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Synthesis and characterization of thermoresponsive diblock copolymer bearing amino acid moieties via click reaction

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Abstract We report here the synthesis of well-defined thermoresponsive diblock copolymer bearing amino acid diamide derivatives, poly(*N*-acryloyl-L-valine *N*′-methylamide)-*b*-poly(ethylene glycol) monomethylether, via click reaction using $CuSO_4$/sodium ascorbate as catalyst in DMF/water mixed solvent. The effects of chain length and water volume fraction in DMF on coupling efficiency were investigated. For those reactions that both reacting polymers have short chain length, the reaction efficiency was less affected by water volume fraction and relatively high conversion was achieved. And the higher molecular weight of reactant polymer performed, the more impressive effects of water content on coupling efficiency appeared. Then, the thermoresponsive property in terms of the lower critical solution temperature (LCST) of the block copolymer was evaluated in aqueous solution by UV/vis spectroscopy. Optically active character was detected by circular dichroism instrument under temperature ranging from 1 to 30 °C, and the spectra undergo one drastic change at the temperature near LCST point.

Keywords Click reaction · LCST · Amino acid

Introduction

Polymers containing amino acid structure have attracted great attentions for many years because of their potential applications in biocompatible and biomedical materials [1–3], in membrane [4], chromatography [5], and electrophoresis [6] for separation of enantiomorphic drugs, and in many other fields such as gene therapy [7], bioassays [8], medical therapy [9], and tissue engineering [10], etc.

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The so-called intelligent or smart polymers are those that can undergo phase separation in response to external stimulations such as temperature, pH, and ion strength, etc. Although the thermoresponsive "smart" polymers bearing amino acid derivatives have many advantages, till now, only several kinds of such well-defined polymers have been reported. With controlled molecular weight and narrow polydispersity, Poly(*N*-acryloyl-L-proline methyl ester) prepared via reversible addition fragmentation chain transfer (RAFT) polymerization in aqueous solution was observed a sharp phase transition at about 20 °C [11, 12]. Poly(*N*-acryloyl-L-valine *N*′-methylamide) (PAVMA), which showed a Lower critical solution temperature (LCST) at 18 °C, was first reported by Dobashi [13]. This polymer was also prepared by RAFT polymerization in our previous study, using a novel alkynyl functionalized 2-dodecylsulfanylthiocarbonylsulfanyl-2-methylpropionic acid propargyl alcohol ester (DMPE) as chain transfer agent (CTA). It showed different LCST values with various molecular weights [14]. We found that the hydrophobic groups played a pronounced impact on the LCST value, but the effects of hydrophilic chains with different length on PAVMA have not been investigated yet.

Recently, click chemistry, especially the most popular Cu (I)-catalyzed 1,3-dipolar cycloaddition reaction between an azide and an alkyne, has been widely used in polymer synthesis and material fabrication owing to its high reaction efficiency, high functional group tolerance, and solvent insensitivity under less stringent experimental conditions [15]. Sharpless and co-workers showed in their pioneered work that, for a click reaction, the reaction time for a heterogeneous mixture of reactants and water is dramatically shorter than that of the same homogeneous solution of reactants in water [16]. Fu and his colleagues reported a quick, efficient copper-catalyzed click chemistry between water insoluble aliphatic/aryl azides and alkynes "on water" at room temperature [17]. These findings show that the addition of water will have an obvious impact on the chemistry during the preparation of low molecular organic compounds. Very recently, we reported the role of water on copper-catalyzed azide-alkyne click chemistry for preparation of polymers [18]. It revealed that the coupling efficiency increased dramatically near the water volume fraction where one or both of the reacting polymers began to precipitate, while further increases in water content caused the click reaction efficiency decreased once again when the polymers began to undergo phase separation. However, some papers [19] also reported high click coupling efficiency even without any water addition. So, the effects of different length of polymer chains, as well as various water contents, on the click coupling efficiency need to be investigated.

