On the abnormal "forced hydration" behavior of P(MEA-co-OEGA) aqueous solutions during phase transition from infrared spectroscopic insights†

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Turbidity, DLS and FTIR measurements in combination with the perturbation correlation moving window (PCMW) technique and 2D correlation spectroscopy (2Dcos) analysis have been utilized to investigate the LCST-type transition of a oligo ethylene glycol acrylate-based copolymer (POEGA) in aqueous solutions in this work. As demonstrated in turbidity and DLS curves, the macroscopic phase separation was sharp and slightly concentration dependent. Moreover, individual chemical groups along polymer chains also display abrupt changes in temperature-variable IR spectra. However, according to conventional IR analysis, the C=O groups present obvious dehydration, whereas C=O and C–O–C groups exhibit anomalous "forced hydration" during the steep phase transition. From these analyses together with the PCMW and 2Dcos results, it has been confirmed that the hydrophobic interaction among polymer chains drove the chain collapse and dominated the phase transition. In addition, the unexpected enhanced hydration behavior of C=O and C–O–C groups was induced by forced hydrogen bonding between polar groups along polymer chains and entrapped water molecules in the aggregates, which originated from the special chemical structure of POEGA.

1. Introduction

In the last few decades, thermosensitive polymers that demonstrate a lower critical solution temperature (LCST) in water have attracted great interest for biologically relevant applications including tissue engineering, switchable cell adhesion, biomolecule separation, controlled drug delivery and sensors.1–4 Among them, poly(N-isopropylacrylamide) (PNIPAM), showing an abrupt coil-to-globule phase transition close to physiological temperature upon heating in water, has been by far the most frequently studied LCST-type polymer. Nevertheless, PNIPAM is discovered to possess some inherent disadvantages like strong bioadhesion with proteins, non-ignorable hysteresis and production of toxic amines during hydrolysis.5 More recently, an emerging class of thermosensitive polymers based on acrylate or methacrylate bearing short oligo ethylene glycol (OEG) side chains have been developed.6–9 These polymers exhibit reversible phase transitions in water with LCSTs conveniently adjusted over a wide range of temperature by several methods, such as changing the number of ethylene glycol units in the side chains and copolymerizing PEG monomers of different hydrophilicities.10,11 Moreover, as compared with PNIPAM, they present obviously better biocompatibility and thus are considered potential alternatives to PNIPAM.

Though methacrylate based OEG polymers (POEGMA) are more common and have been extensively investigated, OEG acrylate polymers (POEGA) hold several advantages. Due to the less hydrophobic acrylate backbone, it could open up a new range of possible thermosensitive monomers with shorter OEG side chains, which would be easier to synthesize or are even commercially available. Acrylate polymers tend to be more convenient to end-functionalize than methacrylate polymers.12 Moreover, acrylates can also be polymerized by nitroxide mediated polymerization (NMP), whereas it is not the case with methacrylates (without adding small amounts of styrenic comonomers).13 Therefore, studies on the synthesis and characterization of thermosensitive POEGA have been increasing.10,14–16 As the most hydrophobic OEGA monomer, 2-methoxethyl acrylate (MEA) exhibits the lowest cloud point temperature ($T_{CP}$) ranging from 0 to 5 °C depending on the end groups.17 By mixing MEA with more hydrophilic monomers, the $T_{CP}$ could be regulated in a wide range. For instance, the copolymerization of MEA and hydroxyethyl acrylate (HEA) allows fine-tuning of the $T_{CP}$ in between 10 and 100 °C, demonstrating a fascinating property.18 Besides, PMEA has been
reported to have excellent blood compatibility with significantly suppressed platelet adhesion and spreading as compared to other poly(meth)acrylates, which could be related to the water structure in PMEA.19–21 Thus, thermosensitive POEGA containing MEA has been widely utilized for the construction of functional polymers, hydrogels, nanogels, star polymers and biohybrid amphiphiles.22–24

