



Preparation of an ethylene glycol-based block copolymer consisting of six different temperature-responsive blocks

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Abstract

An ethylene glycol-based hexa-block copolymer with six different temperature-responsive blocks was prepared via a reversible addition-fragmentation chain transfer (RAFT) polymerization. 2-(2-methoxyethoxy)ethyl methacrylate (MEO₂MA) and oligo(ethylene glycol) methacrylate (OEGMA) were selected as the ethylene glycol-based monomers. Each block of the hexa-block copolymer has a different OEGMA content and thus exhibits a different lower critical solution temperature (LCST). The structures of the block copolymers are similar to gradient copolymers. The molecular weights of the copolymers increased with the number of blocks (first block (P(MEO₂MA)): 2800 g/mol, di-block copolymer: 7400 g/mol, tri-block copolymer: 12,000 g/mol, tetra-block copolymer: 18,500 g/mol, penta-block copolymer: 26,800 g/mol, and hexa-block copolymer: 40,400 g/mol) with relatively narrow molecular weight distributions (1.18–1.42). Each block exhibits a different LCST in aqueous solution owing to their different OEGMA contents. To the best of our knowledge, this is the first report of a block copolymer containing more than four types of temperature-responsive blocks. The temperature-responsive properties of the prepared block copolymers were investigated by UV-vis spectroscopy, micro-differential scanning calorimetry, and scanning electron microscopy.

Introduction

Smart or stimuli-responsive polymers are a new generation of polymers that exhibit reversible physicochemical changes in response to changes in their environment, such as temperature, pH, light, and magnetic field variations or the addition of other molecules [1, 2]. Among such materials, temperature-responsive polymers undergo changes in response to the solution temperature. This ability, especially in aqueous solutions (i.e., reversible hydrophilic/hydrophobic changes), has been applied in a wide range of fields, such as cell cultures, drug carriers, chromatography, microchannels, protein preservation, and detections in cell

biology [3–8]. These polymers typically contain a temperature-responsive block, and the temperature responsiveness is limited to a single structural feature (i.e., hydrophilicity and hydrophobicity). To fine tune the structural properties, block copolymers with two (or more) different temperature-responsive blocks have been reported [9–11]. Three kinds of structural features, i.e., hydrophilic/hydrophilic, amphiphilic, and hydrophobic/hydrophobic structures, can be prepared from dual temperature-responsive block copolymers via modification of the individual block properties. Sugihara et al. prepared a triple temperature-responsive block copolymer by living cationic polymerization [12]. Weiss and Laschewsky prepared tri-block copolymers with three temperature-responsive blocks, and 12 types of structures were obtained by hydrophilicity/hydrophobicity variations and using different combinations of the three blocks in different sequences [13]. To the best of our knowledge, no reports exist on temperature-responsive block copolymers with more than four

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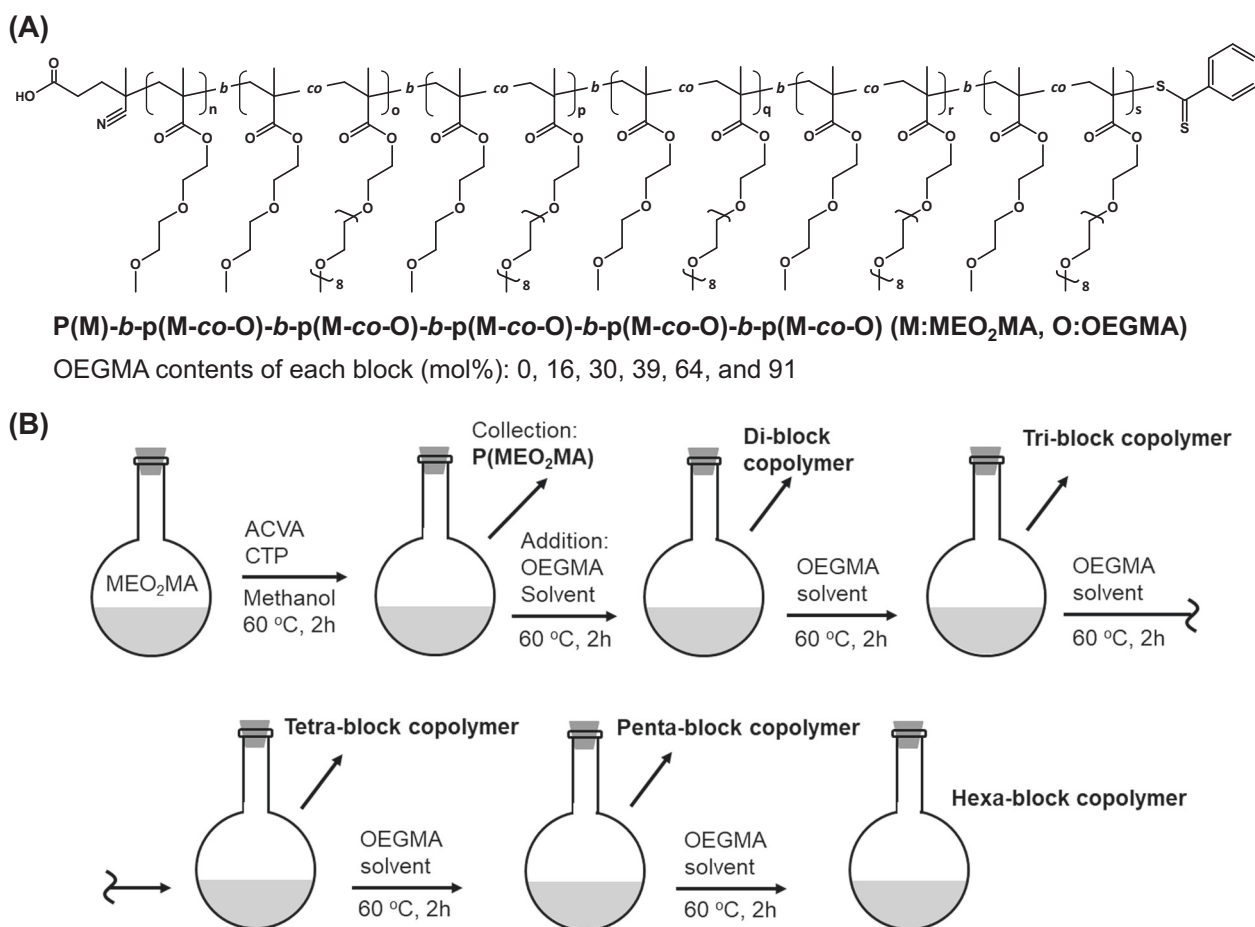


