Diels–Alder Reaction in Water for the Straightforward Preparation of Thermoresponsive Hydrogels

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Received 15 October 2009; accepted 1 August 2010
DOI 10.1002/app.33116
Published online 8 November 2010 in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: Because the properties and applications of hydrogels are determined by the formation principle and conditions of the hydrogels, novel methods for preparing hydrogels have increasingly triggered scientists' interest. Here the Diels–Alder reaction was applied to the preparation of hydrogels. For the resultant polymeric diene and dienophile, the Diels–Alder reaction could be performed in water. The gelation time was found to be closely related to the temperature. The gelation time decreased with the temperature increasing. Moreover, the hydrogels were stable in water, and the retro-Diels–Alder reaction could be performed in *N,N*-dimethylformamide easily. A study of the swelling ratio indicated that the hydrogels were responsive to the temperature. The hydrogel formation method described here provides several advantages, such as mild reaction conditions, no initiator or catalyst, a tunable gelation rate, and thermal reversibility, and it has great potential for the preparation of biomaterials.
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Key words: biomaterials; gelation; hydrogels

INTRODUCTION

Hydrogels have attracted the interest of many investigators because of scientific interest and potential applications.[1–3] Because the properties and applications of hydrogels are determined by the formation principle and conditions of the hydrogels, novel methods for preparing hydrogels have increasingly triggered scientists' interest. In the process of exploring possible reactions for preparing hydrogels, researchers have paid more and more attention to the Michael reaction, the click reaction,[4,5] and the Diels–Alder (DA) reaction. The DA reaction generally provides simple, efficient, and clean procedures for generating new bonds by intermolecular or intramolecular coupling and represents one of the most useful synthetic methods in organic chemistry.[6] On the one hand, the DA reaction possesses atomic economy and generally requires no catalyst or initiator. On the other hand, the DA 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.[7,8] In addition, the DA reaction opens the way to the preparation of novel materials bearing mendable, recyclable, and thermally reversible features.[9] Therefore, the DA reaction is one promising reaction for preparing hydrogels and especially smart hydrogels and injectable hydrogels. Although a great number of reports on polymer preparation by the DA reaction have been released,[10–18] there is very little work on the preparation of hydrogels via the DA reaction in water. Recently, we reported a novel gelation process based on the aqueous DA reaction of poly(*N,N*-dimethylacrylamide-*co*-furfuryl methacrylate) (PDMAFM) and *N*-[4-(formyl polyethylene glycol ester) bismaleimide. We found that water could accelerate the DA reaction, whereas *N,N*-dimethylformamide (DMF) could accelerate the retro-Diels–Alder (retro-DA) reaction. The gelation time decreased with the temperature increasing. Swelling/shrinking kinetics indicated that the as-prepared hydrogels had high swelling ratios and could respond to the temperature.[19] More recently, in order to improve the biocompatibility of hydrogels, after a more detailed characterization of PDMAFM, we used a dienophile-terminated poly(ethylene glycol) (PEG) in place of *N*-[4-(formyl polyethylene glycol ester) bismaleimide as a dienophile, and some similar results were found. We think that this system is more meaningful because of the biocompatibility and chirality of *N*-maleoyl alanine (AMI).

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Contract grant sponsor: National Natural Science Foundation of China; contract grant number: 50773018.
Contract grant sponsor: Education Department of Henan Province; contract grant number: 2008A430003.
Contract grant sponsor: Science and Technology Department of Henan Province; contract grant number: 102300410119.

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EXPERIMENTAL

Materials

Furfuryl methacrylate (FM; 95.0%) and *N*,*N*-dimethylacrylamide (DMA; 98.0%) were purchased from TCI (Shanghai, China). *N*,*N*′-Dicyclohexylcarbodiimide (DCC; 99%) was purchased from Sigma–Aldrich Trading Co., Ltd. (Shanghai, China). Maleic anhydride (analytical reagent) and l-alanine (analytical reagent) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). 2,2′-Azobisisobutyronitrile (analytical reagent) was produced by Shanghai Shanpu Chemical Co., Ltd. (Shanghai, China). Acetic acid (analytical reagent), ethyl acetate (analytical reagent), DMF (analytical reagent), and toluene (analytical reagent) were obtained from Tianjin Kermel Chemical Reagent Co., Ltd. (Tianjin, China). Poly(ethylene glycol) 2000 (PEG2K) was imported from Japan and distributed domestically.