In previous investigations, some specific properties of POEGA have been discovered. For example, An et al.14 have reported the development of thermosensitive copolymers based on poly(MEA-co-ethylene glycol)methyl ether acrylate) (P(MEA-co-OEGA180)) and their use for nanogel synthesis by reversible addition-fragmentation chain transfer (RAFT) dispersion polymerization in water. The synthesized nanogels exhibit an unprecedented linear relationship between nanogel size and temperature. Copolymerization of HEA and MEA has been performed via RAFT polymerization by Hoogenboom et al.,18 and it was discovered that the T_{cp} of synthesized copolymers increases with increasing chain length, and such a phenomenon is consistent with the findings in other POEGA systems.25 Nevertheless, the observed trend herein is opposite to general findings for POEGMA and poly(N-vinylcaprolactam) (PVCL), where a decrease in the chain length leads to an increase of T_{cp} as expected according to the Flory–Huggins theory. Then, it has been proposed that the balance between the less hydrophobic backbone and the more hydrophilic side chain might contribute to the opposite trend for POEGA.10 Under such circumstances, the regularities obtained in POEGMA could not be applied directly to POEGA systems.

It is generally believed that IR spectroscopy is a quite suitable technique to observe the variations in hydrated states or hydrogels of individual chemical groups of thermosensitive polymers.26–28 With regard to POEAGMA, Maeda et al.29 have investigated and compared the thermosensitive phase separation of aqueous solutions of poly(2-(2-methoxyethoxy)methyl acrylate) (PMEO2MA) and poly(2-(2-methoxyethoxy)ethyl methacrylate) (PMEO2MA) by IR and confocal micro-Raman spectroscopy and found that though free C–O and C–O–C groups exhibit the hydration process Q exists in both polymer systems at low polymer concentrations and below the phase separation temperature, the average number of hydrogen bonds in PMEO2MA was lower than in PMEO2MA, probably due to the steric effects of the α-methyl groups. The thermally reversible hydration behavior of P(MEO2MA-co-ethylene glycol)methacrylate) P(MEO2MA-co-OEGA MA173) in water was studied by our group mainly by applying IR spectroscopy and it was shown that polymer chains successively experience four consecutive conformation changes, i.e. hydrated chains-dehydrated chains-loosely aggregated micelles-densely aggregated micelles, during the whole phase transition procedure.30 However, as far as we know, no integrated dynamic mechanism of the phase transition of linear POEGA in water has ever been clarified.

Recently, we have noticed a “forced hydration” behavior, that is, C=C–O and C–O=C groups exhibit the hydration process upon heating, in the thermally induced phase transition of POEGA-based star polymers in water, which was supposed to be rooted in the thermosensitive P(MEA-co-OEGA480) arms.31

![Chemical structure of P(MEA-co-OEGA480)](image)

Then, we have employed IR spectroscopy in combination with the perturbation correlation moving window (PCMW) technique and two-dimensional correlation spectroscopy (2Dcos), which have been proved to be powerful tools for deducing group motions of thermosensitive polymers during the phase transition from the molecular level,32–36 to investigate the LCST-type transition of P(MEA-co-OEGA480) in aqueous solution. Thus, detailed information on the microscopic changes of complicated interactions within P(MEA-co-OEGA480) chains during the LCST-type transition process could be extracted and the “forced hydration” related to C–O and C–O=C groups could be elucidated. What’s more, a deeper understanding of the relationship between thermosensitive properties and structures of polymers can be obtained.

2. Experimental section

2.1 Materials

P(MEA-co-OEGA480) (chemical structure shown in Fig. 1) was synthesized via RAFT copolymerization of MEA and OEGA (n = 8–9, M_n = 480 g mol⁻¹) monomers according to previous reports.14 Herein, the feed ratio of [MEA]/[OEGA] was fixed at 90:10 and the degree of polymerization (DP) was designed to be 100. GPC measurements (elution phase: DMF; standard: monodisperse poly methyl methacrylate) were carried out to determine the number-average molecular weight ($M_n$) and polydispersity index (PDI = $M_w/M_n$) of P(MEA-co-OEGA480). The obtained copolymer has $M_n = 1.8 \times 10^4$ g mol⁻¹ and PDI = 1.21. D₂O was purchased from Cambridge Isotope Laboratories Inc. (D-99.9%). The concentration of P(MEA-co-OEGA480) in D₂O was fixed at 10 wt% and the solution was placed at 4 °C for a week to ensure complete dissolution for FTIR measurements.