Since some interesting problems of the thermoresponsive polymer of PAVMA have not been solved, in this paper, diblock copolymer poly(*N*-acryloyl-L-valine *N*′-methylamide)-*b*-poly(ethylene glycol) monomethylether (PAVMA-*b*-MPEG) was synthesized under various conditions and the impacts of molecular weight of MPEG ended with azide group and PAVMA capped with alkyne group on the click coupling efficiency under various water contents were discussed. Besides, the thermosensitive properties of the diblock copolymer with different hydrophilic contents and its characters of optical activity were also discussed.

Experimental section

Materials

Poly(ethylene glycol) monomethylether (MPEG, $M_n = 1k, 2k, 5k, 98.0\%$) was purchased from Aldrich and used as received. 2-bromopropionyl bromide (BPB, 99+%) purchased from Acros was distilled prior to use. Sodium azide (NaN_3, 99.5%) was purchased from Tianjin Kemiou Chemical Reagent Co. Ltd and used as received. $CuSO_4 \cdot 5H_2O$ (99.0%) from Guangzhou Xinbo Chemical Co. Ltd and sodium ascorbate (99.6%) from Shanghai Re-found Chemical Co. Ltd were both recrystallized in water twice. Toluene (99.0%), diethyl ether (99.5%), and tetrahydrofuran (99.5%), all from Shanghai Chemical Reagent Co. Ltd, were refluxed over sodium and distilled before use. *N*,*N*-dimethylformamide (DMF, 99.5%), which was purchased from Tianjin Chemical Reagent Development Center, was dried with $MgSO_4$ overnight and distilled under reduced pressure before use. Dialysis bag was purchased from Shanghai Green Bird Science & Technology. The synthesis protocols for monomer, *N*-acryloyl-L-valine *N*′-methylamide (AVMA), and DMPE, and the corresponding homopolymer with different molecular weight were followed the procedure reported in our previous paper [14].

Measurements

^{1}H-NMR spectra were recorded at 20 °C on a Bruker DMX-400 spectrometer and the sample concentration in deuterated solvent was kept at 1–3 wt%.

Size-exclusion chromatography (SEC) was performed using a Waters 150C instrument equipped with a refractive index and UV–Visible absorption detector at 25 °C. The eluant used was *N*,*N*-dimethylforamide containing LiBr at 3.0 mg/mL. The 5×10^4 Å, PSS 1000 Å, and PSS 100 Å columns that used were calibrated by monodisperse polystyrene (PS) standards.

Lower critical solution temperature evaluated by monitoring the transmittance of the sample in aqueous solution at 500 nm on a Xinmao instrument UV-7504 (China) spectrophotometer equipped with a circulating water bath for controlling the temperature. The temperature at 55% light transmittance (55% T) of the polymer solution was defined as the LCST.

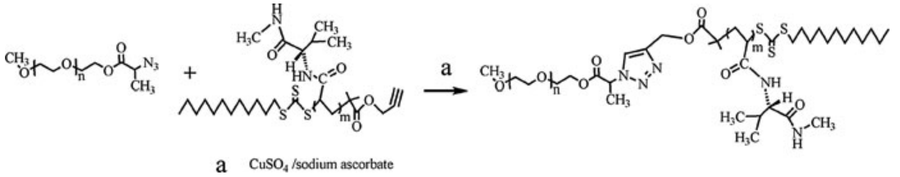
Circular dichroism (CD) spectra were recorded between 190 and 500 nm on a Jasco 810 A dichrograph in a cylindrical quartz cells with a path length of 1.00 cm. Different temperature spectra were conducted by first set Jasco 810 A dichrograph at a certain point, then the pre-dissolved PAVMA solution at the same temperature was introduced. After balancing for 5 min, the spectrum was depicted by scanning the sample for eight times. The consecutive temperature was increased at a rate of 0.2 °C/min, and keeping for 3 min at the pre-setting point before scanning.

