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Thermosensitive hydrogels synthesized by fast Diels–Alder reaction in water

Hong-Liang Wei*, Zhe Yang, Li-Mei Zheng, Yan-Min Shen

School of Chemistry and Chemical Engineering, Henan University of Technology, Zhengzhou 450001, PR China

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ABSTRACT

Novel thermosensitive hydrogels have been synthesized by aqueous Diels–Alder reaction of poly(N,N-dimethylacrylamide-co-furfuryl methacrylate) and N-[4-(formyl polyethylene glycol ester) bismaleimide. The gelation time was measured by a vial inversion method and the swelling behavior of dried hydrogel was studied by a general gravimetric method. It was found that water can accelerate Diels–Alder reaction while DMF can accelerate retro-Diels–Alder reaction. The gelation time decreases with the increase of temperature. Swelling/shrinking kinetics indicates that the as-prepared hydrogels have high swelling ratio and can respond to temperature. The strategy described here provides several advantages for the hydrogel formation including mild reaction conditions, no initiator or catalyzer, no organic solvent, tunable gelation rate, and thermal reversibility.

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1. Introduction

Hydrogels are hydrophilic and crosslinked polymer networks that can absorb plenty of water while maintaining their dimensional stability [1]. They are solids on the macroscopic scale: they have definite shapes and do not flow. At the same time, they behave like solutions on the molecular scale: water-soluble molecules can diffuse in hydrogels with various diffusion constants reflecting the diffusant size and shape [2]. Because of the similarity between the highly hydrated three-dimensional networks and the hydrated body tissues, hydrogels have been widely used in biomedical fields [3,4]. Hydrogels were the first biomaterials designed for use in the human body [5]. There are two highlight research fields in hydrogels nowadays: one is injectable hydrogels and the other is intelligent (or smart) hydrogels. The two research fields not only have difference but also have close relationship, which have been widely studied in recent years. Injectable polymeric biomaterials have great advantages as they can be applied directly to fill tissue defects via in situ hardening or fabricated into preformed tissue-engineering scaffolds, which may be one important direction of development of the future biomaterials [6,7]. Smart hydrogels have been used in diverse applications, such as in making artificial muscles, chemical valves, controlled cell attachment–detachment, and concentrating dilute solutions in bio-separation [8–10].

Hydrogels can be divided into two categories according to their formation principle: physically and chemically crosslinked hydrogels. The hydrogels formed by physical crosslink can carry through a reversible gel–sol transition without any catalyzers or initiators, but their mechanical strength is lower than the hydrogels formed by chemical crosslink. The hydrogels formed by chemical crosslink usually contain a 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. This results in poorly defined materials and increases the difficulty in relating the network structure to the final physical properties of the gel. For these reasons, it would be highly desirable to develop alternative chemistries for the formation of crosslinked hydrogel materials which combine the best features of traditional strategies with an increased level of chemical control and diversity [11]. In search of ideal reactions of preparing hydrogels, Michael reaction [12], click reaction [11], and Diels–Alder reaction (DA reaction) are paid great attention. The DA reaction involves a ring-forming coupling between a dienophile and a conjugated diene which can be described by a symmetry-allowed concerted mechanism without forming biradical or zwitterionic intermediates [13]. The Diels–Alder approach, which involves a diene and a dienophile not present in any biomolecule, allows for a chemoselective reaction without the need for protecting groups, and water has an extraordinary rate-accelerating effect on the reaction process [14–16]. In addition, DA reaction is thermally reversible, whose reaction degree can be controlled by temperature [17–26]. Thermoreversible polymerization processes have the potential to provide materials of varied and unique utility [27]. Therefore, DA reaction is one promising reaction to prepare hydrogels, especially to prepare

* Corresponding author. Tel.: +86 371 67756849; fax: +86 371 67756718.
E-mail address: wtroy68@yahoo.com.cn (H.-L. Wei).

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smart hydrogels and injectable hydrogels. A great deal of work on utilization of DA reaction in polymer crosslinking reactions to build up the polymer networks has been done [17–30], but there are very few reports on applying Diels–Alder reaction to preparation of hydrogels in water.

