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Amphiphilic graft copolymers: Effect of graft chain length and content on colloid gel

Kyohei Nitta[1], Atsushi Kimoto[2], Junji Watanabe[*2] and Yoshiyuki Ikeda[2]

[1]Department of Life and Functional Material Science, Graduate School of Natural Science, Konan University, 8-9-1 Okamoto, Higashinada-ku, Kobe 658-8501, Japan
[2]Department of Chemistry of Functional Molecules, Faculty of Science and Engineering, Konan University, 8-9-1 Okamoto, Higashinada-ku, Kobe 658-8501, Japan

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Abstract. A series of amphiphilic graft copolymers were synthesized by varying the number of graft chains and graft chain lengths. The polarity of the hydrophobic graft chain on the copolymers was varied their solution properties. The glass transition temperature of the copolymers was in the low-temperature region, because of the amorphous nature of poly (trimethylene carbonate) (PTMC). The surface morphology of the lyophilized colloid gel had a bundle structure, which was derived from the combination of poly(*N*-hydroxyethylacrylamide)(poly(HEAA)) and PTMC. The solution properties were evaluated using dynamic light scattering and fluorescence measurements. The particle size of the graft copolymers was about 30-300 nm. The graft copolymers with a higher number of repeating units attributed to the TMC (trimethylene carbonate) component and with a lower macromonomer ratio showed high thermal stability. The critical association concentration was estimated to be between 2.2×10^{-3} and 8.9×10^{-2} mg/mL, using the pyrene-based fluorescence probe technique. These results showed that the hydrophobic chain of the graft copolymer having a long PTMC segment had a low polarity, dependent on the number of repeating units of TMC and the macromonomer composition ratio. These results demonstrated that a higher number of repeating units of TMC, with a lower macromonomer composition, was preferable for molecular encapsulation.

Keywords: poly(trimethylene carbonate); amphiphilic graft copolymer; colloid gel; critical association concentration; molecular incorporation

1. Introduction

Biomaterials must be versatile, displaying properties such as good biocompatibility, biodegradability, mechanical strength, functionality, specific surface properties, and non-toxicity. A great deal of research is being pursued in order to achieve these properties. Biocompatible polymers such as poly(L-lactic acid) (PLA) and poly(ε-caprolactone) (PCL) have been widely studied for use as biomaterials (Kim *et al*. 2009, Kim *et al*. 2006, Amsden *et al*. 2004) As an aliphatic polycarbonate, poly(trimethylene carbonate) (PTMC) is one of the hydrophobic polymers that have been widely investigated for biomedical applications (Zhang *et al*. 2006, Andronova

∗Corresponding author, Professor, E-mail: junjiknd@konan-u.ac.jp

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et al. 2006, Terao *et al*. 2012, Nederberg *et al*. 2007a, b, Watanabe *et al*. 2007, Watanabe *et al*. 2008, Atthoff *et al*. 2006, Srivastava *et al*. 2007).

PTMC has a number of advantageous properties compared with PLA and PCL, including good biocompatibility, excellent biodegradability, and an amorphous structure. In addition, trimethylene carbonate (TMC), the cyclic monomer of PTMC, is widely available as an industrial reagent. TMC is easily polymerized by ring-opening polymerization (ROP) using various catalysts such as organometallic compounds, organic compounds, enzymes, and acids (Watanabe *et al*. 2008, Cho *et al*. 2008, Mindemark *et al*. 2007, Shibasaki *et al*. 2000, Zheng *et al*. 2004, Bisht *et al*. 1998, Hyun *et al*. 2008). However, recently, concerns have been raised regarding the biosafety of organometallic catalysts. Therefore, we attempted to use 1,8–diazabicyclo[5.4.0]undec–7–ene (DBU) as a basic organic catalyst because of its reduced cytotoxic potential (Dove 2012).

Amphiphilic polymers with hydrophobic and hydrophilic segments self-assemble to form a hydrophobic domain in aqueous media. Thus, amphiphilic copolymers have enormous potential as drug delivery systems (DDS) to enhance drug-loading efficiency (Siegel and Pitt 1995). DDS vehicles in solution consist of a stable core-shell structure formed by the aggregation of polymer chains. In many studies, biocompatible amphiphilic block copolymers have been designed using poly(ethylene glycol), poly(methacrylic acid), and poly(2-methacryloyloxyethyl phosphorylcholine) as the hydrophilic segment, and PLA, PCL, or PTMC as the hydrophobic segment (Kim *et al*. 2009, Kim *et al*. 2006, Nederberg *et al*. 2007, Nam *et al*. 2002, Tosaki *et al*. 2011, Tyson *et al*. 2009, Ishihara *et al*. 1999).