Synthesis of MPEG-N_3

To prepare MPEG-N_3 that with different molecular weight, MPEG-OH (Mn = 1k, 2k, 5k) was initially reacted with BPB in order to introduce a terminal bromide group individually. Then, the product was reacted with NaN$_3$ to obtain the final polymer. The detailed procedure was reported in a previous paper [18], and the final yields of MPEG-N$_3$ were 51.8, 72.5, and 90.0%, respectively. ^{1}H-NMR results for MPEG-Br. ^{1}H-NMR (D$_2$O, 400 MHz) δ: 1.75 (3H, CHBr–CH_3), 3.32 (3H, CH_3–O), 3.64(4n-4H, –(CH_2–CH_2–O)$_{n-1}$–), 4.32 (2H, –CH_2–O–CO–), 4.71(1H, CHBr–CH$_3$). ^{1}H-NMR results for MPEG-N$_3$. ^{1}H-NMR (D$_2$O, 400 MHz) δ: 1.40 (3H, –CHN$_3$–CH_3), 3.32 (3H, CH_3–O), 3.64(4n-4H, –(CH_2–CH_2–O)$_{n-1}$–), 4.21(1H, –CHN$_3$–CH$_3$), 4.31 (2H, –CH_2–O–CO–).

Synthesis of PAVMA-b-MPEG

PAVMA-*b*-MPEG diblock copolymer with different molecular weight was prepared similarly via click coupling reaction between MPEG-N$_3$ and PAV-CCH in DMF with a variety of amount of water content. The synthetic route was shown in scheme 1. In a representative run, PAVMA-CCH (1.0002 g, 0.10 mmol) and MPEG-N$_3$ (0.6502 g, 0.13 mmol) were mixed in 30 mL of DMF and bubbled with argon for 30 min. 0.0025 g (0.010 mmol) of CuSO$_4$·5H$_2$O and sodium ascorbate (0.0043 g, 0.020 mmol) was dissolved in 3 mL of water individually, followed by bubbling with argon for 10 min. Then, the CuSO$_4$ aqueous solution and sodium ascorbate solution was separately dropped into the flask in sequence via syringe under vigorous stirring at room temperature. The flask was then placed in an oil bath preheated at 80 ± 1 °C for 20 h before cooled down to RT and exposed to air. After that, the solution was concentrated by rotary evaporation to 15 ml under vacuum, and then 30 mL of water was dropped under vigorous stirring. To remove MPEG-N$_3$ and other low molecular impurities, the dispersion was dialyzed against water, using a regenerated cellulose bag with a cut-off about 50,000. The water was changed with fresh water every 6 h over 3 days. The dispersion inside the dialysis bag was then centrifuged, and the supernatant was collected, followed by concentrating and drying under vacuum at RT before re-dissolved in MeOH and precipitated out in diethyl ether. Finally, the precipitation was dried under vacuum at RT for 72 h. Yield: 1.30 g (86.7%). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 0.81–0.93 ((CH_3)$_{2m}$–, 6mH), 1.22 (–(CH_2)$_{10}$–, 20H), 1.34–1.65 –(CH_2CH)$_m$–, 2mH), 1.75 (–CH$_2$–O–CO–CH(CH_3)–, 3H), 1.95 (br, –(CH$_3$CH$_3$CH)$_m$– and –(CH$_2$CH)$_m$–2mH), 2.59 (br, CH_3NH–, 3mH), 3.23 (CH_3–(CH$_2$–CH$_2$–O)$_n$–, 3H), 3.49–3.68(–(CH_2–CH_2–O)$_n$–, 4nH), 3.99 (br, –CHCO–, mH), 4.23 (–CH_2–O–CO–CH(CH$_3$)–, 2H), 4.57 (–CH$_2$–O–CO–CH(CH$_3$)–,1H), 5.16 (–CH_2C, 2H), 8.06 (br, CH$_3$NH–, mH), 8.55 (br, –CONHCH–, mH).



