

Complexation of Poly(2-ethyl-2-oxazoline)-*block*-poly(ϵ -caprolactone) Micelles with Multifunctional Carboxylic Acids

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ABSTRACT: Polymeric micelles derived from amphiphilic block copolymers of poly(2-ethyl-2-oxazoline) (PEtOz) and poly(ϵ -caprolactone) (PCL) were prepared in an aqueous phase. Dynamic light scattering and TEM experiments showed PEtOz–PCL micelles have a single core–shell structure with a mean hydrodynamic diameter range of 18–25 nm. The complex formation behavior of PEtOz–PCL micelles with low molecular weight multifunctional carboxylic acids such as malonic acid (MA), tricarballic acid (TCA), and 1,2,3,4-butanetetracarboxylic acid (BTCA) was investigated using dynamic light scattering in an aqueous media. As the molar concentration ratio of COOH group to EtOz repeating unit ($[\text{COOH}]/[\text{EtOz}]$) in the block copolymer increased, MA and TCA began to induce intermicellar aggregation at $[\text{COOH}]/[\text{EtOz}] = 160$, which resulted in the formation of large aggregates with a diameter range 96–146 nm. As $[\text{COOH}]/[\text{EtOz}]$ continued to increase, the aggregate size became larger. On the other hand, the mixture of micellar solution and BTCA began to undergo intermicellar aggregation even at $[\text{COOH}]/[\text{EtOz}] = 4$. The complex formation behavior of PEtOz–PCL micelles with poly(carboxylic acid)s was also investigated. Addition of poly(acrylic acid) (PAA) with $M_w = 2000$ into the PEtOz–PCL micellar solutions induced the formation of complex precipitates at $[\text{COOH}]/[\text{EtOz}] = 2.5$. The block copolymer micelles formed complex precipitates with PAA2000 ($M_w = 2000$) and PAA5000 ($M_w = 5000$) at $\text{pH} < 3.3$. The higher the molecular weight of PAA utilized for the complex formation, the higher the yield of complex precipitates. On the other hand, the block copolymer micelles formed complex precipitates with poly(methacrylic acid) (PMAA) with $M_w = 6400$ at $\text{pH} < 4.9$. The complex formed from micelles and PAA or micelles and PMAA could be reversibly redispersed as micelles at $\text{pH} > 3.6$ or $\text{pH} > 5.2$, respectively. The reversible micelle releasing behavior from the complex was investigated in aqueous media. The micelle release rate was highly dependent on the pH of release medium, molecular weight, and chemical structure of poly(carboxylic acid)s.

Introduction

Polymeric micelles formed in aqueous phase through self-association of amphiphilic block copolymers have attracted great interest.^{1–3} In particular, the unique characteristics of polymeric micelles in an aqueous phase such as nanosize, thermodynamic stability, and their core–shell structure have found numerous application fields including separation technology and the area of drug delivery.^{1–22} The hydrophilic block constructing the micellar outer shell in an aqueous media plays a critical role in the interaction with the surrounding environment and hence determines the solution properties and the stability of micelles.⁸ So far, most micelle-forming nonionic block copolymers are based on hydrophilic poly(ethylene oxide) (PEO), and the structural variation has been made mainly with hydrophobic blocks including polystyrene, poly(amino acids), poly(propylene oxide), and polyalkanes.^{2,3,9,23,24} Therefore, the systematic design and modification of the hydrophilic block of amphiphilic block copolymers have become one of important issues to diversify the structures of micelle-forming block copolymers, thereby expecting unique functions of micellar outer shells.^{25,26}

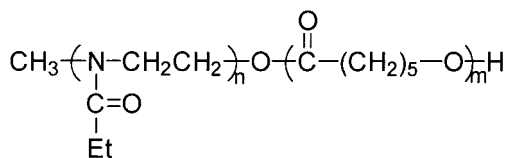
In recent reports, we described some unique characteristics of micelles based on diblock copolymers of poly-

(2-ethyl-2-oxazoline) (PEtOz) and aliphatic polyesters such as poly(L-lactide) (PLA) or poly(ϵ -caprolactone) (PCL) in an effort to systematically investigate the micellar characteristics of PEtOz-based amphiphilic block copolymers.^{27,28} These block copolymers in an aqueous media self-associated to form micelles, in which hydrophobic PLA or PCL constructed an inner core of micelles. The focus has been made on the investigation of their micellar characteristics mainly such as micelle size, shape, solubilizing capacity for pyrene, local viscosity, and specific polarity of micellar inner core.

PEtOz homopolymer is known to undergo pH-sensitive hydrogen bonding with poly(methacrylic acid) (PMAA) and poly(acrylic acid) (PAA) in an acidic aqueous media.^{29–31} In this study, we extend the scope of complexation of PEtOz homopolymer to the self-organized polymeric micelles with PEtOz outer shell. It is expected that the complex formation between micellar outer shell and poly(carboxylic acid)s could provide an opportunity for the functional variations of PEtOz-based polymeric micelles. In a preliminary study, we have demonstrated that the micelles of PEtOz–PLA and PEtOz–PCL diblock copolymers could form complex precipitates with poly(acrylic acid).^{27,32} In this work, we describe a comprehensive study on the reversible complexation of PEtOz–PCL micelles with poly(carboxylic acid)s via hydrogen bonding. In addition, the complex formation characteristics of PEtOz–PCL micelles with

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low molecular weight multifunctional carboxylic acids are described.



PEtOz-PCL

For the low molecular weight multifunctional carboxylic acids, malonic acid (MA), tricarballic acid (TCA), and 1,2,3,4-butanetetracarboxylic acid (BTCA) were employed. As poly(carboxylic acid)s, poly(acrylic acid) (PAA) and poly(methacrylic acid) (PMAA) were used for the complexation with PEtOz-PCL micelles.

