Structural investigation of thermo-responsive poly(2-isopropyl-2-oxazoline) hydrogel across the volume phase transition†

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The deswelling and swelling behaviors of poly(2-isopropyl-2-oxazoline)-based hydrogel synthesized by a one-pot microwave-assisted solvent-free reaction were investigated. A distinct hydrophobic collapse of the hydrogel compared with the corresponding aqueous solution was observed by FT-IR spectroscopy combined with two-dimensional correlation spectroscopy (2DCOS) and perturbation–correlation moving-window (PCMW) analyses. The volume phase transition (VPT) temperature of 35 °C during heating and the transition temperature range of 41–30 °C during cooling were determined, indicating different dynamic transition mechanisms during heating and cooling. Water expulsion starting from the benzene ring-centered hydrophobic spots to the surroundings was revealed during deswelling. However, during swelling, although the rebuilding of cross-linking hydrogen bond bridges provided a channel-like microstructure to reswell the hydrogel gradually, a slow, unusual recovery of the amide hydrogen bonds to water molecules was observed.

Introduction

Poly(2-alkyl-2-oxazoline)s and corresponding derivatives, which possess structural similarities with polypeptides, low cytotoxicity, high biocompatibility and thermo-responsiveness, have been widely investigated in recent years because of their potential applications as thermo-responsive biomaterials1–3 and for the easy preparation of defined amphiphilic structures for self-assembly.4,5 Poly(2-isopropyl-2-oxazoline) (PIPOZ), which has a repeat unit isomeric with poly(N-isopropylacrylamide) (PNIPAM), has been widely investigated because of its unique lower critical solution temperature (LCST) phase transition behavior accompanied by irreversible crystallization in aqueous solution.6–9 For instance, Katsumoto et al. investigated the thermo-sensitive phase transition and crystallization behavior of PIPOZ in aqueous solution via vibrational spectroscopy and molecular orbital (MO) calculations. The conformational change was accompanied by the gradual dehydration of the polymer chains and it finally adopted a mainly all-trans conformation.7

In addition to synthetic thermo-sensitive copolymers with various topological structures, poly(2-oxazoline)-based hydrogels have been designed and fabricated for in vivo applications in controlled drug delivery, cosmetic surgery, and tissue engineering, because of their good biocompatibility and degradability, high stability, and lack of toxic by-products during degradation.10–14 Depending on the cross-linking method, hydrogels can be permanent (via chemical bonds) and undergo swelling/shrinking volume phase transitions (VPT), or reversible (via weak interactions, such as hydrogen bonds, van der Waals or ionic interactions) and the sol–gel transition is observed.8 Cova-lently cross-linked poly(2-oxazoline)-based hydrogels were first synthesized by copolymerization of poly(2-ethyl-2-oxazoline) (PEtOx) and poly(ε,ε-l-lactide).15 Based on hydrogels bearing aromatic units, Wiesbrock et al. investigated the relationship between the swelling degree and the cross linking degree and monomer ratio.16 By incorporating a hydrolytically cleavable dithiol cross-linker, Hoogenboom et al. prepared degradable poly(2-oxazoline)-based hydrogels that formed homogeneous networks under UV irradiation.17 In addition, redox and pH responsive hydrogels were also fabricated from partially hydrolyzed PEtOx with disulfide linkages at the cross-linking points.18 Nevertheless, although various smart stimuli-responsive poly(2-oxazoline)-based hydrogels have been prepared for facial synthesis and promising applications in biomaterials,17–20 the VPT behavior of poly(2-oxazoline)-based hydrogels has seldom been obtained accurately. Recently, David et al. reported a fast deswelling poly(2-oxazoline)-based hydrogel and attributed the fast deswelling rate compared with PNIPAM hydrogels to the presence of a channel-like microstructure.21 However, as far as we are concerned, the VPT behavior and precise thermally induced swelling/deswelling mechanism of poly(2-oxazoline) based...
hydrogel observed in PNIPAM hydrogels\textsuperscript{22–29} have not yet been clarified.