Scheme 1 Synthetic route for diblock copolymer PAVMA-*b*-MPEG by click chemistry

Results and discussion

Polymer preparation

To synthesize block copolymers by click coupling reactions, the existence of functional groups of $-N_3$ and $-C\equiv C$ in homopolymers is the principal requirement. The introduction of $-N_3$ at the end of MPEG was confirmed by ^{1}H-NMR spectrum, in which the chemical shift of –CH– that associated with –Br changed from $\delta = 4.7$ to $\delta = 4.2$ after the reaction. Meanwhile, the $-N_3$ absorption peak appearing in the FT-IR spectrum at 2113 cm^{-1} also demonstrates the successful displacing of –Br by $-N_3$. By using DMPE that contains the $-C\equiv C$ in the end as CTA, the happening of following click reactions is insured. The ^{1}H-NMR spectrum shows the peak of $-CH_2-$ that adjacent to alkynyl group appears at $\delta = 4.81$, which confirms the successful introduction of alkynyl group into the PAVMA chains. Furthermore, according to the ^{1}H NMR spectrum, the molecular weight of PAVMA can be calculated by contrasting the peak areas of dodecyl groups in DMPE with that of –CH– in PAVMA. The detailed properties of the azide or alkyne-end-functionalized polymers used in this study are shown in Table 1.

In our previous study, we have discovered that the efficiency of click chemistry between $PAVMA_{-2}$ and $MPEG_{-5k}$ with long polymer chain could be drastically increased with the addition of an optimum amount of water into the reaction system [18]. Therefore, in this article, we focused our research on the impact of length of polymer chains on the click coupling efficiency. PAVMA-CCH with the molecular weight of 4210 and 10460 and $MPEG\text{-}N_3$ with 1000, 2000, and 5000 were thus used. In our experiments, with $CuSO_4$/sodium ascorbate as the catalyst, the diblock copolymer PAVMA-*b*-MPEG was prepared by coupling PAVMA-CCH with 1.3 equivalent mole of $MPEG\text{-}N_3$ at 80 ± 1 °C for 20 h in DMF with different water contents. The results of the coupling efficiency under various conditions were summarized in Table 2, in which f_{v,H_2O} represents the volume fraction of water in DMF. As is clearly shown, when the water content is 2.0, 5.0, and 10.5, respectively, all the mixture solution is clear; while with 13.5, 18.6, 12.1, and 16.7% water contents, the appearance of the reaction mixture is micro-turbidity; and when

Table 1 Molecular characteristics of PAVMA-CCH and $MPEG\text{-}N_3$ used in this study

Sample	$M^a_{n,NMR}$ (g/mol)	$M^b_{n,GPC}$ (g/mol)	M_w/M^b_n	^{1}H-NMR^a (DP)	$[\alpha]^{25c}_D$
$MPEG_{-1k}$	1,027	1,600	1.09	23	–
$MPEG_{-2k}$	2,039	3,500	1.11	46	–
$MPEG_{-5k}$	5,075	7,100	1.10	115	–
$PAVMA_{-1}$	4,210	6,800	1.16	23	−46.5
$PAVMA_{-2}$	10,460	14,900	1.19	56	−41.7

[a] From ^{1}H NMR spectrum (400 MHz) in DMSO-d_6 or D_2O at 20 °C

[b] From size-exclusion chromatography (SEC) using polystyrene standards

[c] Absolute optical value in unit deg dm^{-1} g^{-1} cm^3 was measured by WZZ-1 home-made digital polarimeter at the concentration of 1.00 g/dL at 25 °C

the water content is 27.6 and 22.3%, respectively, the appearance of the reaction mixture is cloudy.

Three trends can be revealed from Table 2. First, the coupling efficiency of PAVMA$_{-1}$ + MPEG$_{-1k}$ and PAVMA$_{-1}$ + MPEG$_{-2k}$, in which the reacting polymers are all with short chains, kept over 86% no matter the reaction systems were clear or micro-turbidity. Second, the results are similar for PAVMA$_{-1}$ + MPEG$_{-5k}$, PAVMA$_{-2}$ + MPEG$_{-1k}$, and PAVMA$_{-2}$ + MPEG$_{-2k}$, in which only one polymer has long chains. In these systems, the coupling conversion of those reactions with little water contents was over 50% and it increased to over 80% when the water content increased to make the reaction system become micro-turbidity. This trend is basically consistent with PAVMA$_{-2}$ + MPEG$_{-5k}$, in which both the reacting polymers have relatively long chains, except for the reaction of PAVMA$_{-2}$ + MPEG$_{-5k}$ under clear solutions, the coupling efficiency of which is less than 25%. Third, all reaction conversions were less than 15% when the reaction system was cloudy, in which most of PAVMA were precipitated out.