In our previous study, we proposed and prepared an amphiphilic graft copolymer (Nitta *et al*. 2012a, b). We evaluated the polarity of the hydrophobic domain formed by poly(*N*–hydroxyethyl acrylamide) (poly(HEAA)) grafted with PTMC (PHT) in aqueous solution. The driving forces for the self-assembly are hydrogen bonding and hydrophobic interaction, and these properties can be adjusted by changing the composition ratio and the chain length of the macromonomer. Thus, PHT copolymers in aqueous solution could spontaneously form aggregates because of these driving forces (Nitta *et al*. 2012b).

In this study, we prepared three kinds of amphiphilic graft copolymers. Two of these had similar hydrophobic chain lengths but different monomer composition ratios. The other combination had a similar total chain length, but different lengths of the hydrophobic segments. Thus, the effect of each molecular force such as hydrogen bonding between HEAA units and hydrophobic interaction by PTMC graft chains could be evaluated in terms of polymer colloid formation for molecule encapsulation.

We studied the properties of the PHT copolymers as well as their critical association concentrations (CAC) and partition equilibrium constants (K_v). The process of aggregation changes the solution properties, such as surface tension, turbidity, and light scattering intensity. Therefore, determining the values of CAC and K_v is important for DDS, as they help in gauging the drug-loading ability of the aggregates. CACs and K_v of the PHT copolymers were calculated by a fluorescence probe technique using the hydrophobic molecule pyrene, which is commonly employed to evaluate hydrophobic environments in aggregated structures (Shibasaki *et al*. 2000, Hyun *et al*. 2008, Kim *et al*. 2000, Mattanavee *et al*. 2009). The resulting data regarding the aggregate formation are extremely important for *in vivo* studies of this DDS vehicle in biomedical applications.

2. Experimental methods

2.1 Materials

In order to synthesize the macromonomer, the conventional ROP was performed. Trimethylene carbonate (TMC) was purchased from Boehringer Ingelheim GmbH (Ingelheim, Germany). HEAA was provided by KOHJIN Co., Ltd., Tokyo, Japan. DBU (Kanto Chemical Co., Ltd., Tokyo, Japan) was used as a basic organocatalyst. The termination reaction of the ROP was performed using benzoic acid (Wako Pure Chemical Industries Co., Ltd., Osaka, Japan). To synthesize amphiphilic graft copolymers with the oligo PTMC segments, radical polymerization was carried out using 2,2′–azobis(isobutyronitrile) (AIBN; Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) as the initiator. All organic solvents were used as received.

2.2 Instruments

Proton nuclear magnetic resonance (^{1}H NMR) measurements were performed using a Unity INOVA AS 500 MHz spectrometer (Varian Technologies Japan Co., Ltd., Tokyo, Japan) and were used to confirm the chemical structures, degree of polymerization (DP), and composition ratios. For the ^{1}H NMR measurements, the macromonomer was dissolved in deuterated chloroform ($CDCl_3$), and the PHT copolymers were dissolved in deuterated dimethyl sulfoxide (DMSO-d_6). Chemical shifts were recorded downfield from 0.0 ppm using tetramethylsilane as an internal standard. The average molecular weight and molecular weight distribution of the PHT copolymers were determined using gel permeation chromatography (GPC; Showa Denko Co., Ltd., Tokyo, Japan) and compared with polystyrene standard. All polymer samples were dissolved in *N*,*N*–dimethylformamide (DMF) at a concentration of 1 mg/mL in the presence of 10 mmol/L lithium bromide. GPC measurements were performed with a Shodex column (SB-804HQ, Showa Denko Co., Ltd., Tokyo, Japan) with a DMF eluent flow rate at 1.0 mL/min. The morphologies of the lyophilized copolymers were observed using field-emission scanning electron microscopy (FE-SEM; JSM-6340FB, JEOL Co., Ltd., Tokyo, Japan), after sputter-coating with platinum (JFC-1600 AUTO FINE COATER, JEOL Co., Ltd). The thermal properties of the copolymer were monitored using differential scanning calorimetry (DSC; Thermo Plus DSC8230, Rigaku Co., Ltd., Tokyo, Japan). The polymer aggregate size was determined by dynamic light scattering measurements (DLS; Zetasizer Nano ZS, Malvern Instruments Co., Ltd., Malvern, UK). Fluorescence measurements were performed on a fluorescence spectrophotometer (F-2500, Hitachi Co., Ltd., Tokyo, Japan). Pyrene was used to evaluate the CACs and partition equilibrium constants (K_v).

2.3 Preparation of amphiphilic graft copolymers using macromonomer method

The graft copolymers in this study were synthesized using a macromonomer method. Conventional ROP of TMC from HEAA was first performed to obtain HEAA–PTMC macromonomer according to a previously reported procedure (Nitta *et al*. 2012a). The DP of PTMC in the macromonomer was calculated from the ^{1}H NMR spectrum. The synthesized HEAA–PTMC macromonomers contained 10 or 50 TMC units. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.1 (*m*, 2H, –CH_2–CH_2–CH_2–), 3.6 (*q*, 2H, –CH_2–CH_2–O–), 3.7 (*t*, 2H, –NH–CH_2–CH_2–), 4.2 (*t*, 4H, –CH_2–CH_2–CH_2–), 5.6 (*d*, 1H, *H*–CH=CH–), 6.1 (*q*, 1H, CH_2=C*H*–CO–), 6.2 (*br*, 1H, –CO–N*H*–CH_2–), 6.3 (*d*, 1H, H–C*H*=CH–).