Herein, a water-soluble polymer possessing furan pendants (dienes) was prepared by free radical copolymerization of N,N-dimethylacrylamide and furfuryl methacrylate. And a novel water-soluble polymer bearing maleimide terminals (dienophiles) was prepared by the coupling of N-4-(chlorocarbonyl)phenyl] maleimide(*p*-CPMIC) with polyethylene glycol (PEG). PEG was selected as the main structural component of the polymer due to their excellent physical and biological properties, including solubility in water and organic solvents, lack of toxicity, and absence of immunogenicity [31,32]. The as-prepared polymers possessing dienes and dienophiles can carry out DA reaction to synthesize hydrogels in water at a low temperature with a fast rate. The hydrogels prepared in this way have not been reported yet to the best of our knowledge.

2. Experimental

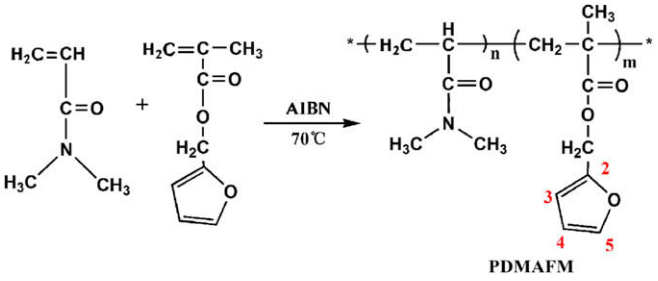
2.1. Chemicals

Furfuryl methacrylate (>95.0%) and N,N-dimethylacrylamide (>98.0%) were purchased from TCI, Japan. Maleic anhydride (AR) and *p*-aminobenzoic acid (AR) 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 (PEG 2K) was imported from Japan and distributed domestically.

2,2′-Azobisisobutyronitrile (AIBN) was purified by crystallization from methanol (mp 104 °C). N,N-Dimethylformamide(DMF) and toluene were distilled then dried over anhydrous magnesium sulphate for 2 d. Triethylamine was distilled, then dried over KOH. All other reagents used were of analytical grade.

2.2. Synthesis of poly(N,N-dimethylacrylamide-co-furfuryl methacrylate) (PDMAFM)

PDMAFM was synthesized by copolymerization of furfuryl methacrylate (FM) and N,N-Dimethylacrylamide (DMA), as shown in Scheme 1. FM and DMA were charged into a round-bottomed flask with a magnetic stirrer under high pure nitrogen, with toluene as a solvent. Monomer and initiator concentrations were 0.5 and 5×10^{-3} mol/L, respectively. The flask was immersed in an oil bath held at the required temperature of polymerization. After the prescribed reaction time, the flask was removed from the bath and the contents were immediately poured into a large excess of diethyl ether. The precipitated samples were washed with the precipitant and dried under vacuum until constant weight was attained. Yield: 65%. For the sake of expression, the copolymer prepared here is denoted PDMAFM-n, where n stands for a feeding molar ratio of N,N-Dimethylacrylamide to furfuryl methacrylate. IR (KBr, thin film, cm^{-1}): 1746, 1638 (C=O), 2931 (CH_3, CH_2), 1347 (C–N), 3113, 1497 (HC=CH), 1141 (C–O). ^1H NMR (400 MHz, D_2O, δ, ppm): 7.3 (H^5 of furan group), 6.20–6.31 (H^3, H^4 of furan group), 4.8 (O–CH_2), 2.4–2.8 (N–CH_3), 1.4(C–CH_2–C), 0.9 (α-CH_3).



Scheme 1. Synthesis of PDMAFM.

2.3. Synthesis of N-[4-(formyl polyethylene glycol ester) bismaleimide (PEG-DMI)

PEG-DMI was prepared from PEG 2K and N-4-(chlorocarbonyl)phenyl] maleimide (*p*-CPMIC), and *p*-CPMIC was synthesized according to a reference with some modification [33]. Typically, PEG-DMI was synthesized by the below three steps, as shown in Scheme 2.