In the present study, a PIPOZ-based hydrogel was prepared and clear differences between the hydrogel and aqueous solution were observed based on a previous integrated study of PIPOZ solution.\textsuperscript{7} We also examined the inherent nature of the VPT behavior of the PIPOZ-based hydrogel at the molecular level by FT-IR combined with two-dimensional correlation spectroscopy (2DCOS) and perturbation–correlation moving-window (PCMW) analysis. 2DCOS is a mathematical method, the basic principles of which were first proposed by Noda,\textsuperscript{30} and it has been widely used to enhance spectral resolution. PCMW is a new technique that relies on principles originating in the conventional moving window technique proposed by Thomas and Richardson,\textsuperscript{31} and later improved by Morita \textit{et al.}\textsuperscript{32} Morita brought the external perturbation variable into the correlation equation to broaden its applicability. Except for determining transition points as in the conventional moving window technique, PCMW can also monitor complicated spectral variations along the perturbation direction. FT-IR spectroscopy combined with 2DCOS and PCMW techniques has been widely used to monitor complicated spectral variations along the perturbation direction and to detect microscopic variations of complex interactions. This combination was used to investigate the inherent nature of the VPT behavior of PIPOZ-based hydrogel at the molecular level. This may be helpful for understanding the VPT behavior of poly(2-oxazoline)-based hydrogels and for further research on the release kinetics of drugs.

**Experimental**

**Materials**

2-Isopropyl-2-oxazoline (IPOZ) and 1,4-phenylenebisoxazoline (1,4-PBO) were purchased from Tokyo Chemical Industry Co. Ltd. An aqueous solution of PIPOZ was prepared via the cationic ring-opening polymerization of IPOZ according to the literature.\textsuperscript{33} Unless otherwise specified, all other reagents were purchased from commercial suppliers and used as received.

**Hydrogel synthesis**

Hydrogels were prepared from IPOZ and 1,4-PBO under solvent-free conditions with methyl p-toluenesulfonate as the initiator (Fig. 1a).\textsuperscript{34–35} Microwave-assisted polymerization sealed in vials was performed at 140 °C for 1 h at a power of 150 W.\textsuperscript{16} A series of hydrogels were obtained with molar fractions of 1,4-PBO ranging from 0.01 to 0.05. The resultant hydrogels were dialyzed with deionized water for a week and freeze-dried to a dry gel before further characterization. Considering the cross-linking degree and swelling rate, the hydrogel with a molar fraction of 1,4-PBO of about 0.02 was discussed as an example. Micrographs of freeze-dried PIPOZ hydrogel are shown in Fig. S1.† The \(^1\)H NMR spectrum and the assignment of various proton resonances for the PIPOZ hydrogel in D\(_2\)O are shown in Fig. 1b.

**Instruments and measurements**

The \(^1\)H NMR experiments were carried out on Mercury plus 400 MHz NMR instrument in D\(_2\)O. Thermal analysis of PIPOZ hydrogel was performed on Mettler-Toledo differential scanning calorimeter (DSC) in a nitrogen atmosphere at a heating rate of 1 °C min\(^{-1}\) from 20 to 55 °C. The morphology of the freeze-dried PIPOZ hydrogel was observed with field emission scanning electron microscope (FE-SEM; Hitachi, S-4800). The sample was coated with gold before the FE-SEM observations.

The hydrogel was swollen in excess D\(_2\)O at 4 °C for a week to ensure complete deuteration and sufficient swelling before the FT-IR measurements. The hydrogel in D\(_2\)O was sealed between two ZnS tablets and the FT-IR spectra at different temperatures were recorded on a Nicolet Nexus 6700 spectrometer. A resolution of 4 cm\(^{-1}\) and 32 scans were used to achieve an acceptable signal-to-noise ratio. Temperatures were controlled with an electronic cell holder at a rate of 0.5 °C min\(^{-1}\) with an increment of 1 °C between 20 and 60 °C. Perturbation–correlation moving-window (PCMW) analysis and 2D correlation analysis were performed using 2D Shige, ver. 1.3 (Shigeki Morita, Kwansei-Gakuin University, Japan). The contour maps were plotted with Origin, ver. 8.6, with red and yellow as the positive intensities and blue as the negative intensities. An appropriate window size (\(2m + 1 = 11\)) was chosen to generate good quality PCMW spectra.

