



Article

Amphiphilic Block Copolymers Bearing Hydrophobic γ -Tocopherol Groups with Labile Acetal Bond

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Abstract: High concentrations of γ -tocopherol (γ TCP) tend to show antioxidant, anti-inflammatory, and anticancer effects. In this study, we prepared polymer micelles under acidic conditions with a controlled release of γ TCP due to the decomposition of pendant acetal bonds. First, a precursor diblock copolymer composed of poly(ethylene glycol) (PEG) and acrylic acid (AA) was prepared. This was followed by the synthesis of an amphiphilic diblock copolymer (PEG₅₄-P(AA/VE6/ γ TCP29)₁₄₀), incorporated into hydrophobic γ TCP pendant groups attached to the main chain through an acetal bond. The prepared PEG₅₄-P(AA/VE6/ γ TCP29)₁₄₀ was further dispersed in water to form polymer micelles composed of hydrophobic cores that were generated from a hydrophobic block containing γ TCPs and hydrophilic shells on the surface. Under acidic conditions, γ TCP was then released from the core of the polymer micelles due to the decomposition of the pendant acetal bonds. In addition, polymer micelles swelled under acidic conditions due to hydration of the core.

Keywords: γ -tocopherol; acetal bond; amphiphilic diblock copolymer; polymer micelles

1. Introduction

Amphiphilic diblock copolymers in water form polymer micelles consisting of a hydrophobic core and hydrophilic shell [1–3]. This serves as an effective route to encapsulate hydrophobic anticancer drugs into the core, thereby improving the solubility of the reagents while limiting side effects [4]. As such, the passive targeting of drug-loaded polymer micelles of 10–100 nm average size can enhance the permeability and retention (EPR) effect [5,6]. In general, polymer micelles with a size of several hundred nanometers are unable to penetrate normal vascular walls, while polymer micelles around tumor tissue can penetrate through defected vascular walls (enhanced permeability). This is due to incomplete neovascularization around the tumor and gaps that form between vascular endothelial cells. In addition, polymer micelles cannot be completely removed from the lymphatic tissue around the tumor due to the immaturity of the lymphatic tissue. Therefore, the leaked polymer micelles from tissue vesicular walls tend to accumulate around tumor tissue (enhanced retention). Such a property generated by the accumulation of polymer micelles around tumor tissue is the EPR effect [7]. However, in the passive targeting mechanism, the amount of drugs for delivery to the affected area is low, making the evaluation of the side effects of anticancer drugs non-negligible [8,9].

Recently, cancer-cell apoptosis has been reported using food-derived natural products [10,11]. γ -tocopherol (γ TCP) is a type of vitamin E, contained in vegetable oils such as canola, soybean,

and corn oils. This substance possesses antioxidative and anti-inflammatory effects, and demonstrates the potential to improve cardiovascular disease and prostate cancer [12,13]. According to the literature, an increase in γ TCP concentrations in plasma may decrease the risk of prostate cancer [14]. However, it is well established that the accumulation of excess amounts of vitamin E in the body by a normal diet is impossible due to the low absorption rate of vitamin E that is in the range of 21%–29% [15]. Nevertheless, by local administration of more highly concentrated vitamin E, cancer-cell apoptosis can be induced without the use of anticancer drugs.

According to previous studies, the pH of cancer and healthy cells was determined as 5 and 7.4, respectively [16]. Acetal bonds under acidic conditions can also be readily cleaved into suitably functional moieties [17]. pH-responsive nanoparticles containing acetal linkage within the chemical structure for the controlled release of drugs under acidic conditions have been developed in the last two decades [18–21]. Simo et al. [22] reported that acetal linkage-containing hydrophilic *N*-(2-(tetrahydro-2H-pyran-2-yl)oxy)ethyl acrylamide (HEAmTHP) was polymerized via reversible addition-fragmentation chain transfer (RAFT) radical polymerization using a hydrophobic poly(2-hydroxyethyl acrylate) (PHEA) macro-chain transfer agent (CTA) to prepare an amphiphilic diblock copolymer (PHEA-PHEAmTHP). In a subsequent study [23], the encapsulation of a hydrophobic antibiotic substance in the hydrophobic domain of formed PHEA-PHEAmTHP micelles in water was performed. Another study by Gold et al. investigated the effects of attached Amphotericin B to the cell membrane of micro-organisms, which showed antibiotic property by breaking membrane structures [24]. Under acidic conditions, Amphotericin B was released from the polymer-micelle duet that cleaved the acetal linkages. Feng et al. [25] prepared hyperbranched polyesters (S-hbPE) containing multiple acetal linkages via condensation of 2,2'-(propane-2,2-diylbis(oxy)) diethanol, phosphoryl chloride, and poly(ethylene glycol) monomethyl ether (m-PEG₄₅-OH). Chlorin e6 a photosensitizer for photodynamic therapy (PDT) was encapsulated into S-hbPE to prepare nanoparticles. Chlorin e6 was then released under acidic conditions around tumor tissue attributed to the decomposition of the acetal linkages. As expected, the release of chlorin e6 under acidic conditions around tumor tissue showed the high efficiency of PDT with reduced side effects. Thus, nanocarriers containing acid labile acetal linkages for delivery systems via endosome/lysosome possess advantages such as solubilization of hydrophobic guest molecules, and controlled release in the acidic conditions around tumor tissue.