Next, the graft copolymer was synthesized according to our previously reported method (Nitta *et al*. 2012b). The composition ratio of HEAA to HEAA–PTMC macromonomer in the graft copolymer was calculated from the ^{1}H NMR measurement. The number of graft chains in the copolymer was found to be approximately 1 to 10mol%. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.1−1.6 (*br*, 3H, –CH_2–CH–CO–), 1.9 (*m*, 2H, –CH_2–CH_2–CH_2–), 3.4−3.5 (*br*, 4H, –NH–CH_2–CH_2–), 4.1 (*t*, 4H, –O–CH_2–CH_2–CH_2–O–), 7.2−7.9 (*br*, 1H, –CO–NH–CH_2–).

2.4 Observation of lyophilized colloid gel morphology

The resulting graft copolymer was dissolved in DMF and dialyzed in deionized water for 48 h (molecular weight cut-off was 3.5 kDa). The water was replaced every 2 h for the first 6 h and then at longer intervals. The final aqueous solution of the polymer was then lyophilized using liquid nitrogen. For the SEM observation, the lyophilized PHT copolymer was fixed by carbon tape to a sample stage, and then electro-conductive paste (DOTITE; Fujikura Kasei Co., Ltd., Tochigi, Japan) was spotted onto the corner of the sample. All samples were sputter-coated with platinum prior to observation.

2.5 Thermal analysis

The thermal properties of the graft copolymer were investigated using DSC. The glass transition temperature (T_g) of the lyophilized graft copolymer was recorded from −50 to 150℃ using liquid nitrogen at a scanning rate of 10℃/min.

2.6 Particle size and their thermal stability

The synthesized graft copolymers spontaneously formed aggregates in aqueous media. In order to measure the size of these aggregates, the lyophilized graft copolymer was dissolved in water, with ultrasonic agitation, for a short time, and then was filtered (pore size 0.8 μm). The copolymer solution was adjusted to a concentration at 1 mg/mL and DLS measurements were performed at temperature ranging from 20 to 70℃.

2.7 Determination of CAC and partition equilibrium constant (K_v)

In order to dissolve the hydrophobic pyrene probe in an aqueous solution, it was first dissolved in THF at 1.2×10^{-3} mol/L (Cho *et al*. 2008, Hyun *et al*. 2008). This solution was then added drop wise to water (6.0×10^{-7} mol/L) and vigorously stirred. THF was removed by rotary evaporation at 40℃ for 2 h. A solution of the graft copolymer containing pyrene was then prepared. The final concentration of pyrene was 6.0×10^{-7} mol/L. Several graft copolymer solutions were prepared, with concentrations varying in the range of 10^{-5} to 1 mg/mL. The excitation spectrum of pyrene was measured using a fluorescence spectrophotometer at room temperature. The emission was measured at 373.0 nm with a slit-width of 5.0 nm and a scan speed of 300 nm/min.

3. Results and discussion

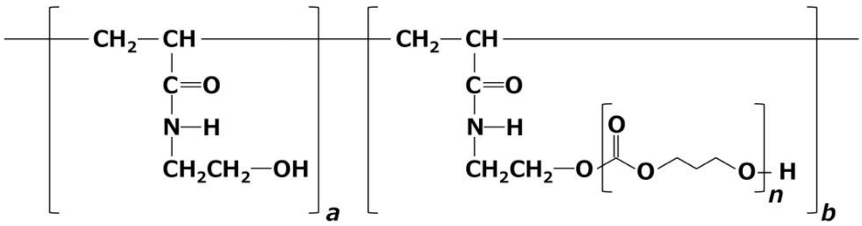
3.1 Preparation of macromonomer and amphiphilic graft copolymer

Table 1 Synthetic result for graft copolymers

Sample	DP of PTMC	Composition ratio[a)] (mol%)		Molecular weight by GPC			Yield (%)
		HEAA	HEAA–PTMC	M_n	M_w	M_w/M_n	
Poly(HEAA)	—	100	0	1.8×10^4	3.7×10^4	2.0	93.9
$PH_{99}T_{11}$	11	99.0	1.0	2.5×10^4	4.7×10^4	1.9	83.0
$PH_{92}T_9$	9	91.6	8.4	8.0×10^4	2.5×10^5	4.2	63.8
$PH_{99}T_{52}$	52	99.1	0.9	3.7×10^4	9.4×10^4	2.6	84.7

