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Disparate polymerization facilitates the synthesis of versatile block copolymers from poly(trimethylene carbonate)

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ABSTRACT

A disparate polymerization technique is utilized for preparing versatile block copolymers from poly(trimethylene carbonate) (poly(TMC)). In this study, 4-(chloromethyl)benzyl alcohol (CBA) is used for the disparate polymerization. The hydroxyl group of CBA is involved in ring-opening polymerization and the benzyl chloride group is involved in incorporating dithiocarbamate for pseudo-living radical polymerization. First, TMC is polymerized from the hydroxyl group of CBA by using an organocatalyst. The benzyl chloride group in CBA is modified using a dithiocarbamate, and then vinyl and methacrylate monomers are polymerized by photo-driven pseudo-living radical polymerization. The resulting block copolymers are versatile and the molecular weight distribution is reasonably narrow. In the present study, *N*-isopropylacrylamide, acrylamide glycolic acid, and 2-hydroxyethyl methacrylate are used for the disparate polymerization. The resulting block copolymers could be well dissolved in water by incorporation of hydrophilic segment into hydrophobic poly(TMC). The solution property is characterized in terms of hydrophobic domain formation and phase transition under ambient conditions. Moreover, enzymatic degradation is evaluated by using a copolymer-coated substrate. The block copolymer synthesis technique is considerably versatile, and the resulting polymer function can be freely designed. The disparate polymerization technique is a promising approach that provides universal materials for integrating biodegradable polyesters and functional polymers.

1. Introduction

Poly(trimethylene carbonate) (poly(TMC)) is a biodegradable polymer, and several investigations have been conducted to study their suitability as biomaterials [1–4]. Poly(TMC) provides some advantages such as (i) excellent mechanical property with low glass transition temperature, (ii) high molecular motion under appropriate conditions, and (iii) easy and precise synthesis by ring-opening polymerization. Another advantage is degradation products could not contain any acidic compounds. In the case of conventional poly(lactic acid), acidic degradation product is produced; and it causes serious damage in our body. The design of block copolymers is crucial in terms of not only industrial products but also biomedical devices. Block copolymers allow the resulting materials to enhance their self-assemble behavior [5,6], phase separation behavior [7], thermo responsive behavior [8], and surfactant property [9,10]. Cyclic monomers such as TMC and lactide are easily polymerized by employing conventional ring-opening polymerization techniques that use organometallic catalysts. The resulting poly(TMC) is highly inert in terms of reactivity with another compound via functional groups. Only hydroxyl group exists at the terminal of poly(TMC). The lower reactivity is a fatal disadvantage in polyester-based materials such as poly(TMC) and poly(lactic acid). To improve the low reactivity, a copolymerization technique, which uses substituted-cyclic monomers such as depsipeptide and cyclic carbonate [11–14], is proposed. However, further alteration is required in terms of not only polymer synthesis but also material functionalities. A novel technique proposed by Waymouth Hedrick is employed for the synthesis of versatile block copolymers comprising poly(TMC) and poly(lactic acid) segments [15,16]. Reversible addition–fragmentation chain transfer (RAFT) compounds with hydroxyl group at the terminal are designed. RAFT compounds allow the synthesis of versatile vinyl and methacrylate monomers by means of living radical polymerization [17,18]. The resulting polymer contains a hydroxyl group on the terminal, and it is capable of further polymerization with cyclic monomers such as TMC and lactide. This alternative polymerization technique is called disparate polymerization [15].

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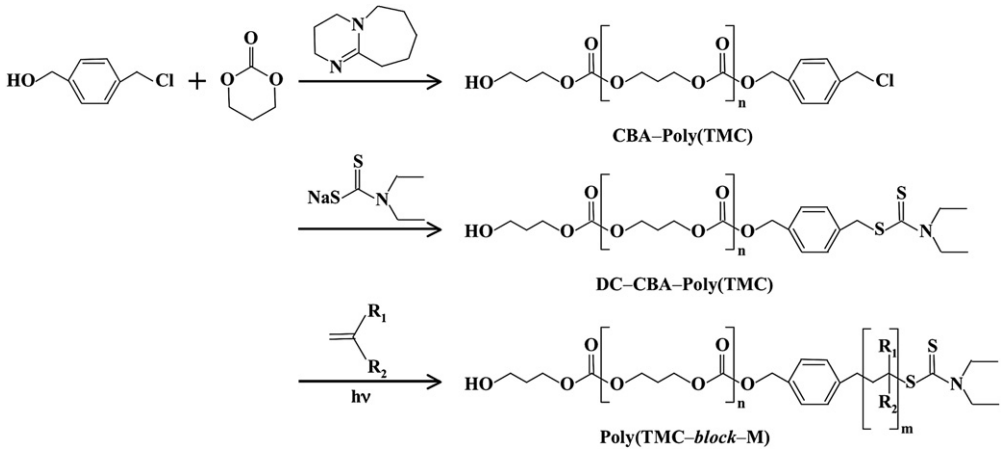

Fig. 1. Synthetic route of disparate polymerization using photoiniferter modified poly(TMC).