Experimental Section

Materials and Equipment. 2-Ethyl-2-oxazoline (Aldrich) was dried and distilled over calcium hydride. ϵ -Caprolactone (Aldrich) was vacuum distilled over calcium hydride. Methyl *p*-toluenesulfonate (Aldrich) was purified by vacuum distillation. Stannous octoate (Sigma), pyrene (Aldrich), malonic acid (MA) (Aldrich), tricarballic acid (TCA) (Aldrich), and 1,2,3,4-butanetetracarboxylic acid (BTCA) (Aldrich) were used as received. Poly(acrylic acid) with M_w of 2000 (Aldrich) and 5000 (Wako Pure Chemical Industries, Ltd.) and poly(methacrylic acid) with M_w of 6400 (Aldrich) were used as received. Acetonitrile and chlorobenzene were distilled over calcium hydride and calcium chloride, respectively. The water was purified by distillation, deionization, and reverse osmosis (Milli-Q Plus). Other solvents such as THF and diethyl ether were used without further purification. Molecular weights and block compositions of the diblock copolymers were determined by the analysis of ^1H NMR spectra. ^1H NMR spectra were obtained on a Bruker AC 250 spectrometer at 250 MHz. Molecular weight distributions were determined using a GPC equipped with a Waters Associates 410 RI detector, 510 HPLC pump, and μ -Styragel columns with pore sizes of 10^2 , 500, 10^3 , and 10^4 Å. The eluent was THF, and the molecular weights were calibrated with polystyrene standards.

Preparation of Amphiphilic Diblock Copolymers. The diblock copolymers of 2-ethyl-2-oxazoline/ ϵ -caprolactone (PEtOz-PCL) were prepared following a procedure reported previously.²⁷ As a representative example, the block copolymer, PEtOz-PCL-0.19, which has 0.19 molar ratio of ϵ -caprolactone to 2-ethyl-2-oxazoline, was synthesized by the following procedure: A solution of 2-ethyl-2-oxazoline (70.00 g, 706.0 mmol) and methyl *p*-toluenesulfonate (2.19 g, 11.8 mmol) in acetonitrile (300 mL) was stirred at reflux for 30 h under nitrogen. After cooling to room temperature, 0.1 N methanolic KOH was added to introduce hydroxyl groups at the end of the PEtOz chain. The product, PEtOz-OH, was isolated following a reference procedure.²⁷ At room temperature, ϵ -caprolactone (2.30 g, 20 mmol) was added under nitrogen to an azeotropically dried chlorobenzene solution of PEtOz-OH (10.00 g). The temperature was raised at reflux, and stannous octoate (33 mg, 1/20 of [PEtOz-OH]) was added under nitrogen. The reaction was maintained for 30 h. The block copolymer was purified by a repeated precipitation from methylene chloride into diethyl ether. Other block copolymers which have different chain lengths of hydrophobic PCL block, PEtOz-PCL-0.37 and PEtOz-PCL-0.46, were synthesized in an identical manner except that different feed molar ratios of ϵ -caprolactone to the oxazoline unit of PEtOz-OH were employed.

Fluorescence and Light Scattering Measurements. Pyrene fluorescence spectra were recorded on an ISS K2 spectrofluorometer (ISS, Champaign, IL). The concentration of sample solution containing 6.0×10^{-7} M of pyrene was varied from 5×10^{-5} to 5 g/L. For the measurement of

excitation spectra, emission and excitation slit widths were set at 2 and 0.5 nm, respectively. For emission intensity of pyrene, emission and excitation slit widths were set at 0.5 and 2 nm, respectively. The excitation wavelength was 336 nm, and $\lambda_{\text{em}} = 393$ nm. The spectra were accumulated with an integration of 3 s/nm. The sample solutions were prepared following a reference method.²⁷ Dynamic light scattering measurements were performed using a Brookhaven BI-200SM goniometer and BI-9000AT digital autocorrelator. All the measurements were carried out at 25 °C. The scattered light of an argon ion laser (Lexel Laser model 95) operated at 488 nm was measured at an angle of 90° and collected on an autocorrelator. The hydrodynamic diameters (d) of micelles were calculated using the Stokes-Einstein equation $d = k_B T / 3\pi\eta D$, where k_B is the Boltzmann constant, T is the absolute temperature, η is the solvent viscosity, and D is the diffusion coefficient. The polydispersity factor, represented as μ_2/Γ^2 , was calculated by the cumulant method.³³ CONTIN algorithms were used in the Laplace inversion of the autocorrelation function to obtain micelle size distribution.

Transmission Electron Microscopy. Transmission electron microscopy (TEM) was performed on a Philips CM 200, operating at an acceleration voltage of 80 kV. For the observation of size and distribution of micellar particles, a drop of sample solution (concentration = 1 g/L) was placed onto a 200 mesh copper grid coated with carbon. About 2 min after deposition, the grid was tapped with a filter paper to remove surface water, followed by air-drying. Negative staining was performed by using a droplet of a 5 wt % uranyl acetate solution. The samples were air-dried before measurement.³²

Complex Formation of PEtOz-PCL Micelles with Low Molecular Weight Multifunctional Carboxylic Acids. PEtOz-PCL aqueous solutions (1 mL) with concentrations of 1 g/L were mixed with MA, TCA, and BTCA by varying a molar concentration ratio ([COOH]/[EtOz]) of COOH group of each carboxylic acids to EtOz repeating unit in the block copolymer. The mixture was vortexed for 10 min and was allowed to stand for 1 day before dynamic light scattering measurements.