[a)]Determined by ^{1}H NMR

The polymerization of TMC was initiated from the hydroxyl end-group of HEAA, and then the synthesized HEAA–PTMC macromonomer was used in a typical radical polymerization. The chemical structure of the graft copolymer is shown in Scheme 1. Table 1 shows the results of the polymer synthesis. The sample code of $PH_{99}T_{11}$ refers to the compound with an HEAA monomer composition ratio of 99mol% with 11 repeating units of TMC in the macromonomer; the same system of nomenclature was applied to $PH_{92}T_9$ and $PH_{99}T_{52}$. Both the number of repeating units of TMC in the macromonomer and the composition ratio of the graft copolymer were calculated from the ^{1}H NMR spectra. $PH_{99}T_{11}$ and $PH_{92}T_9$ were similar in terms of the number of repeating units of TMC, whereas $PH_{99}T_{11}$ and $PH_{99}T_{52}$ were similar in macromonomer composition. $PH_{92}T_9$ had a higher molecular weight and a larger M_w/M_n than the others. The GPC result showed the effect of the interaction between the graft copolymers, because of the Tyndall phenomenon was observed on $PH_{92}T_9$ solution in DMF. Particularly, the interaction of $PH_{92}T_9$ was much enhanced.



Scheme 1 Chemical structure of amphiphilic graft copolymer (PHT)

3.2 Characterization of graft copolymer

In Fig. 1, SEM images show the surface morphology of each copolymer. In the case of poly(HEAA), the punched-sheet morphology was observed. The diameter of the observed fibrils was approximately 2 μm. The pore size was roughly 2-5 μm, and the pores were spread over the entire area. In contrast, the surface morphology of the graft copolymer hydrogel appeared as a fibrous entangled structure of aggregates derived from PTMC.

On the other hand, $PH_{92}T_9$ showed bundles of random entanglements. The surface morphologies are formed from the aggregate due to its modification by a stronger interaction among the PTMC segments (Mattanavee *et al*. 2009, Chandler-Temple *et al*. 2010). Because the hydrophobic PTMC prevented the invasion of water molecules, there is no pore on the surface.

As an amorphous polymer, PTMC has a low T_g, below 0℃ (Kim *et al*. 2006, Amsden *et al*. 2004, Terao *et al*. 2012, Tyson *et al*. 2009). For the graft copolymers, the values of T_g were higher (Fig. 2), with poly(HEAA), $PH_{99}T_{11}$, $PH_{92}T_9$, and $PH_{99}T_{52}$ demonstrating endothermic transitions at 61.3, 52.3, 36.6, and 36.3℃, respectively. The flexible PTMC segment showed to be in micro-

Brownian motion, so that $PH_{99}T_{11}$, $PH_{92}T_9$, and $PH_{99}T_{52}$ displayed lower T_g values than did poly(HEAA). The T_g was influenced by the DP of PTMC, rather than the composition ratio of the macromonomer, in the copolymers with a similar number of TMC units. $PH_{92}T_9$ and $PH_{99}T_{52}$ showed approximately equal values of T_g. We concluded that this is because the strength of the interaction in both polymer segments was similar. Additionally, the T_g data indicated good compatibility between the poly(HEAA) and PTMC segments.

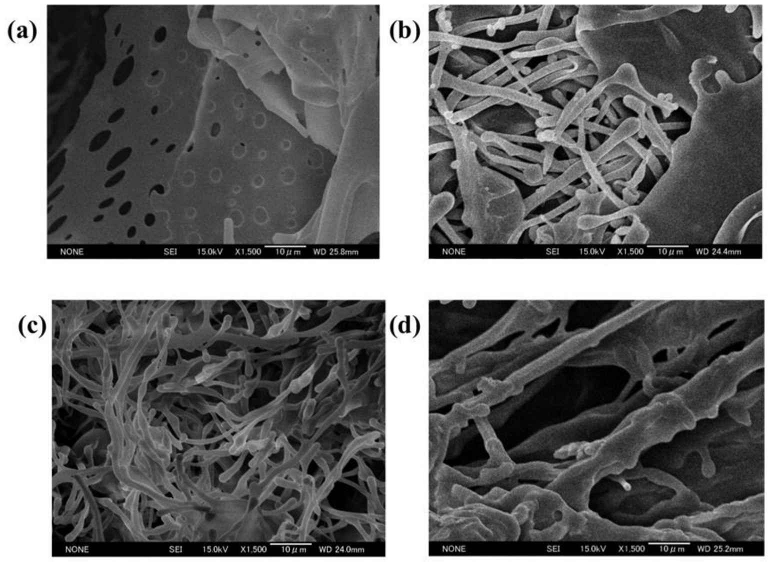


Fig. 1 SEM images of the freeze-dried polymers (Scale bar, 10 μm): (a) Poly(HEAA), (b) $PH_{99}T_{11}$, (c) $PH_{92}T_9$, (d) $PH_{99}T_{52}$