We have already synthesized a block copolymer composed of poly(ethylene glycol) and poly(TMC); the reflexive function of the polymer was reported on the basis of surface enrichment of the hydrophilic segment [19]. In this study, a hydroxyl group of poly(ethylene glycol) monomethyl ether (mPEG) was utilized for the ring-opening polymerization of TMC. In order to synthesize a versatile block copolymer using a poly(TMC) segment, ring-opening polymerization from the hydroxyl group on another functional polymer is a lack of diversity. An alternative approach is required, for example, the disparate polymerization is a good method for the design of versatile block copolymers. Therefore, we proposed a versatile polymerization technique, which involves vinyl and methacrylate monomers from the terminal of poly(TMC), for the production of block copolymers.

In the present study, photo-driven pseudo-living radical polymerization was carried out by using the terminal functional group on poly(TMC). A photoiniferter group was incorporated into the terminal of poly(TMC). The iniferter group is one of the compounds that allows pseudo-living radical polymerization. Originally, Otsu proposed the general iniferter-based polymerization technique; the term iniferter refers to a chemical compound that possesses the functions of an initiator, transfer agent, and terminator [20–22]. A typical iniferter compound is benzyl dithiocarbamate. As shown in Fig. 1, 4-(chloromethyl)benzyl alcohol (CBA) was selected as the connecting unit for the disparate polymerization. CBA provides two types of functional groups: the hydroxyl group for ring-opening polymerization and the benzyl chloride group for incorporation of the iniferter group. First, TMC was polymerized from the hydroxyl group of CBA by using an organocatalyst. The resulting benzyl chloride was modified using dithiocarbamate in order to incorporate the iniferter group. Finally, vinyl and methacrylate monomers were polymerized by photo-driven pseudo-living radical polymerization via the terminal iniferter group. In the present study, *N*-isopropylacrylamide, acrylamide glycolic acid, and 2-hydroxyethyl methacrylate were used for the disparate polymerization (Fig. 2). The function that is easily imagined from the structure was characterized as one example. The resulting copolymers based on poly(TMC) were characterized in terms of an amphiphilic property, a thermo responsive function, and surface enrichment of the hydrophilic segment.

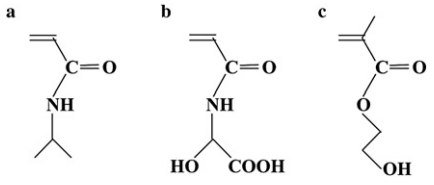

Fig. 2. Chemical structures of vinyl monomers for disparate polymerization; (a) *N*-isopropylacrylamide, (b) acrylamide glycolic acid, and (c) 2-hydroxyethyl methacrylate.

2. Experimental

2.1. Materials

For ring-opening polymerization, trimethylene carbonate (TMC) was purchased from Boehringer Ingelheim GmbH, Ingelheim, Germany. 4-(Chloromethyl)benzyl alcohol (CBA) was purchased from Sigma–Aldrich Corp., MO, USA. 1,8-Diazabicyclo[5.4.0]-7-undecene (DBU) (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) was used as a basic organocatalyst. For terminal modification of poly(TMC), sodium *N*,*N*-diethyldithiocarbamate trihydrate (Wako Pure Chemical Industries, Ltd, Osaka, Japan) was used. For photo-induced pseudo-living radical polymerization, *N*-isopropylacrylamide (NIPAAm) (KOHJIN Co., Ltd., Tokyo, Japan), 2-acrylamidoglycolic acid monohydrate (AGA) (Sigma–Aldrich Corp.), and 2-hydroxyethyl methacrylate (HEMA) (Wako Pure Chemical Industries) were used. The conventional ring-opening polymerization technique that involved the use of stannous octoate (tin(II) 2-ethyl hexanoate) (Wako Pure Chemical Industries) was performed in order to compare the synthetic result. Other organic solvents of extra pure grade were used, and they were purified by the usual method. A property of the polymer solution was evaluated by using a hydrophobic fluorescence probe, sodium 8-anilino-1-naphthalenesulfonate (ANS) (Tokyo Chemical Industry Co., Ltd.), and the critical micelle concentration was estimated. To examine enzymatic degradation, Dulbecco's phosphate buffered saline (PBS, pH 7.1, #14200-075, Invitrogen Corp., CA, USA) was used. As an enzyme, lipase (Wako, pig pancreas, 10 unit/mg) was used with a concentration of 10 mg/mL in PBS, and its supernatant was used in enzymatic degradations.

