have positive impact on phase transition process which could neutralize limitation of the caprolactam rings, suppressed sustained dehydration process and brought about stable dehydrated states at higher temperature. Additionally, the C=O–C band showed an S-shaped red shift at T_p of 39 °C, reconfirming its special hydration process. During the cooling process, perfect resilience was observed compared with PVCL homopolymer which showed obvious hysteresis both in C@H and C@O groups.11

Integral area of v(C@O) was present in Figure 5(c,d) in the regions of 1645 to 1630 cm⁻¹ and 1610 to 1546 cm⁻¹, which were assigned to free C@O and v(hydrated C@O), respectively. Gradual pseudolinear trend in 1645 to 1630 cm⁻¹ region was observed while an inflection point was presented in 1610 to 1546 cm⁻¹ region. Maeda et al. had reported that less localized cis-amide group in cyclic caprolactam rings could be easily affected by the groups were hydrogen bonded states, which would result in a larger number of components of carbonyls in PVCL compared with PNIPAM.10 Spevacek et al. found the existence of water bound in PVCL globular structures in semidilute solutions. Fast exchange between bound and free water molecules with 2.1ms was presented in comparison with other thermosensitive polymers. Their FT-IR results showed that the amount of fully dehydrated C=O in PVCL homopolymer was rather small. Water molecules hydrogen bonded to C=O groups could serve as intermediary of interactions between polymer segments.14 Previous study on the hydration had also proved that the intensity of IR bands

**FIGURE 4** Temperature-dependent FT-IR spectra of PEO-b-PVCL during the heating (a–c) and cooling (d–f) processes with an interval of 1 °C in the regions of C–H (3000–2837 cm⁻¹), C=O (1645–1546 cm⁻¹) and C=O–C (1100–1056 cm⁻¹). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
decreased at 1610 and 1592 cm\(^{-1}\), which indicated that the carbonyl groups were hydrogen bonded with one and two water molecules.\(^{11}\) In short, \(\text{C=O}\) group which formed more than two hydrogen bonds with water directly could be attributed to hydrated \(\text{C=O}\). If \(\text{C=O}\) formed a hydrogen bond with one water molecule, it was hydrating state. When there was one water molecule in the middle of two \(\text{C=O}\), forming hydrogen bonds with them separately, it was dehydrating state. Once there was no hydrogen bond, \(\text{C=O}\) got complete dehydrated. Similarly, for \(\text{C=O}\) regions, lower frequencies were applied to more hydrated states. Once \(\text{C=O}\) released one water molecule, it became hydrating states. In general, the changes of amide I during the heating process could be explained by the transformation from hydrated \(\text{C=O}\) to free \(\text{C=O}\). When the temperature came close to the LCST, more water molecules were expelled outside resulting in a discontinuous decrement of hydrated \(\text{C=O}\) area. However, in the present system, it was inferred that although PVCL dehydrated, a part of hydrated \(\text{C=O}\) released only one water molecule and formed incomplete dehydrated states, which could not be counted into the free \(\text{C=O}\). Therefore, the area of free \(\text{C=O}\) showed a pseudo-linear change without an obvious transition temperature point. Insufficient dehydration process of the carbonyl groups caused the gradual phase transition process, corresponding to the wide transition temperature ranges and resulting in a distribution gradient of water molecules inside of core-shell assembly structures. During the cooling process, the integral area of hydrated \(\text{C=O}\) experienced a mild excessive reversion, which could be explained that water absorbed by PEO shell together with free water outside of the assembly structures flowed into the core and lead to more hydrated \(\text{C=O}\) in the cooling process.

**Perturbation Correlation Moving Window (PCMW) Analysis**

PCMW is a newly developed technique which was introduced by Thomas and Richardson\(^{43}\) and improved by Morita et al.\(^{44}\) Based on this method, not only specific and characteristic perturbation variable could be revealed, but also the transition points together with intervals along the perturbation direction could be determined. In PCMW analysis, the spectral intensity increment was defined as positive synchronous correlation while negative correlation indicated the spectral intensities decrement.\(^{43,44}\) The spectral variations along temperature perturbation could be monitored with the signs of synchronous and asynchronous spectra, which was consistent with above analysis in one-dimensional FT-IR spectrum. PCMW synchronous and asynchronous spectra of PEO-b-PVCL copolymer in D\(_2\)O during the heating and cooling cycles were presented in Figure 6. The transition temperature of bands at 2911 and 2849 cm\(^{-1}\) was about 36 °C. For 2948, 2867, 1610, and 1080 cm\(^{-1}\), the temperature was about 38 °C, while the temperature point appeared at about 39 °C for 1090 cm\(^{-1}\) band. PCMW results implied that the
C–H groups had an earlier response than C=O and C–O–C and the hydrophobic alkyl groups underwent dehydration process firstly. The transition temperature region could also be determined by the asynchronous spectra. It was concluded that the copolymer experienced phase transition mainly between 31 and 41 °C during the heating process.