Fig. 1 **a** Structures of the hexa-block copolymer with different OEGMA contents. **b** Preparation methods of the block copolymers

different temperature-responsive blocks. There are two main reasons for this: (1) it is difficult to synthesize block copolymers using the available synthesis techniques and (2) it is difficult to obtain more than four types of monomers with different LCSTs. With recent developments in polymerization techniques, block copolymers consisting of more than 10 blocks have been reported [14]. Zhang et al. prepared multi-block glycopolymers for the inhibition of lectin and the gp120 proteins in human immunodeficiency virus (HIV) [15].

In this study, a hexa-block copolymer consisting of six types of temperature-responsive blocks was prepared using two ethylene glycol-based monomers (Fig. 1). The homopolymers of (2-(2-methoxyethoxy)ethyl methacrylate (MEO₂MA) and oligo(ethylene glycol) methacrylate (OEGMA)) exhibit lower critical solution temperatures (LCSTs) of 28 and 90 °C, respectively [16]. Therefore, the LCSTs of the P(MEO₂MA-co-OEGMA) copolymers can be designed to be between 28 and 90 °C by varying the monomer composition [17]. The hexa-block copolymer was prepared by simple syringe-based method in 12 h, and each block was prepared with a different OEGMA content to

obtain different LCSTs. To the best of our knowledge, this is the first report of a block copolymer consisting of more than four kinds of temperature-responsive blocks. The physicochemical properties of the block copolymers were investigated using ¹H nuclear magnetic resonance (¹H NMR) spectroscopy, gel permeation chromatography (GPC), UV-vis spectroscopy, micro-differential scanning calorimetry (micro-DSC), and scanning electron microscopy (SEM).

Experimental section

Materials and methods

2-(2-Methoxyethoxy)ethyl methacrylate (MEO₂MA) and oligo(ethylene glycol) methacrylate (OEGMA, $M_n = 475$ g/mol) were purchased from Sigma-Aldrich and purified by passage through a basic alumina column. All other chemicals and solvents were used as received. The distilled water used in this study was purified with a Millipore Milli-Q system.

Preparation of the ethylene glycol-based hexa-block copolymer

A reversible addition-fragmentation chain transfer (RAFT) polymerization was employed to synthesize block copolymers with a narrow molecular weight distribution. 4-Cyanopentanoic acid dithiobenzoate (CTP) was selected as the RAFT agent. The hexa-block copolymer was prepared from blocks containing different contents of OEGMA. The first block, i.e., P(MEO₂MA), was polymerized in a polymerization tube. MEO₂MA (2.26 g, 12.0 mmol), CTP (13.97 mg, 0.05 mmol), and 4,4'-azobis-4-cyanovaleric acid (ACVA, 5.5 mg, 0.022 mmol) ([MEO₂MA]₀/[CTP]₀/[ACVA]₀ = 240/1/0.44) were dissolved in 5 mL of methanol. After degassing with nitrogen for 30 min, the mixture was allowed to polymerize for 2 h at 60 °C. Then, one quarter of the polymerization solution was removed as P(MEO₂MA) with a syringe, and an OEGMA solution (602.8 mg in 0.85 mL of methanol) was added to the polymerization tube to synthesize the di-block copolymer (P(MEO₂MA)-*b*-P(MEO₂MA-*co*-OEGMA)). The extracted P(MEO₂MA) was purified by dialysis against ethanol and acetone and dried under reduced pressure. After 2 h, one quarter of the polymerized solution (i.e., P(MEO₂MA)-*b*-P(MEO₂MA-*co*-OEGMA)) was again extracted, and an OEGMA solution (532.0 mg in 0.92 mL of methanol) was added in the same way for the polymerization of the tri-block copolymer. After six steps of such polymerizations, a hexa-block copolymer made of blocks with different OEGMA contents was synthesized. Different OEGMA solutions were added to the polymerization tube to obtain the tetra-block (578.0 mg in 0.87 mL of methanol), penta-block (670.0 mg in 0.78 mL of methanol), and hexa-block (822.0 mg in 0.63 mL of methanol) copolymers. The composition of each block was calculated using ¹H NMR and GPC analyses. For example, the monomer units in P(M₁₃) (i.e., P(MEO₂MA)) were calculated as shown below.