In this study, we first prepared a precursor diblock copolymer (PEG₅₄-PAA₁₄₀) composed of biocompatible poly(ethylene glycol) (PEG) and poly(acrylic acid) (PAA) blocks. This was followed by the preparation of a diblock copolymer (PEG₅₄-P(AA/VE35)₁₄₀) via incorporation of vinyl ether (VE) groups to the pendant carboxylic acid groups of the PAA block through esterification. Finally, a diblock copolymer (PEG₅₄-P(AA/VE6/ γ TCP29)₁₄₀) was prepared via introduction of hydrophobic γ TCP groups onto the pendant VE groups by acetal linkage (Figure 1). The plane and subscript numbers express the content (mol%) and degree of polymerization (DP), respectively. In addition, aqueous solutions of PEG₅₄-P(AA/VE6/ γ TCP29)₁₄₀ were formulated to produce polymer micelles via hydrophobic interactions of pendant γ TCP (Figure 2). Under acidic conditions, partial release of γ TCP occurred due to the decomposition of the pendant acetal linkages. This was also attributed to the slight swelling of the polymer micelles since hydrophobicity of the micelles decreased with the release of hydrophobic γ TCP. This suggests that the release of highly concentrated γ TCP by the polymer micelles under acidic conditions may lead to cancer-cell apoptosis without the use of anticancer drugs.

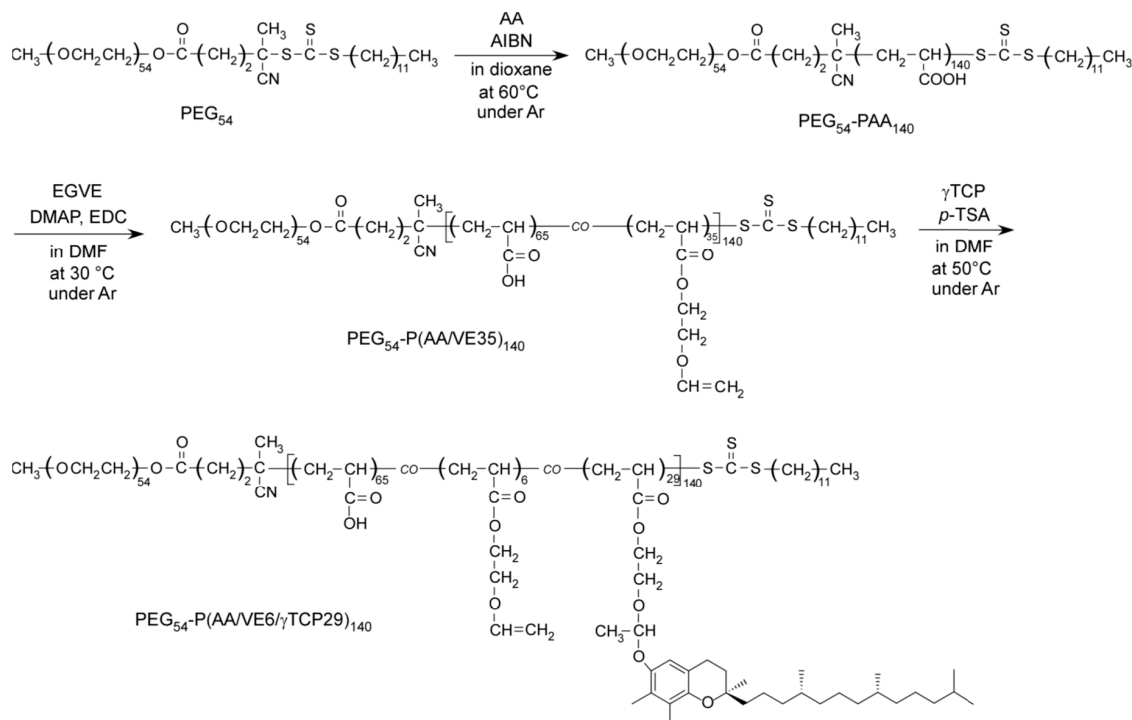


Figure 1. Synthesis of PEG₅₄-PAA₁₄₀, PEG₅₄-P(AA/VE35)₁₄₀, and PEG₅₄-P(AA/VE6/ γ TCP29)₁₄₀.