Complex Formation of PEtOz-PCL Micelles with Poly(carboxylic acid)s. The PEtOz-PCL micellar solutions (10 mL, concentration = 5 g/L) were mixed with poly(acrylic acid)s with M_w of 5000 (PAA5000), 2000 (PAA2000), and poly(methacrylic acid) with M_w of 6400 (PMAA6400), respectively. The pH of the mixture of micellar solutions and PAA was then adjusted in the range 1.8–3.2. For the mixture of micellar solutions and PMAA, the pH was adjusted in the range 1.8–4.8. The precipitated complex of micelles and PAA or PMAA were recovered, vacuum-dried at 40 °C for 48 h, and pressed as a disk with a radius of 1 cm and a thickness range of 0.8–1.0 mm. The yields were calculated by the ratio of the weight of the recovered complex to the total weight of the initial block copolymer and poly(carboxylic acid)s.

Micelle Release from the Complex. For the micelle releasing experiment, pyrene-loaded micelles were prepared as follows: For pyrene-loaded micellar solutions with concentration of 5 g/L, distilled water (145 mL) was added dropwise to a vigorously stirred THF solution of block copolymer (0.725 g) containing 3.0×10^{-6} M of pyrene. Then, THF was removed on a rotary evaporator at 30 °C. The final pyrene concentration was 6.0×10^{-7} M. All the samples were sonicated for 10 min and allowed to stand for 1 day. The release behavior of pyrene-loaded micelle from the complex was investigated in a phosphate-buffered saline (PBS) solution (pH 7.4) and phosphate buffer solutions (pH 6.5 and pH 5.0). The concentrations of all of these release media were 100 mM. The disk-shaped complex was placed in a screw-capped tube containing 10 mL of release media. The tube was placed in a shaker bath maintained at 37 °C and shaken horizontally at 100 rpm. At predetermined time intervals, 5 mL of solutions was taken from the release media and replaced with an equal volume of fresh media. The samples were assayed using a spectrofluorophotometer. For quantitative analysis for the micelle release, a calibration curve for pyrene fluorescence intensities vs block copolymer concentration (0.125–5 g/L) was used. The pyrene-loaded micelle solutions were mixed with poly(carboxylic acid)s

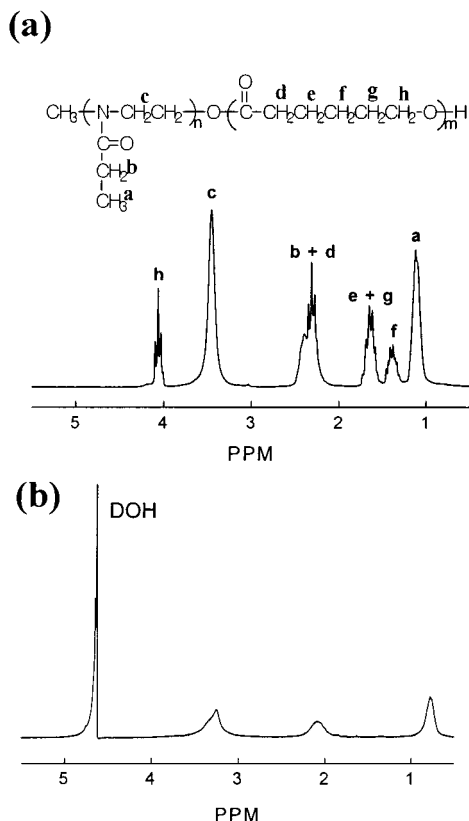


Figure 1. ¹H NMR spectra of PEtOz-PCL micelles: (a) PEtOz-PCL-0.46 in CDCl₃ and (b) PEtOz-PCL-0.46 micelles in D₂O.

directly after calibration to ensure the identical intensity of pyrene entrapped in micelles before and after complex formation.

Results and Discussion

Preparation of Amphiphilic Block Copolymers.

The synthesis and characterization were performed following a literature procedure.²⁷ The block copolymers were prepared by varying the length of the hydrophobic PCL block, while that of the hydrophilic PEtOz block ($M_n = 6200$) was fixed. The molecular weights and block compositions of the block copolymers were determined by the analysis of ¹H NMR spectra in Figure 1a. The molar ratios of repeating units in PEtOz and PCL blocks were determined by the peak integration ratios of methyl protons (1.10 ppm) in PEtOz and CH₂OCO (4.05 ppm) in the PCL block. The block copolymers were denoted as PEtOz-PCL-0.19, PEtOz-PCL-0.37, and PEtOz-PCL-0.46, which have 0.19, 0.37, and 0.46 molar ratios of ϵ -caprolactone to 2-ethyl-2-oxazoline, respectively. All the copolymers have identical PEtOz blocks with $M_n = 6200$ and $M_w/M_n = 1.08$. The block compositions and molecular weights of PEtOz-PCLs are summarized in Table 1.

Micelles of Block Copolymers. The micelle formation of block copolymers in an aqueous phase was confirmed by NMR spectroscopy and a fluorescence technique using pyrene as a probe.^{3,4,34} Figure 1 shows the ¹H NMR spectra of PEtOz-PCL-0.46 micelles in CDCl₃ (a) and D₂O (b).