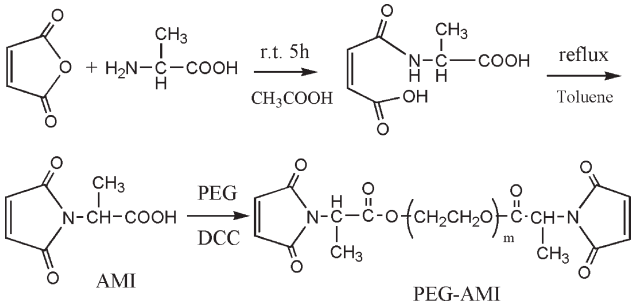
2,2′-Azobisisobutyronitrile was purified by crystallization from methanol. DMF and toluene were distilled and then dried over anhydrous magnesium sulfate for 2 days. Triethylamine was distilled and then dried over KOH. All other reagents were analytical-grade.

Synthesis of PDMAFM

PDMAFM was synthesized according to ref. 19, and its synthesis can be described briefly as follows. A mixture solution of FM and DMA in toluene was charged into a three-neck, round-bottomed flask with a magnetic stirrer under highly pure nitrogen. The monomer and initiator concentrations were 0.5 and 5 $\times$ 10^{-3} mol/L, respectively. The flask was immersed in an oil bath held at 70°C. After 24 h of stirring, the flask was removed from the bath, and the contents were immediately poured into a large excess of diethyl ether. The copolymer was filtered and then dried *in vacuo* until a constant weight was attained. To purify the copolymer further, the copolymer was dissolved in tetrahydrofuran, precipitated into an excess of diethyl ether, filtered, and then dried under reduced pressure three times. The prepared copolymers are labeled PDMAFM-*n*, where *n* represents the feed molar ratio of DMA to FM. For a direct comparison, the homopolymers of DMA and FM [poly(*N*,*N*-dimethylacrylamide) (PDMA) and poly(furfuryl methacrylate) (PFM)] were also prepared.

Synthesis of dienophile-terminated PEG (PEG–AMI)

AMI was synthesized according to the literature, and its synthetic route is presented in Scheme 1.[20,21] Typically, maleic anhydride (11.77 g, 0.12 mol) and l-alanine (8.91 g, 0.10 mol) were charged into a 250-mL, round-bottomed flask, dissolved in 170 mL of acetic acid, and stirred at room temperature for 5 h. The white precipitate was filtered and dried *in vacuo*. Crystallization from methanol afforded pure maleamic acid (17.8 g, yield = 95%, melting point = 141–142°C). Afterwards, maleamic acid (4.68 g, 0.025 mol) was suspended in dry toluene (150 mL) and treated with Et_3N (7 mL, 0.05 mol). This solution was refluxed with vigorous overhead stirring for 1.5 h with concomitant removal of formed water via a water separator. The toluene solution was decanted away from an orange oil. The toluene was removed by evaporation *in vacuo* to produce a hygroscopic solid. The solid was acidified to pH 2 with hydrochloric acid, extracted with ethyl acetate, and dried with $MgSO_4$. The ethyl acetate was removed by evaporation *in vacuo* to produce AMI (1.69 g, yield = 40%, melting point = 99–100°C).



Scheme 1 Synthetic route to PEG–AMI (r.t. = room temperature).

^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm): 9.6 (1H), 6.7 (s, 2H), 4.8 (q, 1H), 1.6 (d, 3H).

PEG–AMI was prepared by the coupling of AMI to PEG2K under DCC.[22] Typically, the synthesis of PEG–AMI was conducted as follows. PEG2K (2.00 g, 1 mmol) and AMI (0.41 g, 2.4 mmol) were dissolved in dry dichloromethane. After cooling to 0°C, a solution of DCC (0.54 g, 2.6 mmol) in dichloromethane was added dropwise, and the mixture was stirred at room temperature for 24 h under a nitrogen atmosphere. The resulting mixture was filtered and washed with dichloromethane. The filtrate was precipitated in a large amount of diethyl ether. The precipitate was filtered out and dried *in vacuo* to a constant weight.