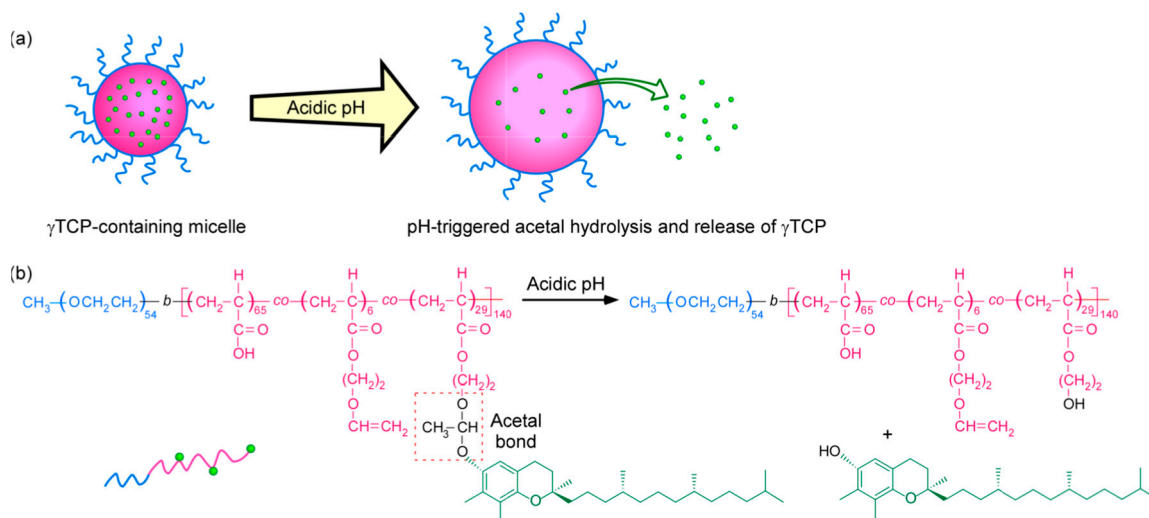


Figure 2. (a) Micelle formation and release of γ TCP in acidic aqueous solution; (b) chemical structure of PEG₅₄-P(AA/VE6/ γ TCP29)₁₄₀.

2. Materials and Methods

2.1. Materials

Poly(ethylene glycol) methylether (4-cyano-4-pentanoate dodecyltrithiocarbonate) (PEG₅₄, $M_n = 2.40 \times 10^3$ g/mol) and γ -tocopherol (γ TCP, 96%) were purchased from Sigma Aldrich (St. Louis, MO, USA); 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC, 98%) and *N,N*-dimethyl-4-aminopyridine (DMAP, 99%) were procured from Fujifilm Wako Pure Chemicals (Osaka, Japan). Ethylene glycol mono-vinyl ether (EGVE, 98%) and *p*-toluenesulfonic acid (*p*-TSA, 98%) were supplied by Tokyo Chemical Industry (Tokyo, Japan). All reagents above were used as received without further purification. We used 2,2'-azobisisobutyronitrile (AIBN, 98%) from Sigma Aldrich (St. Louis, MO, USA) after recrystallization using methanol. Acrylic acid (AA, 98%), 1,4-dioxane

(99%), and *N,N*-dimethylformamide (DMF, 99%) from Fujifilm Wako Pure Chemicals (Osaka, Japan) were used after vacuum distillation. Water was purified with an ion-exchange column system. Other reagents were used as received.

2.2. PEG₅₄-PAA₁₄₀ Synthesis

PEG₅₄ (2.00 g, 0.833 mmol), AA (9.00 g, 124 mmol), and AIBN (30.5 mg, 0.186 mmol) were dissolved in 1, 4-dioxane (50 mL). The solution was deoxygenated by purging argon gas for 30 min. Polymerization was carried out at 60 °C for 16 h. The conversion of AA estimated from ¹H NMR was 98.2%. The polymerization mixture was dialyzed against pure water for 2 days. The polymer (PEG₅₄-PAA₁₄₀) was recovered by a freeze-drying method (8.70 g, 79.1%). DP for PAA block and number-average molecular weight (M_n) for PEG₅₄-PAA₁₄₀ were estimated using ¹H NMR as 140 and 1.26×10^4 g/mol, respectively. While, M_n and molecular-weight distribution (M_w/M_n) were calculated as 1.25×10^4 g/mol and 1.36, respectively, using gel-permeation chromatography (GPC).

2.3. Synthesis of PEG₅₄-P(AA/VE35)₁₄₀

We have used the conventional esterification method using EDC [26]. PEG₅₄-PAA₁₄₀ (134 mg, 1.50 mmol of COOH unit), DMAP (91.8 mg, 0.751 mmol), and EDC (860 mg, 4.49 mmol) were dissolved in DMF (20 mL). The solution was stirred at 30 °C for 10 h under an argon atmosphere, and then EGVE (0.667 mL, 7.55 mmol) was added to the solution. The solution was stirred at 30 °C for 24 h. Once the reaction was complete, the solution was dialyzed against pure water for three days. The prepared polymer (PEG₅₄-P(AA/VE35)₁₄₀) was recovered by a freeze-drying method (0.15 g, 56.6%). The M_n of PEG₅₄-P(AA/VE35)₁₄₀ estimated from ¹H NMR was determined as 1.67×10^4 g/mol. M_n and M_w/M_n estimated using GPC were determined as 7.02×10^3 g/mol and 1.38, respectively. The exchange ratio from the pendant carboxylic acid in the PAA block to the VE group was 35.0% estimated from ¹H NMR.

