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Facile preparation of poly(*N*-isopropylacrylamide)-based hydrogels via aqueous Diels–Alder click reaction

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ABSTRACT

The study reports a facile method of preparing poly(*N*-isopropylacrylamide)- based hydrogels by means of the Diels–Alder reaction. First, polymeric dienes were synthesized by free radical copolymerization between *N*-isopropylacrylamide (NIPA) and furfuryl methacrylate (FM), with 2, 2′-azobisisobutyronitrile (AIBN) as an initiator, and polymeric dienophile was obtained by a coupling reaction of poly(ethylene glycol) (PEG) and *N*-maleoyl-L-leucine (LMI) under *N*, *N*′-dicyclohexylcarbodiimide (DCC). Afterwards, the resultant dienes and dienophiles were dissolved in water and put in a refrigerator remaining a temperature of 9 °C, gelation via Diels–Alder reaction was observed after some time. The samples obtained at different steps were characterized by FTIR, NMR, GPC, SEM, CD, etc. It was found that LCST of copolymers decreases with the increase of FM content in copolymers. And the disassembly time of the hydrogels is closely related to the temperature and the solvents used. The swelling behavior study by gravimetric measurement indicates the hydrogels possess thermosensitivity and exhibit considerable swelling in water. Due to the simplicity of synthesis and no need for initiator or catalyzer and organic solvent, the strategy described here could find a promising application in the preparation of hydrogels.

1. Introduction

Hydrogels are a class of cross-linked polymers that can swell in water without dissolving [1]. As is well-known, living organs possess fantastic functions in soft and wet gel-like state. Therefore, hydrogels have been the research focus of many material scientists as excellent wet materials which are suitable for making artificial organs for medical treatments [2]. Especially, stimuli responsive or so-called "smart" polymer gels have been studied extensively during the past decades, which are capable to undergo quite strong and abrupt physical or chemical changes in response to small external changes such as pH, temperature, ionic strength, electric and magnetic field, etc. [3]. By far, the most studied temperature-sensitive polymer gel is cross-linked poly(*N*-isopropylacrylamide) [4,5]. Due to its special properties, poly(*N*-isopropylacrylamide) is a good candidate material for many applications, such as in artificial muscles, drug delivery systems, separation membranes, catalysis substrates, actuators, and chemical valves [6]. Poly(*N*-isopropylacrylamide) gels exhibit a temperature-induced volume phase transition in water upon heating to above its lower critical solution temperature (LCST) at 32 °C, and the LCST can be appropriately adjusted by copolymerizing *N*-isopropylacrylamide with a more hydrophilic monomer or a more hydrophobic monomer [7–11]. Below LCST, the poly(*N*-isopropylacrylamide) hydrogel takes in a lot of water in its network, and thus exhibits a swollen state. But above its LCST, its swollen state collapses and displays an abrupt reduction in volume. And this process of phase transition is thermoreversible [12,13]. These characteristics make poly(*N*-isopropylacrylamide) hydrogels a particularly interesting class of "intelligent" materials. Generally, polymer hydrogels can be divided into two main classes: chemically cross-linked hydrogels, and physically cross-linked hydrogels [14]. The hydrogels formed by physical crosslink can perform a reversible gel–sol transition without catalyzer or initiator, but their mechanical strength is lower than that of the hydrogels formed by chemical crosslink and the loss in vivo is unavoidable due to humoral scour. Therefore, much attention has been paid to the hydrogels formed by chemical crosslink. But there are still some drawbacks in the traditional preparation methods of hydrogels. One is that the hydrogels formed by chemical crosslink usually contain catalyzer or initiator, which is difficult to be gotten rid of and debases the biocompatibility of materials. Moreover, traditional hydrogel synthesis relies upon uncontrolled crosslinking methods, such as radical chemistry, which leads to poorly defined materials and increases the difficulty in correlating the network structure with the final physical properties of the gel [15]. Therefore, methods of preparing hydrogels

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have been constantly explored so as to improve the properties of hydrogels [16,17]. The Diels–Alder reaction is a chemoselective reaction and water has an extraordinary rate-accelerating effect on the reaction process [18–21]. On the other hand, Diels–Alder reaction is thermally reversible, whose reaction degree can be controlled by temperature [22–28]. It is also one representation of "click"-typed reactions [29]. Furthermore, Diels–Alder reaction can be applied to the preparation of chiral polymer [30]. Based on these properties of Diels–Alder reaction, it can be speculated that Diels–Alder reaction is a promising reaction for the preparation of hydrogels. Chujo et al. reported the first example of a thermally reversible hydrogel through the covalent bond, and the hydrogel was prepared by means of intermolecular Diels–Alder reaction between furan-modified poly(*N*-acetylethylenimine) (PAEI) and maleimide-modified PAEI [31]. Later, they prepared polymer hybrids utilizing the Diels–Alder reaction [26,32]. Liu et al. reported gel formation through Diels–Alder in *N*, *N*-dimethylacetamide from polymers based on maleimide-containing polyamides and a tri-functional furan compound [33]. More recently, we demonstrated a novel gelation process based on the aqueous Diels–Alder reaction of poly(*N*, *N*-dimethylacrylamide-co-furfuryl methacrylate) and *N*-[4-(formyl polyethylene glycol ester) bismaleimide. It was found that water can accelerate Diels–Alder reaction while DMF can accelerate retro-Diels–Alder reaction. Swelling/shrinking kinetics indicates that the as-prepared hydrogels have high swelling ratio and can respond to temperature [34]. But its thermosensitivity is lower, so we replaced *N*, *N*-dimethylacrylamide by *N*-isopropylacrylamide, a most used monomer to prepare thermosensitive hydrogel, to improve its thermosensitivity. In order to enhance the biocompatibility, we use *N*-maleoyl-L-leucine (LMI) to synthesize polymeric dienophiles, which possess chirality and can be used to prepare chiral hydrogels. As a result, thermosensitive chiral hydrogels were obtained in our laboratory.