Firstly, maleic anhydride (9.8 g, 0.1 mol), toluene (40 mL) and DMF (10 mL) were charged into three-neck flask with a water separator and a magnetic stirrer. After dissolving, 13.7 g (0.1 mol) of *p*-aminobenzoic acid was added slowly. After reacting at room temperature for 2 h, 0.2 g (1.2 mmol) of *p*-toluenesulfonic acid was added. The mixture was refluxed and dehydrated for 4 h. The resulting solution was poured into a large amount of water to give crude *p*-CPMI. The crude *p*-CPMI was filtered, washed with water, dried, recrystallized three times from acetone, and dried under vacuum until constant weight was attained. Yield: 80%; mp 243.5–244.8 °C (lit. mp 244 °C) [33]. FTIR (KBr, thin film, cm^{-1}): 3100 and 1398 (=C–H), 1719 and 1694 (C=O), 1603 (C=C), 1316 (C–N), 1150 (C–O), 694 (*cis*-CH=CH). ^1H NMR (400 MHz, DMSO-d_6, δ, ppm): 13.05 (1H, COOH), 8.06 and 7.51 (4H in phenyl), 7.22 (2H, CO–CH=CH–CO).

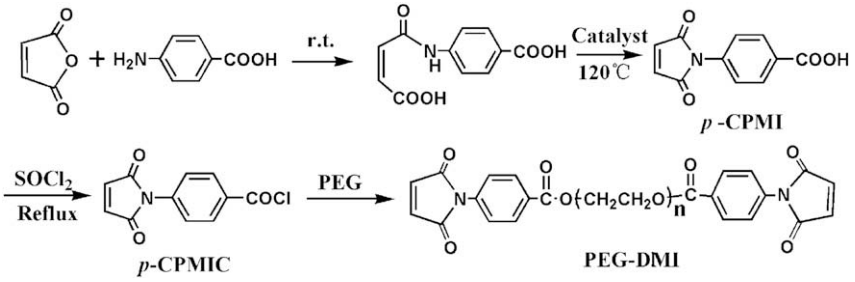
A mixture of *p*-CPMI (15 g, 0.07 mol), thionyl chloride (120 g, 1.01 mol), and hydroquinone (0.02 g, 0.18 mmol) was refluxed for 3 h. Unreacted thionyl chloride was evaporated out, and then the residual product was recrystallized from benzene to obtain pure *p*-CPMIC. Yield: 78%; mp 167.7–168.5 °C (lit. mp 168–169 °C) [33].

FTIR (KBr, thin film, cm^{-1}): 1775 and 1713 (C=O), 1599 (C=C), 1391 and 1373 (=C–H), 1304 (C–N), 1180 (C–O), 723 (*cis*-CH=CH).

Finally, water-soluble polymeric dienophile was prepared by the coupling of *p*-CPMIC with PEG. As a typical example, its synthesis was conducted as follows. In order to remove trace of water, 15 g of PEG 2K was dissolved in 150 mL of toluene in a round-bottomed flask with a magnetic stirrer. About 80 mL of toluene were distilled off using a water separator. After being cooled to room temperature, a precisely weighed amount of 1.9 mL triethylamine was added, then a solution of *p*-CPMIC was added dropwise and the mixture was stirred for 24 h at room temperature under nitrogen atmosphere. The product was precipitated in a large amount of diethyl ether. The precipitate was filtered out and dried under vacuum to constant weight. Yield: 94%. FTIR (KBr, thin film, cm^{-1}): 2883 (CH_2), 1720 (C=O), 1651 (C=C), 1362 (=C–H), 1279 (C–N), 1115 (C–O–C), 702(*cis*-CH=CH). ^1H NMR (400 MHz, D_2O, δ, ppm): 7.99–8.02 (4H in phenyl group), 7.32–7.35 (4H in phenyl group), 3.39–3.57 (m, OCH_2CH_2O), 6.84–6.87(4H, CO–CH=CH–CO).

