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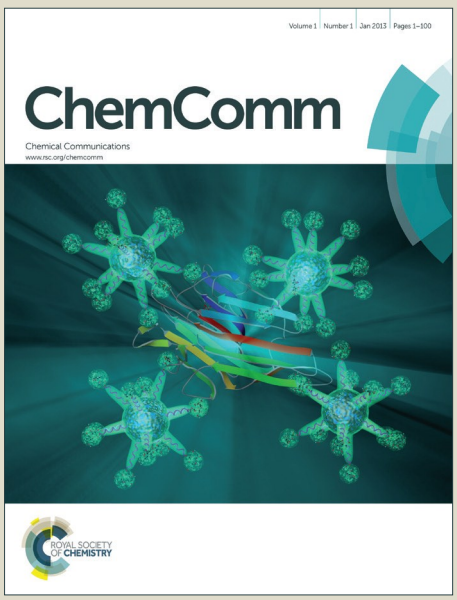

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One-step hydrothermal route to programmable stimuli-responsive hydrogels

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A hydrothermal route to regulate the swelling and responsiveness properties of poly (*N*-isopropylacrylamide) (PNIPAM) hydrogels is reported. During the process, water is the only reactive medium used, and the hydrogel properties can be programmed effectively.

Stimuli-responsive hydrogels, which can change their properties in response to external stimuli such as temperature and pH have increasingly important applications in various fields.[1] Poly(*N*-isopropylacrylamide) (PNIPAM), a temperature-responsive polymer, is widely used in the fabrication of responsive hydrogels.[2] PNIPAM hydrogel can undergo a reversible volume phase transition near its lower critical solution temperature (LCST), which is close to physiological temperature.[3] Because of this unique property, PNIPAM hydrogels are attractive for various applications, such as smart actuators,[4] microfluidic valves,[5] micro-lens optical systems,[6] soft biomimetic machines,[7] and drug delivery vehicles.[8] Moreover, long-term cell culture experiments have demonstrated that PNIPAM hydrogels are biocompatible to several cell types and can be potentially used in the field of tissue engineering.[9] Despite their application potential, PNIPAM hydrogels prepared using the conventional redox initiation method exhibit uncontrollable swelling and an untunable LCST, which limits their applications. Moreover, tuning the composition of *N*,*N*-methylenebisacrylamide (MBA) crosslinkers and/or NIPAM monomers during synthesis results in minimal improvement in the regulation of hydrogel properties. To overcome these limitations, approaches such as copolymerizing hydrophilic components,[10] grafting side chains,[11] and introducing porogen[12] have been developed. However, these strategies require either physical mixing or covalent linking with other chemical components, which has several disadvantages, such as the presence of toxic chemical residues, the use of a complicated multistep synthetic processes and impairment of the stimuli responsiveness.

In this study, MBA-crosslinked PNIPAM hydrogels were first synthesized using a conventional redox initiation method and then subjected to hydrothermal treatments to program their swelling, LCST and responsiveness. Hydrothermal treatments are performed in closed systems of relatively high temperatures and internal pressures, where water was the only reactive medium. With elevated temperatures, water exists in subcritical state having strong electrolytic solvent power and high ion molecules. Consequently, hydrothermal routes can prompt a variety of chemical reactions such as hydrolysis, dehydration and polymerization, using only water as the reactive medium.[13] Because of the aqueous nature of PNIPAM hydrogels, hydrothermal treatment of the hydrogels may provide a convenient approach to tune their properties, where the hydrogel networks can be hydrolyzed and/or polymerized. Herein, an increase in the hydrothermal reaction time resulted in a steady increase in the swelling ratio of the PNIPAM hydrogels from 600% to 20,000%, and the LCST was simultaneously tuned to designated values of 32 ℃, 34 ℃, 38 ℃ and 42 ℃. Moreover, an integrated core-shell hydrogel with discrete properties was also obtained via hydrothermal treatment of a partially crosslinked PNIPAM hydrogel.