While the resonance peaks from both PEtOz block and PCL block were clearly observed in CDCl₃, the peaks of PCL block disappeared in D₂O, which indicated the limited molecular motion of PCL block surrounded by

Table 1. Molecular Weights and Compositions of the Block Copolymers

block copolymers ^a	feed ratio ^b [CL]/[EtOz]	composition ratio ^d	wt % of PCL ^e	M_n / M_n^f
PEtOz-PCL-0.19	0.20	0.19	18	1.13
PEtOz-PCL-0.37	0.40	0.37	30	1.16
PEtOz-PCL-0.46	0.50	0.46	35	1.21

^a All the samples were prepared using PEtOz-OH with M_n of 6200. ^b Molar feed ratio of ϵ -caprolactone to the repeating unit of PEtOz-OH. ^c Estimated by ¹H NMR. ^d Molar composition ratio of the repeating units of PCL to that of PEtOz by ¹H NMR analysis. ^e Weight percentage of hydrophobic PCL block in the block copolymers. ^f Estimated by GPC.

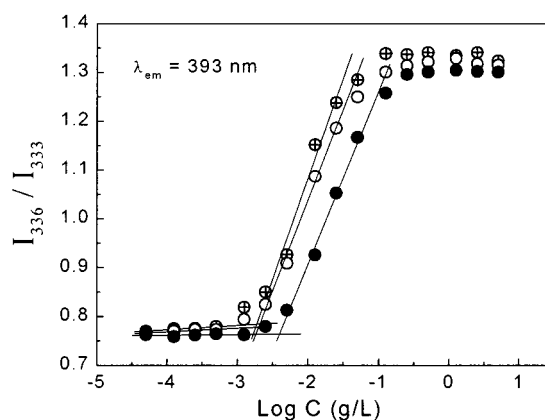


Figure 2. Plot of I_{336}/I_{333} (from pyrene excitation spectra) vs $\log C$ for PEtOz-PCL-0.19 (●), PEtOz-PCL-0.37 (○), and PEtOz-PCL-0.46 (⊕).

Table 2. Properties of PEtOz-PCL Block Copolymer Micelles

block copolymers	cmc ^a (mg/L)	d^b (nm) (μ_2/Γ^2) ^c	d^d (nm) (μ_2/Γ^2)	d^e (nm) (μ_2/Γ^2)
PEtOz-PCL-0.19	3.9	20 (0.13)	22 (0.11)	24 (0.19)
PEtOz-PCL-0.37	2.0	18 (0.14)	25 (0.14)	29 (0.10)
PEtOz-PCL-0.46	1.8	25 (0.04)	29 (0.14)	34 (0.24)

^a Measured at 25 °C. ^b Mean diameters of micelles in distilled water at 25 °C. ^c Polydispersity factor. ^d Mean diameters of micelles in PBS solution at 25 °C. ^e Mean diameters of redispersed micelles from the complex with PAA5000 in PBS solution at 25 °C.

the hydrophilic PEtOz. This result confirms the micelle formation of PEtOz-PCL block copolymers in an aqueous phase. When the micelles are formed in an aqueous phase, pyrene molecules preferably locate inside or close to the hydrophobic microdomain of micelles, and consequently their photophysical characteristics are changed, compared to pyrene molecules in an aqueous phase.^{3,35-37} The characteristic feature of the pyrene excitation spectra, (0,0) band shift from 333 to 336 nm upon pyrene partition into micellar hydrophobic microdomain, was utilized to determine the cmc value of PEtOz-PCLs. Figure 2 shows the intensity ratio (I_{336}/I_{333}) of pyrene excitation spectra vs logarithm of PEtOz-PCL block copolymer concentration.

The cmc value was determined from the threshold concentration, where the intensity ratio I_{336}/I_{333} begins to increase markedly. Therefore, the interception of two straight lines in the low concentration range is determined as cmc. The cmc values of PEtOz-PCLs, depending on the block composition, were in the range 1.8–3.9 mg/L (Table 2). As the length of hydrophobic PCL block increases, the cmc value decreases. The mean hydrodynamic diameters of micelles from dynamic light

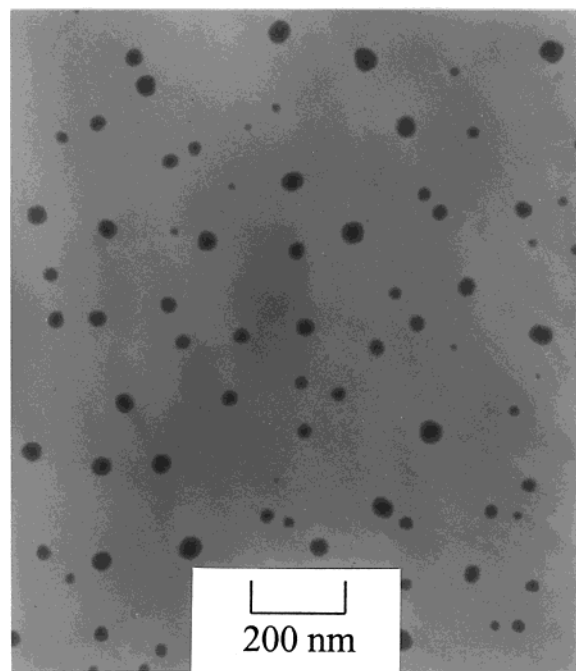


Figure 3. TEM image of PEOz-PCL-0.37 micelles.

scattering were in the range of 18–25 nm as listed in Table 2. Considering the block length of PEOz-PCLs and the micelle size, it can be suggested that the micelles of PEOz-PCLs have a simple core-shell structure, rather than a multicore structure of several hundreds of nanometers, which is formed by the association of individual micelles. In particular, Eisenberg's group recently reported that the formation of large aggregates of individual micelles was more pronounced in the case of micelles with a shell from a relatively short PEO block (M_n = ca. 2000), which could not evade the hydrophobic interaction and van der Waals interaction between exposed hydrophobic cores of micelles.³⁸ In our previous report, micelles of multicore structure with a size range of 108–192 nm were observed when PEOz homopolymer with M_n of 4400 was employed in the synthesis of block copolymers.²⁷ Consequently, it is interesting to note that the increase in the number of EtOz repeating unit from 44 (M_n = 4400) to 63 (M_n = 6200) can avoid the intermicellar aggregation by constructing densely packed hydrophilic PEOz shell. The micelle size was not significantly affected by the change of the polymer concentration in the range 0.5–5 g/L. The micelles of PEOz-PCL block copolymers were visualized by TEM as shown in Figure 3. The micelles of PEOz-PCL block copolymers were generally spherical.