**Two-Dimensional Correlation Analysis (2Dcos)**

For getting more detailed and additional useful information about the microdynamic mechanism, 2Dcos analysis of conventional IR spectra was applied to study the motions of each band during the phase transition process. Due to the different response of species to external variations, resolution of spectra could be significantly enhanced and supplementary valuable information about the molecular motions or conformation which could not be obtained straight from conventional one-dimensional spectrum might be obtained.45 This technique has been utilized in a series of thermo-responsive polymers researches successfully.46-48 Synchronous and asynchronous maps of PEO-b-PVCL copolymer in D2O during the heating and successive cooling process which were generated from all the FT-IR spectra between 25 and 50 °C with an interval of 1 °C were shown in Figure 7. In the 2Dcos analysis, the synchronous spectra reflected the relative degree of in-phase response between two given wavenumbers while asynchronous one illustrated out-phase response similarly. In more detail, the bands at 2950, 2867, 1609, and 1088 cm⁻¹ had positive cross-peaks, reflecting that the bands had similar response to temperature perturbation and decreased during the heating process. On the contrary, the bands at 2928, 2857, 1621, and 1082 cm⁻¹ had negative cross-peaks, indicating the diverse changing direction. The bands during the heating process in 2Dcos analysis and corresponding assignments were presented in Table 1 while the bands in cooling were presented in Supporting Information Table S1.

Specific order taking place under external temperature perturbation was discerned by 2Dcos analysis. According to Noda’s rule: if the cross-peaks (ν₁ and ν₂, and assumed ν₁ > ν₂) were in synchronous and asynchronous spectra with the same sign, the changes at ν₁ happened prior than ν₂, and vice versa.49 The specific order of PEO-b-PVCL copolymer in D2O during the heating process was deduced as follows (→ means before): 2857 cm⁻¹ → 1082 cm⁻¹ → 1591 cm⁻¹ → 1609 cm⁻¹ → 1598 cm⁻¹ → 2950 cm⁻¹ → 1638 cm⁻¹ → 2867 cm⁻¹ → 1617 cm⁻¹ → 1621 cm⁻¹ → 1088 cm⁻¹ → 2928 cm⁻¹, or ν₂(dehydrated CH₂) → ν₁(hydrated C=O–C) → ν₁(hydrated C=O) → ν₁(hydrated CH₂) → ν₂(hydrating C=O) → ν₁(dehydrating C=O) → ν₁(hydrated C=O) → ν₁(hydrating CH₂). Considering separately C=O relative vibrations, we had 1591 cm⁻¹ → 1609 cm⁻¹ → 1598 cm⁻¹ → 2950 cm⁻¹ → 1638 cm⁻¹ → 1617 cm⁻¹ → 1621 cm⁻¹. It was noted that both of 1591, 1609 and 1598 cm⁻¹ corresponded to hydrated C=O. With rising temperature, hydrated C=O released only one water molecule, and then less hydrated C=O with higher wavenumbers (1638, 1617, and 1621 cm⁻¹) responded, forming incomplete dehydrated states. In other word, a part of hydrated C=O released partial water molecules and became incomplete dehydrated states. Breaking of hydrogen bonds occurred gradually during the heating process, corresponding with the pseudo-linear changes of free C=O integral area in Figure 5(c). Neglecting the differences in the stretching modes of the chemical groups, the special order could be expressed as CH₂ → C–O–C → C=O.

Since the hydrophobic C–H groups responded before temperature elevating than the relative hydrophilic C=O and C–O–C groups, it was deduced that the cooperative dynamic assembly process was predominated by the hydrophobic interactions among copolymer chains. Notably, the sequence of copolymer was different from PVCL aqueous solution in which the phase transition was driven by hydrogen bonding transformation of amide groups and followed by the hydrophobic dehydration of C–H group.11 Due to the incompatibility of PEO and PVCL under LCST, a small number of aggregations had existed far below the transition temperature which was proved by the earlier change of C–H group. In contrast, periphery PEO shell absorbed water from PVCL core, played as water absorption sponge layer and kept water inside of the assembly structures, which lead to the gradient distribution of water.
With similar method, the sequence order of PEO-b-PVCL copolymer in the cooling process was obtained as follows:

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2857 \text{ cm}^{-1} \rightarrow 2930 \text{ cm}^{-1} \rightarrow 1635 \text{ cm}^{-1} \rightarrow 1620 \text{ cm}^{-1} \rightarrow \\
1089 \text{ cm}^{-1} \rightarrow 2869 \text{ cm}^{-1} \rightarrow 1617 \text{ cm}^{-1} \rightarrow 2951 \text{ cm}^{-1} \rightarrow \\
1609 \text{ cm}^{-1} \rightarrow 1083 \text{ cm}^{-1}, \text{ or } \nu_s(\text{dehydrated CH}_2) \rightarrow \nu_{as}(\text{dehydrated CH}_2) \rightarrow \\
\nu(\text{free C}=-\text{O}) \rightarrow \nu(\text{dehydrating C}=-\text{O}) \rightarrow \nu(\text{hydrating C}=-\text{O}=-\text{C}) \rightarrow \\
\nu(\text{hydrating C}=-\text{O}) \rightarrow \nu(\text{hydrating C}=-\text{O}=-\text{C}).
\]

Considering separately C=O relative vibrations, we had 1635 cm\(^{-1}\) → 1620 cm\(^{-1}\) → 1617 cm\(^{-1}\) → 1609 cm\(^{-1}\), indicating the gradual formation of hydrogen bonds during the cooling process. Neglecting the differences in the stretching modes of the chemical groups, the special order could be expressed as CH\(_2\) → C=O → C=O=C. The sequence order in the cooling was the roughly opposite process of the former heating process, except 2857 cm\(^{-1}\) which responded firstly both in the heating and cooling process. The 2857 cm\(^{-1}\) band in the heating process responded to earlier changes of main chains under LCST which was attributed to aggregations. While in the cooling process, its disaggregation occurred firstly as well.

In general, comprehensive self-assembly mechanism of PEO-b-PVCL copolymer was depicted in Figure 8. Under LCST, PEO, and PVCL segments were both hydrophilic and well soluble in water, although a small number of large aggregations
absorption sponge layers. Water molecules transferred from carbonyl groups to ether groups and finished the whole phase transition assembly process. In the cooling process, denser assembled structures dissociated through relaxation of main chains, and carbonyl bonds reformed hydrogen bonds with water molecules. The PEO sponge layers released absorbed water to the PVCL and finished the heating-cooling cycles of PEO-\(b\)-PVCL copolymer with perfect resilience.

**CONCLUSIONS**

In summary, thermoinduced phase transition assembly behavior and mechanism of PEO-\(b\)-PVCL block copolymer were explored. With increment of concentration from 0.1 wt % to 10 wt %, the phase transition temperature decreased from 40 °C to 36 °C. Discontinuous changes of dehydration process were put forward based on asymmetric DSC curves with sharp increment of the heat capacity below LCST and gradual decrement above LCST. Based on the temperature-dependent \(^1\)H NMR experiment, the phase separation degree of copolymer above LCST (0.8) was less than PVCL-D\(_2\)O system (0.9) which indicated that the dehydration process was insufficient compared with homopolymer. Notably, the negative phase separation fraction of PEO main chains underwent a very slight decrement change, and it illustrated unique

![Schematic illustration of the dynamic mechanism of the phase transition of PEO-\(b\)-PVCL copolymer during the heating processes.](image)
feeble hydration process of PEO in the heating process, compared with typical dehydration process of PVCL blocks.

More detailed information about assembly behavior and mechanism was obtained through FT-IR in combination with 2Dcos and PCMW analysis. The C=O band showed bidirectional spectral intensity changes through breakage of hydrogen bonds with water during the heating process. Particularly, C=O–C had an abnormal red shift with rising temperature, verifying that ether groups experienced unusual hydration process during the heating. The S-shaped temperature-dependent frequency shifts of CH2 stretching bands together with less degree of separation fraction demonstrated that PEO chains had a positive impact on phase transition process which neutralized limitation of the caprolactam rings from PVCL segments, suppressed its sustained dehydration process and brought about stable dehydrated states at higher temperature. Interestingly, pseudo-linear changes of the integral area of free C=O were presented compared with an inflection point in hydrated C=O integral area, which proposed that a part of hydrated C=O released only one water molecule and formed incomplete dehydrated states. Further investigation of the transition behavior via 2Dcos analysis indicated that the hydrophobic interactions among copolymer chains predominated the heat-induced phase transition process and were responsible for the cooperative dynamic assembly behavior. The earlier change of C–H group certified the occurrence of incompatibility-induced aggregations under LCST. Periphery PEO shell absorbed water from PVCL core, played as water absorption layer and kept water in the assembly structures. During the cooling process, the PEO layers released absorbed water to the PVCL and finished the heating-cooling cycles of PEO-b-PVCL copolymer with perfect resilience.

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REFERENCES AND NOTES