2.2 Instruments and measurements

Turbidity measurements were performed on a Lambda 35 UV-vis spectrometer at 500 nm using deionized water as a reference (100% transmittance). Using a water-jacketed cell holder, temperatures were manually regulated at the rate of ca. 0.4 °C min⁻¹ and the interval was 1 °C. Before each measurement, the temperature was maintained for 2 min to ensure the thermal equilibrium of the sample cell. DLS measurements were conducted on a Malvern Zetasizer Nano ZS instrument (scattering angle: 173°) at a heating rate of ca. 0.4 °C min⁻¹ with an interval of 1 °C and the temperature was also maintained for 2 min.
before each measurement for equilibration of the sample. For FTIR measurements, the sample of P(MEA-co-OEGA480) D2O solution was sealed between two ZnS tablets. All the temperature-resolved FTIR spectra were recorded utilizing a Nicolet Nexus 6700 FTIR spectrometer equipped with a DTGS detector. In order to obtain an acceptable signal-to-noise ratio, 32 scans at a resolution of 4 cm⁻¹ were accumulated. The temperatures were under programmed control using an electronic cell holder at a rate of ca. 1 °C/3 min. The interval was 0.5 °C (accuracy: 0.1 °C). Baseline correction was performed using the OMINIC 6.1a software.

2.3 Investigation method

Perturbation correlation moving window (PCMW). All the FTIR spectra of 10 wt% P(MEA-co-OEGA480) in D2O collected during heating with an interval of 0.5 °C were selected to conduct PCMW analysis. Primary data processing was carried out by the method provided by Morita, and further correlation calculation was performed by applying the software 2D Shige, ver. 1.3 (Shigeaki Morita, Kwansei-Gakuin University, Japan, 2004–2005) with an appropriate window size (2m + 1 = 11). The final contour maps were plotted using Origin Program ver. 8.0, with the red color representing positive intensities and the green color representing negative intensities.

Two-dimensional correlation spectroscopy (2Dcos). Applying the temperature-dependent FTIR spectra of 10 wt% P(MEA-co-OEGA480) in D2O obtained during heating with an interval of 0.5 °C, 2Dcos analysis was conducted utilizing the same 2D Shige software, ver. 1.3 (Shigeaki Morita, Kwansei-Gakuin University, Japan, 2004–2005). Then, they were plotted as contour maps using Origin Program ver. 8.0. In the contour maps, the red color represents positive correlation intensities and the green color represents negative correlation intensities.

3. Results and discussion

3.1 Turbidity and DLS measurements

Firstly, turbidity and DLS measurements are performed to provide a macroscopic vision of the LCST-type transition of POEGA in water. Fig. 2a displays the turbidity curves of P(MEA-co-OEGA480) in water upon heating at two concentrations. The cloud points are taken as the initial turn points in the transmittance vs. temperature curves. As expected, the transmittance of the polymer solutions undergoes a sharp decrease at the cloud points, where polymer chain aggregation and macroscopic phase transition take place. Considering the effect of concentration, it is observed that the cloud point temperature at 0.1 wt% (33 °C) is slightly higher than that at 1 wt% (31 °C). Moreover, the transition profile becomes more gradual after dilution from 1 to 0.1 wt%. These observations are in accordance with what has been reported previously, where the LCST-type phase transition of POEG(M)A in water is slightly concentration dependent. The results are further confirmed by DLS curves, as shown in Fig. 2b. The hydrodynamic diameter (Dh) exhibits an abrupt increase at the cloud points which are almost the same as that observed in turbidity curves at two concentrations. In addition, more details related to polymer chain aggregation could be obtained. In a relatively dilute solution (0.1 wt%), Dh increases continuously from 312 nm at 33 °C to 586 nm at 46 °C, corresponding to the gradual increase in the size of aggregates by binding more polymer chains, while at 1 wt%, the polymer chains easily find each other, leading to a more abrupt increase in Dh and much bigger aggregates (4856 nm). Herein, a slight decrease of Dh from 40 to 46 °C could be attributed to the continuous dehydration and compaction of the aggregates.