Yield: 91%. Fourier transform infrared (FTIR; KBr, thin film, cm^{-1}): 2884 (CH_3 and CH_2), 1713 and 1748 (C=O), 1214 (C=N), 1654 (C=O). ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm): 6.8 (4H, CO—C*H*=C*H*—CO), 5.6 [2H, N—C*H*(CH_3)], 4.2–3.4 (182H, —OCH_2CH_2O—), 1.4 [6H, N—CH(CH_3)].

The circular dichroism (CD) spectra of AMI and PEG–AMI are shown in Figure 1. The broad peaks around 320 nm were produced by the conjugative system in AMI and PEG–AMI, and this indicated that AMI and PEG–AMI possessed chirality.

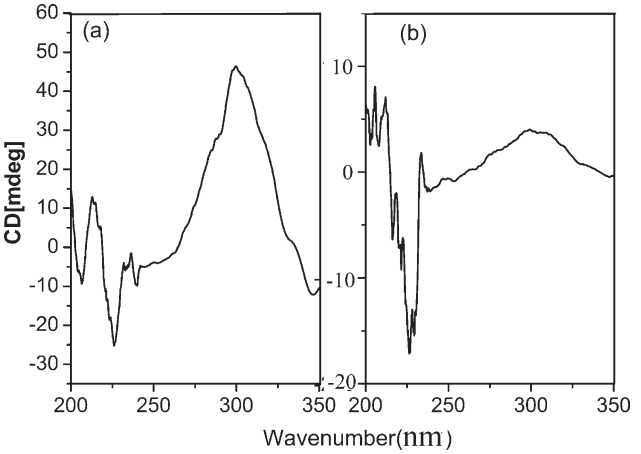
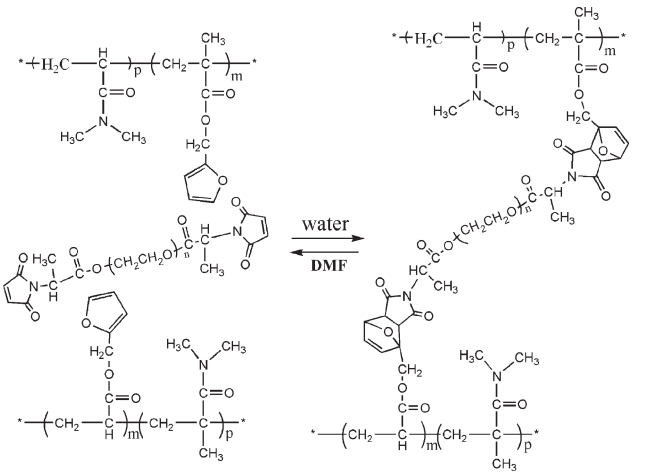


Figure 1 CD spectra of (a) AMI and (b) PEG–AMI in $CHCl_3$.



Scheme 2 Chemical structures of the hydrogels formed by the DA reaction.

DA reaction and retro-DA reaction

An aqueous PDMAFM-5 solution (1 mL, 10 wt %) was mixed with 1 mL of an aqueous solution containing stoichiometric PEG–AMI. After 5 min of stirring, the resulting mixture was put into a thermostatic bath at different temperatures, and the gelation time was visually determined when the mixture did not flow with the inversion of the vials (Scheme 2). For the preparation of the hydrogels for the study of the swelling behavior, the hydrogels were prepared with the same process at 47°C.

Two 50-mL flasks were charged with 10 mL of DMF and water, respectively. The dry gel (0.2 g) was added to each flask. After they were heated to different temperatures, the disassembly time based on the retro-DA reaction was visually determined when the gel disappeared.

Measurements

FTIR spectra were measured with a Shimadzu IR Prestige-21 FTIR spectrometer (Shimadzu, Japan) at room temperature from 4000 to 500 cm^{-1} at a resolution of 2 cm^{-1} with 20 scans. Samples were prepared by the good dispersion of the complexes in KBr and the compression of the mixtures into disks. The ^{1}H-NMR spectra were recorded at room temperature on a Bruker DPX-400 NMR instrument (Bruker, Germany) with tetramethylsilane as an internal standard. Gel permeation chromatography (GPC) analysis was carried out with a Shimadzu LC-10AVP chromatographic system equipped with a Shimadzu Shim-Pack GPC-803 chromatographic column. Tetrahydrofuran was used as an eluent at a flow rate of 1.0 mL/min. Monodispersed polystyrene standards were used to obtain a calibration curve. Scanning electron microscopy (SEM) experiments were performed with a model S-4800 field emission scanning electron microscope (Hitachi High-Technologies Corp., Japan). CD spectra were recorded on a Jasco J-810 spectrophotometer (Jasco International Co., Ltd., Tokyo, Japan) at room temperature. Before the measurements, AMI and PEG–AMI were dissolved in water.