2. Experimental

2.1. Materials

Furfuryl methacrylate (>95.0%) was purchased from TCI, Japan. *N*, *N*′-Dicyclohexylcarbodiimide (DCC) and *N*-isopropylacrylamide (NIPA) were purchased from Sigma–Aldrich (Shanghai) Trading Co., Ltd. Maleic anhydride (99%) and L-leucine (98%) were obtained from Sinopharm Chemical Reagent Co., Ltd, China. 2, 2′-Azobisisobutyronitrile (AR) was produced by Shanghai Shanpu Chemical Co., Ltd, China. Poly(ethylene glycol) 2000 (PEG2k) was imported from Japan and distributed domestically. 2, 2′-Azobisisobutyronitrile (AIBN) was purified by crystallization from methanol 1, 4-Dioxane was distilled then dried over molecular sieve for 2 d. Triethylamine was distilled then dried over KOH. All other reagents used were of analytical grade.

2.2. Synthesis of poly(N-isopropylacrylamide-co-furfuryl methacrylate) (PIPAFM)

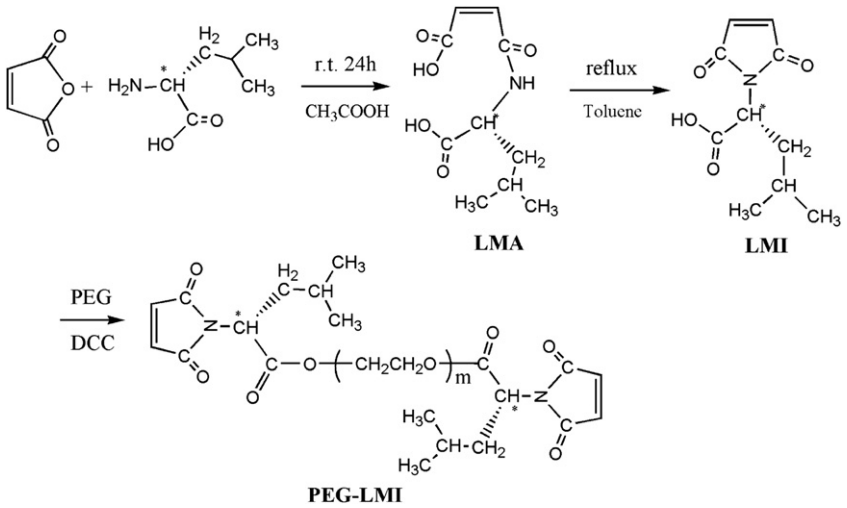
PIPAFM was synthesized by copolymerization of FM and NIPA. In brief, FM and NIPA were charged into a round-bottom flask with a magnetic stirrer under high pure nitrogen, with1, 4-dioxane as a solvent. Monomer and initiator concentrations were 0.3 and 5×10^{-3} mol/L, respectively. The flask was immersed in an oil bath held at 70 °C for 24 h. Afterwards, the flask was removed from the bath and the contents were immediately dropped into an excess of diethyl ether to afford the precipitates. The precipitated samples were washed with the precipitant and dried under vacuum until constant weight was attained. For the sake of expression, the copolymers prepared here are denoted PIPAFM-*n*, where *n* stands for a feed molar ratio of NIPA to FM. To afford a direct comparison, the homopolymers of NIPA and FM, denoted PNIPA and PFM, respectively, were also prepared.

2.3. Synthesis of N-maleoyl-L-leucine polyethylene glycol ester bismaleimide (PEG–LMI)

A water-soluble dienophile of PEG–LMI was prepared by the coupling reaction of PEG and *N*-maleoyl-L-leucine (LMI). LMI was synthesized by following the literature method [35]. Typically, PEG–LMI was synthesized by the below three steps, as shown in Scheme 1.

Firstly, maleic anhydride (9. 9 g, 0.1 mol), acetic acid (50 mL) and L-leucine (13.1 g, 0.1 mol) were charged into one-neck flask with a magnetic stirrer. After being stirred for 24 h at room temperature, the reaction mixture was distilled under reduced pressure to remove acetic acid and then dried under vacuum, (*S*)-*N*-maleamic acid-L-leucine (LMA) was obtained as a white powder. mp: 133–137 °C. (lit. mp 134–136.5 °C) [35].

Secondly, LMA (5.0 g, 22 mmol) was suspended into dry toluene (200 mL) in a there-neck flask with a mechanical agitator, then H_3PO_4 (85%, 0.7 mL) and triethylamine (6.5 mL, 47 mmol) were added in sequence. The resultant mixture was refluxed for



Scheme 1. Synthesis route of water-soluble dienophile (PEG–LMI).

1.5 h with vigorous stirring. Afterwards, the mixture was cooled to room temperature and organic solution was concentrated. The obtained residue was neutralized with 0.5 N HCl until pH = 3, and extracted with ethyl acetate. The organic phase was fully washed with water and saturated NaCl aqueous solution, then dried over Na_2SO_4, and concentrated under reduced pressure, crystallized in *n*-hexane to give (S)-*N*-maleoyl-L-leucine (LMI) as a white powder 2.0 g (Yield: 43%). mp: 89–91 °C. (lit. mp 88–89.5 °C) [35]. FTIR (KBr, thin film, cm^{-1}): 1736 and 1709 (C=O); 1607 (C=C); 1402 and 1387 (=C–H); 1273 (C–N); 1176 (C–O); 698 (cis-CH=CH).

^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 6.75 (s, 2H, –CH=CH–), 4.82–4.78 (dd, H, –CH), 2.31–1.83 (m, 2H, $-CH_2$), 1.58 (m, 1H, –CH), 0.94–0.91 (dd, 6H, $-CH_3$).

Finally, a novel water-soluble dienophile was prepared by the coupling of LMI with PEG2k under DCC. PEG was selected as the main structural component of dienophile due to its hydrophilicity and biocompatibility [36,37]. As a typical example, synthesis process of PEG–LMI was conducted as follows. PEG2k (4.00 g, 4 mmol) and LMI (1.86 g, 8.8 mmol) were dissolved in dry dichloromethane. After being cooled to 0 °C, a solution of DCC in dichloromethane was added dropwise, and the mixture was stirred at room temperature for 24 h under nitrogen atmosphere. The resulting mixture was filtered, washed with dichloromethane. The filtrate was precipitated in a large amount of diethyl ether. The precipitate was filtered out and dried under vacuum to constant weight (Yield: 90%).