2.2. Instrument for characterization

The chemical structure of the compounds was confirmed by ^{1}H NMR (JNM-GSX, 400 MHz, JEOL, Tokyo, Japan) measurements. Size exclusion chromatography (SEC) measurements were performed on an HLC-8120GPC (Tosoh Corp., Tokyo, Japan) with a TSKgel SuperH4000 column (Tosoh Corp.) in order to determine the molecular weights and their distributions. As an eluent, dimethylformamide (DMF) was used. The flow rate was 0.6 mL/min and a universal calibration procedure based on polystyrene standards (Shodex Standard SM-105, Showa Denko K.K., Tokyo, Japan) was performed. To evaluate the solution property, a fluorescence

spectrophotometer (FP-6500, JASCO Corp., Tokyo, Japan) was used. Moreover, a UV–vis spectrophotometer (V-550, JASCO Corp.) was used for monitoring the changes in the transmittance of the polymer solution. To monitor the changes in weight of a block copolymer-coated substrate, a quartz crystal microbalance (QCM) substrate was used. The frequency of the quartz crystal resonator was 9 MHz (USI Co., Ltd, Fukuoka, Japan), and the change in frequency was monitored by using a frequency counter (53131A, Agilent Technologies, Santa Clara, CA, USA).

2.3. Synthesis of poly(TMC) and incorporation of the iniferter group

To achieve disparate polymerization, CBA comprising both hydroxyl and chloromethyl groups was used. CBA and DBU were dissolved in methylene chloride (2 mL). TMC was dissolved in methylene chloride, and then their solutions were mixed together. The final concentration of TMC was adjusted as 2 mol/L. The solutions were mixed together, and the resulting mixture was stirred for 8 h at room temperature. All the procedures were carried out under nitrogen atmosphere. To terminate the polymerization, benzoic acid (20 times the amount of CBA) was added to the solution. The crude product was added to the large amount of methanol in order to precipitate the poly(TMC). The final product was dried under reduced pressure, and the composition was confirmed by ^{1}H NMR and SEC measurements. ^{1}H NMR (400 MHz, $CDCl_3$) (ppm): 1.86 (m, 2H, –*CH*$_2$–CH$_2$–OH), 2.05 (m, 2H, –CH$_2$–*CH*$_2$–CH$_2$–), 3.72 (q, 2H, –*CH*$_2$–OH), 4.25 (t, 2H, –O–C*H*$_2$–CH$_2$–), 4.58 (s, 2H, Cl–C*H*$_2$–Ph), 5.12 (s, 2H, –O–C*H*$_2$–Ph), 7.38 (dd, 4H, aromatic).

Alternatively, the conventional ring-opening polymerization was carried out by using stannous octoate. The typical procedure was as follows. TMC and CBA were added to a round-bottom flask equipped with a magnetic stir bar. Stannous octoate (14.5 μL of a toluene solution (0.33 mol/L)) was added, and the flask was evacuated with a vacuum pump for 20 h. The flask was heated in an oil bath, and the polymerization was allowed to continue. After cooling, the mixture was dissolved in chloroform and poured into a large amount of methanol to obtain poly(TMC). The product was dried under reduced pressure, and the product was confirmed by ^{1}H NMR measurements.

To incorporate the iniferter group, the terminal chloromethyl group was reacted with sodium *N*,*N*-diethyldithiocarbamate trihydrate. Poly(TMC) with the chloromethyl group on the terminal was dissolved in anhydrous acetone under a nitrogen atmosphere. Sodium *N*,*N*-diethyldithiocarbamate trihydrate was added to the solution, and it was stirred at 0 °C for 1 h. The mixture was stirred for another four days at room temperature. The crude product was poured into ultrapure water, and the precipitate was recovered and dried under reduced pressure. The final product was confirmed by ^{1}H NMR measurements. ^{1}H NMR (400 MHz, $CDCl_3$) (ppm): 1.25 (t, 3H, –N–CH$_2$–*CH*$_3$), 1.86 (m, 2H, –*CH*$_2$–CH$_2$–OH), 2.05 (m, 2H, –CH$_2$–*CH*$_2$–CH$_2$–), 3.72 (q, 2H, –*CH*$_2$–OH), 4.25 (t, 2H, –O–*CH*$_2$–CH$_2$), 4.54 (s, 2H, –S–C*H*$_2$–), 5.12 (s, 2H, –O–C*H*$_2$–Ph), 7.27–7.47 (m, 4H, aromatic).

2.4. Disparate polymerization by the macro-iniferter

Disparate polymerization was carried out by using conventional vinyl monomers. The poly(TMC) macro-iniferter was dissolved in DMSO (for AGA) or THF (for NIPAAm or HEMA), and a given amount of vinyl monomer was added. In this study, NIPAAm, AGA, and HEMA were used. The synthetic condition in feed was summarized in Table 2. After nitrogen bubbling for 15 min, photo-induced polymerization was carried out by using a high-pressure mercury lamp (UVL-400P, Riko, Chiba, Japan) for 90 min at room temperature. The distance between the lamp and a flask was adjusted to 10 cm. Wavelength of the irradiation light was over 280 nm, and the irradiation output was 400 W. The crude product was poured into a large amount of methanol (or ethanol) to precipitate the resulting polymer. The synthesized copolymers were abbreviated as follows: poly(TMC-*block*-NIPAAm), poly(TMC-*block*-AGA), and poly(TMC-*block*-HEMA). The resulting block copolymers were dried under reduced pressure and were confirmed by ^{1}H NMR measurements.