From the results of the click reaction between PAVMA and MPEG, we can conclude that the water contents and the length of reactant polymer chains played very important effects on the coupling efficiency, and this result might be attributed to the exposure of the functional groups of –N_3 and –C≡C [18].

Figure 1 shows SEC curves of the homopolymer PAVMA and the corresponding diblock copolymer PAVMA-*b*-MPEG samples prepared by click chemistry. After purification by dialysis against water to remove MPEG-N_3 and centrifugation to remove little amount of unreacted PAVMA-CCH, it clearly shows that SEC peaks are essentially symmetrical unimode without distinct unreacted homopolymer shoulders, and that SEC peaks for higher molecular weight of diblock copolymer PAVMA-*b*-MPEG appeared at less retention time points, suggesting that the click coupling reaction was well controlled and pure copolymer was obtained.

Shown in Fig. 2 is the ^{1}H-NMR spectrum of the copolymer sample of PAVMA$_{-2}$-*b*-MPEG$_{-5k}$ and its verified structure. The peaks corresponding to PAVMA-*b*-MPEG can be clearly observed, and the integrations of the appropriate

Table 2 Conditions used and results for the click reactions

	Reaction samples	Water content (f_{v,H_2O}) (%)					
		Clear solution			Micro-turbidity		Cloudy
	f_{v,H_2O} (%)	2.0	5.0	10.5	13.5	18.6	27.6
Yield (%)[a]	PAVMA$_{-1}$ + MPEG$_{-1k}$	91.7	89.6	90.5	91.5	88.9	14.2
	PAVMA$_{-1}$ + MPEG$_{-2k}$	88.6	87.5	89.2	89.6	86.2	13.3
	PAVMA$_{-1}$ + MPEG$_{-5k}$	53.6	58.7	62.5	86.7	89.2	11.3
	f_{v,H_2O} (%)	2.0	5.0	10.5	12.1	16.7	22.3
Yield (%)[a]	PAVMA$_{-2}$ + MPEG$_{-1k}$	68.5	70.3	71.8	83.8	89.3	11.2
	PAVMA$_{-2}$ + MPEG$_{-2k}$	65.3	68.7	70.2	81.5	87.6	10.5
	PAVMA$_{-2}$ + MPEG$_{-5k}$	12.3	19.8	23.5	46.2	86.7	9.1

[a] Calculated from ^{1}H-NMR

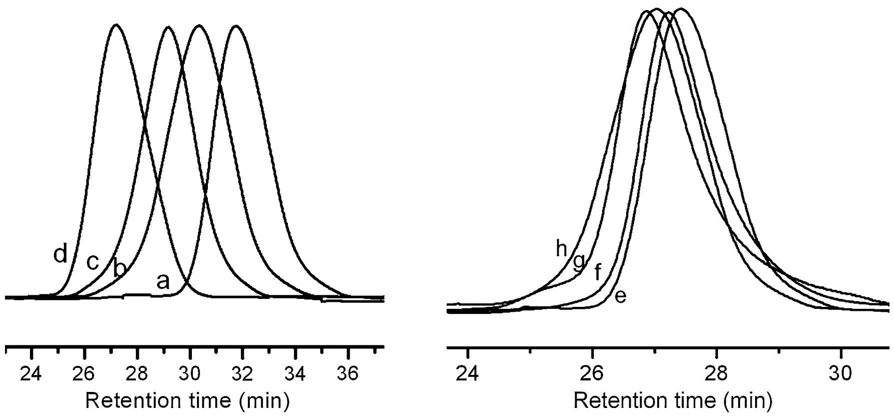


Fig. 1 SEC traces of (*a*) PAVMA$_{-1}$, (*b*) PAVMA$_{-1}$-*b*-MPEG$_{-1k}$, (*c*) PAVMA$_{-1}$-*b*-MPEG$_{-2k}$, (*d*) PAVMA$_{-1}$-*b*-MPEG$_{-5k}$; (*e*) PAVMA$_{-2}$, (*f*) PAVMA$_{-2}$-*b*-MPEG$_{-1k}$, (*g*) PAVMA$_{-2}$-*b*-MPEG$_{-5k}$, (*h*) PAVMA$_{-2}$-*b*-MPEG$_{-2k}$ recorded at 25 °C using DMF (containing 0.3 wt% tetra-*n*-butylammonium bromide) as eluant and polystyrene as standard