2.4. Gelation by Diels–Alder reaction

A certain quantity of PEG–LMI was added into a prescribed amount of PIPAFM-*n* aqueous solution, and the total concentration of the admixture was 15 wt%. The resulting mixture was stirred to make the PEG–LMI dissolved, and then put into refrigerator at 9 °C and viewed the change of sol–gel (Scheme 2).

2.5. Disassembly by retro-DA reaction

5 mL of Dimethylformamide (DMF), dimethylsulphoxide (DMSO), xylenes, 1, 4-dioxane, and water were put into five 10 mL test-tubes, respectively, then 0.2 g of dry gel was added to each tube. After heated to different temperatures, disassembly time based on retro-DA reaction was determined when the gel disappeared.

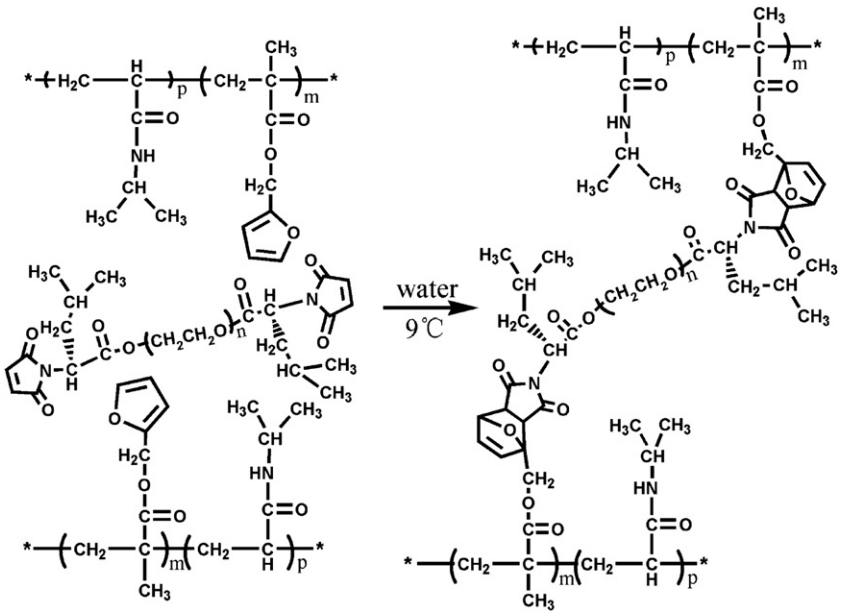
Table 1
Characterization of some copolymers.

Copolymer	NIPA/FM molar feed	NIPA/FM molar ratio in copolymer (by ^{1}H NMR)	Yield (%)	$\overline{M_n}$	$\overline{M_w}$	PDI
PIPAFM-5	5	4.3	82	9071	21 045	2.32
PIPAFM-10	10	5.8	90	8625	19 149	2.22
PIPAFM-15	15	7.4	86	10 695	21 926	2.05
PIPAFM-20	20	10.4	80	18 791	34 200	1.82
PIPAFM-25	25	14.5	89	10 450	20 482	1.96

2.6. Characterization

FTIR spectra were measured using Shimadzu IR Prestige-21 FTIR spectrometer at room temperature, in the range from 4000 to 500 cm^{-1}, with a resolution of 2 cm^{-1} and 20 scans. Samples were prepared by well dispersing the complexes in KBr and compressing the mixtures to form disks. The ^{1}H NMR spectra were recorded at room temperature on a Bruker DPX-400 NMR instrument with tetramethylsilane (TMS) as internal standard. Gel permeation chromatography (GPC) analysis was carried out with an SHIMADZU LC-10AVP chromatographic system equipped with a (SHIMADZU) shim-pack GPC-803 chromatographic column. THF was used as eluent at a flow rate of 1.0 mL/min. Monodispersed polystyrene standards were used to obtain a calibration curve. Scanning electron microscopy experiments were performed by a model S-4800 field emission scanning electron microscope (Hitach: HIGH-TECHNOGIES CORPORATION, Japan). After freeze-drying, samples were placed in a sputter coater and covered by a thin gold film. Afterwards, they were fixed in the microscope support by a carbon tape. Working distance, spot size, pressure and accelerating voltage were adjusted for each sample in order to obtain the best resolution.

The thermal behavior or LCST of polymer aqueous solutions was observed by transmittance measurements using a TU-1900 dual-beam UV–visible spectrophotometer (Beijing purkinje general



Scheme 2. Chemical structure of hydrogels formed by DA reaction.

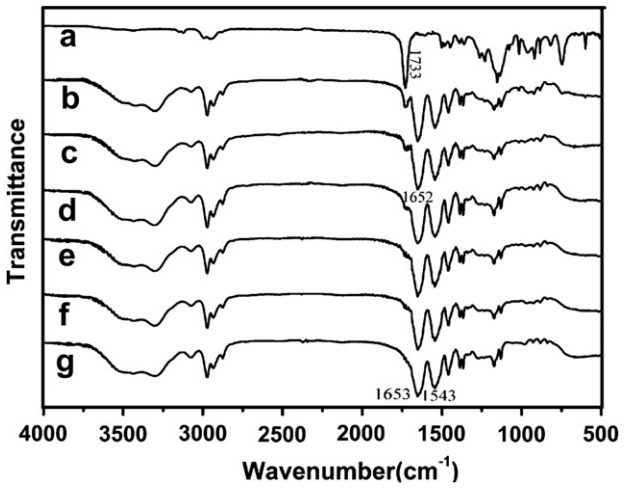


Fig. 1. FTIR spectra of polymers: (a) PFM; (b) PIPAFM-5; (c) PIPAFM-10; (d) PIPAFM-15; (e) PIPAFM-20; (f) PIPAFM-25; (g) PNIPA.

instrument Co., Ltd., China) equipped with a temperature controller. The samples for the UV–vis experiments were prepared in quartz colorimetric cell (10 mm in thickness) with a concentration of 1 g/L. As a reference we used a colorimetric cell filled with doubly distilled water. All extinction measurements were performed at a wavelength of 500 nm. The transmittance of the copolymer solutions was recorded as a function of temperature. The temperature was varied at a step width of 0.5 K/10 min. LCSTs of the polymer solutions at different concentrations were defined as the temperature at which the optical transmittance of the solution reduced to 10% of its original value.