2.4. PEG₅₄-P(AA/VE6/ γ TCP29)₁₄₀ Synthesis

Conjugation of γ TCP onto PEG₅₄-P(AA/VE35)₁₄₀ via an acid-labile acetal bond was prepared according to a previously reported method [17]. PEG₅₄-P(AA/VE35)₁₄₀ (62.5 mg, 180 μ mol of VE unit), γ TCP (25.0 mg, 60.0 μ mol), and *p*-TSA (0.56 mg, 2.94 μ mol) were dissolved in DMF (10 mL). Molecular sieves 4A (1.00 g) was added to the solution. The reaction was carried out at 50 °C for 4 days under an argon atmosphere. Molecular sieves were removed by filtration, and the solution was dialyzed against pure water for 2 days. The polymer (PEG₅₄-P(AA/VE6/ γ TCP29)₁₄₀) was recovered by a freeze-drying method (80.9 mg, 92.5%). M_n for PEG₅₄-P(AA/VE6/ γ TCP29)₁₄₀ using ¹H NMR was estimated as 1.96×10^4 g/mol. M_n and M_w/M_n estimated using GPC were determined as 1.02×10^4 g/mol and 1.47, respectively. The exchange ratio from the VE to γ TCP was calculated as 82.9% from ¹H NMR.

2.5. Measurements

¹H NMR spectra were measured with a Bruker DRX-500 (Billerica, AM, USA) at room temperature. The block copolymer sample solutions for the ¹H NMR measurements were prepared in dimethylsulfoxide-*d*₆ (DMSO-*d*₆). GPC measurements were performed using Shodex (Tokyo, Japan) Asahipak GF-1G guard column and GF-7M HQ column at 40 °C with a refractive index detector and yielded a phosphate buffer solution (PBS) of pH 9 containing 10% *v/v* of acetonitrile at 0.6 mL/min in the developing solvent. Determination of M_n and M_w/M_n according to GPC were calibrated using standard sodium poly(styrene sulfonate)s. Hydrodynamic radius (R_h) and scattered light intensity (LSI) were estimated using a Malvern (Malvern, UK) Zetasizer Nano ZS-ZEN3600 equipped with a He-Ne laser source (4 mW at 632.8 nm) at 25 °C. The sample solutions for light-scattering measurements were filtered through a filter with pore-size diameter of 0.8 μ m. Static light scattering (SLS) measurements were carried out with Otsuka Electronics (Osaka, Japan) DLS 7000 at 25 °C. The He-Ne laser (10 mW at 632.8 nm) was used as a light source. Weight average molecular weight (M_w) and radius of gyration (R_g) were calculated from a Debye plot at 1 polymer concentration. The Rayleigh ratio of toluene was

used in instrument calibration. Refractive index increment against the polymer concentration (dn/dC_p) at 633 nm was determined using Otsuka Electronics DRM-3000 differential refraction meter at 25 °C. TEM observations were performed with a JEOL (Tokyo, Japan) JEM-2100 at an accelerating voltage of 160 kV. Prior to TEM analysis, the sample was prepared by placing 1 drop of the aqueous solution on a copper grid coated with Formvar. Excess water was blotted using filter paper. Samples were stained by sodium phosphotungstate and dried under vacuum for a period of 1 day.

3. Results and Discussion

3.1. PEG₅₄-P(AA/VE6/γTCP29)₁₄₀ Preparation

The conversion of AA by RAFT polymerization using PEG₅₄ macro-CTA was determined as 98.2% estimated from the decrease of ¹H NMR integral area intensity ratio of the vinyl group in AA after polymerization. The DP of the PAA block was calculated to be 140 from the area integral intensity ratio of peaks attributed to ethylene oxide protons in PEG₅₄ at 3.7 ppm and the main chain protons in PAA at 2.3 ppm (Figure 3a). The introduction of the pendant vinyl groups in PEG₅₄-P(AA/VE35)₁₄₀ were confirmed by the observed ¹H NMR signal at 6.5 ppm attributed to VE (Figure 3b). The reaction rate from the pendant carboxylic acid to the vinyl groups, calculated as 34.8%, was estimated from the integral intensity ratios of the attributed peaks to the main chain protons of PAA₁₄₀ at 2.3 ppm and the vinyl protons in EGVE at 6.5 ppm. This result indicated that about 49 pendant vinyl groups were introduced into one polymer chain of PEG₅₄-P(AA/VE35)₁₄₀. The protons attributed to the terminal methyl at 0.8 ppm, benzyl at 2.0 ppm, and phenyl protons at 6.3 ppm in the pendant γTCP could be observed from a PEG₅₄-P(AA/VE6/γTCP29)₁₄₀ ¹H NMR spectrum (Figure 3c). The introduction rate of γTCP in PEG₅₄-P(AA/VE6/γTCP29)₁₄₀ was calculated as 83.3% estimated from the integral intensity ratio of the unreacted pendant vinyl protons in PEG₅₄-P(AA/VE35)₁₄₀ at 6.5 ppm and phenyl protons in γTCP at 6.3 ppm [27]. This result indicated that about 41 pendant γTCPs were introduced into a single polymer chain of PEG₅₄-P(AA/VE6/γTCP29)₁₄₀. The theoretical M_n of each polymer was calculated using the following equation:

$$M_n(\text{theo}) = \frac{[M]_0}{[CTA]_0} \times \frac{p}{100} \times M_m + M_{CTA}, \quad (1)$$

where $[M]_0$ and $[CTA]_0$ are the initial molar concentrations of monomer and CTA, respectively, p is the conversion of the monomer, and M_m and M_{CTA} are the molecular weights of the monomer and CTA, respectively.