$$\text{Units} = ((M_n \text{ of P(MEO}_2\text{MA) from GPC}) - (M_n \text{ of RAFT agent})) / (M_n \text{ of MEO}_2\text{MA})$$

The composition of the di-block copolymer (i.e., P(M₁₃)-*b*-P(M_{17-*co*-O₃))) was calculated as shown below.}

The molar ratio of MEO₂MA (f_M) and OEGMA (f_O) ($f_M + f_O = 1$) in the di-block copolymer was measured by ¹H NMR. Therefore, the total units can be calculated using the M_n (from GPC) and the ratio.

The total units:

$$((M_n \text{ of di-block copolymer}) - (M_n \text{ of RAFT agent})) / ((M_n \text{ of MEO}_2\text{MA}) \times (f_M) + (M_n \text{ of OEGMA}) \times (f_O)) = 33 \text{ units (MEO}_2\text{MA: 30 units, and OEGMA: 3 units).}$$

For the first block of P(MEO₂MA), 13 units of MEO₂MA were used. Therefore, the composition of the di-block copolymer was calculated based on P(M₁₃)-*b*-P

(M_{17-*co*-O₃)). In addition, the OEGMA content (16 mol%) in the P(M_{17-*co*-O₃)) block was calculated using the numbers of units. The compositions of the tri-, tetra-, penta-, and hexa-block copolymers were calculated using same methods.}}

Characterizations

¹H NMR spectra of copolymers were recorded on a JNM-GSX300 spectrometer (300 MHz, JEOL, Tokyo, Japan) to confirm the successful synthesis and determine the chemical composition of the synthesized copolymers.

The molecular weight and polydispersity of the synthesized copolymers were determined by GPC at 40 °C (DMF containing 10 mM LiBr, 1 mL/min) with a TOSOH TSK-GEL α-2500 and α-4000 columns (Tosoh, Tokyo, Japan) connected to an RI-2031 refractive index detector (JASCO International Co., Ltd., Tokyo, Japan). Poly(ethylene glycol)/poly(ethylene oxide) standards were used to prepare a calibration curve.

The transmittance of the copolymer solution at 500 nm was continuously recorded at a heating rate of 1.0 °C/min on a UV-Vis spectrometer V-550 or V-650 (JASCO International Co., Ltd., Tokyo, Japan) to determine the LCST. The synthesized copolymers were dissolved in an aqueous solution at a given concentration. The LCSTs of the copolymers were measured at 50% transmittance.

The specific heat capacity (C_p) of the hexa-block copolymer solution (0.1 wt% in water) was determined by differential scanning calorimetry (MicroCal VP-DSC). The baseline was stabilized for 12 h with milliQ water prior to sample measurement. The heating rate was 1.0 °C/min.

The morphology of the nanoparticles was observed by SEM (JSM-IT 100, JEOL, Tokyo, Japan) at an accelerating voltage of 15.0 kV. A drop of the sample solution (0.1 wt% in water at the desired temperature) was placed on a silicon wafer by spin coating and allowed to dry under atmospheric pressure. The sample was then sputter-coated with Au.

Results and discussion

The block copolymers were synthesized by RAFT polymerization. MEO₂MA and OEGMA were selected as the monomers for the target block copolymers. The homopolymers of P(MEO₂MA) and P(OEGMA) present lower critical solution temperatures (LCSTs) of 28 and 90 °C, respectively [17]. The LCSTs of P(MEO₂MA-*co*-OEGMA) copolymers can be controlled by varying the monomer composition. Copolymers polymerized using Z-C(=S)-S-R-type RAFT agents have Z and R groups at the chain ends [18]. The end groups of the copolymers can thus be controlled by careful selection of the RAFT agent, which in