**Results and discussion**

**Conventional FT-IR analysis**

Temperature-dependent FT-IR spectra of PIPOZ hydrogel in D\(_2\)O during the heating and cooling cycle from 20 to 60 °C with an interval of 1 °C were collected to investigate dehydration and hydration. For clarity, the \(\nu(C–H)\) band at 3040–2840 cm\(^{-1}\) and the \(\nu(C––O)\) band at 1685–1540 cm\(^{-1}\) between 25 and 55 °C were used for detailed analysis (Fig. 2). D\(_2\)O was used as the solvent instead of H\(_2\)O to eliminate the overlap of the \(\delta(O–H)\) band of H\(_2\)O around 1640 cm\(^{-1}\) with the \(\nu(C––O)\) of PIPOZ hydrogel, as well as the overlap of the broad \(\nu(O–H)\) band of H\(_2\)O around 3300 cm\(^{-1}\) with the \(\nu(C–H)\) bands of PIPOZ hydrogel because the effect of the isotopic substitution on the transition temperatures can be ignored.\textsuperscript{7} Notably, the different responses of the \(\nu(C–H)\) band and the \(\nu(C––O)\) band with temperature variation were observed, suggesting that the hydrogel underwent different mechanisms during the heating–cooling cycle. Meanwhile, no isosbestic point was observed in the spectral variations of the two investigated regions during the
heating–cooling cycle, indicating that the chain collapse of the hydrogel occurred in some intermediate states, which is different from PNIPAM hydrogel.25

To show the variation and different response of C–H and C=O groups in PIPOZ hydrogel and aqueous solution clearly, the spectral comparison of the FT-IR spectra and the second derivative spectra at different temperatures during heating are shown in Fig. 3. Band shifts to lower frequencies for CH3 (2979 to 2972 cm⁻¹) and CH2 (2942 to 2937 cm⁻¹) in PIPOZ hydrogel are visible in Fig. 3a, indicating the gradual dehydration of the C–H groups during heating.27 At the same time, the intensity of the hydrated C=O groups in the hydrogel system shows a decrease during heating, and then a relatively free C=O (around 1655 cm⁻¹) absorption gradually appears. As shown in Fig. 3b, the C–H stretching band frequencies in PIPOZ hydrogel remain slightly lower than those in aqueous solution at the same temperature, which suggests that the hydration degree in the hydrogel was lower than that in the solution due to the hydrophobicity and steric hindrance from the cross-linking agent. Furthermore, the overall peak intensity of the C–H groups in the PIPOZ hydrogel decreased gradually from 45 to 60 °C while a drastic decrease in the intensity of CH3 (around 2976 cm⁻¹) was observed. This indicates that a large collapse happened to the CH3 groups and they were wrapped in hydrophobic chains during the VPT.

The effect of the cross-linking network on the phase transition was observed by comparing the FT-IR spectra of the hydrogel and aqueous solution in the C=O region. Based on previous work,7,18,19 two types of hydrogen bonds coexist in PIPOZ aqueous solution: polymer–water (1605 and 1597 cm⁻¹) and polymer–water–polymer (1631 cm⁻¹), as shown in Fig. S2. The second derivative spectra (blue and green lines) in Fig. 3b show that the band at 1632 cm⁻¹ (cross-linking hydrogen bonds C=O···D–O–D···O=) is larger in the hydrogel than aqueous solution spectrum at 25 °C, which could be explained by the preference of forming intra-chain polymer–water–polymer hydrogen bonds in the surrounding constrained network structure.