A fully-crosslinked PNIPAM hydrogel was formed with modified procedure as described previously.[14] Briefly, 100 mg of NIPAM, 10 mg of MBA and 10 mg of APS were dissolved in 1 ml of DI H_2O and filled into a shaped mold maintained at 4 ℃. The polymerization was then initiated by adding 10 μl of 0.1% aqueous TEMED solution. The polymerization was performed at 4 ℃ for 3 h to form the PNIPAM hydrogel. The hydrogels were then peeled off and placed in 8 L of H_2O under stirring at 4 ℃ for 20 days to completely remove the un-reacted components (ESI†-II). The PNIPAM hydrogels were subsequently subjected to hydrothermal treatment at 180 ℃ for different durations. At designated time intervals of 0, 5, 15 and 25 h, the hydrogels were removed and re-swollen in H_2O for analysis (Fig. 1a). The hydrothermal reaction temperature was set to 180 ℃, since substantial hydrolysis was initiated at this temperature,[15] which can be used to hydrolyze amide bonds and de-crosslink the PNIPAM hydrogel network. Figure 1b shows the morphologies of hydrogels hydrothermally treated for different durations. As the reaction time was increased, the volumes of the hydrogels expanded (Fig. 1b). The length of the untreated PNIPAM hydrogel was approximately 0.75 cm, whereas the lengths of the hydrogels hydrothermally treated for 5 h, 15 h and 25 h increased to approximately 1 cm, 1.75 cm and 2.5 cm, respectively. The interior morphologies of hydrogels were investigated using SEM (ESI†-II Figs. S1a, S1b). The cross-section of the untreated hydrogel (HT 0 h) was dense and compact, whereas the treated hydrogel (HT 25 h) exhibited an interconnected macroporous structure, indicating the increased porosity of hydrothermally treated PNIPAM hydrogel.

The swelling ratio and LCST of the hydrogels were conveniently programmed via adjusting hydrothermal reaction time (Figs. 1c, 1d). The untreated hydrogel reached a maximum swelling ratio of 600% at 80 min, while the hydrogels hydrothermally treated for 5, 15 and 25 h required 15 min to reach maximum swelling ratios of 1000%, 9500% and 20,000%, respectively, indicating that the hydrothermally treated hydrogels exhibit faster swelling kinetics and have higher water contents than the untreated sample. Meanwhile, the untreated hydrogel exhibited an LCST of 32 ℃, whereas the hydrogels treated for 5, 15 and 25 h exhibited elevated LCSTs of 34 ℃, 38 ℃ and 42 ℃, respectively; these results reflect the hydrothermally programmable nature of PNIPAM hydrogels (Fig. 1e). The untreated PNIPAM hydrogel shrunk from 100% to 50% of its original volume at 34 ℃, whereas the hydrogels hydrothermally treated for 5, 15 and 25 h shrunk from 100% to 50% of their original size at 36 ℃, 42 ℃ and 46 ℃, respectively, indicating that the hydrothermally treated hydrogels have a higher phase change temperature than the untreated hydrogel (ESI†-IV Fig. S3).