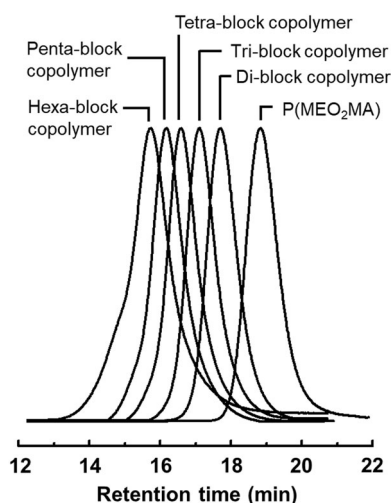


Fig. 2 GPC chromatograms of the block copolymers

this work was 4-cyanopentanoic acid dithiobenzoate (CTP). The hexa-block copolymer was prepared from blocks containing different OEGMA contents. The first block, i.e., P(MEO₂MA), was polymerized in a polymerization tube for 2 h (Fig. 1b). Then, one quarter of the polymerized solution was removed as P(MEO₂MA) via a syringe, and an OEGMA solution was added to the polymerization tube to synthesize the di-block copolymer (P(MEO₂MA)-*b*-P(MEO₂MA-*co*-OEGMA)). After 2 h, another quarter of the polymerized solution (i.e., P(MEO₂MA)-*b*-P(MEO₂MA-*co*-OEGMA)) was removed, and an OEGMA solution was again added in the same way for the polymerization of the tri-block copolymer. Six such polymerization steps afforded a hexa-block copolymer composed of blocks with different OEGMA contents.

Figure 2 shows the GPC chromatograms for each block copolymer. The retention time of the GPC peaks decreased as the number of blocks increased from the P(MEO₂MA) (the first-block) to the hexa-block copolymer. Table 1 shows the OEGMA composition, molecular weight (M_n), and molecular weight distribution (M_w/M_n) of each block copolymer. The OEGMA compositions were calculated using the ¹H NMR and GPC data. The M_n and M_w/M_n of P(MEO₂MA) were 2800 g/mol and 1.18, respectively. The M_n of the di-block copolymer P(MEO₂MA)-*b*-P(MEO₂MA-*co*-OEGMA) was 7400 g/mol and its M_w/M_n was 1.18. The OEGMA content in the P(MEO₂MA-*co*-OEGMA) block of the di-block copolymer was 16 mol%. The M_n of the tri-block copolymer was 12,000 g/mol, and the OEGMA content of the third block of the P(MEO₂MA-*co*-OEGMA) fragment was 30 mol%. The M_n of the tetra- (18,500 g/mol), penta- (26,800 g/mol), and hexa-block (40,400 g/mol) copolymers increased with the number of blocks, and the OEGMA contents of the fourth, fifth, and sixth block were 39, 64, and 91 mol%, respectively. The M_w/M_n values of all the block copolymers were

Table 1 Characterization of the block copolymers

Code	Monomer units ^a	OEGMA content in each block ^b (mol%)						M_n^b (g/mol)	M_w/M_n^b (-)
		1st block	2nd block	3rd block	4th block	5th block	6th block		
P(MEO ₂ MA)	P(M ₁₃)	0	—	—	—	—	—	2800	1.18
Di-block copolymer	P(M ₁₃)- <i>b</i> -p(M ₁₇ - <i>co</i> -O ₃)	0	16	—	—	—	—	7400	1.18
Tri-block copolymer	P(M ₁₃)- <i>b</i> -p(M ₁₇ - <i>co</i> -O ₃)- <i>b</i> -p(M ₁₂ - <i>co</i> -O ₃)	0	16	30	—	—	—	12,000	1.20
Tetra-block copolymer	P(M ₁₃)- <i>b</i> -p(M ₁₇ - <i>co</i> -O ₃)- <i>b</i> -p(M ₁₂ - <i>co</i> -O ₃)- <i>b</i> -p(M ₁₃ - <i>co</i> -O ₈)	0	16	30	39	—	—	18,500	1.27
Penta-block copolymer	P(M ₁₃)- <i>b</i> -p(M ₁₇ - <i>co</i> -O ₃)- <i>b</i> -p(M ₁₂ - <i>co</i> -O ₃)- <i>b</i> -p(M ₁₃ - <i>co</i> -O ₈)- <i>b</i> -p(M ₈ - <i>co</i> -O ₁₄)	0	16	30	39	64	—	26,800	1.30
Hexa-block copolymer	P(M ₁₃)- <i>b</i> -p(M ₁₇ - <i>co</i> -O ₃)- <i>b</i> -p(M ₁₂ - <i>co</i> -O ₃)- <i>b</i> -p(M ₁₃ - <i>co</i> -O ₈)- <i>b</i> -p(M ₈ - <i>co</i> -O ₁₄)- <i>b</i> -p(M ₃ - <i>co</i> -O ₂₈)	0	16	30	39	64	91	40,400	1.42