2.4. Gelation by DA reaction

1 mL of PDMAFM-n aqueous solution (15 wt.-%) was mixed with 1 mL of aqueous solution containing a stoichiometric amount of PEG-DMI in a vial (20 mm in diameter and 45 mm in length). The resulting mixture was stirred for 4 min, and then put into thermostatic bath at different temperature until the gelation to occur (Scheme 3). The gelation time of the mixture was measured by



Scheme 2. Synthetic route for PEG-DMI.

a vial inversion method. It was visually determined when the mixture did not flow by inverting the vials.

2.5. Depolymerization by retro-DA reaction

0.5 g of dry gel was put into 40 mL of DMF in a flask, then heated to different temperature, depolymerization time based on retro-DA was determined when the gel disappeared.

2.6. Characterization

FTIR spectra were measured by 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 ^1H NMR spectra were recorded at room temperature on a Bruker DPX-400 NMR instrument with D_2O or DMSO as solvent and tetramethylsilane (TMS) as internal standard. Gel permeation chromatography (GPC) analysis was carried out with a chromatographic system equipped with a Waters 1515 isocratic HPLC pump and a Waters 2414 refractive index detector. THF was used as eluent at a flow rate of 1.0 mL/min. Monodispersed polystyrene standards were used to obtain a calibration curve.

The swelling behavior of dried hydrogels was studied by a general gravimetric method. A certain amount of dry hydrogels 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 moistened 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 2 h after increase by 10 °C each time, and then the weight of shrunk hydrogel was measured. The swelling ratio (SR) was calculated by the following equation:

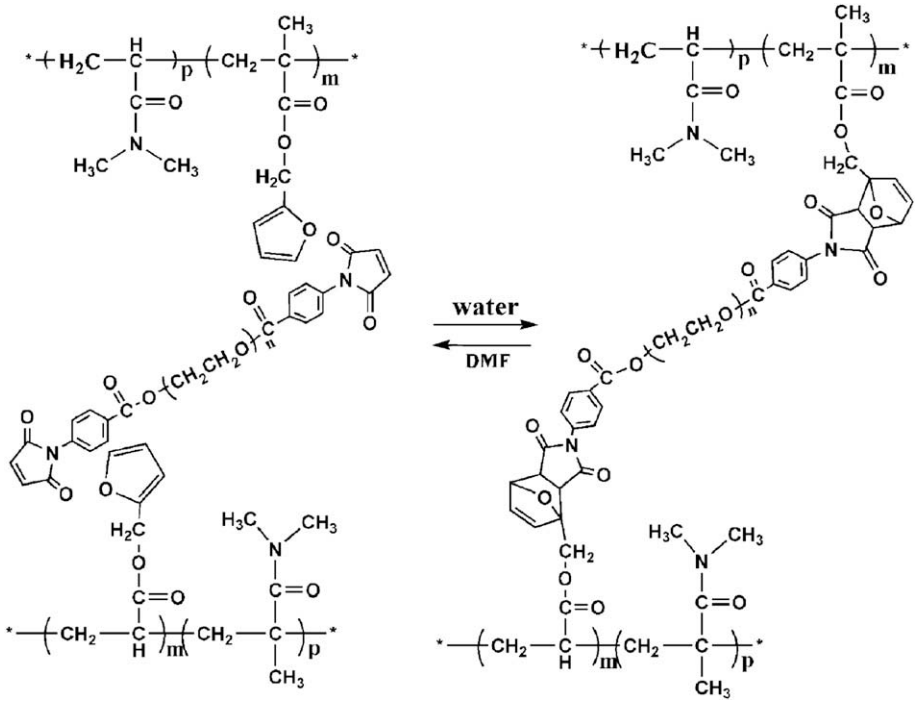
$$SR = (m_1 - m_0) \times 100\%/m_0$$

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

3. Results and discussion

3.1. Synthesis of PDMAFM

PDMAFM was synthesized by free-radical polymerization of furfuryl methacrylate (FM) and N,N-dimethylacrylamide (DMA)



Scheme 3. Chemical structure of hydrogel formed by DA.