2.5. Characterization of the resulting copolymers for biomaterials

In this study, the resulting block copolymers (poly(TMC-*block*-NIPAAm) and poly(TMC-*block*-AGA)) were characterized by evaluating the following biomaterial aspects: critical micelle concentration (CMC), lower critical solution temperature (LCST), and enzymatic degradation.

In the case of CMC evaluation, the block copolymers of poly(TMC-*block*-NIPAAm) were dissolved in ultrapure water to form a 10 mg/mL stock solution. Alternatively, the concentration of poly(TMC-*block*-AGA) was adjusted to 1 mg/mL due to the lower amount of the compound. The stock solution was sequentially diluted to obtain a concentration of 10^{-4}–1 mg/mL. The fluorescence probe (ANS) was dissolved in the polymer solution, and the final concentration was adjusted to 0.1 wt%. The maximum fluorescence wavelength was measured by using a fluorescence spectrometer ($\lambda_{Ex} = 385$ nm).

The LCST measurement of poly(TMC-*block*-NIPAAm) was carried out as follows. The copolymer was dissolved in ultrapure water to form a 0.2 wt% solution. The solution was set in a quartz cuvette, and the transmittance at 500 nm was monitored by using a UV–vis spectrometer, and the solution temperature was regulated by using a Peltier heating system (1 °C/min).

Enzymatic degradation of the polymer-coated surface (poly(TMC-*block*-AGA)) was estimated by using a QCM with a parent frequency of 9 MHz. The change in the frequency shift was correlated to the weight loss by using Sauerbrey's equation (1 Hz corresponds to roughly 1.15 ng) [23]. The change in frequency was monitored by a frequency counter. Then, the mass per unit area was determined by measuring the change in frequency of the quartz crystal resonator. The QCM substrates were treated three times with a piranha solution (concentrated H_2SO_4/H_2O_2 (30 wt% aqueous solution) = 3/1, v/v) for 1 min, followed by rinsing with ultrapure water. The poly(TMC-*block*-AGA) and poly(TMC) homopolymers were dissolved in a DMSO/chloroform mixed solvent to form a 0.2 wt% polymer blend solution. The polymer solution was coated onto the QCM substrate by spin coating (1500 rpm, 60 s). The resulting substrates were immersed in PBS lipase solutions (100 unit/mL) at 37 °C. The QCM substrate was taken out from the lipase solution in 15 min interval; the substrate was mildly rinsed by ultrapure water. After drying with nitrogen gas, the frequency of the QCM substrate was monitored by a frequency counter. After the measurement of frequency, the QCM substrate was immersed in the lipase solution again. This procedure was repeated for 135 min. After a certain amount of time, the QCM substrate was rinsed in ultrapure water and then dried with N_2 gas. The frequency was monitored and plotted as a function of the degradation time.

3. Results and discussion

3.1. Synthesis of block copolymers

For the synthesis of block copolymers using disparate polymerization, ring-opening polymerization of TMC and photo-driven pseudo-living radical polymerization of vinyl and methacrylate monomers were performed. The key compounds for the polymerization were the hydroxyl and benzyl chloride groups. The

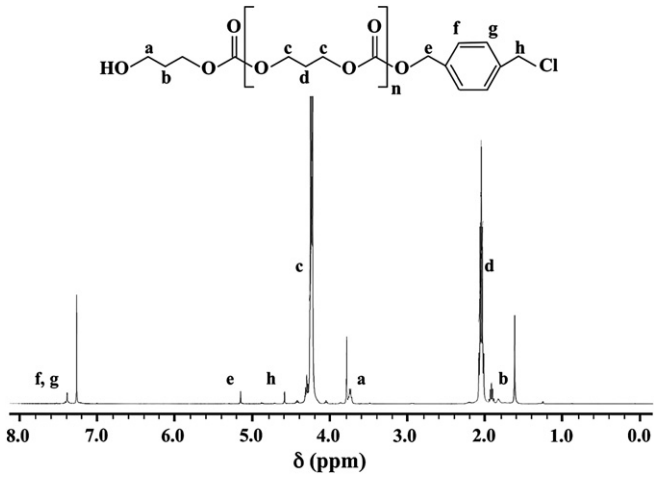

Fig. 3. ^{1}H NMR spectrum of CBA–poly(TMC).