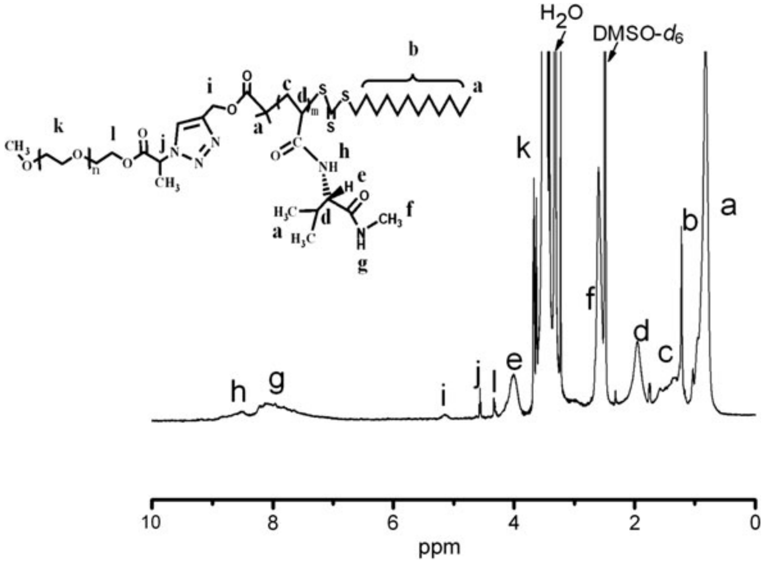


Fig. 2 ^{1}H-NMR spectrum of PAVMA$_{-2}$-*b*-MPEG$_{-5k}$ recorded in DMSO-d_6

peaks in PAVMA and MPEG give a composition that is in agreement with those expected. The coupling efficiency was calculated from the product before purification by dialysis, by calculating the changes of the intensity of the peak –CH_2– which related to alkyne and changed from $\delta = 4.81$ to 5.16 ppm after cycloaddition with azide group.

LCST properties

Bearing diamide groups in the side chains of PAVMA makes the diblock copolymer a balance of hydrophobicity and hydrophilicity through the interactions between the intramolecular hydrogen bonds and polymer chains with water at different temperatures, exhibiting the lower critical solution temperature. Figure 3 shows the transmittance versus temperature plots for PAVMA-*b*-MPEG with different chain length of MPEG, which were monitored by UV at 500 nm, with a heating rate of 0.5 °C/min. The figure demonstrated that, as MPEG was coupled to PAVMA, the phase transition point (55% T) increased from 6.5 to about 20 °C. Similar results that LCSTs are prone to increase with the increase of hydrophilic portion in polymer are also reported by Matyjaszewski [20]. However, the LCSTs of PAVMA-*b*-MPEG with the MPEG chain length of 1k, 2k, 5k only changed from 18.7 to 19.6 °C, which was relatively quite little compared with the changes from homopolymer of PAVMA to diblock copolymer.

CD measurement

Owning LCST properties of PAVMA-*b*-MPEG, we invested the secondary structure under the consecutive temperature change from 1 °C, at which the polymer was absolutely dissolvable in water, to 30 °C, at which the polymer coiled. When setting the dilute aqueous solution of PAVMA-*b*-MPEG at 0.01 mg/mL, the transparency monitored by UV at 500 nm was found to be constant regardless of the temperature. Above the LCST, low concentration makes the solution non-coacervated but only coiled for single polymer chains. We then recorded the CD spectra of PAVMA$_{-2}$-*b*-MPEG$_{-5k}$ at the same conditions, except for different temperatures.