To quantitatively describe the degree of dehydration and the VPT during heating and cooling, temperature-dependent frequency shifts of νas(CH3) and νas(CH2), and the integral area of the C=O groups in the hydrogel are plotted in Fig. 4. A gradual change in the frequency shifts of νas(CH3) and νas(CH2) in the hydrogel is visible compared with PIPOZ aqueous solution, indicating the gradually expulsion of water molecules during the heating of the hydrogel. No obvious VPT region was found, and PCMW was required to obtain it. Typically, asymmetric sigmoid curves of νas(CH3) and νas(CH2) for heating generally match those for cooling, indicating that there is almost no hysteresis. In contrast, in the PNIPAM hydrogel, hysteresis of about 2 °C was observed.26

The clear difference between heating and cooling can be observed in the C=O stretching region. The heating line shows a steady decrease, although the C=O groups show an apparent hysteresis in the initial stage of cooling. This may originate from the breakage of polymer–water–polymer hydrogen bonds between different chain segments when they are shrunk at high temperatures. The hydrogen bond bridges can be gradually rebuilt during cooling, but cannot reform quickly during the initial stage of cooling. Therefore, water molecules cannot enter the hydrophobic hydrogel structure and hysteresis is detected.
However, during cooling from 40 to 20 °C, a very sharp retrieval is observed, indicating that the rapid recovery of water molecules entering the hydrogel structure happens after the rebuilding of the cross-linking hydrogen bond bridges.

Interestingly, the retrieval of C=O groups during cooling is slightly higher than during the initial heating, whereas the opposite is true for C–H groups. During cooling the C–H groups cannot return to their initial frequency immediately; therefore, we propose that the hydrogel undergoes a swelling VPT mechanism during cooling that is different from the shrinking mechanism during heating. This requires further confirmation.

**Perturbation–correlation moving window (PCMW)**

To obtain more detailed spectral variations and an accurate VPT temperature of PIPPOZ hydrogel, PCMW analysis provides synchronous and asynchronous correlation spectra between the spectral variable axis and the perturbation variable axis. In the case of variable increments, a spectral intensity increase indicates a positive synchronous correlation, whereas a negative correlation produces a decrease in spectral intensities. A positive asynchronous correlation corresponds to a positive synchronous variation, whereas a negative asynchronous correlation corresponds to a concave variation.

Fig. 5 presents PCMW synchronous and asynchronous spectra of PIPPOZ hydrogel in D₂O during heating and cooling between 25 and 55 °C. PCMW synchronous spectra are very helpful for finding transition points, whereas asynchronous spectra can be used to determine the transition temperature region from the peaks with the strongest intensity. During heating, there are five transition points in the C–H region. The bands at 2966, 2933 and 2872 cm⁻¹ are all positive and appear from 31–38 °C. The temperature transition points are all around 35 °C. This means that the CH₃ and CH₂ groups in the ordered dehydrated state increase from around 31 to 38 °C, and the sharp changes in temperature appear around 35 °C. There are two bands at 2978 and 2941 cm⁻¹ that are negative in Fig. 5, although the decrease occurs throughout heating, and no obvious start or end points are observed. This is because the CH₃ and CH₂ groups in the disordered hydrated state dehydrate and disappear gradually during heating, and this accompanies the formation of ordered dehydrated CH₃ and CH₂ groups. PCMW in the C–H region reveals that C–H groups undergo dehydration and conformational change.

In the C=O region, the transition points for hydrated C=O groups and relatively free C=O groups are at the same temperature as the C–H groups at around 35 °C. However, the transition temperature region is from 32 to 39 °C, which shows a slight difference from C–H. The C–H groups responded before the C=O groups in the VPT during heating. The details of the change were confirmed by the following 2DCOS analysis.

Cooling shows great differences from heating. We can obtain the following transition points (→ denotes ‘before’): 2881 cm⁻¹ at 43 °C → 2943 cm⁻¹ at 40 °C → 2980 cm⁻¹ at 39 °C → 1655 cm⁻¹ at 38 °C → 1610 and 2872 cm⁻¹ at 34 °C → 2931 cm⁻¹ at 32 °C → 2964 cm⁻¹ at 31 °C. This indicates that the number of groups in the disordered hydrated state decreases during heating and they appear first during cooling; during swelling, the C–H groups in the disordered state absorb water first. Next, the numbers of relatively free C=O groups at 1655 cm⁻¹ start to decrease, which increases the numbers of hydrated C=O groups. Therefore, the apparent hysteresis for C=O groups shown in Fig. 4 could be qualitatively analyzed as follows. Water molecules cannot enter the hydrogel below 42 °C. At 41 °C, water molecules start forming hydrogen bonds with partially free C=O. As more of these hydrogen bond bridges form, they produce a channel-like microstructure for water molecules to penetrate the hydrogel network system gradually and allow swelling. Then, C–H groups in the ordered dehydrated state begin to decrease from 36 to 30 °C.