3.2 Conventional IR analysis

To further understand the dynamic LCST-type transition mechanism, the temperature-dependent FTIR spectra of P(MEA-co-OEGA480) in 10 wt% D2O solution were collected during heating between 25 and 46 °C, as presented in Fig. 3. It is noted that D2O, rather than H2O, is used as the solvent here so that the influence of broad ν(O–H) bands related to H2O at around 3300 cm⁻¹ on ν(C–H) bands as well as the overlap of δ(O–H) of H2O at around 1640 cm⁻¹ with ν(C=O) of P(MEA-co-OEGA480) could be eliminated.

Three spectral regions are specifically focused on herein, that is, C–H stretching region (3010–2808 cm⁻¹), C=O stretching region (1760–1690 cm⁻¹) and C–O–C stretching region (1110–1050 cm⁻¹). Thus, we will be able to trace almost all the group motions of P(MEA-co-OEGA480) in water during the phase transition. As displayed in Fig. 3, all the C–H stretching bands demonstrate red shifts upon heating. It has been reported that water clathrates, where water molecules hold a well-ordered structure, exist around the hydrophobic moieties of water-soluble polymers. Then, it can be deduced that hydrophobic C–H groups along P(MEA-co-OEGA480) chains experience the dehydration process with increasing temperature, which is a normal phenomenon in the phase transition of LCST-type polymers. With regard to the ν(C=O) region, a bidirectional spectral intensity variation was observed. All the ν(C=O) bands can be roughly divided into two parts: the higher frequency moiety and the lower one. It is shown in the spectra that the intensity of the higher frequency moiety decreases, while that of the lower one increases during the heating process, demonstrating a transformation among different states of C=O groups in the phase transition of P(MEA-co-OEGA480) in water. Referring to the bands of C–O–C groups, they present an obvious frequency decrease upon heating. For ν(C=O) and ν(C–O–C), it is generally believed that lower

Fig. 2 Turbidity (a) and DLS (b) curves of P(MEA-co-OEGA480) in water during heating at two concentrations.
frequency implies stronger interaction within the groups. Thus, considering the situation in this system, it can be deduced from the variation tendency of temperature-dependent FTIR spectra that C=O and C–O–C groups experienced formation/strengthening of hydrogen bonds with water molecules during the phase transition process. Such an observation is, to some extent, abnormal and goes against the phase separation process, where dehydration is the predominant process of the LCST-type transitions of polymers in water.41–44

For more critical information on the changes in the interactions between polymer chains and water molecules, some contrast FTIR spectra of P(MEA-co-OEGA$_{480}$) in D$_2$O before and after the phase transition as well as the corresponding second-derivative curves are plotted in Fig. 4. The sharpened minima in a second-derivative curve are related to the maxima in the original absorption spectrum. In the C–H stretching region, an apparent red shift of all bands after the phase transition has been observed, demonstrating the dehydration of hydrophobic alkyl groups. In the C=O stretching region, it is noticed that a broadening of the band occurs after heating above the transition points, which can be further elucidated by the related second-derivative curves. In particular, two peaks located at around 1733 and 1716 cm$^{-1}$ are identified in both second-derivative curves of $\nu$(C=O), indicating that there mainly exist two kinds of C=O groups in the system, and they may roughly correspond to relatively free C=O and hydrated C=O. This finding, to some degree, accords well with previous studies on the kinds of C=O groups in POE(G)MA aqueous solution, where free C=O exists even at temperatures lower than the LCST.29,45 After phase transition, two trends in $\nu$(C=O) can be observed in the second-derivative FTIR spectra: firstly, the higher frequency part decreases and shifts to an even higher frequency, suggesting that the relatively free C=O decreases in amount and becomes more free; secondly, the lower frequency part increases obviously, revealing the increasing amount of hydrated C=O. Then, the responses of C=O groups in P(MEA-co-OEGA$_{480}$) during phase transition in water can be described as follows: upon heating, a portion of relatively free C=O transforms into hydrated C=O, while the other portion turns into more free ones. In the C–O–C stretching region, the bands present strong lower frequency shifting after the phase separation process, revealing the hydration behavior of all the C–O–C groups.