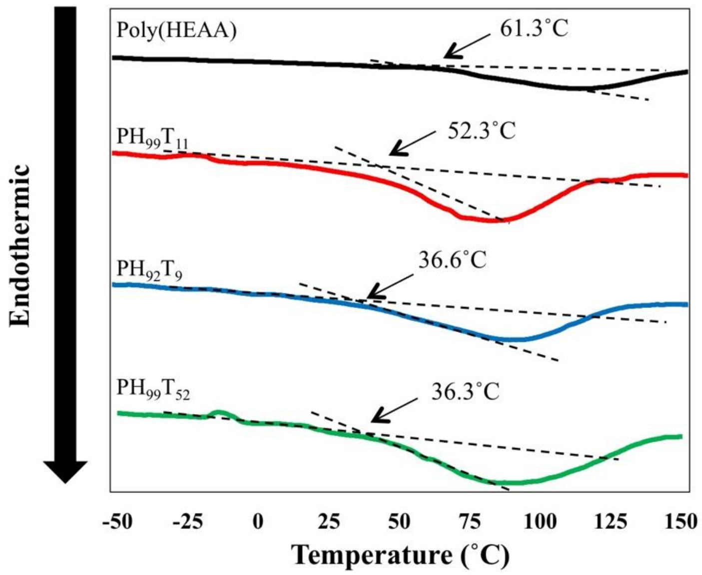


Fig. 2 DSC thermograms of lyophilized poly(HEAA) and PHT copolymers

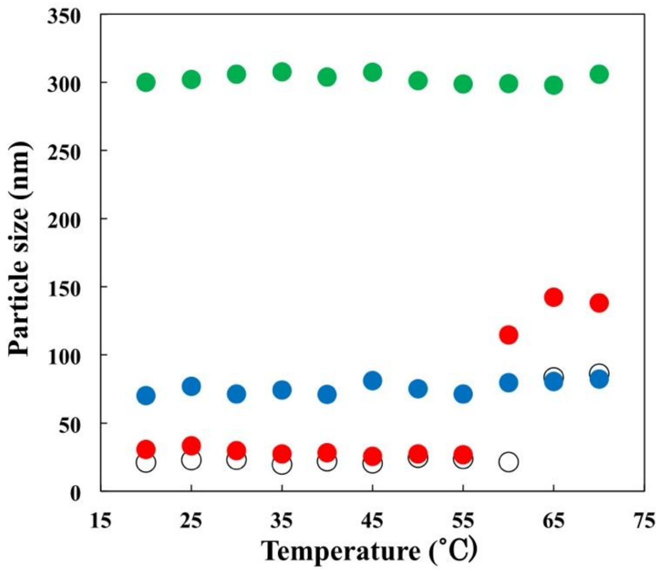


Fig. 3 Change in particle size over the temperature range 20 to 70˚C. Poly(HEAA): ○, $PH_{99}T_{11}$: ●, $PH_{92}T_{9}$: ●, $PH_{99}T_{52}$: ●

3.3 Thermal stability of graft copolymer aggregates in aqueous media

The amphiphilic PHT graft copolymers spontaneously formed aggregates in aqueous media, driven by the hydrophobic interactions among the PTMC segments and the hydrogen bonding derived from HEAA. These aggregates consisted of a shell, covered with hydrophilic poly(HEAA) segments, and a core of PTMC domains. The particle size of the PHT copolymers at a concentration of 1 mg/mL in aqueous media was analyzed by DLS measurements. The particle sizes of $PH_{99}T_{11}$, $PH_{92}T_{9}$, and $PH_{99}T_{52}$ were approximately 30, 75, and 250 nm at 25℃, respectively. For each PHT copolymer, the total amount of PTMC segments was different. For $PH_{99}T_{11}$ and $PH_{92}T_{9}$, the HEAA–PTMC macromonomer contents were significantly different, although the DP of the PTMC segment was almost the same. Therefore, these two copolymers should have different hydrophobic properties. By comparison with $PH_{99}T_{11}$, the $PH_{99}T_{52}$ copolymer had a PTMC segment whose DP was five times higher, although the macromonomer content was quite similar. This result indicated that the larger particle size was observed as a result of the higher hydrophobicity caused by the PTMC environment.