Complexation of Micelles with Low Molecular Weight Multifunctional Carboxylic Acids. PEOz constructing the outer shell of PEOz-PCL micelles has two possible sites, the nitrogen and the carbonyl oxygen, for the hydrogen bonding with carboxyl groups, which would provide an opportunity for the self-aggregation of micelles with multifunctional carboxylic acids. The complex formation behavior of PEOz-PCL micelles with multifunctional carboxylic acids such as malonic acid (MA), tricarballic acid (TCA), and butanetetracarboxylic acid (BTCA) was investigated using dynamic light scattering in an aqueous media. Figure 4 shows the dependence of mean hydrodynamic diameters of micelles on the molar concentration ratio ([COOH]/

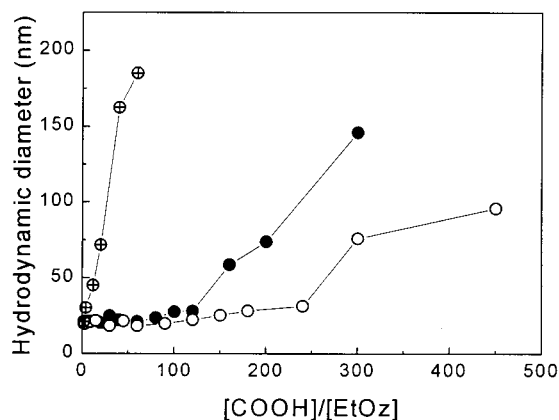


Figure 4. Dependency of hydrodynamic diameters of PEOz-PCL micelles as a function of molar concentration ratio ([COOH]/[EtOz]): ●, MA; ○, TCA; ⊕, BTCA.

[EtOz]) of COOH groups of each molecule to EtOz repeating unit in PEOz-PCL-0.37.

The pH ranges of all the solutions were below 2.7, where carboxylic acid groups of multifunctional acids could form hydrogen bonding with PEOz repeating units. As [COOH]/[EtOz] increased, MA and TCA began to induce intermicellar aggregation at [COOH]/[EtOz] of 160. At the very initial stage of intermicellar aggregation by MA, the micelle size distribution became bimodal, i.e., ca. 25 nm for unaggregated micelles and ca. 240 nm for intermicellar aggregates. For intermicellar aggregation by TCA, bimodal size distribution of micelles, ca. 25 nm for unaggregated micelles and ca. 150 nm for intermicellar aggregates, was also observed. As the intermicellar aggregation proceeded, the aggregate size became larger with unimodal distribution. In addition, it is interesting to note that the mixture of micellar solution and BTCA with four carboxylic groups underwent more drastic intermicellar aggregation, which could be observed even at [COOH]/[EtOz] of 4, suggesting that enhanced intermicellar aggregation is induced by the compound with more carboxyl groups. The principle of this type of complexation of PEOz-PCL micelles with multifunctional carboxylic acids could be applied to that with polymeric carboxylic acids.

Complexation of Micelles with Poly(carboxylic acid)s. Poly(2-ethyl-2-oxazoline) is known to undergo pH-sensitive hydrogen bonding with poly(methacrylic acid) (PMAA) or poly(acrylic acid) (PAA) in an acidic water.^{29–31} At a low pH (<5.0), PEOz was reported to form hydrogen bonding with PMAA to produce precipitates of the polymer complex, which then redissolves at a high pH (>5.4) due to the disappearance of hydrogen bonding. In preliminary studies, we have demonstrated that the micelles of PEOz-PLA and PEOz-PCL diblock copolymers could form complex precipitates with poly(acrylic acid) at pH < 3.5.^{27,32} These precipitates could be redispersed as micelles in water at pH > 3.8. The concept of complex formation between PEOz-PCL micelles and poly(carboxylic acid)s is illustrated in Figure 5.

Figure 6a shows yields of complex precipitates of PEOz-PCL micelles with PAA2000 at various pHs.

It is clearly shown that complex precipitates are formed at pH below 3.3. As the content of hydrophobic PCL became higher, the higher the yield of complex precipitates, probably because the micelles with higher hydrophobicity show a higher tendency toward precipitation.³² The yield of complex precipitates of micelles