^aThe monomer units were calculated from the GPC and ¹H NMR data

^bDetermined by GPC using 10 mM LiBr in DMF at 40 °C

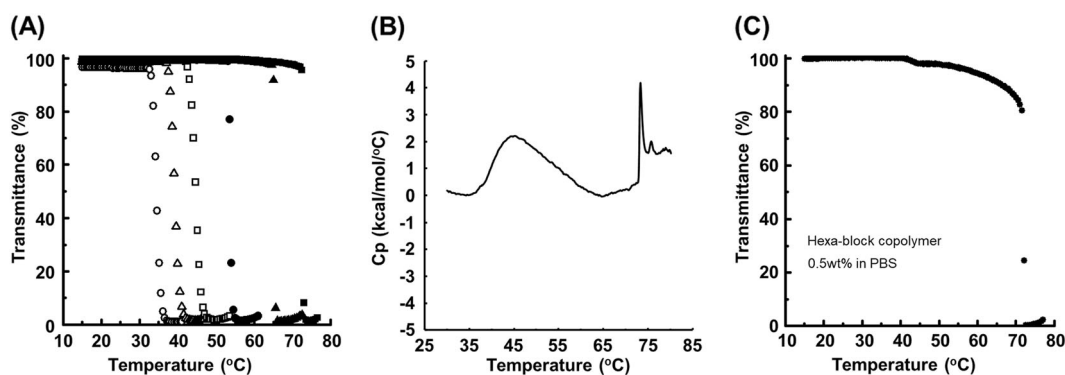


Fig. 3 **a** Transmittance change of 0.1 wt% solutions of block copolymers in pH 7.4 PBS as a function of temperature (○: P(MEO₂MA), △: di-block copolymer, □: tri-block copolymer, ●: tetra-block copolymer, ▲: penta-block copolymer, and ■: hexa-block copolymer). **b**

Micro-DSC curve of 0.1 wt% solution of the hexa-block copolymer in milliQ water as a function of temperature. **c** Transmittance change of a 0.5 wt% solution of the hexa-block copolymer in pH 7.4 PBS as a function of temperature

relatively narrow (1.18–1.42). The degree of polymerization of the hexa-block copolymer was P(M₁₃)-*b*-p(M_{17-co}-O₃)-*b*-p(M_{12-co}-O₅)-*b*-p(M_{13-co}-O₈)-*b*-p(M_{8-co}-O₁₄)-*b*-p(M_{3-co}-O₂₈), where the numbers denote the number of monomer units (M: MEO₂MA and O: OEGMA) calculated from the molecular weight and OEGMA content. Moreover, another hexa-block copolymer was synthesized to confirm the polymerization method (Table S1, Figures S1 and S2). To increase the molecular weight of the first block (P(MEO₂MA)), the polymerization time was increased from 2 to 3 h. The polymerization for the other five blocks was 2 h. The M_n of the P(MEO₂MA) was 4200 g/mol, which is higher than that of P(MEO₂MA) in P(M₁₃)-*b*-p(M_{17-co}-O₃)-*b*-p(M_{12-co}-O₅)-*b*-p(M_{13-co}-O₈)-*b*-p(M_{8-co}-O₁₄)-*b*-p(M_{3-co}-O₂₈). The hexa-block copolymer was thus identified as P(M₂₁)-*b*-p(M_{13-co}-O₃)-*b*-p(M_{13-co}-O₅)-*b*-p(M_{9-co}-O₇)-*b*-p(M_{6-co}-O₁₂)-*b*-p(M_{2-co}-O₂₁).

Next, the temperature-responsive properties of the block copolymers were determined. Figure 3a shows the changes in the transmittance of the block copolymers with changing temperature. All the block copolymers were dissolved in pH 7.4 phosphate-buffered saline (PBS) at 0.1 wt%. The LCST of the first block of P(MEO₂MA) was ~34 °C. The temperature of the strong decrease in transmittance increased with the number of blocks (tri < tetra < penta < hexa). In the di-block copolymer, the transmittance drop was observed at ~39 °C. The di-block copolymer contains two different temperature responsive blocks, and thus it is expected to exhibit two transmittance reductions via the dehydrations of both P(M₁₃) and P(M_{17-co}-O₃) blocks. Indeed, the dual temperature-responsive block copolymer showed two different transmittance changes due to conformational changes, i.e., water-soluble hydrophilic/hydrophilic block copolymers, amphiphilic block copolymers (nanoparticles), and hydrophobic/hydrophobic block copolymers (nanoparticle aggregates/precipitates) [19–22]. The di-block copolymer exhibits a single transmittance drop up to 1 wt