The GPC-determined M_n values of PEG₅₄-PAA₁₄₀, PEG₅₄-P(AA/VE35)₁₄₀, and PEG₅₄-P(AA/VE6/γTCP29)₁₄₀ were 1.25×10^4 , 7.02×10^3 , and 1.02×10^4 g/mol, respectively (Figure 4). Due to the unexpected interactions between polymers within the GPC column, and the difference between structures of the measured polymer and the standard polymer, accurate molecular weight could not be estimated [28,29]. The M_w/M_n values of PEG₅₄-PAA₁₄₀, PEG₅₄-P(AA/VE35)₁₄₀, and PEG₅₄-P(AA/VE6/γTCP29)₁₄₀ were relatively narrow, obtained as 1.36, 1.38, and 1.47, respectively. Table 1 summarizes the molecular weight and M_w/M_n of each polymer.

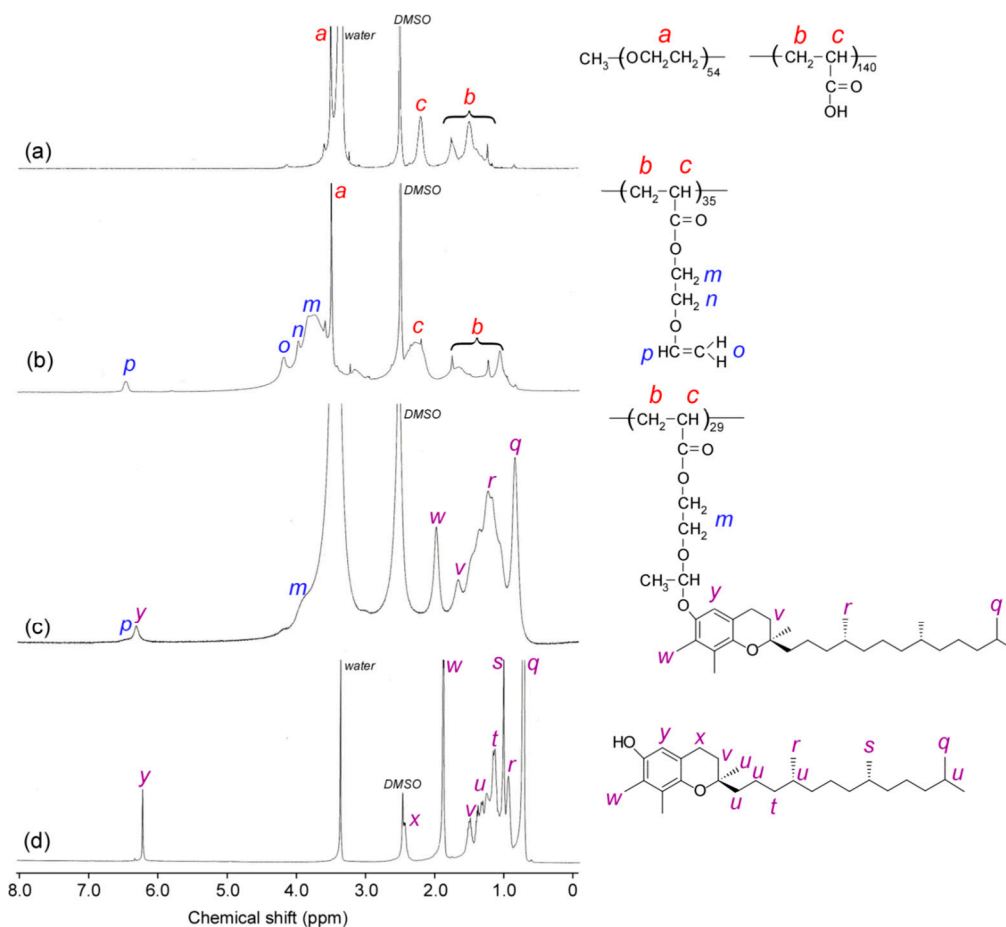


Figure 3. ¹H NMR spectra of (a) PEG₅₄-PAA₁₄₀, (b) PEG₅₄-P(AA/VE35)₁₄₀, (c) PEG₅₄-P(AA/VE6/γTCP29)₁₄₀, and (d) γTCP in DMSO-*d*₆.