In order to obtain the details on the anomalous hydration behavior of C=O and C–O–C groups in the phase transition process, the temperature-variable content of relatively hydrated C=O as well as the frequency shift of C–O–C bands has been quantitatively examined. According to the second-derivative curves, the $\nu$(C=O) bands can be divided into two Gaussian sub-bands at 1733 and 1716 cm$^{-1}$, and they are assigned to relatively free C=O and relatively hydrated C=O, respectively. With the assumption of 1:1 conversion between the free C=O groups and relatively hydrated ones, when the integral peak areas of relatively hydrated C=O at around 1716 cm$^{-1}$ are plotted against the integral peak areas of the component at
around 1733 cm\(^{-1}\) (relatively free C=O), the slope of the fitted line provides the ratio of the molar absorption coefficient (\(\epsilon_{1716}/\epsilon_{1733}\)), which is 1.12 herein. Fig. 5a displays the temperature-dependent molar fraction of relatively hydrated C=O, defined as \(A_{1716}/[A_{1716} + A_{1733} \times (\epsilon_{1716}/\epsilon_{1733})]\), of P(MEA-co-OEGA\(_{480}\)) during heating. It is shown that a gradual decrease of the content of relatively hydrated C=O takes place at temperatures below the thermal transition point, revealing the dehydration of C=O groups at temperatures below the LCST. On the other hand, when the transition point is reached, an abnormal hydration process of the C=O groups takes place immediately and abruptly, as reflected by the sharply increased fraction of the relatively hydrated C=O. After the drastic phase transition is complete, the C=O groups resume the dehydration process upon further heating. Meanwhile, in the temperature-dependent frequency shift of the C=O-C stretching bands, a similar dehydration–hydration–dehydration trend has been detected, as presented in Fig. 5b.

Until now, the enhanced hydration of C=O and C-O-C groups during the heat-induced phase transition of P(MEA-co-OEGA\(_{480}\)) has been described in detail. As mentioned above, extensive literature studies have reported that the dehydration process dominates the phase transition of LCST-type thermosensitive polymers (including POEGMA) in water upon heating and the observed hydration behaviour of polar groups on the POEGA chains herein is anomalous. In view of the fact that the phase separation (polymer aggregation) still occurs after heating above the LCST, which is supported by turbidimetry and DLS results, the anomalous enhanced hydration of C=O and C-O-C parts does not disturb the overall phase separation procedure. Moreover, the anomalous hydration only takes place in the abrupt transition temperature region. We tentatively propose that during the abrupt phase transition, part of the water molecules that have been entrapped in the collapsed polymer chains are “forced” to have stronger interactions (hydrogen bonding) with relatively polar groups on the polymer chains within the compressed space of aggregates. Considering that such behavior has not been observed in POEGMA polymer systems (temperature-dependent FTIR spectra of POEGMA in D\(_2\)O are shown in Fig. S1, ESI†), the “forced hydration” could be specific to POEGA. In particular, in comparison with common POEGMA, POEGA studied in this work holds a similar chemical structure except for the lack of \(\alpha\)-methyl groups on the backbone and the monomer MEA. Then, the distinctive “forced hydration” behavior of C=O and C-O-C groups in POEGA could be interpreted from the following aspects. Firstly, due to the weakening of the steric effect brought by \(\alpha\)-methyl groups on the backbone, POEGA chains would be more flexible than POEGMA chains. Therefore, during the abrupt phase transition, the collapse and aggregation of POEGA chains would be faster than that of POEGMA chains, resulting in harder “escape” of water molecules out of collapsed chains or aggregates. Herein, the collapse and aggregation rates of POEGA and POEGMA in water could be qualitatively compared and confirmed in DLS curves (Fig. S2, ESI†), where the transition temperature window of the POEGA system is narrower than that of the POEGMA system. Moreover, \(\alpha\)-methyl groups on the backbone can change the hydrophobic/hydrophilic balance, resulting in less hydrophobic POEGA chains than POEGMA chains and more water molecules surrounding POEGA chains even at temperatures higher than transition points. Thus, there would be more chances for POEGA chains (especially polar groups on the chain) to interact with water molecules. Secondly, though hydrophobic, PMEA has been reported to have strong interactions with water molecules via hydrogen bonds between its polar groups and water molecules.\(^{19,20}\) Then, after heating above the transition temperature, the formation of a hydrogen bonding structure between C=O and C-O-C groups in MEA units and water molecules would be much more possible. Thirdly, the formation of hydrogen bonds is exothermic, which could lower the free energy of the system. Then, it will benefit the phase transition process. Hence, the abnormal “forced hydration” specific for the POEGA system is, to some extent, reasonable.