Chirality of the hydrogels was characterized by circular dichroism (CD). CD spectra were recorded on a JASCO J-810 spectrophotometer at room temperature. Before measurement, LMI and PEG–LMI were dissolved in water respectively and the xerogels were prepared to be thin films.

The gelation time of the mixture was measured by a vial inversion method. Aqueous solutions containing a prescribed amount of PIPAFM and PEG–LMI were put into a refrigerator maintaining at a temperature of 9 °C until the gelation occurred. The gelation time was visually determined when the mixture did not flow by inverting the vials.

The swelling behavior of dried hydrogels was studied by a general gravimetric method. A certain amount of dry gel was incubated in distilled water at 37 °C, and the swollen weight for each sample was recorded at regular time intervals after excess surface water was blotted carefully with filter paper. The procedure was repeated until there was no further weight increase. While the temperature increased gradually the swollen hydrogels began to shrink. The temperature was maintained constant for 12 h after increased by each 3 °C, and then the weight of shrunk hydrogel was measured. The swelling ratio (SR) was calculated by the following equation:

$$SR = (m_1 - m_0) \times 100\%/m_0$$

where m_0 stands for the initial weight of dried gel, and m_1 stands for the weight of the swelling gel at a particular temperature and a prescribed time interval.

3. Results and discussions

3.1. Synthesis of PIPAFM

When using toluene as a solvent of copolymerization, the solution become cloudy after reacting for some time, so we use 1,4-dioxane as a solvent in order to keep the solution transparent during polymerization. In order to suppress the gelation which may be attributed to lability of the carbon-5 of the furan ring during polymerization of furan derivatives, we reduce the initial total concentration of monomers to 0.3 mol/L, which is obtained from the reference and our experiment results [34,38]. The GPC data show that all the obtained macromers have an unimodal peak with a polydispersity index (PDI) around 2 (Table 1). Molar feed is different from molar ratio in copolymer, which is relevant to reactivity ratios of NIPA and FM and detailed research on this aspect is ongoing.

FTIR spectra of PFM (a), PIPAFM-5 (b), PIPAFM-10 (c), PIPAFM-15 (d), PIPAFM-20 (e), PIPAFM-25 (f), PNIPA (g) are presented in Fig. 1.

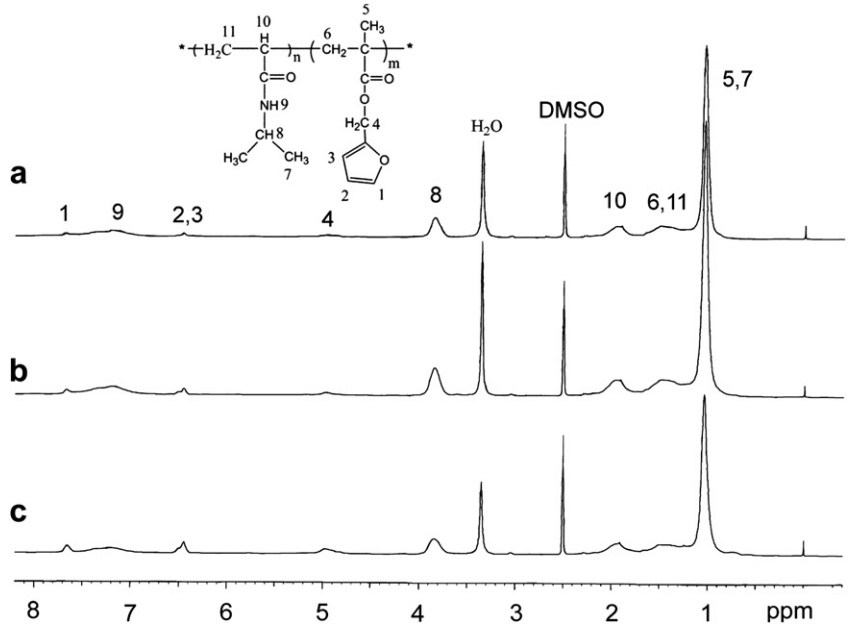


Fig. 2. ^{1}H NMR spectra of PIPAFM-25 (a), PIPAFM-15 (b), PIPAFM-5 (c) in DMSO-d_6.

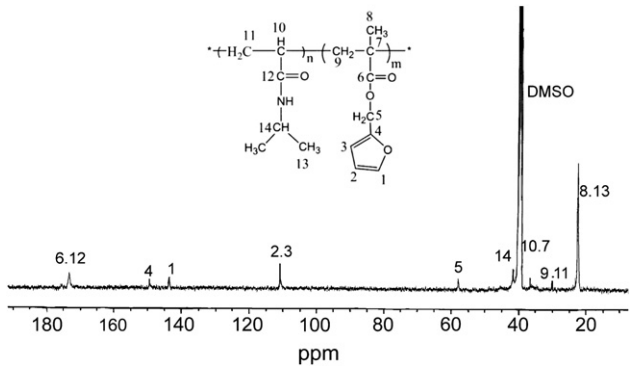


Fig. 3. ^{13}C NMR spectrum of PIPAFM-5 in DMSO-d_6.

The PFM spectrum shows the band at 1733 cm^{-1} assigned to the C═O stretching mode and the bands at 1266 cm^{-1},1223 cm^{-1} and 1157 cm^{-1} attributed to the symmetric and asymmetric C–O–C stretching mode. In the PNIPA spectrum, the amide I band (C═O stretch) emerges at 1653 cm^{-1}, the amide II band (N–H vibration) at 1543 cm^{-1}, and the methyl groups (in isopropyl group) at 1362 and 1386 cm^{-1}. The broad peak at the range from 3200 to 3600 cm^{-1} belongs to the N–H or O–H vibration. Moreover, the characteristic absorptions of NIPA and FM emerge in the spectra of copolymers (curve b, c, d, e, f) with a little shift. It can be seen that the intensity of the peak at about 1733 cm^{-1} becomes weaker and the intensity of the peak at about 1653 cm^{-1} becomes stronger from curve b to curve f as the NIPA to FM molar ratio augments.