% (Figure S3). In the dual temperature-responsive block copolymer, the temperature-responsive properties of one block are strongly affected by the presence of the other block. The chain length of the P(M₁₃) block is shorter than that of P(M_{17-co}-O₃). The temperature-responsive behavior of the P(M₁₃) block might be coupled to that of the P(M_{17-co}-O₃) block, or the behavior might be due to the gradient-like structure of the copolymers. The di-block copolymer of P(M₂₁)-*b*-p(M_{13-co}-O₃) (Table S1) contains a longer P(M₂₁) block because of the longer polymerization time employed in its synthesis. The transmittance change of P(M₂₁)-*b*-p(M_{13-co}-O₃) is shown in Figure S4. A transmittance drop was observed at approximately 33 °C and 1 wt%. These results made us wonder if each block exhibits temperature-responsive properties. Figure 3b shows the micro-DSC results for the hexa-block copolymer in milliQ water. DSC presents the temperature-responsive properties in the form of endothermic peaks [23]. First, the endothermic peak of the hexa-block copolymer increased from 35 to 65 °C with a broad curve. The starting temperature (35 °C) was similar to the LCST of the first block of P(MEO₂MA). The temperature range of the broad curve includes the LCSTs of the second, third, and fourth blocks. Moreover, the endothermic peak also increased from 65 °C, and a sharp peak was observed at 73 °C, which is similar to the LCST value of the sixth block. These results suggest that each block of the hexa-block copolymer exhibits observable temperature-responsive properties. However, it should be noted that the chain lengths of each block are relatively short. In addition, it is difficult to fix the boundaries between adjacent blocks. Moreover, the LCSTs of each block consisting of several tens of OEGMA/MEO₂MA units will be strongly affected by slight changes in the number of OEGMA units. The slight variations will be observed in each block from a probabilistic viewpoint of polymerization.

Sequential dehydrations of the hexa-block copolymer lead to the formation of self-assembled particles owing to

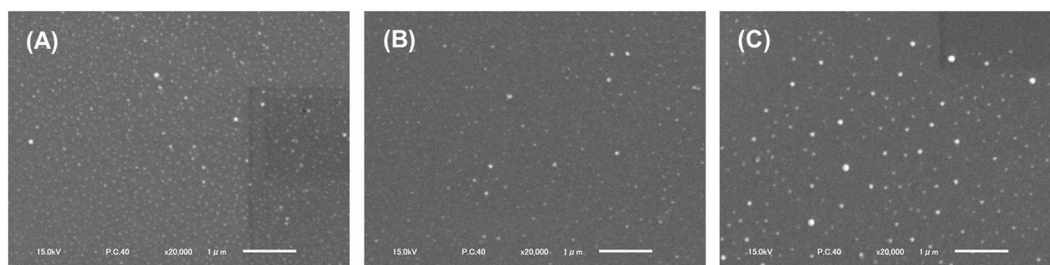
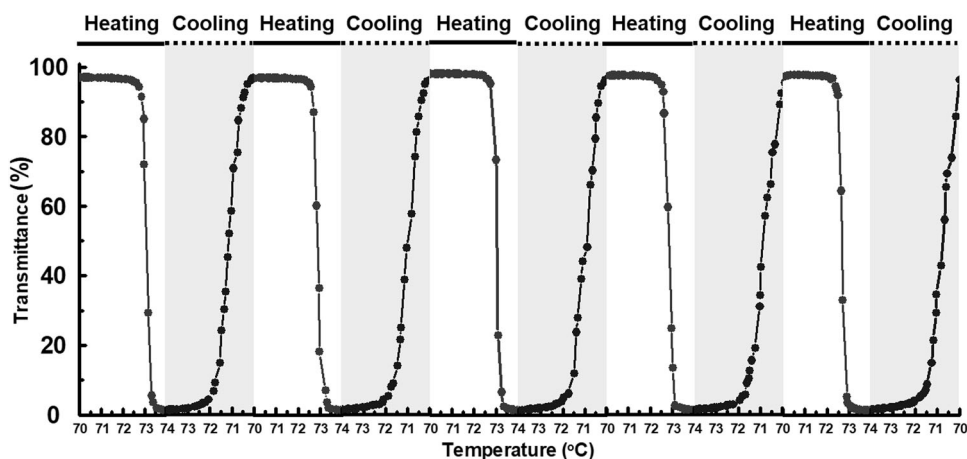


Fig. 4 SEM images of the hexa-block copolymer. The polymer solution (0.1 wt% in pH 7.4 PBS) was maintained at **a** 50 °C, **b** 60 °C, and **c** 70 °C for 15 min before the sample preparation

Fig. 5 Transmittance change during heating–cooling cycles of 0.1 wt% solution hexa-block copolymers in pH 7.4 PBS as a function of temperature



the amphiphilic structure of the copolymer. For example, dual temperature-responsive block copolymers become amphiphilic at temperatures between the LCSTs of each block and form nanoparticles via self-assembly. Therefore, the hexa-block copolymer is also expected to form nanoparticles in a specific temperature range. Figure 3c shows the transmittance change of 0.5 wt% hexa-block copolymer in PBS. A small transmittance decay (of ~5%) was observed at 45 °C. Then, the transmittance gradually decreased, and a drastic drop was observed at 70 °C. Interestingly, the onset temperature of 45 °C was similar to the response temperature of the third block (P(M₁₂-co-O₅)). These results suggest that the hexa-block copolymer forms nanoparticles between 45 and 70 °C. Figure 4 shows the SEM images of the nanoparticles formed by the hexa-block copolymer. The nanoparticles were kept at 50, 60, and 70 °C for 20 min before sample preparation for SEM measurements. The size of the nanoparticles was thus found to be influenced by temperature. Such size changes may arise from the ratio of hydrophobic and hydrophilic fragments in the hexa-block copolymer. Wei et al. prepared an LCST-LCST-type PEG-*b*-P(NIPAAm)-*b*-P(NIPAAm-co-HEAAm) tri-block copolymer [24]. The tri-block copolymer formed micelles