Figure 4 shows the CD spectra of PAVMA$_{-2}$-*b*-MPEG$_{-5k}$ with a concentration of 0.01 mg/mL under different temperature varying from 1 to 30 °C. As shown in the

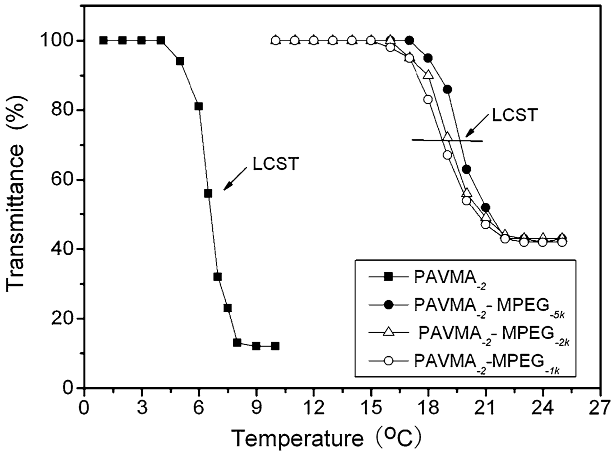


Fig. 3 Temperature dependence on the transmittance at 500 nm for aqueous solutions of PAVMA and PAVMA-*b*-MPEG

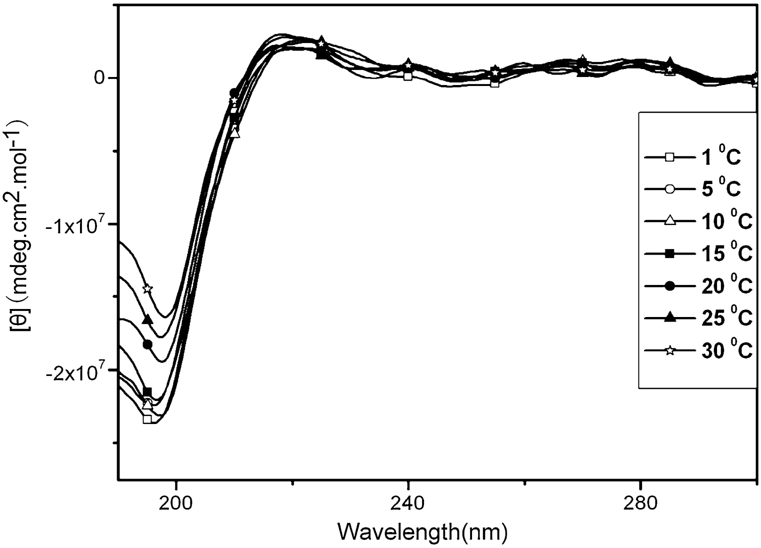


Fig. 4 CD spectra of PAVMA$_{-2}$-*b*- MPEG$_{-5k}$ in water at consecutive temperature rising from 1 to 30 °C with concentration of 0.01 mg/mL

figure, all the CD spectra recorded at different temperature exhibited negative Cotton effects at wavelength around 198 nm and weak positive Cotton effects near 220 nm, which are arising from electronic transition band of n–π* and π–π* of the diamide groups in PAVMA side chains, respectively. This is indicative of a random coil type of conformation at the conditions supplied [21]. The figure also shows that the positive and negative-band strength slightly decreased with the increase of temperature, which is also observed by other reports [22]. However, there is a drastic decrease band which just lies in the range of LCST point between 15 and 20 °C can be observed, indicating the secondary structures of thermosensitive segment of PAVMA shrink from random coil to compact ones. This result was kept in consistent with the LCST results monitored by UV transmittance, which also verified the correction of LCST values.

Conclusions

Well-defined diblock copolymer PAVMA-*b*-MPEG with different molecular weight was synthesized via click chemistry. In the reaction, water volume fraction in the mixed solvent and the length of reacting polymer chains played very important effects on coupling efficiency. When the reacting polymers were all with short chains, high coupling conversions could be achieved no matter the reaction systems were clear or micro-turbidity. When only one or both polymer has long chains, the conversion under micro-turbidity was higher than that in clear solution. The introduction of MPEG to PAVMA makes the LCST values increased obviously from 6.5 to about 20 °C, while different length of MPEG played little effects on LCST at the supplied conditions. CD spectra showed the secondary structure of PAVMA-*b*-MPEG is a random coil type of conformation, and obvious change can be observed near LCST point.

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