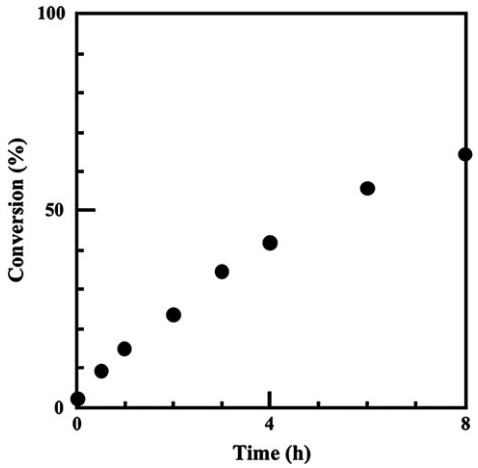

Fig. 5. Time–conversion profile of ring-opening polymerization of TMC.

polymerization occurred as follows: (i) ring-opening polymerization of TMC from the hydroxyl group on CBA, (ii) incorporation of the dithiocarbamate group on benzyl chloride to form the iniferter group, and (iii) photo-driven pseudo-living radical polymerization of the monomers.

First, poly(TMC) was synthesized by using CBA. Generally, cyclic monomers such as TMC and lactide can be easily polymerized by using organometallic catalysts such as stannous octoate. However, the reaction temperature was significantly high to polymerize TMC. We attempted to polymerize TMC by using stannous octoate at 120 °C. It is considered that the side reactions might have proceeded because the benzyl chloride group at the terminal of CBA was not observed. Thus, ring-opening polymerization at mild temperatures was required. Recently, organocatalytic ring-opening polymerization was reported [15,16]. Appropriate quantities of thiourea and basic amine as organocatalyst allow the polymerization of cyclic TMC monomers in a ring-opening fashion. In the present study, DBU was used as the organocatalyst, and it allowed the polymerization at room temperature. Fig. 3 shows the ^{1}H NMR spectrum of poly(TMC) using CBA. Each proton was observed; in particular, methylene proton connected with benzene (proton e and h) was clearly observed and the integral ratio was also similar. Taking this into account, the ring-opening polymerization was successfully achieved by using the organocatalyst. Moreover, we have checked the time–conversion profile to confirm safely polymerizing in the ring-opening fashion. Fig. 4 shows the confirmation of the polymerization by ^{1}H NMR spectrum. The proton NMR spectrum of $CDCl_3$ solution shows that the signals of methylene protons (neighbors of oxygen) on the TMC monomer appear at 4.4 ppm; the chemical shift in the protons occurs at 4.2 ppm. After polymerization for 5 min, a large peak attributed to the TMC monomer is observed at 4.4 ppm, and the methylene proton in poly(TMC) appears. The peak of the methylene proton increases with the polymerization time; in particular, the peak is clearly observed after polymerization for 3 h. The polymerization profile is evaluated by using the time–conversion profile as shown in Fig. 5. The conversion is calculated from the integral ratio of each methylene proton. The time–conversion profile is well correlated within 4 h. After 4 h, the conversion becomes slightly saturated due to the lower concentration of the TMC monomer. From the time–conversion profile, it is determined that the conversion of ring-opening polymerization at room temperature reaches roughly 70%. In the present study, two types of poly(TMC) are prepared as shown in Table 1. The terminology is abbreviated to CBA–poly(TMC60), which indicates CBA-terminated poly(TMC) with 60 repeating units of TMC. The feed ratio of DBU and CBA is 0.5–1.0, and the resulting polymers have an appropriate molecular weight distribution.

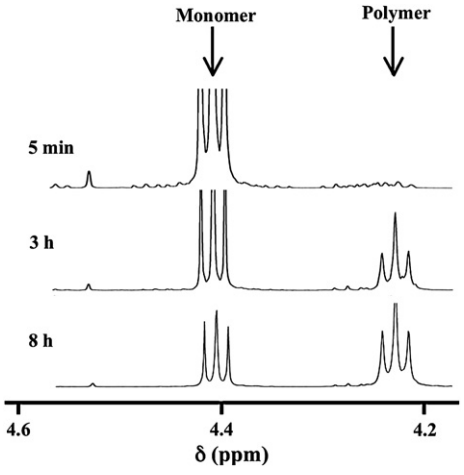

Fig. 4. Changes in the peaks regarding TMC monomer and TMC unit of poly(TMC).