The swelling behavior of the dried hydrogels was studied with a general gravimetric method.[23] A certain amount of each dry hydrogel was immersed in distilled water at 37°C, and the swollen weight of 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. When the temperature was increased gradually, the swollen hydrogels began to shrink. The temperature was kept constant for 2 h after it was increased 10°C, and then the weight of the shrunken hydrogel was measured. All the experiments were conducted in triplicate.

TABLE I
GPC Data and Yields of Some Copolymers

Sample	Feed molar ratio (DMA/FM)	M_n	M_w	Polydispersity index	Yield (%)
PDMAFM-5	5	8,225	23,440	2.50	85
PDMAFM-10	10	4,415	9,624	2.18	91
PDMAFM-15	15	3,665	9,236	2.52	92
PDMAFM-20	20	2,547	5,144	2.02	90

M_n = number-average molecular weight; M_w = weight-average molecular weight.

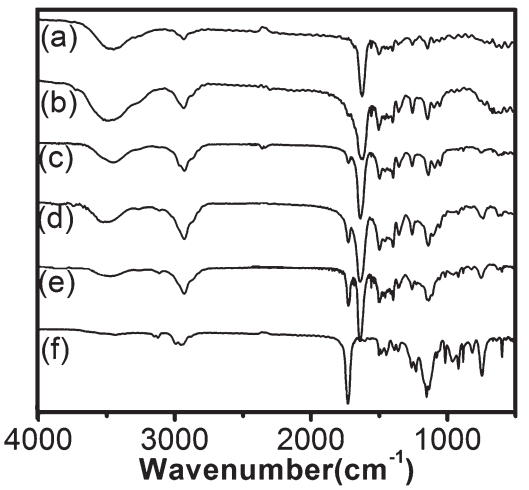


Figure 2 FTIR spectra of (a) PDMA, (b) PDMAFM-25, (c) PDMAFM-20, (d) PDMAFM-10, (e) PDMAFM-5, and (f) PFM.

The swelling ratio was calculated with the following equation:

$$\text{Swelling ratio} = \frac{m - m_0}{m_0} \times 100\%$$

where m_0 is the initial weight of the dried gel and m is the weight of the swelling gel at a particular temperature and a prescribed time interval.

RESULTS AND DISCUSSION

Synthesis and characterization of PDMAFM

PDMAFM was synthesized by the free-radical polymerization of FM and DMA at 70°C with 2,2′-azobisisobutyronitrile as the initiator. A low concentration of the monomer (0.5 mol/L) was selected on the basis of the literature and our former experimental results.[19,24] To obtain a water-soluble copolymer, we controlled the feed molar ratio of DMA to FM to be not less than 5. On the basis of Table I, the following conclusion can be drawn: the copolymer yields were high and the copolymer molecular weights increased as the FM content of the copolymers decreased.

FTIR spectra of PDMA, PDMAFM-25, PDMAFM-20, PDMAFM-10, PDMAFM-5, and PFM are presented in Figure 2. The PDMA spectrum shows a band at 1627 cm^{-1} corresponding to the C=O stretching mode and a band at 1254 cm^{-1} due to C—N stretching, whereas the band at 2931 cm^{-1} belongs to the C—H stretching mode in the backbone. The PFM spectrum shows a band at 1733 cm^{-1} assigned to the C=O stretching mode and bands at 1266, 1223, and 1157 cm^{-1} attributed to the symmetric and asymmetric C—O—C stretching mode. The characteristic peaks of DMA and FM are shown in the spectra of the copolymers [Fig. 2(b–e)] with a little shift. The peak at 1743 cm^{-1} becomes weaker and the peak at 1638 cm^{-1} becomes stronger from Figure 2(e) to Figure 2(b), and this agrees with the feed molar ratios.