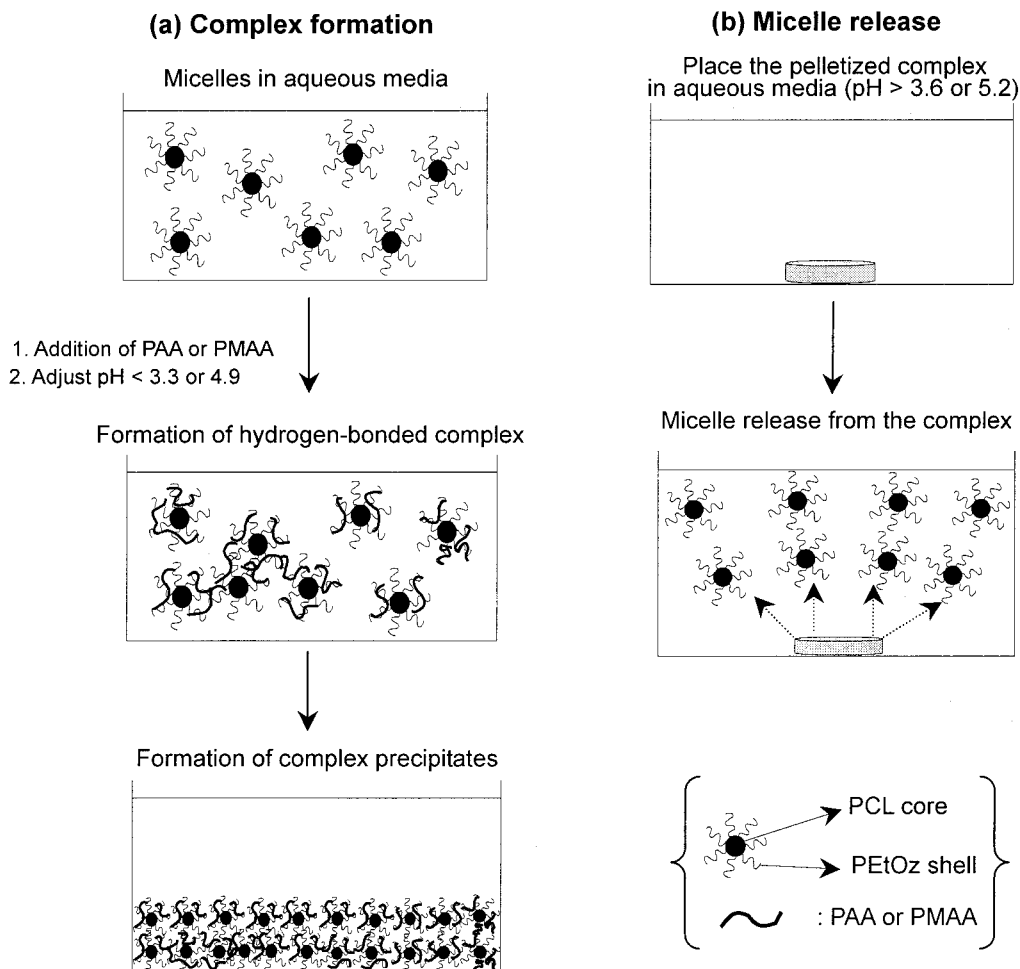


Figure 5. Conceptual illustration of (a) complexation of micelles with poly(carboxylic acid)s and (b) micelle release from the complex.

with PAA2000 was in the range 10.5–58.6% depending on the pH of medium. The dependence of yields of complex precipitates of micelles with PAA5000 on the pH of medium is shown in Figure 6b. As in the case of the complex of micelles with PAA2000, complex precipitates are formed at pH below 3.3. However, it is interesting to note that yields of complex precipitates (25.0–78.5%) are higher than those for complex of micelles with PAA2000 at overall pH range. This result could be ascribed to the long-range intermolecular hydrogen bonding, which may be more pronounced for PAA5000 with more carboxyl groups in a chain than PAA2000 as was observed with low molecular weight multifunctional carboxylic acids. Therefore, it could be considered that the poly(carboxylic acid) with longer chain length would be more effective in terms of complex formation. Figure 7 shows the yields of recovered complex precipitates of PEtOz–PCL micelles and PMAA depending on pH change of aqueous media.

There are two noticeable features of these plots. First, micelles formed complex precipitates with PMAA at pH below 4.9, which is much higher than the case of micelles with PAA. Second, the yields of complex precipitates reach to about 100% at equilibrium pH range. For the comparison with complex from PAA5000 in Figure 6, PMAA with a similar number of repeating units was utilized to exclude the effect of molecular weight on the yields of complex. The former result can be explained by considering the degree of dissociation of carboxylic groups in PMAA and PAA. The pK_a values

obtained from the Henderson–Hasselbach equation are 7.3 for PMAA and 5.6 for PAA.³⁹ This indicates that, at a certain pH, the degree of dissociation of carboxyl groups of PAA to carboxylates would be higher than that of PMAA. Consequently, PMAA could form complex precipitates at higher pH than PAA did. The latter phenomenon could be ascribed to the difference in the hydrophobic character of PMAA and PAA. PMAA is more hydrophobic than PAA due to α -methyl groups in the chain. Therefore, it is likely that the hydrophobic interaction between PMAA and PEtOz shell of micelles participates in the formation of complex precipitates in cooperation with hydrogen bonding, which is the main interaction force. Therefore, it might be suggested that these cooperative interactions between micelles and PMAA generated higher yield in the recovery of complex precipitates. The recovered yields of complex at pH 2.4 were monitored by varying the weight feed ratio of poly(carboxylic acid)s to the block copolymer, PEtOz–PCL-0.46 (Figure 8).

At a wide range of feed ratio, it was found that PMAA generated higher yield than PAA. This complex formation characteristics could be useful in a drug delivery application. For example, drug-loaded PEtOz-based micelles could be recovered by using simple complex formation of the micelles with poly(carboxylic acid)s rather than employing conventional dialysis and freeze-drying process.