In addition, the small shoulder of the particle size distribution indicated that the unimer was formed due to the intramolecular aggregation of PHT. The thermal stability of the particle as a function of temperature was measured using DLS (Fig. 3). The concentration of the polymer solution was 1 mg/mL and the temperature range was from 20 to 70℃. Both $PH_{92}T_{9}$ and $PH_{99}T_{52}$ remained highly stabile and unchanging, but the particle size of poly(HEAA) and $PH_{99}T_{11}$ increased in the interval from 60 to 70℃. By heating, it showed an unstable aggregate structure due to a dissociation of hydrogen bonding formed by amide and hydroxyl groups on the HEAA unit at higher temperatures. Therefore, in this temperature range, the particle size of poly(HEAA) was about 80 nm. On the other hand, the particle size of $PH_{99}T_{11}$ changed from about 115 to 140

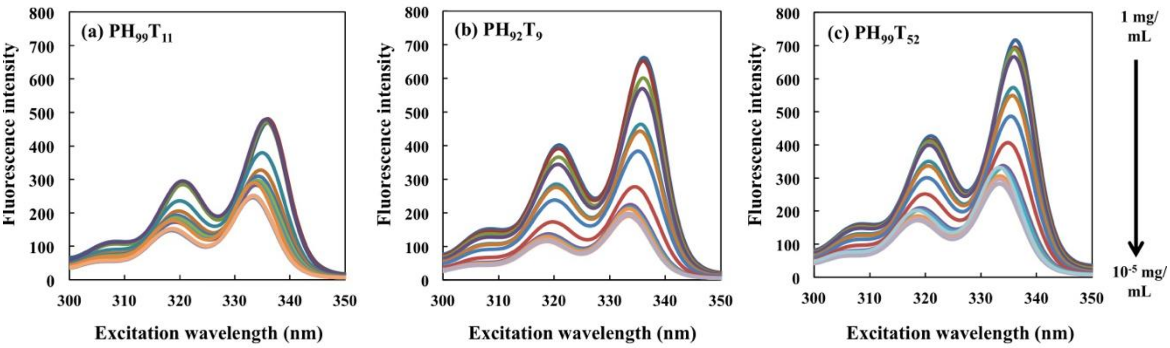


Fig. 4 Excitation spectra of pyrene (6.0×10^{-7} mol/L) as a function of polymer concentration (10^{-5}–1 mg/mL) in aqueous solution at 25℃. (a) $PH_{99}T_{11}$, (b) $PH_{92}T_{9}$, (c) $PH_{99}T_{52}$

nm in the process of forming the aggregate from the hydrophobic interaction of PTMC and its association through hydrogen bonding. This behavior indicated that graft copolymers as colloid gels were in a swelled state. Additionally, the particle size reversibly increased and decreased due to change in temperature. We considered that the physical cross-linking such as hydrogen bonding and hydrophobic interaction was dominant for the colloid gel association and disassociation.

3.4 Evaluation of graft copolymer aggregate by fluorescence measurements

The CAC and K_v values of the PHT copolymers were determined in aqueous media at different concentrations using fluorescence measurements, with pyrene as a hydrophobic fluorescent probe. The fluorescence spectrum of pyrene in solution is known to shift depending on the polarity of the surrounding environment (Kim *et al*. 2000, Wilhelm *et al*. 1991). Fig. 4 shows that by increasing the polymer concentration in aqueous media, the maximum value in the excitation spectra (λ_{ex}) of pyrene shifts from 333.5 to 336 nm, where it was considered that pyrene was incorporated into the hydrophobic PTMC domain. This shift in the excitation spectra was observed for all of the graft copolymers. The total intensities of the spectra varied depending on both the copolymer concentration and the total amount of hydrophobic PTMC segments.

Fig. 5 shows the fluorescence intensity ratio ($I_{336}/I_{333.5}$) in the pyrene excitation spectra at 373 nm versus the logarithm of the PHT concentration. Above the CAC, the fluorescence intensity increased exponentially, as the number of molecules of pyrene increased. On the other hand, below the CAC, absorption only occurred near the surface of the aggregates where the pyrene molecules cohered. The fluorescence intensity increased when the pyrene was solubilized in the hydrophobic domain. The fluorescence intensity also increased with increasing PHT copolymer concentration. The increase in the intensity ratio indicated the onset of aggregate formation. From the result of Fig. 3, the particle size of $PH_{99}T_{11}$ increased over 55℃ due to the dissociation of hydrogen bonding on the HEAA units. Aggregation of the polymer would be enhanced. The CAC of $PH_{99}T_{11}$ was quite high, so hydrophobic interaction was not so strong. Therefore, the CAC can be defined as the intersection of two straight lines in the low concentration range. The CAC values of $PH_{99}T_{11}$, $PH_{92}T_{9}$, and $PH_{99}T_{52}$ were estimated to be approximately 8.9×10^{-2}, 3.2×10^{-3}, and 2.2×10^{-3} mg/mL, respectively (Table 2). The CAC of the graft copolymer decreased with not only the chain length of PTMC, but also with the macromonomer composition ratio. In the case of $PH_{99}T_{11}$, the