Based on the PCMW analysis, the volume transition point is 35 °C and the transition temperature region is between 31 and 39 °C during heating. However, there is no obvious transition point during cooling, and the swelling temperature region is broader, from 41 to 30 °C. The results are roughly in accordance with the DSC experiments shown in the ESI, which served as an important basis for the segmental mode of the following 2DCOS analysis. The reswelling of PIPPOZ hydrogel during cooling is slow and unusual compared with other LCST hydrogels that show quick reswelling.

**Two-dimensional correlation spectroscopy (2DCOS)**

2DCOS, as a mathematical method, has been used to study the motion of groups since its basic principles were first proposed by Noda. The different response of different species to external variables allows spectral resolution enhancement, which means that additional important information about molecular motion or conformational changes that is not readily visible in conventional analysis can be extracted. 2DCOS includes two types of correlation maps: the synchronous spectra, which reflects simultaneous changes between two wavenumbers; and asynchronous spectra, which can greatly enhance the spectral resolution. Auto-peaks are defined as positive peaks along the diagonal, which only appears in the synchronous spectra. The off-diagonal peaks (Φ(r₁, r₂)) are cross-peaks, which appear in both synchronous and asynchronous spectra, which can be positive or negative. In the synchronous spectra, positive cross-

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**Fig. 5** PCMW synchronous and asynchronous spectra of PIPPOZ hydrogel (D₂O) during heating and cooling between 25 and 55 °C. Herein, warm colors (red and yellow) denote positive intensities, and cool colors (blue) denote negative intensities.
peaks indicate changes in same direction for the two wave-numbers under the perturbation, whereas negative cross-peaks indicate the opposite.

Because asynchronous spectra can substantially enhance the spectral resolution, many additional bands that cannot be seen in conventional IR spectra or its second derivative spectra can be obtained by 2DCOS. The synchronous and asynchronous spectra of PIPOZ hydrogel are shown in Fig. 6. The asynchronous spectra of the C=O band region yield the following five peaks: 1657, 1651, 1639, 1610, and 1593 cm⁻¹. Because of the conjugation effects of the benzene ring, free C=O groups next to the benzene ring show a lower wavenumber (1651 cm⁻¹). The effects of hydrophobicity and steric hindrance mean that hydrated C=O groups abutting the cross-linkers possess less water molecules, and exhibit a higher frequency (1610 cm⁻¹) than the strong hydrated C=O bands at 1593 cm⁻¹. The bands at 2962 and 2929 cm⁻¹, which are assigned to the ordered structure of dehydrated C–H groups, can be detected in the asynchronous spectra. The micro changes in wavenumbers from 2976 to 2962 cm⁻¹ and from 2941 to 2929 cm⁻¹ clarify the mechanism of the structural changes of C–H groups. Through 2DCOS method, we can capture more distinct bands for different groups in different states in detail throughout the structural changes, which is important for exploring the phase transition mechanism. All the bands detected in the 2DCOS spectra and their tentative assignments are presented in Table 1.

In addition to enhancing the spectral resolution, 2DCOS can also discern the subtle sequence that occurs under external perturbation. The judging rule of the sequence can be summarized as Noda’s rule; that is, if the cross peaks ($n_1, n_2$, and $n_1 > n_2$) in the synchronous and asynchronous spectra have the same sign, the change at $n_1$ may occur prior to $n_2$, and vice versa. Therefore, the final sequence for the groups during heating can be deduced as follows: $2976 \rightarrow 2941 \rightarrow 2881 \rightarrow 2962 \rightarrow 2929 \rightarrow 2872 \rightarrow 1610 \rightarrow 1651 \rightarrow 1593 \rightarrow 1639 \rightarrow 2981$ cm⁻¹. This means: $n_{as}(\text{CH}_3)$ (disordered and hydrated, branch) $\rightarrow n_{as}(\text{CH}_3)$ (disordered and hydrated,