Now that the “forced hydration” of polar groups along POEGA chains has been illustrated, we still wonder how it works from a more concrete view with regard to group motions and chain movements. Then, further analysis of FTIR spectra has been carried out.

### 3.3 Perturbation correlation moving window (PCMW)

PCMW is a newly developed technique with basic principles dating back to the conventional moving window provided by Thomas et al.\(^{46}\) Then, Morita improved it in 2006.\(^{47}\) The detailed description of PCMW is presented in the ESI.\(^{†}\) By using PCMW, both the specific perturbation variable and the characteristic spectral variable can be easily revealed at the same time. In addition, the transition points as well as the transition intervals along the perturbation direction could be visually presented through PCMW.

Fig. 6 displays the PCMW synchronous and asynchronous spectra of P(MEA-co-OEGA\(_{480}\)) in D\(_2\)O during heating from 25 to 46 °C. As is known, transition points can be conveniently observed in PCMW synchronous spectra. Then, the transition temperatures of C-H, C-O and C-O-C parts can be determined to be ca. 33, 34 and 34 °C, respectively, indicating that C-H groups have an apparent earlier response than C=O and C-O-C groups during heating. Hence, it is deduced that hydrophobic interactions among polymer chains could be the driving force for the self-aggregation process of POEGA in water. PCMW asynchronous spectra are helpful for finding the transition temperature regions. Therefore, for C-H, C-O and C-O-C groups,
we have the LCST-type transition generally located in between 32 and 36 °C.

In addition to determining transition points and intervals, PCMW can be also used to monitor the spectral changes along perturbation by combining the signs of synchronous and asynchronous spectra with the following rules: a positive synchronous correlation means an increase in spectral intensity, whereas a negative one means a decrease in spectral intensity; a positive asynchronous correlation represents a convex spectral intensity variation, whereas a negative one represents a concave variation.47 It is observed that relatively weak peaks, with signs opposite to that of the main transition peaks, exist at temperatures out of the transition region in the PCMW synchronous spectra of \( \nu(C=O) \) and \( \nu(C-O-C) \) bands, indicating that there are slight intensity variations of \( C=O \) and \( C-O-C \) going against the main variation before and after the phase transition. In a sense, such detection is consistent with previously observed “forced hydration” of \( C=O \) and \( C-O-C \) groups, where slight dehydration happens before and after the phase transition and strong abnormal hydration occurs during the phase transition.

3.4 Two-dimensional correlation spectroscopy (2Dcos)

2Dcos is a mathematical method and its basic principles were first introduced by Noda.48,49 Up to now, it has been widely applied to investigate spectral changes of various chemical species under different external perturbations (e.g., temperature, concentration, pressure, time, electromagnetic, etc.).50,51 Thanks to the different responses of varied species to external perturbations, additional fine information on conformational changes or molecular motions can be achieved. The detailed description of 2Dcos is presented in the ESL†

On the basis of the phase transition evolving regions obtained from PCMW, all the spectra of P(MEA-co-OEGA480) between 32 and 36 °C are chosen to perform 2Dcos analysis, and the synchronous and asynchronous spectra are presented in Fig. 7. In this way, the “forced hydration” could be observed from a more specific view of individual group motions, which would be helpful for us to deeply understand the phase transition of P(MEA-co-OEGA480) in water.