^{1}H-NMR spectra of PDMAFM-5, PDMAFM-10, and PDMAFM-20 were recorded in D_2O at room temperature (Fig. 3). The peak at 4.8 ppm has been assigned to the —O—CH_2 protons of FM, the signals at 7.3 and 6.20–6.31 ppm correspond to the protons of the furan ring, and the signals at 2.4–2.8 ppm belong to the —N—CH_3 protons. The copolymer compositions were determined through the comparison of the integrated intensities of resonance signals at 7.3 and 2.4–2.8 ppm, and they agreed with the feed molar ratios. The ^{13}C-NMR spectrum of PDMAFM-5 was also recorded in dimethyl sulfoxide (DMSO) at room temperature. As shown in Figure 4, the signals at 176, 174, 149, 144, 130, 111, 58, 45, 37, 35, and 20 ppm correspond to different carbons of the copolymer.

DA reaction and retro-DA reaction

The solution states of PDMAFM-5 and PEG–AMI before and after the transition are presented in Figure 5. An SEM image of the freeze-dried hydrogels via the DA reaction of PDMAFM-5 and PEG–AMI is shown in Figure 6. The structure was found to be porous, and porous structures are well known to be important for tissue-engineering scaffolds.

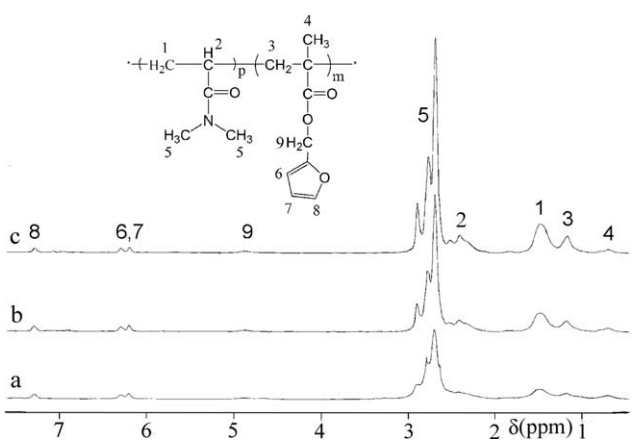


Figure 3 ^{1}H-NMR spectra of (a) PDMAFM-5, (b) PDMAFM-10, and (c) PDMAFM-20 in D_2O.

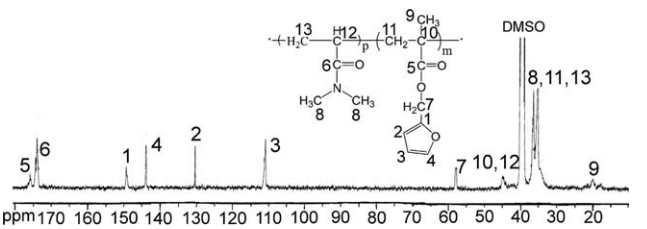


Figure 4 ^{13}C-NMR spectrum of PDMAFM-5 in DMSO-d_6.

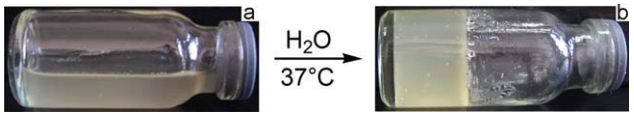


Figure 5 Photographs of the process of gel formation between PDMAFM-5 and PEG–AMI: (a) the gel precursor solution and (b) the gel by the DA reaction. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The gelation times of the mixture solutions of PDMAFM-5 and PEG–AMI are presented in Table II. It could be concluded that the gelation time increased with the FM content decreasing in the copolymer, and this indicated that less active groups led to a slower reaction rate. The gelation times of solutions of PDMAFM-5 and PEG–AMI at different temperatures in water are shown in Figure 7. The gelation time was closely related to the temperature in water; the gelation time decreased with the temperature increasing. Thus, the gelation time could be tuned by changes in the temperature. On the basis of their shorter gelation times, we think that the as-prepared hydrogels are good candidates for injectable hydrogels. However, gelation did not take place in DMF in the control experiment, and this indicates that DMF can suppress the DA reaction, whereas water can accelerate the DA reaction. One reason may be that the hydrophobic interaction results in micelle-like aggregates in an aqueous solution of the polymer, and this leads to higher local concentrations of furan and maleimide groups. When DMF is used as the solvent, the diene and dienophile are separated and do not easily encounter each other; therefore, the reaction is difficult to perform. The other reason may be that there is a hydrogen bond between the solvent and furan and maleimide groups, and this accelerates the DA reaction.[19]