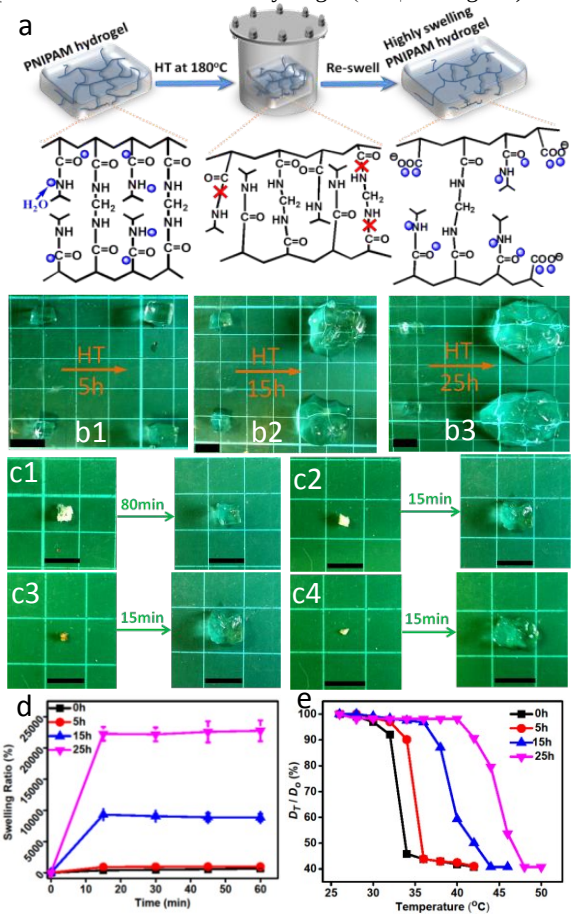

Fig. 1 (a) Hydrothermal (HT) treatment of PNIPAM hydrogels and the resulting changes in chemical structure; (b1-b3) Morphologies of PNIPAM hydrogels hydrothermally treated for different durations, where (b1) shows untreated hydrogel (left) and the hydrogel treated for 5 h (right), (b2) shows untreated hydrogel (left) and hydrogel treated for 15 h (right) and (b3) shows untreated hydrogel (left) and hydrogel treated for 25 h (right); the scale bar is 1 cm. (c1-c4) Dried and swollen states of PNIPAM hydrogels, where (c1) shows untreated hydrogel in dried (left) and equilibrium swelling states (right), (c2) shows hydrogel treated for 5 h in its dried (left) and equilibrium swelling states (right), (c3) shows the hydrogel treated for 15 h in its dried (left) and equilibrium swelling states (right) and (c4) shows the hydrogel treated for 25 h in its dried (left) and equilibrium swelling states; the scale bar is 1 cm. (d) Swelling ratios of hydrogels in H_2O at room temperature. (e) LCST changes of hydrogels, where D_0 is the original diameter and D is the diameter observed under a microscope at a specific temperature.

The volume expansion, rapid swelling and increased LCST of the treated hydrogels may result from the de-crosslinking of their network and the generation of carboxyl groups from the hydrothermal hydrolysis of amide bonds. First, the hydrogel de-swells because of its temperature responsiveness, during which water is expelled and hydrophobic forces become dominant. As reaction progressed, amide bonds in MBA or NIPAM become prone to hydrolysis, which would de-crosslink the hydrogel and generate carboxyl groups in the network (Fig. 1a). To study the reaction kinetic of the hydrothermal process, effects of treating time and treating temperature on the density of hydrogels and pH of the aqueous supernatant were investigated (ESI†-V). Fourier-transform infrared (FTIR) spectrophotometer was used to investigate the chemical structure changes. However, no differences were distinguished between the untreated and hydrothermally treated hydrogels (Fig. 2a). All samples show vibrations of amide bonds (C=O-N-H). Meanwhile, a broad peak at 3500 cm^{-1}, which is characteristic of hydroxyl (–OH) vibrations, was observed for all the samples. The hydroxyl vibrations could be attributed to either the non-freezing water in the hydrogel (H-O-H) or carboxyl groups (-COOH) formed via hydrothermal hydrolysis. To clarify this superimposition, the pH responsiveness of the hydrogels was investigated. The swelling/shrinking cycles of hydrogels between pH 9 and pH 2 at 25 ℃ which is below the LCST values of all the hydrogels were investigated (Fig. 2b). The untreated hydrogel (0 h) exhibited no responsiveness to pH changes, whereas the hydrothermally treated hydrogels (5 h, 15 h and 25 h) exhibited reversible swelling/shrinking in response to pH changes. Specifically, the gels shrunk at pH 2 and re-swelled back to their original size at pH 9, confirming the generation of carboxyl groups in the PNIPAM hydrogel networks after the hydrothermal treatment. The original PNIPAM hydrogel that exhibited only temperature responsiveness was therefore transformed into a hydrogel with temperature/pH dual responsiveness due to the hydrothermal generation of carboxyl groups (Figs. 2c, 2d). The pH responsiveness of carboxyl-bearing hydrogels is due to the protonation/deprotonation process of the carboxyl groups upon pH stimulation. When the pH was 9, hydrogels exhibited high swelling with enlarged volume due to the osmosis and charge repulsion from protonation of carboxyl groups. When the pH was lowered to 2, the hydrogels shrunk because of the deprotonation of carboxyl groups, which resulted in weaker charge repulsion and weaker osmosis.[16] Consequently, longer hydrothermal treatment times result in larger numbers of carboxyl groups, which impart hydrogels higher degree of protonation/deprotonation after pH stimulation and make hydrogels more sensitive to pH changes.