Information on the simultaneous change between two wavenumbers can be provided by 2D synchronous spectra. For instance, almost all the C–H and C–O–C bands show negative synchronous cross-peaks in the synchronous spectra, suggesting that they have opposite sensitivities to temperature perturbation. Then, combined with raw IR data, it can be observed that the intensity of C–H bands decreases, while the intensity of C–O–C bands increases during heating. 2D asynchronous spectra can significantly enhance the resolution of original IR spectra. As shown in the asynchronous spectra, subtle bands located at 1734 and 1718 cm\(^{-1}\), attributed to different states of \( C=O \) as well as 1095 and 1080 cm\(^{-1}\), related to C–O–C groups, can be identified. In addition, hydrated and dehydrated states of C–H groups that are overlapped in the conventional IR spectra are easily recognized. For the convenience of discussion,
all the bands observed in the asynchronous spectra and their corresponding assignments are listed in Table 1. Considering that both the comonomers of P(MEA-co-OEGA) are of the same kind (i.e., both contain an acrylate moiety and a PEG segment), they are not distinguished here in IR spectral assignments.\textsuperscript{11,30}

Besides enhancing spectral resolution, 2Dcos could also provide helpful information on the specific sequence order occurring under external perturbations with regard to different chemical groups. On the basis of Noda’s rule, when cross-peaks ($\nu_1, \nu_2$) in the synchronous and asynchronous spectra hold the same symbol, both positive or both negative, it can be inferred that peak $\nu_1$ changes prior to peak $\nu_2$ with the perturbation, whereas when cross-peaks ($\nu_1, \nu_2$) in the synchronous and asynchronous spectra hold different symbols, one positive and the other negative, it can be deduced that peak $\nu_2$ changes prior to peak $\nu_1$.\textsuperscript{52} A simplified method for the determination of the sequence order has been described in our previous work,\textsuperscript{53} and herein we only present the final sequence order of different chemical groups during the phase transition (→ means earlier than or prior to): 2962 → 2952 → 2920 → 2877 → 2833 → 1095 → 1080 → 1734 → 1718 cm$^{-1}$ or $\nu_{as}$(CH$_2$) (–OCH$_2$CH$_2$O–) (hydrated) → $\nu_{as}$(CH$_3$) (–OCH$_2$CH$_2$O–) (dehydrated) → $\nu_{as}$(CH$_2$) (backbone) → $\nu_s$(CH$_2$) → $\nu_s$(CH$_3$) (–OCH$_2$CH$_2$O–) → $\nu$(dehydrated C–O–C) → $\nu$(hydrated C–O–C) → $\nu$(dehydrated C=O) → $\nu$(hydrated C=O).

Without considering the differences in stretching modes or hydration states of the chemical groups, the overall sequence order can be described as CH$_2$ (–OCH$_2$CH$_2$O–) → CH$_3$ (backbone) → CH$_3$ → C–O–C → C=O. As demonstrated, the change of –OCH$_2$CH$_2$O– displays a prior response in the phase transition, indicating that the dehydration of alkyl groups on OEG side chains plays a key role in the LCST-type transition of POEGA in water. Moreover, the changes of all the C–H groups take place obviously earlier than C=O or C–O–C groups during the phase transition, revealing the increased hydrophobic interaction within alkyl groups along polymer chains to be the driving force for the LCST-type transition. From another point of view, we might say that the change of C=O and C–O–C contributes much less to the phase transition. While taking different stretching modes of C–H groups into account, we observed that the asymmetric stretching vibration of CH$_2$ (–OCH$_2$CH$_2$O–) has an earlier response than the corresponding symmetric stretching vibration.

As has been reported previously, the direction of symmetric stretching vibration is parallel to the chain axis, while that of asymmetric stretching vibration is vertical to the chain axis.\textsuperscript{53} Then, it can be deduced that OEG side chains firstly collapse towards the backbone and then distort to make adjustments during the phase transition. By examining carefully the change sequence of C=O and C–O–C groups, we can discover that they present transformation of dehydrated states into hydrated ones, that is, C=O and C–O–C groups hydrogen bonded with one water molecule, confirming directly the enhanced hydration behavior of C=O and C–O–C groups during the sharp phase transition. Moreover, an earlier response of C–O–C than C=O...
groups has been observed, and this could be attributed to the fact that C–O–C groups are more hydrophilic than C==O groups and the entrapped water molecules interact preferentially with C–O–C during the “forced hydration” process.