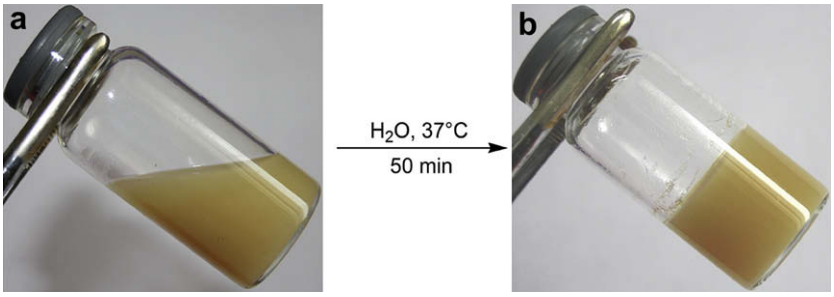


Fig. 1. Photographs in the process of gel formation: (a) hydrogel precursor solution; (b) hydrogel by DA reaction.

with an initiator of AIBN at 70 °C. Crosslinking during polymerization of furan derivatives was noted by some workers who attributed its occurrence to lability of the carbon-5 of the furan ring, facilitating attack by radicals and eventual formation of a crosslinked network structure. In order to suppress the gelation during polymerization, reducing the initial concentration of monomer is necessary [34]. Our observations are in agreement with this view. Therefore, a lower concentration of monomer (0.5 mol/L) was selected in our experiment. In order to get water-soluble copolymer, the molar feed ratio of DMA to FM was controlled to be not less than 5. The polymer of PDMAFM-5 obtained under these conditions exhibits weight-average molecular weight up to 2.34×10^4 g/mol with a polydispersity index of 2.5 in GPC.

3.2. Gelation by DA reaction

When we mixed 1 mL of PDMAFM-5 aqueous solution (15 wt.-%) and 1 mL of aqueous solution containing a stoichiometric amount of PEG-DMI in a vial (20 mm in diameter and 45 mm in length), gelation was observed after a period of time. The solution states before and after transition are shown in Fig. 1.

The gelation time of the as-prepared hydrogels at different temperatures in water and DMF was shown in Table 1. It can be seen that the gelation time is closely related with temperature and solution. In water, the gelation time decreases with the increase of temperature. When the temperature is 37 °C, the gelation time is only 50 min, which is much shorter than the former reports on synthesizing polymers based on DA reaction [22,24]. We think the as-prepared hydrogels is a good candidate for injectable hydrogels, although its gelation time is a little longer when used directly *in vivo*. As shown in Table 1, the gelation doesn't take place in DMF in control experiment, which indicates DMF can suppress DA reaction and water can accelerate DA reaction. Rate acceleration in homogeneous aqueous solution has been attributed to a variety of effects such as hydrophobic aggregation, cohesive energy density, or ground-state destabilization. The importance of hydrogen bonding in the acceleration of Diels–Alder reactions in aqueous solution is supported by both experimental and theoretical studies [35]. Herein, we also make a conjecture that micelle-like aggregates are formed in a solution of polymer carrying hydrophobic group due to hydrophobic interaction, which lead to convergence of furan and maleimide group and then higher local concentrations of dienes and dienophiles result in faster reaction rate. While using DMF as a solvent, diene and dienophile are separate and not easy to encounter each other, therefore, the reaction is difficult to carry out. The same procedure applied to PDMAFM-10 and PDMAFM-15, PDMAFM-20, less in reactive moieties, was also successful in producing gel in water but with a longer gelation time.

Table 1
Gelation time of the hydrogels at different temperatures and solutions.