CBA–poly(TMC) was successfully reacted with sodium *N*,*N*-diethyldithiocarbamate trihydrate to incorporate the iniferter group on the terminal, and the resulting compound was abbreviated to DC–CBA–poly(TMC). Each proton attributed to *N*,*N*-diethyldithiocarbamate ($\delta = 4.54$, s, 2H, –S–CH_2–) and benzyl ($\delta = 5.12$, s, 2H, –O–CH_2–Ph) was confirmed by ^{1}H NMR measurements. The incorporation yield was calculated from the integral ratio above mentioned. Roughly 90% of the terminal CBA group was converted to dithiocarbamate group. Further, pseudo-living radical polymerization was performed using NIPAAm, AGA, and HEMA. Table 2 shows the results obtained for the disparate polymerization. The polymerization was carried out by using aprotic polar solvents such as DMSO for AGA and THF for NIPAAm and HEMA. Fig. 6 shows SEC trace of block copolymers and their precursors. In the case of CBA–poly(TMC), mono distribution of SEC trace was observed. The

Table 1
Synthesis of CBA–poly(TMC)

Sample	Feed ratio [DBU]/[CBA]	Yield[a] (%)	Molecular weight				DP[b]
			M_n[b]	M_n[c]	M_w[c]	M_w/M_n[c]	
CBA–poly(TMC60)	1.0	43	6480	4000	6500	1.6	60
CBA–poly(TMC27)	0.5	30	2800	3600	6400	1.8	27

[a] Methanol insoluble product.
[b] Determined by ^{1}H NMR.
[c] Determined by size exclusion chromatography (polystyrene standard).

Table 2
Synthesis of versatile block copolymers[a,b]

Sample	Monomer in feed (mg)	Molecular weight				DP[c]	Yield(%)
		M_n[c]	M_n[d]	M_w[d]	M_w/M_n[d]		
Poly(TMC60-*block*-NIPAAm55)	170 (1.5 mmol)	12 700	7400	18 500	2.5	55	50[e]
Poly(TMC27-*block*-NIPAAm26)	400 (3.6 mmol)	5700	8500	17 000	2.0	28	47[e]
Poly(TMC27-*block*-AGA29)	580 (3.6 mmol)	7000	5200	8100	1.6	29	57[f]
Poly(TMC27-*block*-HEMA40)	465 (3.6 mmol)	8000	6200	13 000	2.1	40	18[e]

[a] [Monomer]/[DC–CBA–poly(TMC)] = 100.
[b] DC–CBA–poly(TMC60) = 100 mg (15 μmol), DC–CBA–poly(TMC27) = 100 mg (36 μmol) were used.
[c] Determined by ^{1}H NMR.
[d] Determined by size exclusion chromatography (polystyrene standard).
[e] Methanol insoluble product.
[f] Ethanol insoluble product.

elution time was roughly 8.6–8.7 min, and the number average molecular weight was estimated as 4000 and 3600 g/mol in each precursor. After the pseudo-living radical polymerization for synthesis of block copolymer, the elution time shifted earlier than that of precursor. In the case of poly(TMC27-*block*-AGA29), change in the elution time was quite small ($t = 8.64$). However, the molecular weight that determined by ^{1}H NMR was 7000 g/mol, showing much higher than that of the precursor. The molecular weight distribution was 1.6–2.5, and it was slightly larger but within the adequate range for pseudo-living radical polymerization by iniferter technique. The resulting copolymers were well dissolved in ultrapure water at room temperature, relative to the starting DC–CBA–poly(TMC). The solubility varied after the second polymerization. Taking this into account, the disparate polymerization composed of ring-opening polymerization and photo-driven pseudo-living radical polymerization was successfully achieved. The chemical and biological functions are evaluated in the following section.

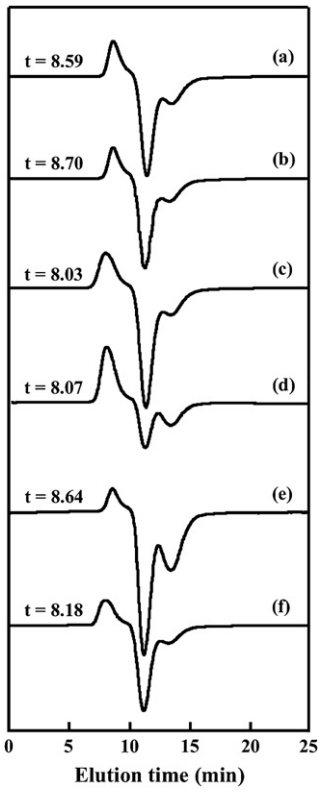

Fig. 6. SEC trace of (a) CBA–poly(TMC60), (b) CBA–poly(TMC27), (c) poly(TMC60-*block*-NIPAAm55), (d) poly(TMC27-*block*-NIPAAm26), (e) poly(TMC27-*block*-AGA29), and (f) poly(TMC27-*block*-HEMA40). Elution time was indicated in each trace.