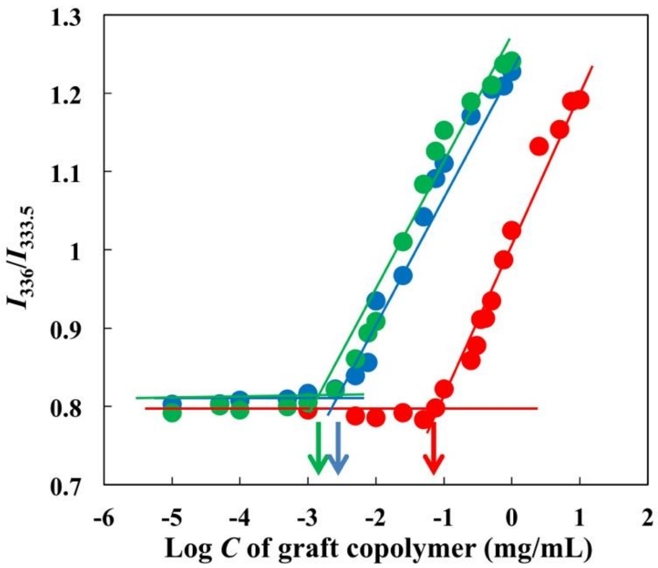


Fig. 5 Plot of $I_{336}/I_{333.5}$ (from the pyrene excitation spectra) versus log C, where C is the concentration of the graft copolymers. $PH_{99}T_{11}$: ●, $PH_{92}T_{9}$: ●, $PH_{99}T_{52}$: ●

CAC had the lowest value among the copolymers, showing lower association forces. The repeating unit of TMC and the macromonomer composition both influenced the formation polymer colloids. In this case, the higher number of repeating units of TMC appears to be dominant; otherwise, a higher macromonomer composition would be necessary. The slope reached a plateau above 1.2 of $I_{336}/I_{333.5}$ for $PH_{92}T_{9}$ and $PH_{99}T_{52}$ samples, indicating that the hydrophobic domain was saturated with incorporated pyrene.

To quantify the separation of pyrene into the hydrophobic domain during aggregation, Wilhelm *et al*. (1991) devised an equation to calculate the K_v value of the hydrophobic domain in graft copolymer aggregates (Wilhelm *et al*. 1991). The concentration of pyrene in the hydrophobic domain of the PHT copolymer was calculated using Eqs. (1)-(4), where $[Py]_A$ and $[Py]_w$ represent the concentrations of pyrene in the aggregated and aqueous phase, respectively. The K_v value for pyrene was calculated from the ratio of the pyrene concentrations ($[Py]_A/[Py]_w$). In this approach, $[Py]_A/[Py]_w$ can be corrected to the volume ratio of each phase: which can be rewritten as

$$[Py]_A/[Py]_w = K_v V_A/V_W \tag{1}$$

Moreover, $[Py]_A/[Py]_w$ can be written as

$$[Py]_A/[Py]_w = K_v x(c\text{–CAC})/1000\rho \tag{2}$$

where x is the weight fraction of the PTMC segment, c is the concentration of the graft copolymer, and ρ is the density of the PTMC aggregation domain, which is assumed to be the value of bulk PTMC (1.0 g/cm^3≅g/mL).

$$[\mathrm{Py}]_A/[\mathrm{Py}]_w = (F - F_{min})/(F_{max} - F) \tag{3}$$

which can be rewritten as

$$K_v = \text{slope} \times 1000\rho/x \tag{4}$$

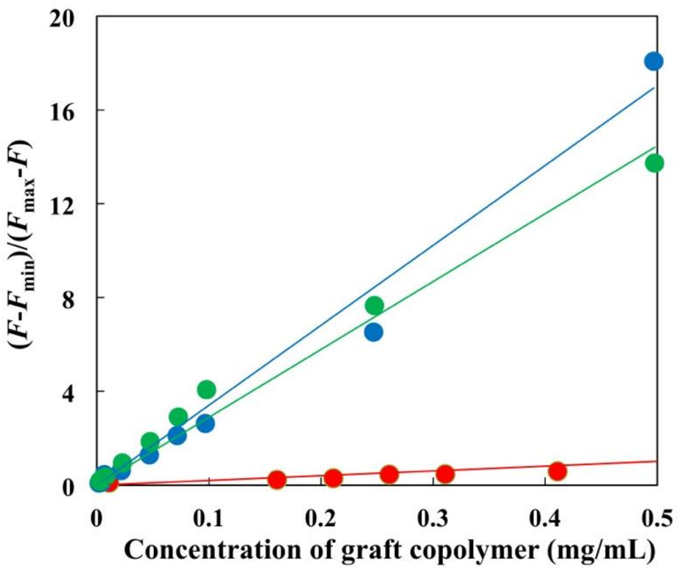


Fig. 6 Plots of $(F\text{-}F_{min}/(F_{max}\text{-}F)$ versus concentration of graft copolymers. $PH_{99}T_{11}$: ●, $PH_{92}T_9$: ●, $PH_{99}T_{52}$: ●