Temperature (°C)	27	37	47	57	67	77
Gelation time in water (min)	160	50	30	20	15	10
Control experiment in DMF for 4 days	No gel	No gel	No gel	No gel	No gel	No gel

3.3. Depolymerization by retro-DA reaction

In order to study retro-DA reaction, 0.5 g of the as-prepared dry gel was put in water and refluxed for 10 h, and the gel didn't dissolve, which indicates its retro-DA reaction is difficult to take place in water. Therefore, we put the dry gel in DMF at different temperatures. The results are shown in Table 2. When temperature was 100 °C, the gel disappeared in 0.4 h in DMF, while the gel did not disappear in water at 100 °C, which indicates DMF can accelerate retro-DA reactions. The time of gel disappearance increases with the decrease in temperature. When temperature was below 70 °C, gels didn't disappear in 12 h. High reaction temperatures promoted the retro-DA reaction to increase the reaction rates and to shorten the time to transfer the polymer gels to clear polymer solutions [22,23]. When DMF was used as a solvent for depolymerization of gel, DMF can trap the polymeric diene and dienophile, and furan and maleimide groups generated by the retro-DA reaction cannot convergence to form micelles again in the sea of DMF to avoid regenerating the networks. While water was used as a solvent for depolymerization of gel, the polymeric diene and dienophile from retro-DA, if any, can react with each other quickly due to an extraordinary rate-accelerating effect of water on DA reaction, so the gel is stable in water.

3.4. Swelling/shrinking kinetics

The swelling ratios of the as-prepared hydrogels were measured in distilled water at 37 °C. After reaching the swelling equilibrium, these hydrogels were left at 37, 47, 57, 67, 77, 87 °C in turn in the thermostatic water bath for 2 h before measuring the swelling ratio changes. As depicted in Fig. 2(a), the hydrogel has a high swelling ratio in water and reach swelling equilibrium in about 10 h. Interestingly, the swelling ratio of the hydrogel is found to have temperature dependence. As shown in Fig. 2(b), the swelling ratio of the hydrogel decreases with the increase in temperature.

Table 2
Depolymerizaion time of the hydrogels in DMF at different temperatures.

Temperature (°C)	60	70	80	90	100
Depolymerizaion time (h)	No[a]	No[a]	5	2	0.4

[a] The gel didn't disappear in 12 h.

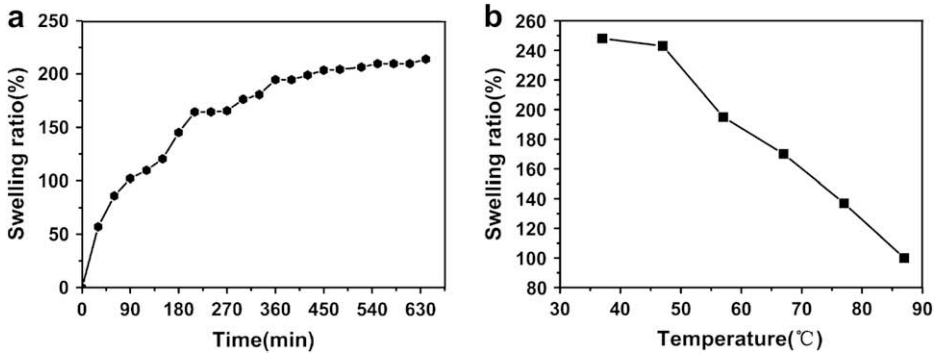


Fig. 2. Swelling curve of the hydrogel: (a) swelling ratios as a function of time; (b) swelling ratios as a function of temperature (from 37 to 87 °C).

4. Conclusion

We have demonstrated a novel and facile approach of preparing thermosensitive hydrogels by utilizing the DA reaction in water between maleimide and furan groups. The hydrogels are stable in water and retro-DA reaction doesn't take place even if temperature is increased to 100 °C, while an organic solution, such as DMF, can accelerate retro-DA reaction. The DA reaction can carry out in mild conditions in the absence of catalyzer with a fast rate. The gelation time can be controlled by temperature. In addition, the swelling ratio of the as-prepared hydrogels decreases with the increase of temperature. This approach holds a potential promise as a platform for preparing smart hydrogels and injectable hydrogels. The versatile studies to the approach will enhance the ability for biomaterial development greatly. The properties and application of this kind of hydrogels are now under investigations.

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