^1H NMR spectra of PIPAFM-25 (a), PIPAFM-15 (b), PIPAFM-5 (c) were recorded in DMSO at room temperature (Fig. 2). The peak at 4.96 ppm is assigned to the $-O-CH_2$ protons of FM, and the signals at 7.65 ppm and 6.44–6.50 ppm correspond to the protons of the furan ring, whereas the broad peak at 7.17–7.32 ppm belongs to the –NH protons. The copolymer compositions were estimated by comparison of the integrated intensities of resonance signals at 7.65 ppm and 3.84 ppm, which varies in accordance with the molar feed ratio (Table 1). ^{13}C NMR spectrum of PIPAFM-5 was also recorded in DMSO at room temperature. As shown in Fig. 3, the signals at 173, 149, 144, 111, 58, 42, 36, 30, 22 ppm correspond to different carbons of the copolymer.

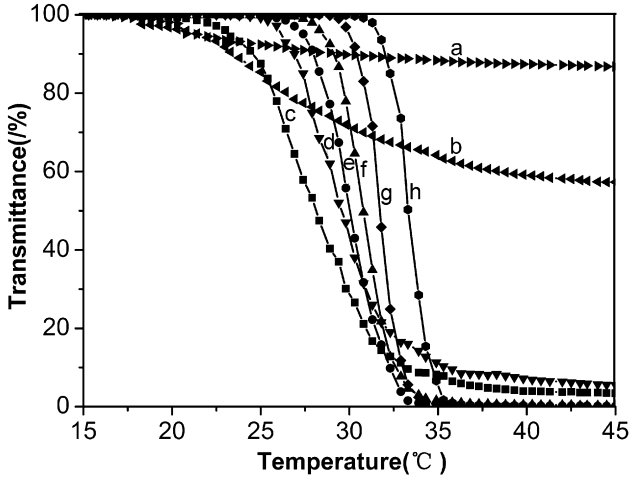


Fig. 4. Transmittance changes for aqueous solutions of polymers as a function of temperature: PIPAFM-10 (a), PIPAFM-15 (b), PIPAFM-20 (c), PIPAFM-25 (d), PIPAM-30 (e), PIPAFM-40 (f), PIPAFM-50 (g), PNIPA (h).

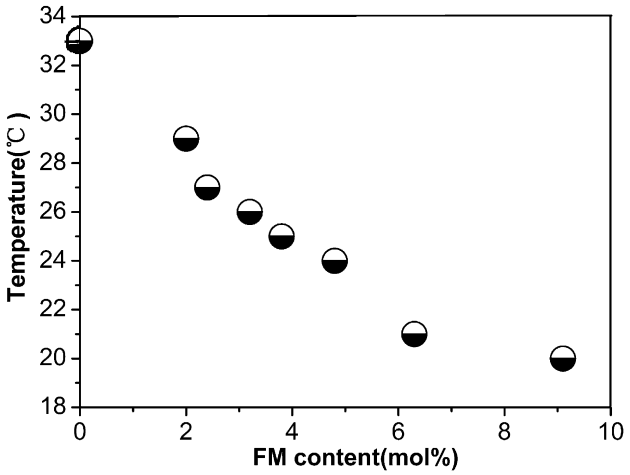


Fig. 5. LCST for aqueous solutions of polymer as a function of FM content.

3.2. Temperature-responsive property of PIPAFM-n and PNIPA

In this study, we selected the most popular thermosensitive poly (*N*-isopropylacrylamide) as main components of polymeric dienes for the purpose of in situ hydrogel formation via Diels–Alder chemistry. In a pure poly(*N*-isopropylacrylamide) system, there generally exists a hydrophilic/hydrophobic balance in the NIPA unit resulting from the hydrophilic (amide group) and hydrophobic (isopropyl group) regions of the PNIPA. The temperature sensitivity of the PNIPA hydrogel is attributed to its uniquely rapid alteration in hydrophilicity and hydrophobicity of the matrix [39]. LCST is the typical feature of temperature-sensitive PNIPA-based materials and it means the critical temperature where hydrophobic interactions of isopropyl groups of PNIPA outweigh the hydrophilic nature of the amide groups on the pendant groups [40].

Below its LCST, the hydrophilic groups in the side chains bond to water molecules through hydrogen bonds, and PNIPA hydrogel subsequently exhibit swelling state. However, as the external

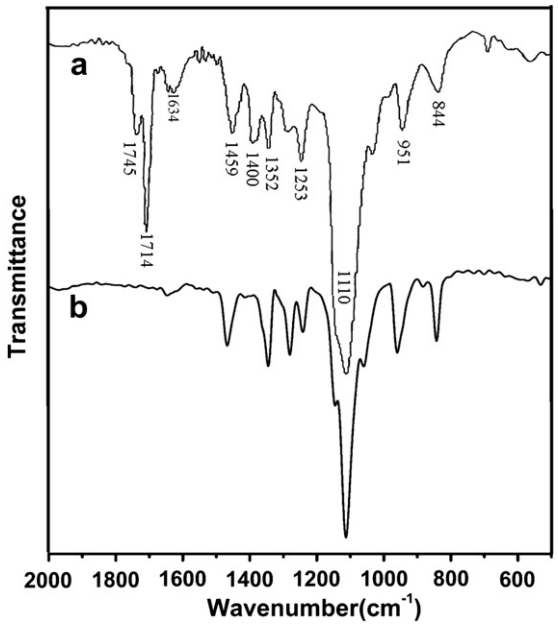


Fig. 6. FTIR spectra of PEG–LMI (a) and PEG (b).

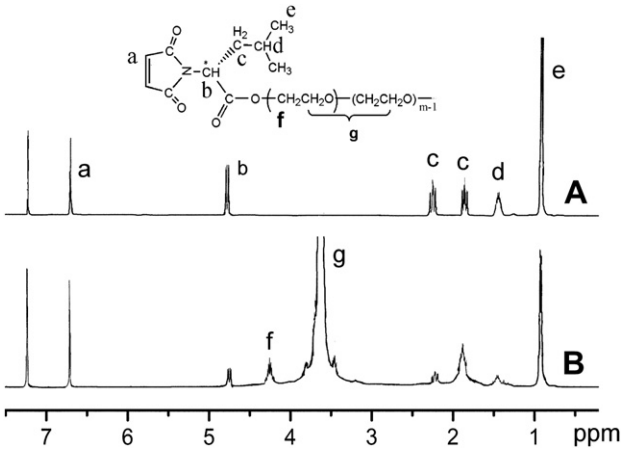


Fig. 7. ^{1}H NMR spectra of LMI (A) and PEG–LMI (B).