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**Fig. 6** 2DCOS synchronous and asynchronous spectra of PIPOZ hydrogel (D$_2$O) from 25 to 55 °C during heating and cooling. Herein, warm colors (red and yellow) denote positive intensities, and cool colors (blue) denote negative intensities.
hydrogels undergo a typical step-by-step dehydration: fully hydrated C=O groups abutting benzene and then it forms free C=O groups. After dehydrating, benzene ring-centered hydrophobic spot, the dehydration spreads to the surrounding areas. The hydrated C=O groups away from the benzene ring undergo gradual dehydration. Water molecules are expelled when the hydrogen bond bridges (C=O⋯D–O⋯D⋯O=C, 1639 cm$^{-1}$) between the polymer chains break. This suggests that the deswelling has ended.

During cooling, the final sequence for the groups is: 2883 → 2943 → 2976 → 2981 → 1657 → 1589 → 1639 → 1610 → 2873 → 2929 → 2962 cm$^{-1}$; or $v(CH_3)$ (disordered and hydrated, branch) → $v(CH_3)$ (disordered and hydrated, backbone) → $v(CH_3)$ (ordered and dehydrated, backbone) → $v(CH_3)$ (ordered and dehydrated, branch) → $v(weakly hydrated C=O)$ (abutting benzene) → $v(free C=O)$ (abutting benzene) → $v(strongly hydrated C=O)$ (away from benzene) → $v(C=O⋯D–O⋯D⋯O=C)$ (polymer–water–polymer) → $v(weakly hydrated C=O)$ (abutting benzene) → $v(CH_3)$ (ordered and dehydrated, backbone) → $v(CH_3)$ (ordered and dehydrated, branch). For PCM analysis, the hydration of C–H groups was used as the starting point for water uptake during cooling. Ignoring the differences in the stretching modes of C–H groups, CH$_3$ → CH$_2$, which suggests that the main chains respond earlier than the branch chains during water absorption and the conformational changes during cooling. For the C–H stretching modes, the symmetric stretching vibration shows an earlier response than the asymmetric stretching vibration during cooling. This means that the vertical direction of the chain axis tunes these groups from an ordered state to a disordered state to make it easy to take up water, and then the polymer chains extend parallel to the chain axis. After the hydration of the C=O groups, the rest of the ordered C–H groups gradually adopt a random state. The behavior of the C–H groups during cooling was different from that during heating.

For the hydration of C=O groups during cooling, water molecules initially entered the hydrophilic areas away from benzene. However, it is hard to penetrate the relatively hydrophobic center of hydrogel until the hydrogen bond bridges between polymer chains are rebuilt. This can explain the hysteresis of C=O groups during the initial stage and the subsequent sharp increase in the curve of the C=O groups during cooling shown in Fig. 4.

The rebuilding of hydrogen bond bridges, discussed in the PCM analysis, provided a channel-like microstructure that allowed the hydrogel to swell. Cooling exhibits a time course that is almost the inverse of heating, except for the absence of the band at 1651 cm$^{-1}$ (free C=O, linked to benzene). This indicates that water molecules cannot penetrate the hydrophobic areas immediately and form hydrogen bonds with free C=O groups linked to the benzene ring during cooling. Moreover, the hydrophobicity and steric hindrance of the benzene ring mean that the transformation of C–H groups cannot return to the original state quickly and water cannot enter the hydrophobic areas immediately. This means that the retrieval of C=O and C–H groups during cooling is different from that during heating. This reveals that the reswelling during cooling is slow and unusual.