(37–45 °C) and vesicles (>50 °C) depending on the proportion of hydrophobic fragments. Figure 5 shows the reversibility of the dispersion and aggregation/precipitation of the nanoparticles in suspension after heating–cooling cycles. A hysteresis of ~2 °C was observed for each heating–cooling cycle. The ethylene glycol-type temperature-responsive copolymers P(MEO₂MA), P(OEGMA), and their copolymers are well known to show a relatively small hysteresis [25], and the hexa-block copolymer contains a sixth p(M₃-co-O₂₈) block located on the surface of the nanoparticles. The chain length of each block is relatively short, and the nanoparticles also bear dithioester groups, originating from the RAFT agent, on the surface. The surface properties strongly affect the dispersion/aggregation of the nanoparticles. Nakayama et al. reported the effect of pH-responsive sulfadimethoxine (SD) end groups on temperature-responsive nanoparticles, and the LCSTs were modified by the end group [26]. A nanogel with a shell of P(MEO₂MA₉₂-co-OEGMA₂₄) with COOH groups on the surface was found to show high dispersion via electrostatic repulsion [27]. The relatively short polymer chain and their surface properties might have led to the observed small hysteresis of the temperature-responsive properties.

Conclusion

In conclusion, an ethylene glycol-based hexa-block copolymer was prepared via RAFT polymerization. The molecular weight (M_n) and OEGMA content increased with the number of blocks (from the first block to the hexa-block copolymer). The M_n and M_w/M_n of the hexa-block copolymer were 40,400 g/mol and 1.41, respectively, and the OEGMA content was zero for the first block (P(MEO₂MA)) and 16, 30, 39, 64, and 91 mol% for each of the additional blocks. Each block of the hexa-block copolymer exhibited its own temperature-responsive behavior upon sequential dehydration, and the size of the resulting polymer nanoparticles was influenced by the temperature. Such a size variation may arise from the ratio of hydrophobic to hydrophilic fragments in the hexa-block copolymer. The nanoparticles in suspension displayed reversible dispersion and aggregation/precipitation under heating-cooling cycles with a hysteresis of ~2 °C. The structure of the hexa-block copolymer is similar to that of gradient-copolymers [28]. In the preparation of the hexa-block copolymer, the required OEGMA monomer was added for the polymerization of each block. The OEGMA concentration in the polymerization tube was increased in batches (not gradually). Therefore, the OEGMA content in each block was expected to be clearly different. However, because of the relatively short chain length of each block, it was difficult to fix the boundaries between adjacent blocks. Very recently, Liu et al. successfully prepared 10-block copolymers (97,000 g/mol) using a combination of enzymatic cascade catalysis and RAFT polymerization [29]. We would like to synthesize a temperature-responsive hexa-block copolymer with longer chain lengths in future. The present multi-temperature-responsive block copolymers exhibit multiple structural changes and can potentially be used as biomimetic materials.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Stuart MAC, Huck WTS, Genzer J, Müller M, Ober C, Stamm M, Sukhorukov GB, Szleifer I, Tsukruk VV, Urban M, Winnik F, Zauscher S, Luzinov I, Minko S. Emerging applications of stimuli-responsive polymer materials. *Nat Mater.* 2010;9:101–13.
2. Hoffman AS. Stimuli-responsive polymers: biomedical applications and challenges for clinical translation. *Adv Drug Deliv Rev.* 2013;65:10–6.
3. Roy D, Brooks WLA, Sumerlin BS. New directions in thermo-responsive polymers. *Chem Soc Rev.* 2013;42:7214–43.
4. Vancoillie G, Frank D, Hoogenboom R. Thermoresponsive poly (oligo ethylene glycol acrylates). *Prog Polym Sci.* 2014;39: 1074–95.
5. Stayton PS, Shimoboji T, Long C, Chilkoti A, Chen G, Harris JM, Hoffman AS. Control of protein-ligand recognition using a stimuli-responsive polymer. *Nature.* 1995;378:472–4.
6. Akimoto J, Nakayama M, Okano T. Temperature-responsive polymeric micelles for optimizing drug targeting to solid tumors. *J Control Release.* 2014;193:2–8.
7. Kikuchi A, Okano T. Intelligent thermoresponsive polymeric stationary phases for aqueous chromatography of biological compounds. *Prog Polym Sci.* 2002;27:1165–93.
8. Yang J, Yamato M, Shimizu T, Sekine H, Ohashi K, Kanzaki M, Ohki T, Nishida K, Okano T. Reconstruction of functional tissues with cell sheet engineering. *Biomaterials.* 2007;28:5033–43.
9. Dimitrov I, Trzebicka B, Müller AHE, Dworak A, Tsvetanov CB. Thermosensitive water-soluble copolymers with doubly responsive reversibly interacting entities. *Prog Polym Sci.* 2007;32: 1275–343.
10. Strandman S, Zhu XX. Thermo-responsive block copolymers with multiple phase transition temperatures in aqueous solutions. *Prog Polym Sci.* 2015;42:154–76.
11. Kotsuchibashi Y, Ebara M, Aoyagi T, Narain R. Recent advances in dual temperature responsive block copolymers and their potential as biomedical applications. *Polymers.* 2016;8:<https://doi.org/10.3390/polym8110380>.
12. Sugihara S, Kanaoka S, Aoshima S. Stimuli-responsive ABC triblock copolymers by sequential living cationic copolymerization: Multistage self-assemblies through micellization to open association. *J Polym Sci Polym Chem.* 2004;42:2601–11.
13. Weiss J, Laschewsky A. Temperature-induced self-assembly of triple-responsive triblock copolymers in aqueous solutions. *Langmuir.* 2011;27:4465–73.
14. Boyer C, Soeriyadi AH, Zetterlund PB, Whittaker MR. Synthesis of complex multiblock copolymers via a simple iterative Cu(0)-mediated radical polymerization approach. *Macromolecules.* 2011;44:8028–33.
15. Zhang Q, Collins J, Anastasaki A, Wallis R, Mitchell DA, Becer CR, Haddleton DM. Sequence-controlled multi-block glycopolymers to inhibit DC-SIGNp120 binding. *Angew Chem Int Ed.* 2013;52:4435–9.
16. Lutz J-F. Polymerization of oligo(ethylene glycol) (meth)acrylates: toward new generations of smart biocompatible materials. *J Polym Sci Polym Chem.* 2008;46:3459–70.
17. Lutz J-F, Hoth A. Preparation of ideal PEG analogues with a tunable thermosensitivity by controlled radical copolymerization of 2-(2-methoxyethoxy)ethyl methacrylate and oligo(ethylene glycol) methacrylate. *Macromolecules.* 2006;39:893–6.
18. Moad G. RAFT polymerization to form stimuli-responsive polymers. *Polym Chem.* 2017;8:177–219.
19. Kotsuchibashi Y, Ebara M, Yamamoto K, Aoyagi T. “On-off” switching of dynamically controllable self-assembly formation of double-responsive block copolymers with tunable LCSTs. *J Polym Sci Polym Chem.* 2010;48:4393–9.
20. Kotsuchibashi Y, Ebara M, Idota N, Narain R, Aoyagi T. A “smart” approach towards the formation of multifunctional nano-assemblies by simple mixing of block copolymers having a common temperature sensitive segment. *Polym Chem.* 2012;3: 1150–7.
21. Kotsuchibashi Y, Takiguchi T, Ebara M, Aoyagi T. The effects of the photo-induced proton generation on the assembly formation of