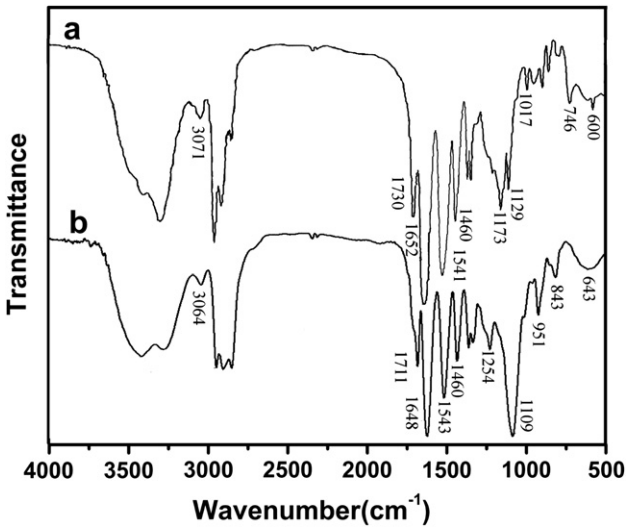


Fig. 9. FTIR spectra of copolymer PIPAFM-10 (a) and the corresponding hydrogel (b).

temperature increase, the hydrogen bonds are broken and the hydrophobic interactions among the hydrophobic groups become dominant. As a result, entrapped water molecules via hydrogen bonds in hydrogel are released from the network, leading to collapse of polymer chains and phase separation of hydrogel [41]. The LCST of PNIPA can be adjusted by copolymerizing NIPA with other comonomers. It is found that the incorporation of ionic hydrophilic moieties into the PNIPA hydrogel networks would enhance its LCST and the gels become sensitive towards pH, whereas hydrophobic moieties decrease the LCST [3,42]. Herein, it can be seen from the following experiment result that the LCST of PIPAFM-*n* is lower than the LCST of PNIPA due to hydrophobicity of PFM.

We estimated the LCSTs of the polymers in water by means of transmittance measurements of the aqueous solutions of polymer. Fig. 4 shows the transmittance change in the polymer solutions. It can be seen that all the polymer solutions show a transmittance change when the temperature increases, which indicates all the polymer solutions are temperature-responsive. In addition, the thermosensitivity increases with the decrease of the FM content in polymer, which shows more NIPA content is of advantage to retain the thermosensitivity of polymer. Fig. 5 shows the relationship between the LCST of the copolymers and the FM content. The LCST of the copolymer solutions shifts to a lower temperature with the increase of FM content because the hydrophobcity of the copolymers can be improved by increasing the FM content in copolymer. Moreover, on the basis of the results in Fig. 5, it is revealed that the LCST of the copolymers could be easily controlled by varying the feed ratio of the comonomers. As is well-known, phase transition

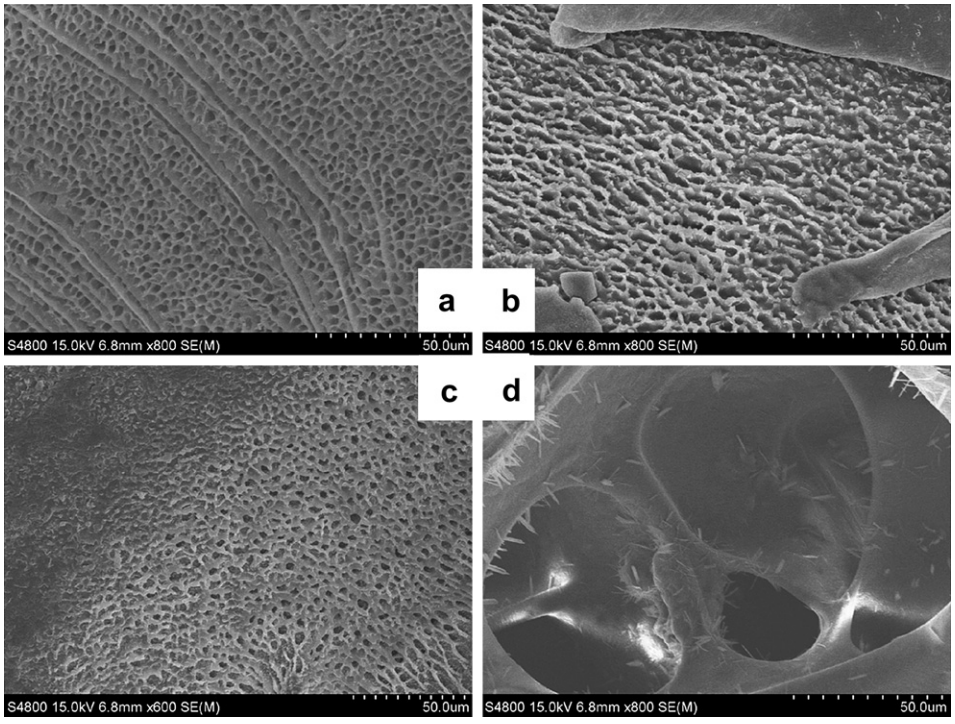


Fig. 8. SEM images of the hydrogel surfaces: (a) PIPAFM-10; (b) PIPAFM-15; (c) PIPAFM-20; (d) PIPAFM-25.

Table 2
Disassembly time of the hydrogels (from PIPAFM-15) at different temperatures and solvents.

Solvents	Water	DMSO	DMF	Xylenes	Dioxane
Dielectric constant [43]	79.7	46.6	36.7	2.3	2.21
Disassembly time (80 °C)	No[a]	No[a]	No[a]	No[a]	No[a]
Disassembly time (100 °C)	No[a]	315 min	600 min	No[a]	No[a]
Disassembly time (135 °C)	–	25 min	35 min	180 min[b]	–

[a] The gel didn't disappear in 600 min.
[b] The gel is most disassembled.

temperature control is very important in some biomedical applications.

3.3. Synthesis of PEG–LMI

The FTIR spectra of PEG–LMI (a) and PEG (b) are shown in Fig. 6. In PEG–LMI sample, the two peaks around 1714 and 1745 cm^{-1} (C=O) are attributed to the carbonyl group stretch in PEG–LMI. The peaks at 1400 and 1352 cm^{-1} are produced by isopropyl. The stretch of C–N (1253 cm^{-1}) and C=C (1634 cm^{-1}) is also observed in the spectrum of PEG–LMI.