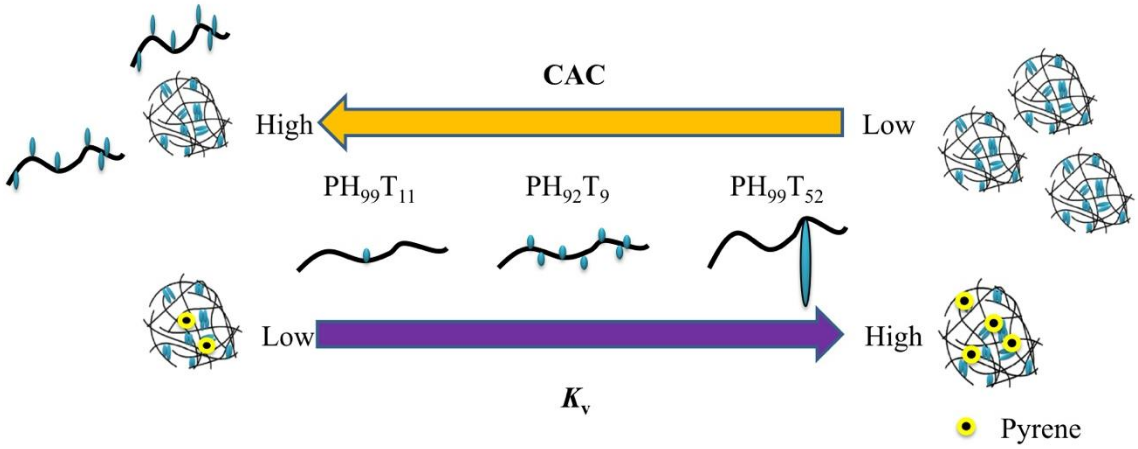


Fig. 7 Schematic illustration of aggregation and pyrene-loading-related CAC and K_v for the graft copolymers

Table 2 CAC and K_v of PHT copolymers (25℃)

Sample	CAC (mg/mL)	$K_v/10^4$
$PH_{99}T_{11}$	8.9×10^{-3}	2.0
$PH_{92}T_9$	3.2×10^{-3}	8.0
$PH_{99}T_{52}$	2.2×10^{-3}	9.8

where F_{min} and F_{max} correspond to the average magnitudes of the peak ratio in the region of high and low concentration ranges shown in Fig. 5, respectively, and F is the fluorescence intensity ratio ($I_{336}/I_{333.5}$) in the intermediate concentration range of the conjugates [Eqs. (2)-(3)]. The slope was determined by a linear approximation and the K_v values were calculated using Eq. (4).

The fluorescence study using pyrene also reflected the polymer structure, including the grafting degree and PTMC chain length. $PH_{92}T_9$ and $PH_{99}T_{52}$ had higher slope, as calculated by Eq. (3), by changing each polymer concentration. The K_v values of $PH_{99}T_{11}$, $PH_{92}T_9$, and $PH_{99}T_{52}$ were estimated to be approximately 2.0×10^{-4}, 8.0×10^{-4}, and 9.8×10^{-4}, respectively (Fig. 6).

The K_v of $PH_{92}T_9$ was four times as large as that of $PH_{99}T_{11}$. We concluded from these results that PTMC segments played a role in both molecular incorporation and cross-linking. Therefore, the K_v of $PH_{92}T_9$ was not in agreement with the theoretical value. Table 2 and Fig. 7 summarize these results. The chain length of PTMC rather than the macromonomer composition ratio decreased the K_v values of graft copolymers. Therefore, the hydrophobic domain in $PH_{99}T_{52}$ proved to have a more significant influence than in $PH_{92}T_9$.

4. Conclusions

Amphiphilic graft copolymers with homogeneous graft chain lengths of PTMC segments were prepared using a macromonomer method. The T_g values of the graft copolymers were measured to be from 35 to 60℃, and the PTMC fraction showed a flexible nature at temperatures close to body temperature. These copolymer associations formed core-shell structures in an aqueous solution. The particle size of the PHT aggregates in aqueous solution was about 30-300 nm and was comparatively stable relative to changes in temperature. In particular, $PH_{99}T_{11}$ and $PH_{92}T_9$ have suitable particle sizes for common DDS. The CAC of the PHT copolymers were in the range of 2.2×10^{-3} to 8.9×10^{-2} mg/mL. The K_v values were dependent to the increase in TMC units. We deduced that the particle size, CAC, and K_v values for the copolymers depended mostly on the chain length of the hydrophobic PTMC. The graft copolymer with a longer PTMC chain length underwent strong hydrophobic interactions, leading to an increase in the particle size, T_g, and K_v. From these results, the function of the hydrophobic PTMC domains seemed to be slightly different according to the number of TMC repeating units. We confirmed that the aggregates formed from the graft copolymers with PTMC domains might be used as potential drug delivery vehicles for loading hydrophobic molecules.

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