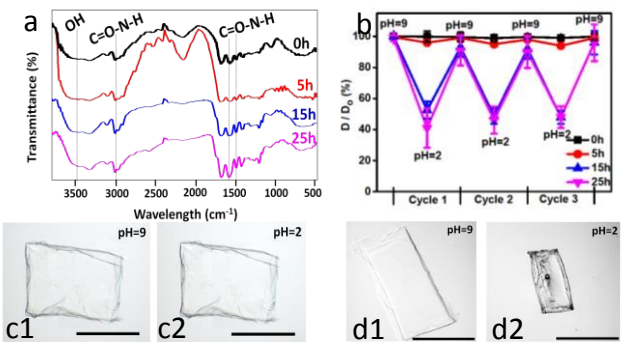

Fig. 2 (a) FTIR spectra of PNIPAM hydrogels hydrothermally treated for different durations; (b) swelling/shrinking responses of hydrogels to pH changes, where D_o is the original diameter and D is the diameter after pH stimulation at 25 ℃. (c-d) Microscopic pictures showing the responses of the untreated PNIPAM hydrogel (c1-c2) and the PNIPAM hydrogel hydrothermally treated for 25 h (d1-d2) when the hydrogels were exposed to buffers of pH 9 and pH 2; the scale bar is 1 cm.

To synthesize core-shell hydrogels, a partially crosslinked PNIPAM hydrogel was first synthesized by interrupting the redox initiation polymerization process. Briefly, 100 mg of NIPAM, 10 mg of MBA and 5 mg of APS were dissolved in 1 ml of H_2O. The polymerization was initiated by adding 2 µl of 0.1% aqueous TEMED solution. The polymerization was performed at 4 ℃ for 1 h; the gels were subsequently peeled off, and directly transferred to a hydrothermal autoclave containing 20 ml of H_2O, heated at 180 ℃ for different durations and re-swollen in H_2O (ESI†-VI and Fig. 3a). After reacting for 3 h, the PNIPAM hydrogel was transformed into a hydrogel with a core-shell structure, where an opaque white core was surrounded by a transparent hydrogel shell (Fig. 3b). The core diameter and shell thickness were adjusted by tuning the hydrothermal reaction time (Fig. 3c). The formation of different core-shell structures may result from the differences in the crosslinking density of the hydrogels, which is controlled by balancing hydrothermal polymerization and hydrolysis. In the initial period, hydrophobic interactions become strong and lead to dehydration of PNIPAM chains in the gel surface layer, where free NIPAM and MBA diffuse out. As temperature increased, the surface layer becomes denser and restrains further diffusion of free NIPAM and MBA. As the reactions progress, NIPAM and MBA molecules retained in the inner part of the hydrogel polymerize rapidly, thereby increasing the crosslinking density. In contrast, polymerization in the gel surface layer hardly occurs due to lack of NIPAM and MBA. As the reaction proceeds further, polymerization reaches completion and hydrolysis of amide bonds starts in the hydrogel surface. Consequently, a core-shell PNIPAM hydrogel with a different crosslinking density is obtained (Figs. 3b, 3c).