By combining 1D IR, PCMW and 2Dcos analyses, the LCST-type transition of POEGA in water and the occurrence of “forced hydration” therein can be illustrated as follows: at temperatures lower than the transition point, POEGA chains dissolve well in water through hydration of the aliphatic groups and hydrogen bonding association of ester and ether groups with water molecules. Even so, it should be noted that there still exist relatively lower than the transition point, POEGA chains dissolve well in water through hydration of the aliphatic groups and hydrogen bonding association of ester and ether groups with water molecules. Even so, it should be noted that there still exist relatively lower than the transition point, POEGA chains dissolve well in water through hydration of the aliphatic groups and hydrogen bonding association of ester and ether groups with water molecules.

As soon as the temperature goes above the LCST, the OEG side chains collapse abruptly to get close to the backbone, which is driven by hydrophobic interactions among polymer chains. As a consequence, the whole polymer chains start to collapse and aggregate, leading to the macroscopic phase separation. Till this moment, the phase transition could be, to some degree, considered finished. However, due to the less hydrophobic backbone and rapid collapse of polymer chains, rooted in the lack of α-methyl groups on the backbone, as well as the strong affinity (hydrogen bonding interactions) of MEA units with water molecules, a portion of water molecules that have been entrapped in collapsed polymer chains are forced to form a hydrogen bonding structure with polar groups (C–O–C and C==O groups) along the polymer chains. In virtue of the difference in hydrophilicity, such “forced hydration” occurs in C–O–C prior to C==O groups. Though the abnormal hydration behavior happens to C–O–C and C==O groups, it does not interfere with the macroscopic phase separation process. After the abrupt phase transition occurs, further heating will lead to gradual dehydration of all chemical groups again, indicating that the space in aggregates is further compressed and the entrapped water molecules interact preferentially with C–O–C during the “forced hydration” process.

This phenomenon could be rooted in the rapid chain collapse and aggregation, the relatively hydrophilic backbone and strong interactions of MEA units with water molecules, all of which are closely related to the chemical structure of POEGA. Though POEGA and POEGMA possess similar chemical structures, their phase transition behavior differs from each other from a microscopic view. Then, the results obtained in this work will help us better understand the relationship between properties and structures of thermosensitive polymers.

4. Conclusion

In this work, we have utilized turbidity, DLS and FTIR measurements as well as the perturbation correlation moving window (PCMW) technique and 2D correlation spectroscopy (2Dcos) to investigate the LCST-type phase transition of P(MEA-co-OEGA) in water. An abrupt macroscopic phase separation, which is slightly concentration dependent, upon heating P(MEA-co-OEGA) aqueous solution has been observed in the turbidity and DLS curves. As shown by temperature-variable FTIR spectra and the corresponding 1D analysis, C–H groups exhibit obvious dehydration, whereas C==O and C–O–C groups present unexpected dehydration–hydration–dehydration during the whole heating process. Under such circumstances, we could conclude that the LCST-type transition of POEGA in water is driven and dominated by hydrophobic interactions among polymer chains. This judgment has been further confirmed by PCMW and 2Dcos analysis, where C–H groups demonstrate an apparent earlier response than C==O and C–O–C groups in the phase transition. Considering that the anomalous hydration behavior only occurs in the sharp phase transition, it can be explained that when a part of water molecules are entrapped in the polymer aggregates, they tend to be forced to form hydrogen bonds with polar groups along the POEGA chains in the compressed space of aggregates. This phenomenon could be rooted in the rapid chain collapse and aggregation, the relatively hydrophilic backbone and strong interactions of MEA units with water molecules, all of which are closely related to the chemical structure of POEGA. Though POEGA and POEGMA possess similar chemical structures, their phase transition behavior differs from each other from a microscopic view. Then, the results obtained in this work will help us better understand the relationship between properties and structures of thermosensitive polymers.

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Notes and references