^{1}H NMR spectra of LMI (A) and PEG–LMI (B) are presented in Fig. 7. It can be seen that the characteristic peaks of LMI emerge in the spectrum of PEG–LMI. The protons in PEG give peaks around 4.43–3.44 ppm. The degree of functionality of PEG–LMI can be estimated by comparison of the integrated intensities of resonance signals at 6.74 ppm and 4.43–3.44 ppm, which is about 63%.

3.4. Gelation by DA reaction

After a certain amount of PIPAFM-*n* was dissolved in 5 mL of water in a vial (20 mm in diameter and 45 mm in length), then to this added a prescribed amount of PEG–LMI, making the total concentration of PIPAFM-*n* and PEG–LMI 15 wt%, gelation was observed after a period of time. It was found that gelation time increased with the decrease of FM content in copolymer and the gelation times of PIPAFM-10, PIPAFM-15, PIPAFM-20, PIPAFM-25 were about 20 h, 35 h, 55 h, 76 h, respectively. The rate of gelation was faster when the content of furanic monomer in the copolymer is higher. SEM images of freeze-dried hydrogels via DA reaction of PIPAFM-*n* and PEG–LMI are shown in Fig. 8. The structure was found to be porous, as is well-known, porous structure is important to tissue engineering scaffold.

The FTIR spectra of the gel show important changes compared with those of the initial polymers, which clearly confirms that the DA reaction has taken place.

FTIR spectra of copolymer PIPAFM-10 (a) and the corresponding hydrogel (b) are shown in Fig. 9. In the spectrum of copolymer PIPAFM-10, the band at about 3071 cm^{-1} is attributed to the =C–H stretching mode, and the band at 1730 cm^{-1} is due to ester carbonyl in FM segments, and the band at 1652 cm^{-1} is from carbonyl stretching vibration in NIPA while the band at 1541 cm^{-1} belongs to bending vibration of N–H. The peaks at 1234 cm^{-1}, 1173 cm^{-1}, 1017 $cm^{-1}$are due to C–H deformation vibration in furan ring, whereas the peaks at 978 cm^{-1}, 922 cm^{-1} are produced by out-of-plane deformation vibration, at 746 cm^{-1} C–H out-of-plane bending vibration, at 600 cm^{-1} cyclic-deformation vibration. After forming gel, the differences are mostly in the carbonyl region and in the low frequency area. The bands at 1652 cm^{-1} and 1730 cm^{-1} shift to 1648 cm^{-1} and 1711 cm^{-1}, respectively, possibly due to peaks of different carbonyl overlapping each other, and the typical bands arising from the presence of the furan rings disappeared. In addition, =C–H stretching also shifts from 3071 cm^{-1} to 3064 cm^{-1} after gelation, which may indicate the disappearance of conjugative double-bond. It was also confirmed that DA reaction took place during gelation via a blank experiment, in which a solution of copolymer or PEG–LMI alone was left in the same experimental conditions, and crosslinking did not occur. Hence it can be inferred with confidence that gelation was due to DA reaction.

3.5. Disassembly by retro-DA reaction

Disassembly times based on retro-DA reaction in different solvents are presented in Table 2. When the temperature is below 80 °C, the gels were not disassembled in all five solvents after 10 h. When the temperature is at 100 °C, the gels were not disassembled in xylenes, dioxane and water, but the gel disappeared in DMSO within 315 min and disappeared in DMF within 600 min. When the temperature is at 135 °C, the gel in DMSO disappeared within 25 min, and the gel in DMF disappeared within 35 min, at the same time the gel in xylenes most disappeared after 180 min. All these facts indicate the gel is stable in water and retro-DA reaction is interrelated with the solvents used. It seems that bigger polarity of

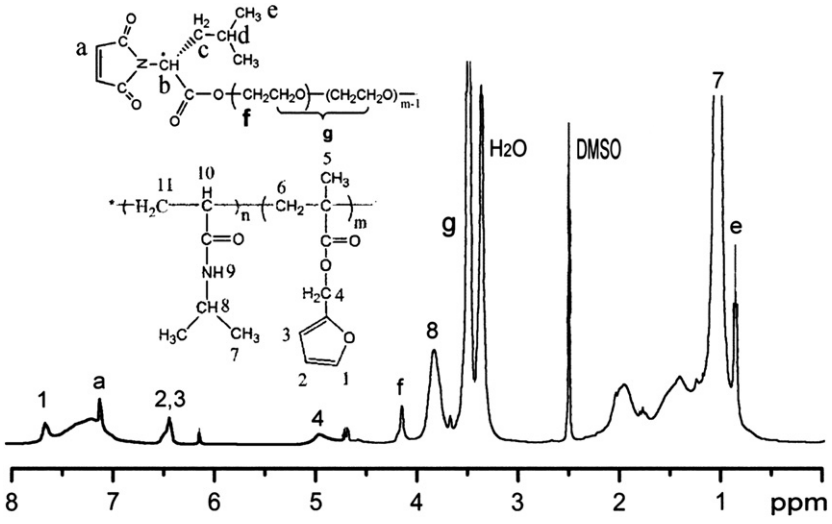


Fig. 10. ^{1}H NMR spectra of the mixture from the disassembly of hydrogel in DMSO-d_6.