Reversible Micelle Release Behavior from the Complex. The recovered complex could be shaped, for

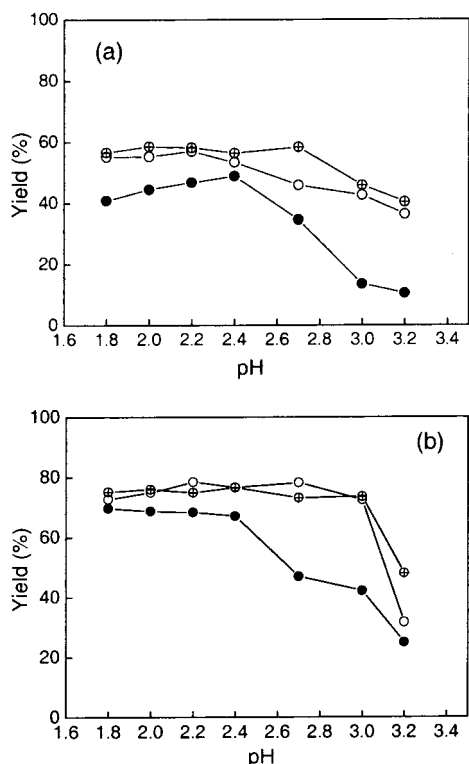


Figure 6. (a) Yield of recovered complex precipitates of block copolymer micelles with PAA2000 as a function of pH. (b) Yield of recovered complex precipitates of block copolymer micelles with PAA5000 as a function of pH. ●, PEToz-PCL-0.19 micelles-PAA complex; ○, PEToz-PCL-0.37 micelles-PAA complex; ⊕, PEToz-PCL micelles-0.46-PAA complex.

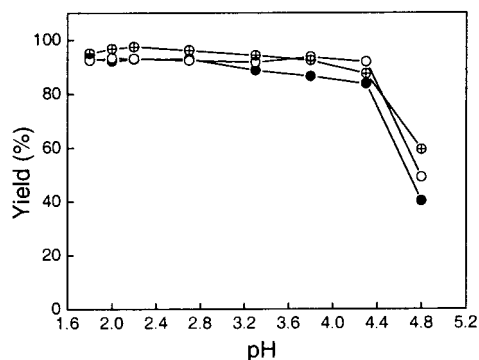


Figure 7. Yield of recovered complex precipitates of block copolymer micelles with PMAA6400 as a function of pH: ●, PEToz-PCL-0.19 micelles-PMAA complex; ○, PEToz-PCL-0.37 micelles-PMAA complex; ⊕, PEToz-PCL-0.46 micelles-PMAA complex.

example, as disks, which can be stored and redispersed as micelles in aqueous media. The conceptual scheme for the micelle release from the disk-shaped complex is illustrated in Figure 4. The complex precipitates from micelles with PAA could be redispersed as micelles in aqueous media at pH > 3.6. For those from micelles with PMAA, the homogeneous dispersion was obtained at pH > 5.2, as a result of breakage of the hydrogen bonding between carboxyl groups and the PEToz outer shell of the micelles. The release of micelles from the complex was confirmed by comparing hydrodynamic diameters of released micelles with those of the micelles before complex formation, in a PBS solution. Parts a and b of Figure 9 respectively show the size distributions of PEToz-PCL-0.37 micelles before complex formation and

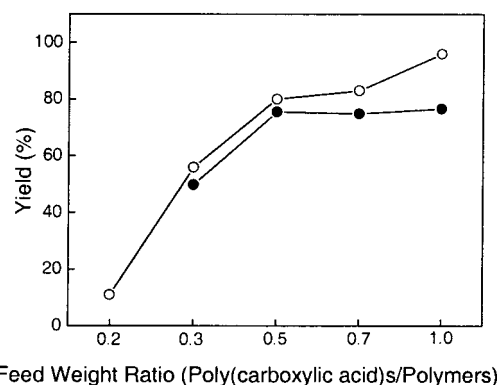


Figure 8. Yield of recovered complex precipitates of PEToz-PCL-0.46 micelles with poly(carboxylic acids) at various weight feed ratios: (●) micelles-PAA5000 complex; (○) micelles-PMAA6400 complex.

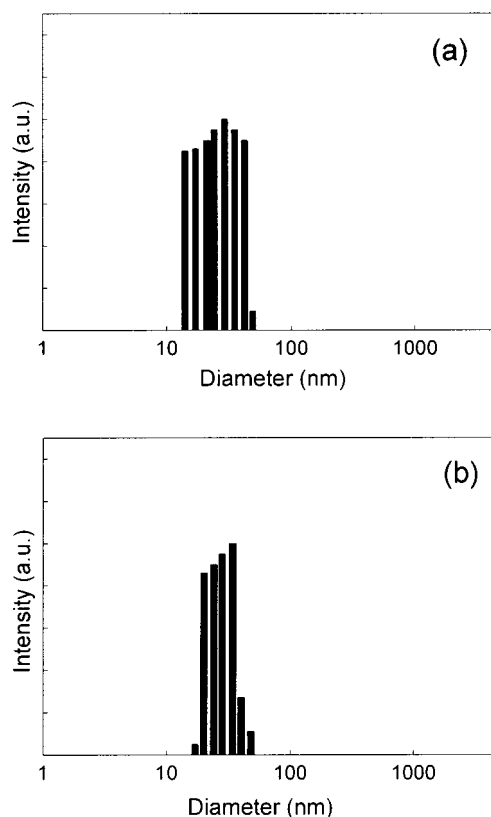


Figure 9. Size distribution of PEToz-PCL-0.37 micelles in PBS (pH 7.4) solution (a) before complex formation and (b) after release from the complex with PAA5000.

after release from the complex with PAA5000, in PBS (pH 7.4) solution.

As obviously shown in the size distribution, the redispersed solution of complex precipitates contains micelles whose hydrodynamic diameters are comparable to those of micelles before complex formation (Table 2). Figure 10 shows the in vitro release of pyrene-loaded PEToz-PCL-0.37 micelles from the complex with PAA2000 in various buffer solutions with pH range from 5.0 to 7.4 at 37 °C.

