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Preparation of methoxy poly(ethyleneglycol)-block-poly(caprolactone) via activated monomer mechanism and examination of micellar characterization

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Summary

The polymerization of ε–caprolactone (CL) in the presence of $HCl \cdot Et_2O$ *via* activated monomer mechanism was performed to synthesize diblock copolymers composed of methoxy polyethyleneglycol (MPEG) and poly(ε–caprolactone) (PCL). The obtained PCLs had molecular weights close to the theoretical values calculated from the CL to MPEG molar ratios and exibited monomodal GPC curves. We successfully prepared MPEG and PCL diblock copolymers by activated monomer mechanism. The micellar characterization of MPEG-PCL diblock copolymers in an aqueous phase was carried out by using NMR, dynamic light scattering, AFM and fluorescence techniques. The diblock copolymers formed micelles with a critical micelle concentration (CMC) ranging 2.07×10^{-2} - 1.16×10^{-3} mg/mL depended on the block lengths of diblock copolymers. The diameters of micelles, measured by dynamic light scattering, were 100-250 nm. Most micelles exhibited a spherical shape in AFM.

Introduction

Poly(ε-caprolactone) (PCL), a biodegradable polyester, is an attractive polymer which can be used as a synthetic biomaterial or a controlled drug release matrix due to its good biodegradability and biocompatibility [1]. However, its high hydrophobicity and crystallinity and slow biodegradation rate have considerably limited its biomedical applications. Therefore, the introduction of hydrophilic poly(ethylene glycol) (PEG) block onto one of the ends of a PCL chain has attracted much attention [2-4]. Many articles have focused on the synthesis of amphiphilic block copolymer composed of PCL and PEG, which are synthesized by ring opening polymerization (ROP) of ε-caprolactone (CL) with stannous octoate or organometallic catalysts, with a PEG terminal hydroxyl groups as an initiator [5-8].

These amphiphilic block copolymers obtained are the focus of numerous investigations in the view of applications in drug delivery systems or self-assembling systems [9-11]. Their unique molecular structure can make them form nanosized

micelles with core-shell architecture in an aqueous medium. The hydrophobic segments of the block copolymers form the inner core of the micelles, whereas the hydrophilic segments form the outer shell of the micelles. These micelles seem to be potential carriers for drug delivery, because the hydrophobic biodegradable core may serve as a hydrophobic drug reservoir and the PEG shell covalently linked to hydrophobic core can greatly increase the micelle stability in the biological environment.

However, some problems have been found in the amphiphilic block copolymers obtained by ROP using organometallic catalysts, including incomplete catalyst removal, as it is difficult to remove the metal contaminant from the resultant polymers [12]. Thus, the use of metal catalysts is the subject of controversy in extending the use of PEG and PCL block copolymers for practical biomedical purpose.

In this article, we describe the synthesis of methoxy polyethyleneglycol (MPEG)-PCL diblock copolymers by a metal-free method *via* living ROP of CL from MPEG in the presence of $HCl \cdot Et_2O$ as a monomer activator. In addition, the characteristics of micelle as a potential drug delivery vehicle, formed from MPEG-PCL diblock copolymer in a function of the length of the MPEG and PCL blocks, were investigated by using fluorescence techniques, NMR, and AFM.

Experimental

Materials

MPEG 550 (E_{13}), 750 (E_{17}), 2000 (E_{45}) $g \cdot mol^{-1}$ (Aldrich), 1000 (E_{23}) $g \cdot mol^{-1}$ (Sunbright), HCl (Aldrich; 1.0 M solution in diethyl ether), and pyrene (Aldrich) were used as received. CL was distilled over CaH_2 under reduced pressure. CH_2Cl_2 was distilled sequentially from $CaCl_2$ and CaH_2 under nitrogen before use.

Instruments

^1H NMR spectra were measured using Bruker 300 MHz instrument with $CDCl_3$ in the presence of tetramethylsilane as internal standard or D_2O for polymeric micelle. Molecular weights and molecular weight distributions of MPEG and MPEG-PCL diblock copolymers were measured by a Futects At-3000 GPC system (Shodex RI-71 detector) using two columns (Shodex K-802 and Shodex Asahipak GF-510). $CHCl_3$ was used as the eluent at a flow rate of $0.6\ mL \cdot min^{-1}$. Dynamic light scattering was measured by ELS-8000, Otsuka Electronics, Japan).

Synthesis of methoxy poly(ethyleneglycol)-block-poly(ε-caprolactone) copolymers (MPEG-PCL).

All glasses were dried by heating in vacuum and handled under a dry nitrogen stream. The typical process for the polymerization to give $E_{45}C_8$ is as follows. MPEG (M_n=2000 $g \cdot mol^{-1}$) (0.812 g, 0.4 mmol) and toluene (30 mL) were introduced into a flask. The MPEG solution was distillated by azeotropic distillation to remove water. Toluene was then distilled off completely. To MPEG was added the CH_2Cl_2 (0.55 mL), followed by the addition of CL (0.274 g, 2.4 mmol) using syringe. The polymerization was initiated by the addition of 1.0 M HCl solution (0.8 mL, 0.8 mmol) at 25 °C. After 24 h, the reaction mixture was poured into *n*-hexane to precipitate a polymer, which was separated from the supernatant by decantation. The

obtained polymer was redissolved in CH_2Cl_2 and then filtered. The polymer solution was concentrated by rotary evaporation and dried in vacuo to give a colorless polymer of quantitative yield. The molecular weight of PCL segment in the block copolymer was determined by the intensity of terminal methoxy proton signal of MPEG at $\delta = 3.38$ ppm and methylene proton signal of PCL at $\delta = 2.31$ ppm in ^{1}H NMR spectroscopy.