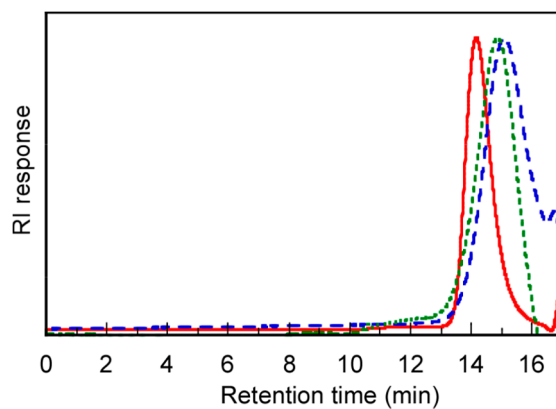


Figure 4. Gel-permeation chromatography (GPC) elution curves of PEG₅₄-PAA₁₄₀ (—), PEG₅₄-P(AA/VE35)₁₄₀ (---), and PEG₅₄-P(AA/VE6/γTCP29)₁₄₀ (···) at 40 °C using a phosphate buffer solution (PBS) at pH 9 containing 10% *v/v* acetonitrile as the eluent.

Table 1. Number-average molecular weight (M_n) and M_n distribution (M_w/M_n).

Sample	Theoretical $M_n \times 10^4$ (g/mol)	$^1\text{H NMR } M_n \times 10^4$ (g/mol)	GPC $M_n \times 10^4$ (g/mol)	M_w/M_n
PEG ₅₄ -PAA ₁₄₀	1.30	1.26	1.25	1.36
PEG ₅₄ -P(AA/VE35) ₁₄₀	1.54	1.67	0.702	1.38
PEG ₅₄ -P(AA/VE6/ γ TCP29) ₁₄₀	1.83	1.96	1.02	1.47

3.2. PEG₅₄-P(AA/VE6/ γ TCP29)₁₄₀ Micelle Formation

The R_h and LSI of the polymers were measured at $C_p = 0.10$ g/L in a PBS buffer of pH 7.4 at 25 °C to confirm aggregation-state changes for PEG₅₄-PAA₁₄₀, PEG₅₄-P(AA/VE35)₁₄₀, and PEG₅₄-P(AA/VE6/ γ TCP29)₁₄₀ (Figure 5). The R_h values of PEG₅₄-PAA₁₄₀ and PEG₅₄-P(AA/VE35)₁₄₀ were 4.4 and 5.8 nm, respectively. The LSI values of PEG₅₄-PAA₁₄₀ and PEG₅₄-P(AA/VE35)₁₄₀ were estimated as 40.4 and 83.0 Kcps, respectively. These polymers may dissolve in PBS to form unimers due to their small R_h and LSI values. On the other hand, the R_h and LSI of PEG₅₄-P(AA/VE6/ γ TCP29)₁₄₀ increased to 84.1 nm and 709 Kcps, suggesting the formation of polymer micelles. These polymer micelles were formed due to the hydrophobic interaction between pendant γ TCP groups, which were composed of a hydrophobic P(AA/VE6/ γ TCP29)₁₄₀ core and hydrophilic PEG₅₄ shells. The end-to-end distance of the PEG₅₄-P(AA/VE6/ γ TCP29)₁₄₀ polymer chain was estimated as 53.4 nm, assuming that the lengths of the vinyl monomer unit and the PEG chain were 0.25 and 18.4 nm, respectively [30–32]. The R_h value of the polymer micelle was calculated as 84.1 nm, which was larger than the end-to-end distance of PEG₅₄-P(AA/VE6/ γ TCP29)₁₄₀ (=53.4 nm). Therefore, polymers could not form simple spherical core-shell structures. The polymers may form intermicellar aggregates or large compound micelles.

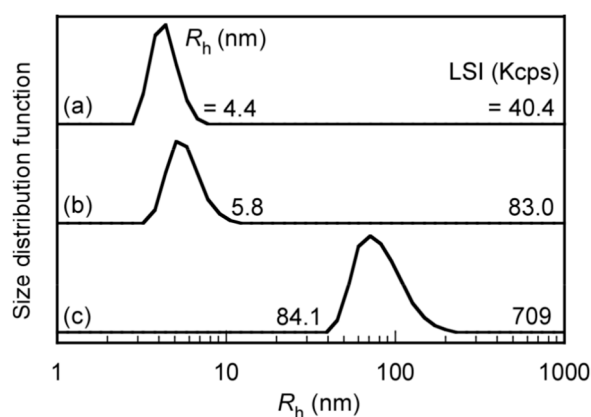


Figure 5. Hydrodynamic-radius (R_h) distributions for (a) PEG₅₄-PAA₁₄₀, (b) PEG₅₄-P(AA/VE35)₁₄₀, and (c) PEG₅₄-P(AA/VE6/ γ TCP29)₁₄₀ in PBS at $C_p = 0.10$ g/L.

To characterize the polymer micelles formed from PEG₅₄-P(AA/VE6/ γ TCP29)₁₄₀ in the PBS buffer, SLS measurements were performed (Figure 6). Using SLS, the apparent M_w and R_g were estimated from the Debye plot (Table 2). The measured dn/dC_p (=1.70 mL/g) of the polymer micelles were then used to estimate the apparent M_w . The aggregation number (N_{agg}), which is the number of polymer chains to form one micelle, was estimated using the following equation:

$$N_{agg} = \frac{M_w(\text{SLS})}{M_w/M_n \times M_n(\text{NMR})} \quad (2)$$

N_{agg} was estimated as 248 using M_w (=7.15 $\times 10^6$ g/mol), M_w/M_n (=1.47), and $M_n(\text{NMR})$ (=1.96 $\times 10^6$ g/mol) determined by SLS. Theoretically, R_g/R_h depends on the shape and polydispersity of the micelles, for instance, hard sphere as 0.775, monodisperse sphere as 1.0, and rodlike shape as

more than 2.0 [33–35]. The R_g/R_h of the polymer micelles formed from PEG₅₄-P(AA/VE6/ γ TCP29)₁₄₀ was calculated as 1.32, which is close to 1.0. Therefore, the polymers formed spherical micelles.