In order to study the retro-DA reaction, we put the as-prepared dry gels (dried to a constant weight at 50°C in an electric blast-drying oven) in water and refluxed them for 12 h. The gels did not dissolve, and this indicates that their retro-DA reaction is difficult to perform in water. Therefore, we put 0.2 g of the dry gels in 10 mL of DMF at different temperatures. The disassembly time based on the retro-DA reaction was determined when the gel disappeared. The results are shown in Table III. When the temperature was 100°C, the gel disappeared in 0.8 h in DMF, whereas the gel did not disappear in water at 100°C; this may indicate that DMF can accelerate retro-DA reactions. The time of the gel disappearance increased with the temperature decreasing. When the temperature was less than 70°C, the gels did not disappear in 12 h. High reaction temperatures could increase the retro-DA reaction rate and shorten the time to transfer the polymer gels into clear polymer solutions. When DMF was used as the solvent for gel disassembly, DMF could trap the polymeric diene and dienophile, and furan and maleimide groups generated by the retro-DA reaction could not form micelles again in DMF. Therefore, the networks could not regenerate. When water was used as the solvent for gel disassembly, the furan and maleimide groups from the retro-DA reaction, if there were any, reacted with each other quickly because of an extraordinary rate-accelerating effect of water on the DA reaction, so the gel was stable in water.[19]

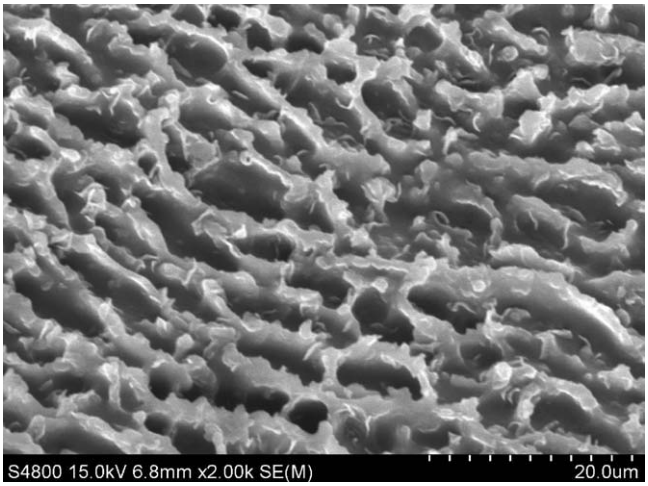


Figure 6 SEM image of the surface of the hydrogel from PDMAFM-5 and PEG–AMI.

Swelling behavior

The swelling ratios of the as-prepared hydrogels were measured in distilled water at 37°C. After the swelling equilibrium was reached, these hydrogels were left at 37, 47, 57, 67, 77, and 87°C in turn in a thermostatic water bath for 2 h before the changes in the swelling ratio were measured. The swelling behavior of the hydrogels prepared from PDMAFM-

TABLE II
Gelation Times for the Mixture Solutions of PDMAFM-*n* and PEG–AMI at 47°C

	PDMAFM-*n*				
	PDMAFM-5	PDMAFM-10	PDMAFM-15	PDMAFM-20	PDMAFM-25
Gelation time (min)	54	62	133	218	253

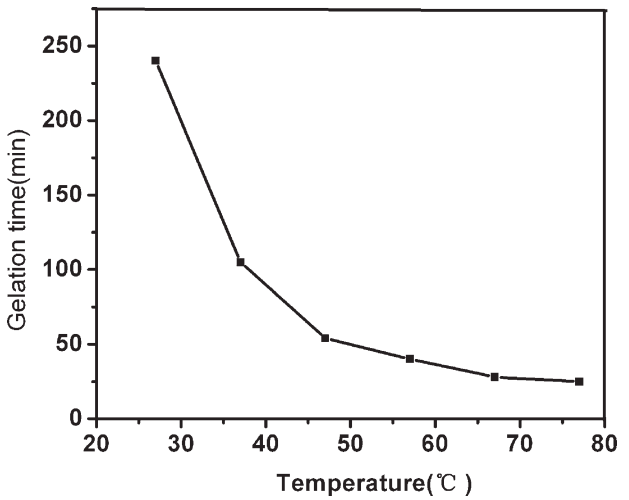


Figure 7 Gelation time of the hydrogels from PDMAFM-5 at different temperatures.