3.2. Solution properties of poly(*TMC*-block-*NIPAAm*) and poly(*TMC*-block-*AGA*)

The polymer morphology in the aqueous solution was characterized in terms of the formation of the hydrophobic domain. ANS is a fluorescence probe used to analyze solution property. The maximum fluorescence wavelength was changeable depending on the hydrophobicity of the domain. In the case of ultrapure water, the maximum fluorescence wavelength was observed at 515 nm ($\lambda_{Ex} = 385$ nm); alternatively, the wavelength shifted to 470 nm in the case of the hydrophobic domain. Therefore, the ANS probe is a type of indicator to confirm the hydrophobic domain in a polymer solution. Fig. 7 shows the changes in the maximum fluorescence wavelength of the ANS in two types of block copolymer solutions. The polymer concentration was adjusted from 10^{-4} to 10 mg/mL. In the case of poly(TMC27-*block*-NIPAAm26), the maximum fluorescence wavelength was observed at 515 nm (10^{-4}–10^{-1} mg/mL). The wavelength suddenly shifted to 470 nm when the concentration exceeded 10^{-1} mg/mL, indicating the formation of the hydrophobic domain in the polymer solution. The change in wavelength depended on the polymer conformation in an aqueous media. To obtain more data, dynamic light scattering of poly(TMC27-*block*-NIPAAm26) was monitored using Zetasizer Nano-ZS, Malvern Ins., Ltd, UK. The size of polymer association was 45 nm at 20 °C (1 mg/mL). Taking these results into account, the block copolymer showed amphiphilic property in an aqueous media. Similar results were observed in the case of poly(TMC27-*block*-AGA29). The maximum fluorescence wavelength for concentrations exceeding 10^{-1} mg/mL was considerably short as compared to that for higher

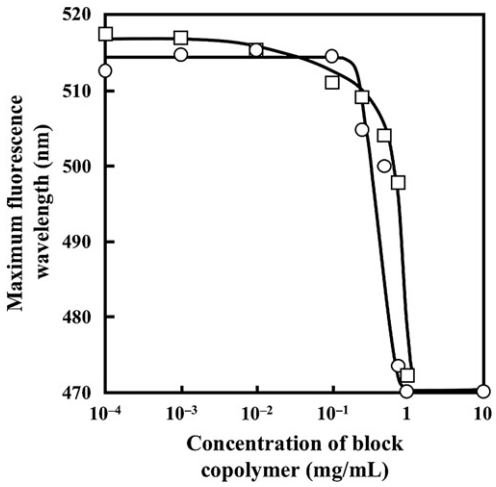

Fig. 7. Change in maximum fluorescence wavelength of ANS in copolymer solutions at 25 °C; poly(TMC27-*block*-NIPAAm26) (open circle) and poly(TMC27-*block*-AGA29) (open square).

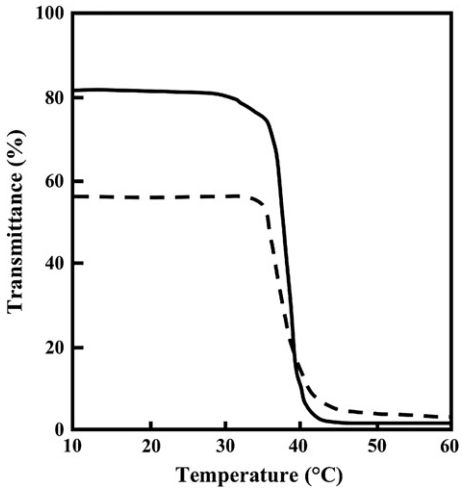

Fig. 8. Change in transmittance of block copolymer (0.2 wt%) as a function of temperature (heating rate 10 °C/min); poly(TMC60-*block*-NIPAAm55) (dotted line) and poly(TMC27-*block*-NIPAAm26) (solid line).

concentrations. Taking this into account, the block copolymers were shown to possess the amphiphilic property, from the results of the solution property determined by using the ANS probe.

The block copolymer comprising the NIPAAm segment might exhibit the thermoresponsive property, thereby indicating change in the transmittance of the solution. Fig. 8 shows the result of the transmittance as a function of temperature. The transmittance is greater than 60% at low temperatures (below 30 °C). The solution was heated, and then the transmittance clearly decreased at around 37 °C, which is the phase transition temperature. The phase transition behavior was similar for each poly(TMC-*block*-NIPAAm) despite the different compositions. However, the higher molecular weight of the block copolymer was slightly turbid even at temperatures below the phase transition temperature. From this result, the resulting copolymers comprising the NIPAAm segment showed clear phase transition and the temperature was close to the physiological conditions.

3.3. *Enzymatic degradation properties of poly(TMC*-block-*AGA)*

In our previous study, poly(TMC)–mPEG was prepared. The hydrophilic segment of mPEG was spontaneously enriched onto the polymer-coated surface under wet conditions, and then enzymatic degradation by lipase was suppressed by the hydrophilic segment [19]. The copolymer, poly(TMC27-*block*-AGA29), contained carboxyl groups on the polymer backbone. The carboxyl groups on poly(AGA) segment were charged anions (p*K*a = 4.5) under physiological conditions. On the other hand, an isoelectric point (p*I*) of the lipase is 5.2; therefore, the lipase is also a charged anion under physiological conditions. In the present study, the poly(AGA) segment was hydrophilic, and the electrostatic repulsion between the lipase and poly(AGA) segment was expected. Unfortunately, poly(TMC27-*block*-AGA29) had higher water solubility, and the block copolymer was blended with the poly(TMC) homopolymer to reduce its dissolution.