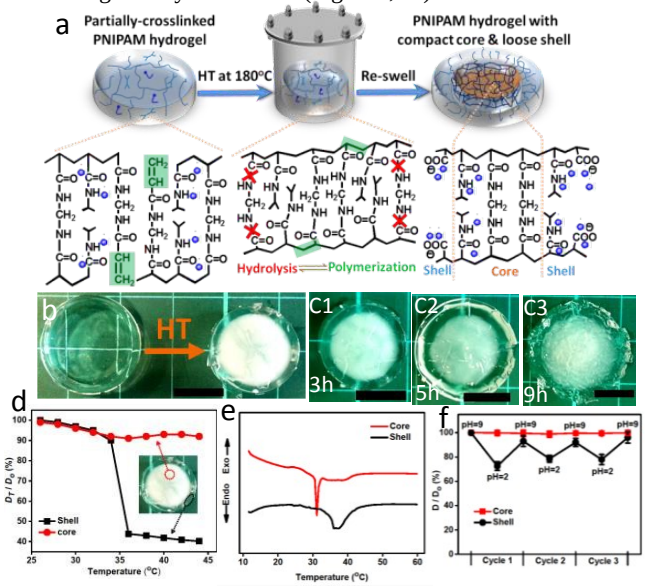

Fig. 3 (a) HT transformation of a partially crosslinked PNIPAM hydrogel into a core-shell hydrogel and the corresponding changes in chemical structure (b-c) Appearance of core-shell hydrogels after hydrothermal transformation of partially crosslinked PNIPAM hydrogels for different durations; the scale bar is 1 cm. (d) Size changes with respect to temperature. (e) DSC curves and (f) size changes of the hydrogel core and hydrogel shell in response to pH stimulation.

The hydrogel core and shell exhibited distinct thermal properties, environmental responsiveness and swelling behaviors. The size changes of the core and shell as the temperature was increased from 26 ℃ to 42 ℃ were studied (Fig. 3d). At 34 ℃, the shell shrank from 90% to 40% of its original size, whereas the core size remained similar. Differential scanning calorimetry (DSC), which can detect enthalpy changes from water dissociation at the LCST, was used to further investigate the hydrogels' thermal properties (Fig. 3e). The endothermic peak of the core appeared at 30 ℃, whereas that of the shell appeared at 36 ℃, indicating that the core is less hydrophilic than the shell. Meanwhile, the peak area of the core was smaller, indicating that the core can dissociate water more rapidly than the shell. The swelling ratios of the core and shell were also studied (Fig. S7). The swelling maximum of the core was 250%, whereas that of the shell was 1100%, indicating that the shell has higher water content than the core. The different swelling behaviors of the hydrogels are due to their different crosslinking densities,[17] where the highly crosslinked core has less chain mobility and therefore swells less and more slowly than the shell. Figure 3f shows the pH responsiveness of the hydrogel core and shell. The core exhibited no responsiveness to pH change, whereas the shell exhibited reversible swelling/shrinking in response to pH changes (Fig. 3f), suggesting the existence of hydrothermally generated carboxyl groups in the hydrogel shell.

In conclusion, a one-step hydrothermal route to programmable PNIPAM hydrogels was reported. A table comparing present technology and other existing works was reported (ESI†-VII). We demonstrated hydrothermal regulation of the swelling, LCST and environmental responsiveness of fully crosslinked PNIPAM hydrogels. Additionally, integrated core-shell structured PNIPAM hydrogels with discrete properties between the core and the shell were also obtained by hydrothermal transformation of partially crosslinked PNIPAM hydrogels.

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Electronic Supplementary Information (ESI) available: Experimental details and Fig. S1-S7. See DOI: 10.1039/c000000x/

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