Determination of critical micelle concentration

The critical micelle concentration (CMC) was determined using pyrene as a fluorescence probe. 1 mL of pyrene solution in THF (1.2 mM) was added to 1000 mL of distilled water. THF was removed by a rotary evaporator at 30 °C for 2 h to give pyrene solution in water (1.2×10^{-6} M). Stock solutions of diblock copolymer were prepared by dissolving the diblock copolymer samples in distilled water, filtered through 0.45 μm membrane filter, under stirring. From the stock solution a series of concentration was prepared by dilution. The pyrene solution was added by the diblock copolymer solution and were allowed to stand overnight at room temperature to equilibrate. The micelle concentration in these experiments varied from 1.0×10^{-7} to 2.0 mg/mL. The pyrene concentration in diblock copolymer solution was 6×10^{-7} M. For the measurements of pyrene excitation spectra scan speed was set at 240 nm/min and, emission and excitation slit widths were set at 2.5 nm. For the excitation spectra, the emission wavelength was 373 nm. Fluorescence intensities of the pyrene entrapped in the micelle core were determined by an F-4500 fluorescence spectrophotometer (I_{ex} 338 nm, Hitachi Co. LTD, Japan) at room temperature.

Atomic Force Microscopy (AFM)

One drop of diblock copolymer solution ($E_{45}C_{23}$) was transferred onto silicone wafer which washed with MeOH. The wafer was quickly placed in liquid nitrogen, followed by the freeze-drying for 3 days. AFM measurements were carried out in the tapping mode with a Nanoscope IV instrument (Digital Instruments Inc.).

Results and discussion

Endo et al and Faust et al investigated the ROP of CL with an alcohol/$HCl \cdot Et_2O$ initiator system [13-15]. Thus, this method may be considered as a candidate to easily give MPEG-PCL diblock copolymers by a metal-free method.

The polymerization of CL was performed with MPEG in the presence of $HCl \cdot Et_2O$ in CH_2Cl_2 at 25 °C for 24 h. Table 1 summarizes the results for MPEG-PCL obtained by ROP. This polymerization gave colorless diblock copolymers with almost quantitative yield after isolation by precipitation in *n*-hexane. The molecular weights of PCLs, calculated by ^{1}H NMR, showed good agreement with those calculated from the feed ratio of CL to MPEG. In addition, the polydispersities of MPEG-PCL increased slightly at complete monomer conversion compared to MPEG as an initiator, but have maintained still narrow one. Scheme 1 represents a plausible polymerization path of CL by MPEG as an initiator. The hydroxyl end group of MPEG could attack the carbonyl carbon of the protonated CL in the presence of $HCl \cdot Et_2O$.

Figure 1A shows the ^{1}H NMR spectrum of diblock copolymer obtained by the polymerization. The diblock copolymer exhibited characteristic peaks of PCL and

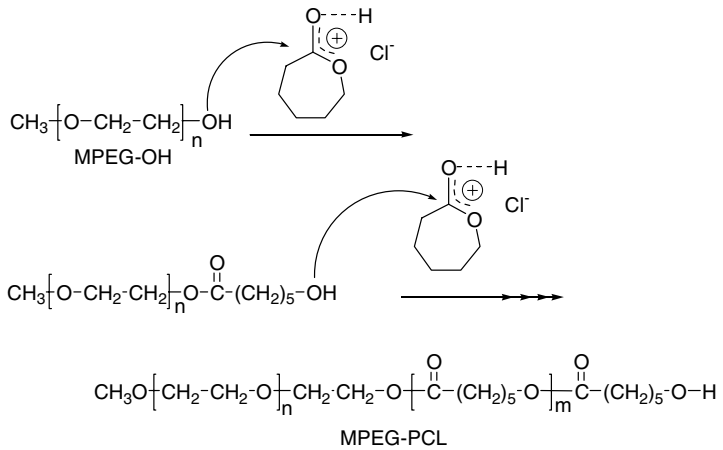
MPEG. Terminal methoxy protons (*a*) and methylene protons (*c*) of MPEG observed at δ = 3.38 and 3.68 ppm, respectively. The signals (1-5) assignable to the sequence of α, β + δ, γ and ε–methylene protons of the ester carbonyl moiety of PCL were also observed at = 2.31, 1.63, 1.39, and 4.07 ppm, respectively. In addition, polymer chain end proton (8) of PCL was observed at 3.88 ppm, and a signal assignable to terminal ω-methylene protons (*d*) of the MPEG blocked by PCL were observed at around 4.22 ppm. Moreover, the integration ratio of the *a* to *d* was exactly 3 to 2, indicating good agreement with the expected value. This indicates that the terminal hydroxyl group of MPEG served as an initiator in this polymerization system.