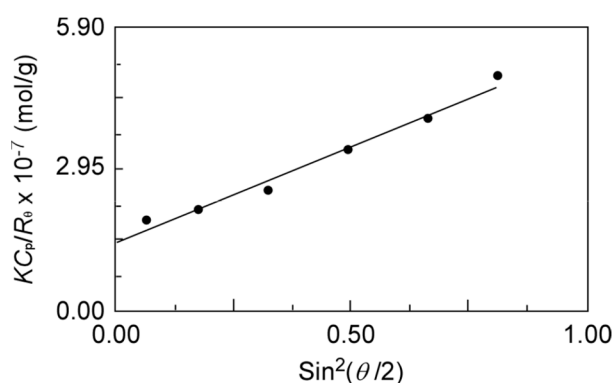


Figure 6. Debye plot for PEG₅₄-P(AA/VE6/ γ TCP29)₁₄₀ in PBS at fixed C_p (=0.10 g/L). K , optical constant; C_p , polymer concentration; R_θ , difference between Rayleigh ratio of solution and solvent; and θ , scattering angle.

Table 2. Dynamic and static light-scattering data of PEG₅₄-P(AA/VE6/ γ TCP29)₁₄₀ in PBS.

Sample	SLS $M_w \times 10^6$ (g/mol)	N_{agg}	R_g (nm)	R_h (nm)	R_g/R_h	dn/dC_p (mL/g)
PEG ₅₄ -P(AA/VE6/ γ TCP29) ₁₄₀	7.15	248	111	84.1	1.32	1.70

3.3. Micelle Decomposition in Acidic Condition

To evaluate the time dependence on micelle size and density formed from PEG₅₄-P(AA/VE6/ γ TCP29)₁₄₀ in acetate buffer of pH 5.2 (0.01 M), R_h and LSI changes were monitored at 25 °C (Figure 7). At constant particle size, density depended on the LSI. As a reference experiment, the time dependence on R_h and LSI of the polymer micelles in a PBS buffer of pH 7.4 (0.01 M) were also monitored. R_h increased with increasing time at pH 5.2. R_h values just after preparation and after 60 h at pH 5.2 were determined as 81.0 and 107.0 nm, respectively. LSI values of just after preparation and after 60 h at pH 5.2 were estimated as 693 and 321 Kcps, respectively. The LSI were observed to decrease with increasing time. At pH 5.2, the pendant acetal bonds decomposed to release the hydrophobic γ TCP from the polymer micelles. As a result, hydroxyl groups were generated at the pendant groups. However, not all pendant acetal bonds were decomposed at pH 5.2. However, an increase in R_h and decrease of the LSI was due to decreasing densities observed at pH 5.2, attributed to the hydration and then swelling of the core of the polymer micelles. Oho et al. [36] reported that γ TCP is relatively stable against acidic conditions. The released γ TCP from the polymer in a buffer at pH = 5.2 may not be decomposed.

At pH 7.4, the R_h values of the micelles just after preparation and after 60 h were calculated as 81.5 and 83.8 nm, respectively. The LSI of the micelles slightly decreased with increasing time. The values of LSI just after preparation and after 60 h were obtained as 695 and 492 Kcps. These observations suggest that the pendant acetal groups slightly decomposed at pH 7.4. Compared to the time dependence of R_h and LSI at pH 5.2 and 7.4, γ TCP was effectively released due to the decomposition of the pendant acetal bonds.

The micelles of PEG₅₄-P(AA/VE6/ γ TCP29)₁₄₀ in PBS of pH 7.4 were observed with Transmission electron microscopy (TEM, Figure S1). The radius (R_{TEM}) estimated from the TEM image was 72.1 ± 12 nm. R_{TEM} was calculated from a randomly selected 50 particles ($N = 50$). R_{TEM} was smaller than the R_h values determined by light-scattering measurements because the aggregates shrank during the drying process in preparation for TEM measurements. The micelles of PEG₅₄-P(AA/VE6/ γ TCP29)₁₄₀ in the acetate buffer of pH 5.2 just after preparation and after 60 h were observed using TEM to confirm

the shape changes of the micelles (Figure 8 and Figure S1). Both samples were close to spherical shapes. The R_{TEM} values for just after preparation and after 60 h were estimated as 77.9 ± 18 and 113 ± 32 nm, respectively. This result suggests that the swollen micelles were due to increasing core hydrophilicity at pH 5.2 after 60 h. The particle-size range of the polymer aggregates after 60 h was larger than that just after preparation. This observation suggests that decomposition of the pendant acetal linkage was not uniform.