The enzymatic degradation was evaluated by using the QCM technique. The block copolymer was coated onto the QCM substrate, and it was then immersed into the lipase solution at a given time. The change in frequency corresponding to the weight loss was monitored. Fig. 9(a) shows the change in frequency of the QCM. In the case of poly(TMC), the frequency drastically decreased from 1300 to 300 Hz, indicating the enzymatic degradation of poly (TMC). On the other hand, no enzymatic degradation was observed without the lipase. Taking this into account, the enzymatic degradation was allowed to proceed and was clearly detectable by using QCM. Poly(TMC27-*block*-AGA29) was blended with poly(TMC) homopolymer, and the blending ratio (homopolymer:block copolymer) was 3:7, 5:5, and 7:3. In the case of 7:3 blending, the blended polymer was coated onto the substrate, and the frequency was observed to be 800 Hz, indicating a thickness of roughly 15 nm. The frequency gradually decreased with increase in incubation time, thereby indicating enzymatic degradation. Moreover, similar results were observed despite the blending ratio (3:7 and 5:5). The time to reach complete diffusion of lipase might be dominant factor in the enzymatic degradation. However, the data shown in Fig. 9 suggest that the diffusion rate was much faster relative to the degradation time. Therefore, the suppression of enzymatic degradation was due to the electrostatic repulsion by hydrophilic segment. Although the hydrophilic poly(AGA) segment could not be enzymatically degraded, the resulting degradation product of poly(TMC27-*block*-AGA29) would be fully dissolved from the QCM substrate. This result indicated that the hydrophilic poly(AGA) segment played an important role in suppression of enzymatic degradation. The raw data obtained for the change in frequency showed a similar trend, and the results were not discussed in detail. Therefore, the change in frequency was normalized by the initial frequency, as shown in Fig. 9(b). The weight loss (%) was plotted as a function of the degradation time. From the normalized result, the

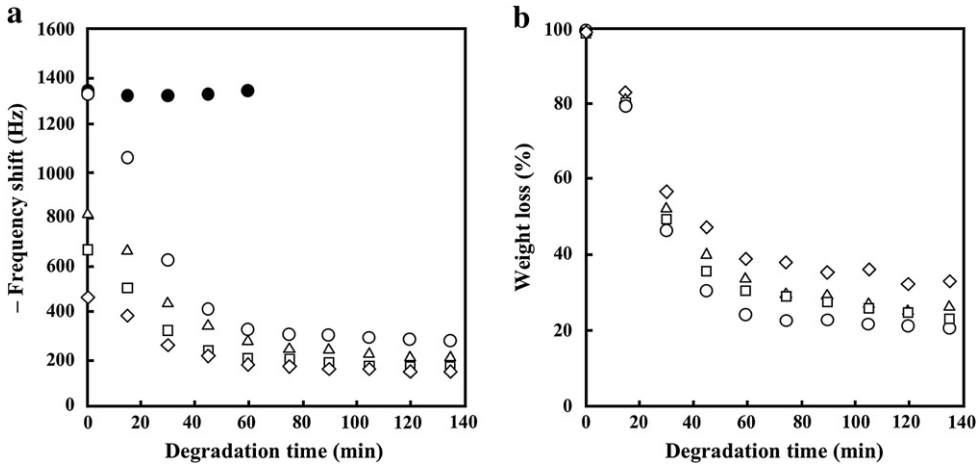

Fig. 9. Enzymatic degradation of poly(TMC-*block*-AGA) on QCM substrate; (a) change in frequency shift and (b) normalized weight loss. Blending ratio between poly(TMC) and poly(TMC27-*block*-AGA29) was changed; 10:0 (open circle), 7:3 (triangle), 5:5 (square), and 3:7 (diamond). Closed circle indicates change in frequency of poly(TMC)-coated QCM without lipase.

effect of blending was observed. In the case of 3:7 blending (higher block copolymer content), the remaining polymer was roughly 40%. The weight loss increased with decrease in the block copolymer content. Taking this into account, the block copolymer suppressed the enzymatic degradation. The dominant factor was considered to be the surface enrichment of the hydrophilic segment in addition to the electrostatic repulsion. The mechanism will be reported in detail in our forthcoming paper.

4. Conclusions

In the present study, the disparate polymerization technique was demonstrated by using ring-opening polymerization of TMC, followed by photo-driven pseudo-living radical polymerization to form a hydrophilic segment. The resulting copolymer was roughly regulated by the repeating unit of monomers, and it showed amphiphilic property. The copolymer comprising the NIPAAm segment provided the phase transition temperature, and anion-charged hydrophilic segment suppressed the enzymatic degradation of the poly(TMC) segment. Hence, the disparate polymerization was successfully achieved and the resulting copolymer showed versatile properties. The disparate polymerization technique is a good candidate for polymer design with desired properties.

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