It was found that the micelle release from the complex was considerably influenced by the pH of media, where the more basic the release medium, the higher the rate of release of micelles. The dissolution of complex into micelles by the breakage of hydrogen bonding was more pronounced in more basic media. Figure 11 shows that

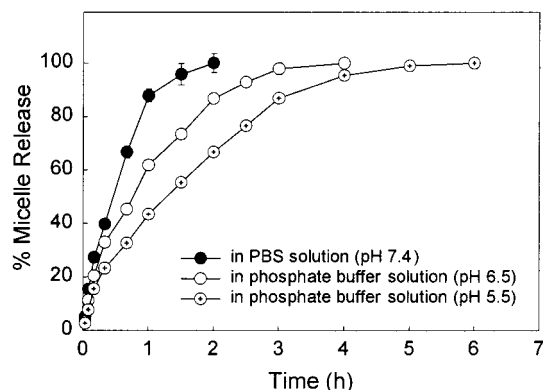


Figure 10. Release profile of PEtOz-PCL-0.37 micelles from the complex with PAA2000 in different pH media at 37 °C.

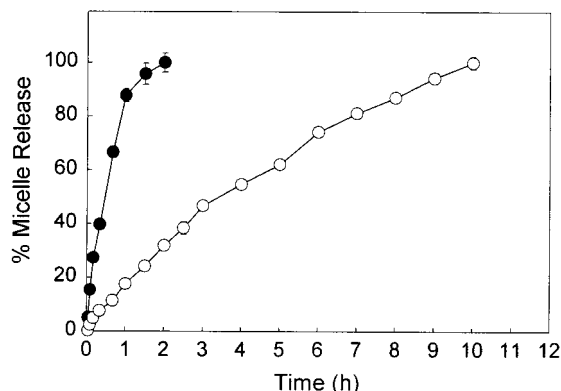


Figure 11. Release profile of PEtOz-PCL-0.37 micelles from (●) the micelles-PAA2000 complex and (○) the micelles-PAA 5000 complex in PBS solution at 37 °C.

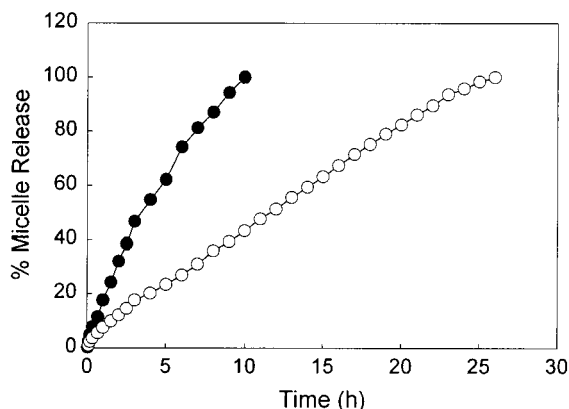


Figure 12. Release profile of PEtOz-PCL-0.37 micelles from (●) the micelles-PAA5000 complex and (○) the micelles-PMAA6400 complex in PBS solution at 37 °C.

the micelle release from the complex can be controlled by the adjustment of molecular weights of poly(carboxylic acid)s utilized for the complex formation.

The micelle release from the complex of micelles with PAA5000 was slower than that from the complex with PAA2000 in PBS solution at 37 °C. Figure 12 shows the comparison of micelle release profiles between the complex with PAA5000 and that with PMAA6400.

It is of interest to note that the micelle release from the complex of micelles with PAA5000 completed in 10 h, but the micelle release from the complex of PMAA5000 micelles was found to continue for 24 h. The unique micelle release characteristics may find application in drug delivery area. For example, in polymeric micellar

carrier systems, the rapid clearance by the reticulo-endothelial system (RES) in the bloodstream has been still problematic after intravenous administration, though stable circulation of some class of polymeric micelles in bloodstream has been reported.⁴⁰ In this micelle-releasing system, the sustained release of drug-containing micelles from the complex is the release of drug carriers rather than the release of free drug. Therefore, the characteristics as the carriers can be expressed over a long period during the release of micelles.

Conclusions

The complexation behavior of PEtOz-based polymeric micelles with multifunctional carboxylic acids such as MA, TCA, BTCA, and poly(carboxylic acid)s in an aqueous phase was investigated. The addition of MA, TCA, and BTCA to PEtOz-PCL micellar solutions induced intermicellar aggregation above a critical molar concentration ratio ($[\text{COOH}]/[\text{EtOz}]$) via hydrogen-bonding-induced complexation. This complexation generated stable dispersion of intermicellar aggregates. On the other hand, the complexation of PEtOz-PCL micelles with poly(carboxylic acid)s did not form a stable dispersion but led to formation of complex precipitates. The PEtOz-PCL micelles could form complex precipitates with PAA or PMAA at pH < 3.3 and pH < 4.9, respectively. The recovered yields of micelle-containing complex could be controlled by adjusting the molecular weight and chemical structure of poly(carboxylic acid)s. The micelles-PAA and micelles-PMAA complex could be redispersed as micelles at pH > 3.6 or pH > 5.2, respectively. The micelle release rate could be conveniently controlled depending on the pH of release medium, molecular weight, and chemical structure of poly(carboxylic acid)s.

These unique complexation characteristics of PEtOz-based micelles with poly(carboxylic acid)s could find a novel applicability in the area of drug delivery, in that the complex of micelles and poly(carboxylic acid) can act as a matrix for a sustained release of drug-containing micelles at a physiological pH.

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