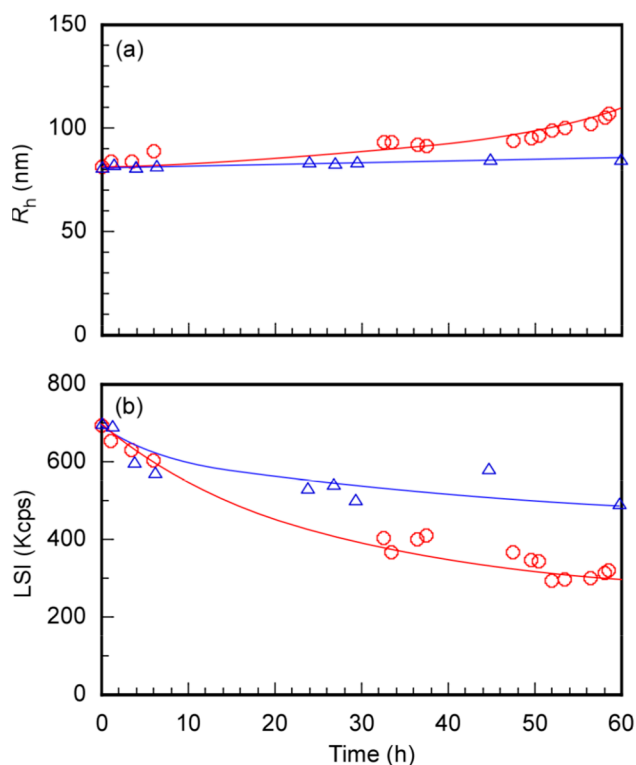


Figure 7. Time dependence on (a) hydrodynamic radius (R_h) and (b) light-scattering intensity (LSI) for PEG₅₄-P(AA/VE6/ γ TCP29)₁₄₀ at $C_p = 0.10$ g/L at 25 °C in acetate buffer of pH 5.2 (○) and in PBS buffer of pH 7.4 (△).

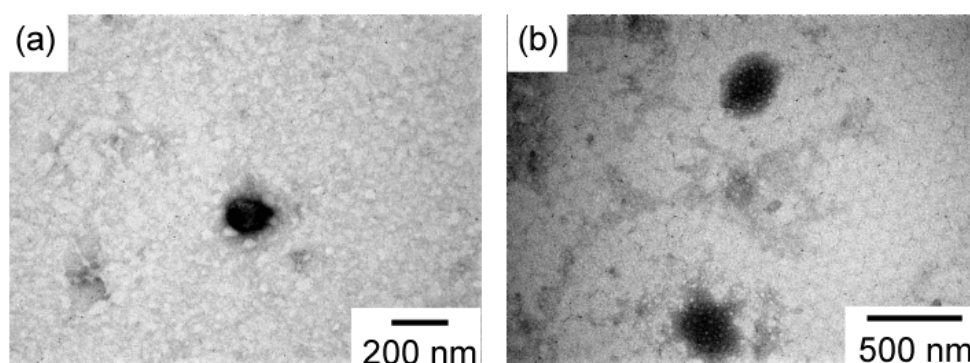


Figure 8. Transmission electron microscopy (TEM) images of PEG₅₄-P(AA/VE6/ γ TCP29)₁₄₀ at $C_p = 0.10$ g/L in acetate buffer of pH 5.2 (a) just after preparation and after (b) 60 h.

4. Conclusions

The amphiphilic block copolymers of PEG₅₄-P(AA/VE6/ γ TCP29)₁₄₀ bearing hydrophobic γ TCP via acetal bond linkage were prepared using RAFT radical polymerization. PEG₅₄-P(AA/VE6/ γ TCP29)₁₄₀ were demonstrated to dissolve in water, forming polymer micelles composed of hydrophobic P(AA/VE6/ γ TCP29)₁₄₀ block cores and hydrophilic PEG₄₅ shells. At pH 5.2, a gradual increase

in micelle size was observed with the gradual increase in time, attributed to the hydration and swelling of the core, which leads to the breakage of pendant acetal bonds. The breaking of acetal bonds generated γ TCP and pendant hydrophilic hydroxyl groups, which were hydrated and swollen to increase micelle size. At pH 7.4, the LSI of the polymer aqueous solutions decreased slightly with increasing time. This observation suggested that acetal bond was slightly degraded, even at pH 7.4. By applying PEG₅₄-P(AA/VE6/ γ TCP29)₁₄₀ as a suitable polymer for cancer treatment, a slow release of γ TCP could be achieved. However, low concentrations of γ TCP show nontoxicity, as well as no side effects for healthy tissue; when γ TCP is released in large amounts around cancer tissue, pendant acetal linkages are effectively broken due to low pH values. Moreover, the high released concentrations of γ TCP from the polymer micelle present an alternative route in the treatment of cancer tissue in the absence of anticancer-drug usage, especially prostate cancer, with reduced side effects.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2073-4360/12/1/36/s1>: Figure S1. (a) TEM images of PEG₅₄-P(AA/VE6/ γ TCP29)₁₄₀ at $C_p = 0.10$ g/L in PBS buffer of pH 7.4, in acetate buffer of pH 5.2 (b) just after preparation and (c) after 60 h.

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