- dual-temperature and pH responsive block copolymers. *Polym Chem.* 2017;8:295–302.
22. Kotsuchibashi Y, Ebara M, Hoffman AS, Narain R, Aoyagi T. Temperature-responsive mixed core nanoparticle properties determined by the composition of statistical and block copolymers in the core. *Polym Chem.* 2015;6:1693–7.
 23. Luo S, Ling C, Hu X, Liu X, Chen S, Han M, Xia J. Thermo-responsive unimolecular micelles with a hydrophobic dendritic core and a double hydrophilic block copolymer shell. *J Colloid Interface Sci.* 2011;353:76–82.
 24. Wei H, Perrier S, Dehn S, Ravariana R, Dehghani F. One-pot ATRP synthesis of a triple hydrophilic block copolymer with dual LCSTs and its thermo-induced association behaviour. *Soft Matter.* 2012;8:9526–8.
 25. Lutz J-F, Akdemir Ö, Hoth A. Point by point comparison of two thermosensitive polymers exhibiting a similar LCST: is the age of poly(NIPAM) over? *J Am Chem Soc.* 2006;128:13046–7.
 26. Nakayama M, Kawahara Y, Akimoto J, Kanazawa H, Okano T. pH-induced phase transition control of thermoresponsive nanomicelles possessing outermost surface sulfonamide moieties. *Colloids Surf B.* 2012;99:12–9.
 27. Kotsuchibashi Y, Narain R. Dual-temperature and pH responsive (ethylene glycol)-based nanogels via structural design. *Polym Chem.* 2014;5:3061–70.
 28. Seno K, Tsujimoto I, Kikuchi T, Kanaoka S, Aoshima S. Thermosensitive gradient copolymers by living cationic polymerization: semibatch precision synthesis and stepwise dehydration-induced micellization and physical gelation. *J Polym Sci Polym Chem.* 2008;46:6151–64.
 29. Liu Z, Lv Y, An Z. Enzymatic cascade catalysis for the synthesis of multiblock and ultrahigh-molecular-weight polymers with oxygen tolerance. *Angew Chem Int Ed.* 2017;56:13852–6.