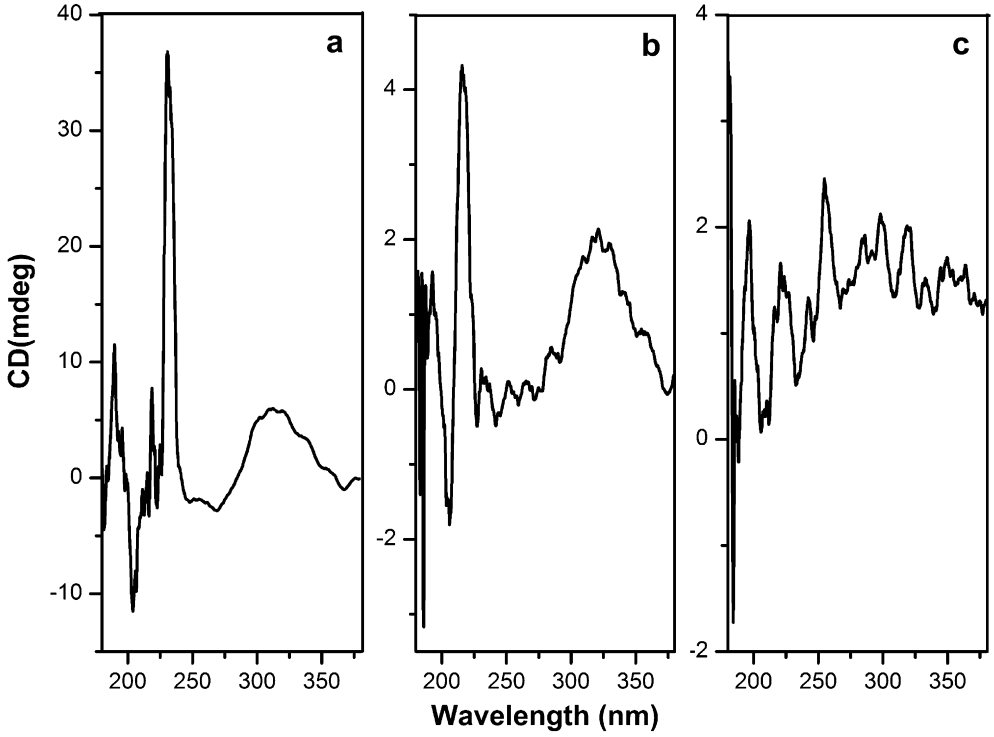


Fig. 11. CD spectra of LMI (a), PEG–LMI (b) and representative hydrogel (c).

organic solvents is of advantage to disassembly of the gel. Additionally, the disassembly time shortens with the increase of temperature. When DMSO-d_6 was used as a solvent of disassembly of the gel, ^{1}H NMR spectrum of the resultant mixture was recorded directly after the gel disappeared. As shown in Fig. 10, the special bands of furans reappear after disassembly, which confirms the occurrence of the retro-DA reaction.

3.6. CD analysis

As is shown in Fig. 11, the CD spectra of LMI and PEG–LMI have similarity in shape and position. The broad peaks around 320 nm are produced from conjugative system in LMI and PEG–LMI. After forming gel by DA reaction, the broad peak disappears because of disappearance of conjugative system. However, from180 nm to 380 nm, the gel produces obvious CD signals (varying between 3.56 and −1.73 mdeg), which indicates chiral structures exist.

3.7. Swelling behavior

The swelling ratios of the as-prepared hydrogels were measured in distilled water at 17 °C. After reaching the swelling equilibrium, these hydrogels were left at 10, 13, 16, 19, 22, 25, 28, 31, 34, 37, 40, 43, 46, 49, 52, 55, 58, 61 °C in turn in the thermostatic water bath for 12 h before measuring the swelling ratio changes. As depicted in Fig. 12, the hydrogels have high swelling ratios in water at 17 °C, which reach swelling equilibrium in about 12 h, and the equilibrium swelling ratio increases with the decrease in cross-link density. In order to study the thermosensitivity of the hydrogels, the swelling behavior of the hydrogels was measured between a broad temperature range from 10 °C to 61 °C. It was found that the swelling ratios of the hydrogels have temperature sensitivity, just as expected. As shown in Fig. 13, when temperature increased from 10 °C to 61 °C, the swelling ratio of the hydrogels decreased. Deswelling rate was faster at the beginning and became slower when temperature was higher than 34 °C, reaching a deswelling limit gradually. It also could be seen from Fig. 13 that the rate of thermosensitivity increased with the decrease in the cross-link density. The reason may be that the less cross-link density has less bondage to PNINA segments, which benefit to bring thermosensitivity of PNIPA into play.

4. Conclusions

In conclusion, thermosensitive hydrogels based on PNIPA were prepared by means of clean and efficient DA reaction in water. The preparation process is facile and the reaction conditions are mild with neither initiator nor organic solvent. The gelation time can be controlled by changing the component of copolymer, whereas swelling behavior can be adjusted by varying temperature and

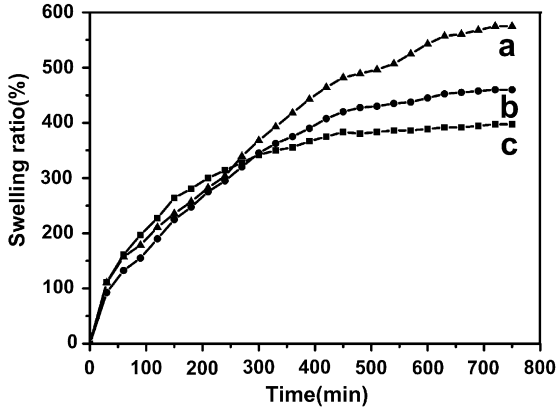


Fig. 12. The swelling ratio of the hydrogels as a function of time at 17 °C from PIPAFM-20 (a), PIPAFM-15 (b), PIPAFM-10 (c).

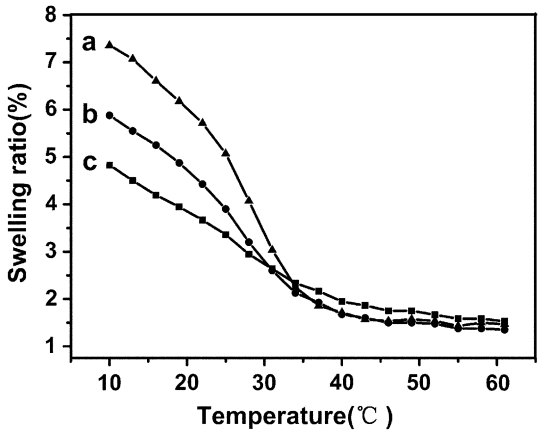


Fig. 13. The swelling ratio of the hydrogels as a function of temperature from PIPAFM-20 (a), PIPAFM-15 (b), PIPAFM-10 (c).

crosslinking density. And the disassembly time of the hydrogels is affected by temperature and solvents used. The method presented here has potential application in biomaterials. The study on the application of the as-prepared hydrogels is currently in progress.

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