TABLE III
Disassembly Times for the Hydrogels from PDMAFM-5 in DMF at Different Temperatures

	Temperature (°C)				
	60	70	80	90	100
Disassembly time (h)	—[a]	—[a]	7	3	0.8

[a] The gel did not disappear in 12 h.

5, PDMAFM-10, and PDMAFM-20 is depicted in Figures 8 and 9. As shown in Figure 8, the hydrogels had a high swelling ratio in water, and the swelling ratio of the hydrogels from PDMAFM-20 was greater than that of the hydrogels from PDMAFM-5 and PDMAFM-10; this suggests that the swelling behavior is directly correlated to the composition of the copolymer. The swelling ratio of the hydrogels

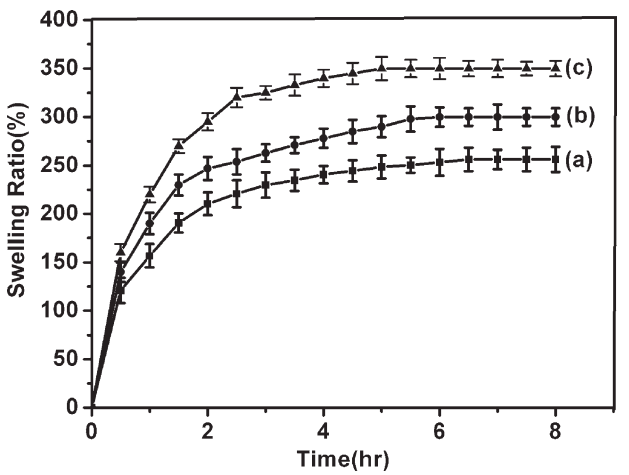


Figure 8 Swelling ratio of the hydrogels as a function of time at 37°C: (a) PDMAFM-5, (b) PDMAFM-10, and (c) PDMAFM-20. Each point represents the mean and standard deviation (n = 3).

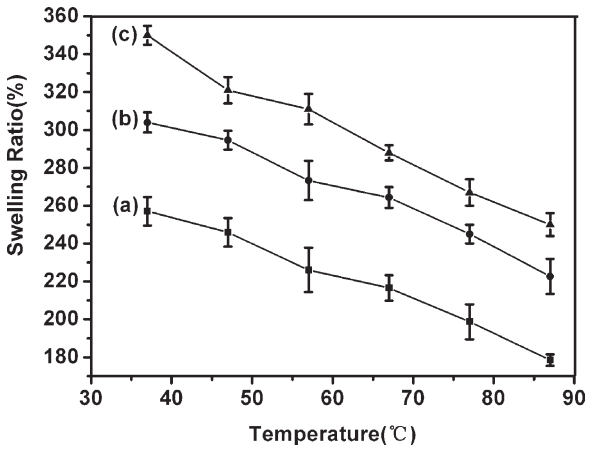


Figure 9 Swelling ratio of the hydrogels as a function of temperature: (a) PDMAFM-5, (b) PDMAFM-10, and (c) PDMAFM-20. Each point represents the mean and standard deviation (n = 3).

decreased with the FM content increasing in the copolymer because more crosslinks were formed with the FM content increasing in the copolymer. The swelling ratios of the as-prepared hydrogels as a function of temperature are shown in Figure 9. The swelling ratios of the hydrogels were temperature-dependent, and a low temperature facilitated swelling. When the temperature was increased from 37 to 87°C, the swelling ratio of the hydrogels decreased obviously.

CONCLUSIONS

The DA reaction in water between PDMAFM and PEG–AMI was applied to the straightforward preparation of thermoresponsive hydrogels. The research results showed that the gelation time could be tuned by the variation of the temperature, and increasing the temperature could decrease the gelation time. The swelling behavior of the hydrogels was influenced by the temperature and the hydrogel components. The swelling ratio increased with the temperature and FM content in the copolymer decreasing. The method described here possesses great potential for the preparation of biomaterials because of the obvious advantages during hydrogel formation. The further characterization and application of the resultant hydrogels are currently in progress and will be reported in forthcoming articles.

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