Fig. 7 shows the proposed molecular mechanism of the PIPOZ hydrogel during the heating and cooling cycle. The following table shows tentative band assignments of PIPOZ hydrogel in D$_2$O and the 2DCOS analysis:

<table>
<thead>
<tr>
<th>Wavenumber (cm$^{-1}$)</th>
<th>Tentative assignments</th>
</tr>
</thead>
<tbody>
<tr>
<td>2981</td>
<td>$v(CH_3)$ (fully hydrated, CH$_3$)</td>
</tr>
<tr>
<td>2976</td>
<td>$v(CH_3)$ (disordered and hydrated, branch)</td>
</tr>
<tr>
<td>2962</td>
<td>$v(CH_3)$ (ordered and dehydrated, branch)</td>
</tr>
<tr>
<td>2941/2943</td>
<td>$v(CH_3)$ (disordered and hydrated, backbone)</td>
</tr>
<tr>
<td>2929</td>
<td>$v(CH_3)$ (ordered and dehydrated, backbone)</td>
</tr>
<tr>
<td>2881/2883</td>
<td>$v(CH_3)$ (ordered and dehydrated, branch)</td>
</tr>
<tr>
<td>2872/2873</td>
<td>$v(CH_3)$ (ordered and dehydrated, branch)</td>
</tr>
<tr>
<td>1657</td>
<td>$v(free C=O)$ (away from benzene)</td>
</tr>
<tr>
<td>1651</td>
<td>$v(free C=O)$ (abutting benzene)</td>
</tr>
<tr>
<td>1639</td>
<td>$v(C=O⋯D–O⋯D⋯O=C)$ (polymer–water–polymer)</td>
</tr>
<tr>
<td>1610</td>
<td>$v(weakly hydrated C=O)$ (abutting benzene)</td>
</tr>
<tr>
<td>1593/1589</td>
<td>$v(strongly hydrated C=O)$ (away from benzene)</td>
</tr>
</tbody>
</table>
conformational change and dehydration of the C–H groups is the starting point for the hydrogel deswelling during heating. The branch chains respond earlier than the backbone chain and the collapse of the main chains parallel to the chain axis occurs prior to the adjustment vertical to the chain axis. This makes the C–H groups transform from a disordered hydrated structure to an ordered dehydrated structure. The dehydration of the C==O groups is controlled by the breakage of hydrogen bonds and the formation of relatively free carbonyl bonds. Water is expelled by the benzene ring-centered hydrophobic spot and it spreads to the surrounding areas. Finally, the water molecules of the hydrogel periphery and the water inside are expelled further as the hydrogen bonds break with fully hydrated CH₃ and hydrogen bond bridges. Overall, the collapse and shrinkage of the hydrophobic carbon skeleton squeezes the water molecules out of the hydrogel through the breakage of the hydrogen bonds during heating.

The hydrogel undergoes a different mechanism during cooling. Firstly, water molecules approach the peripheral C–H groups of the hydrogel and break the ordered state that develops during heating. Secondly, the hydrogen bonds with C==O groups that are far from hydrophobic cross-linking spots are formed. However, water molecules cannot penetrate the hydrophobic areas until the polymer–water–polymer cross-linking hydrogen bonds are recovered, which can provide a channel-like microstructure that swells the hydrogel gradually. Nevertheless, the hydrophobicity and steric hindrance of the benzene ring cause the slow, unusual recovery of the PIPOZ hydrogel network during cooling.

Conclusions

The VPT of a PIPOZ hydrogel in D₂O was investigated by FT-IR spectroscopy combined with PCMW and 2DCOS. The VPT point at 35 °C during heating and the VPT temperature region between 41 and 30 °C during cooling were determined, and occurred in a physiological temperature range. Due to the hydrophobicity and steric hindrance of the cross-linking agent, the hydrogel shows a lower transition point than the aqueous solution of PIPOZ, and it exhibits different shrinking and swelling behavior. The mechanism of the VPT of the hydrogel during heating and cooling is proposed as follows. During heating, the conformational change and dehydration of C–H groups occurs before the dehydration of C==O groups and drives the shrinking procedure. Water expulsion starts at the benzene ring-centered hydrophobic spots and spreads to the surroundings as it shrinks. In contrast, during cooling, water molecules approach the peripheral C–H groups of the hydrogel and cannot penetrate the hydrophobic areas until the polymer–water–polymer cross-linking hydrogen bonds are reformed. A slow, unusual recovery of the hydrogel during cooling is observed, which may be attributed to the hydrophobic cross-linking spots that constrain the network structure. The proposed mechanism may help understand the VPT of PIPOZ hydrogel and support further research on the release kinetics of drugs or macromolecular active agents such as proteins and peptides.
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Notes and references