Table 1. Synthesis of MPEG-PCL diblock copolymers

Initiator (I)[a]	No.	[CL]/[I]	Yield [b] (%)	$M_{n,\ calcd}$	$M_{n,\ NMR}$ [c]	M_w/M_n [d]
MPEG-OH (MW=550)	$E_{13}C_{20}$	14.0	99	550-1600	550-2040	1.17
MPEG-OH (MW=750)	$E_{17}C_{20}$	16.6	99	750-1900	750-2060	1.28
MPEG-OH (MW=1000)	$E_{23}C_{20}$	17.5	99	1000-2000	1000-2130	1.27
MPEG-OH (MW=2000)	$E_{45}C_8$	6.0	99	2000-730	2000-780	1.12
	$E_{45}C_{10}$	9.7	99	2000-1100	2000-1060	1.27
	$E_{45}C_{20}$	17.5	99	2000-2000	2000-1980	1.22
	$E_{45}C_{32}$	24.1	99	2000-2750	2000-3200	1.29
	$E_{45}C_{47}$	37.8	99	2000-4310	2000-4760	1.29
	$E_{45}C_{60}$	52.6	94	2000-6000	2000-6000	1.28

Condition; [HCl]/[Initiator] = 2, [CL]/[CH_2Cl_2] = 0.5 M, Room temperature, 24 h.
[a] MPEG = 550 (M_w/M_n = 1.10), 750 (M_w/M_n = 1.18), 1000 (M_w/M_n = 1.13), 2000 (M_w/M_n = 1.17).
[b] *n*-Hexane insoluble part. [c] Determined by ^{1}H NMR.
[d] Measured by gel permeation chromatography (Based on standard polystyrene).

Scheme 1



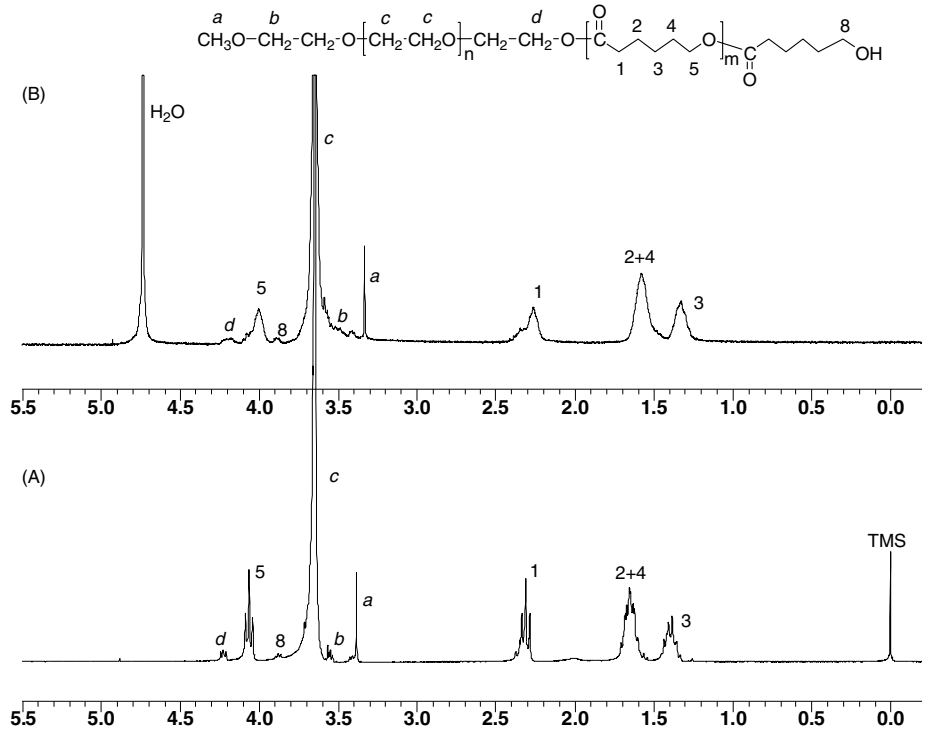


Figure 1. ^{1}H NMR spectra of MPEG-PCL diblock copolymer ($E_{45}C_8$) in (A) $CDCl_3$ and (B) D_2O.

First, the micelle formation of diblock copolymers in an aqueous condition was confirmed by NMR spectroscopy. Figure 1B shows the ^{1}H NMR spectrum of MPEG-PCL diblock copolymer in D_2O. While the resonance peaks from both MPEG block and PCL block were clearly observed in $CDCl_3$, the peaks of PCL block changed broadly in D_2O. This indicates that PCL blocks preferably locate inside core of the micelles and consequently PCL molecular motion are limited by MPEG or water, compared to MPEG blocks located at the outer shell of the micelles in an aqueous phase.

The mean hydrodynamic diameters of micelles from dynamic light scattering (DLS) were in the range of 100-250 nm as listed in Table 2. No large difference of diameters of micelles observed at changes in block length and concentration to make micelle. We measured AFM image for micelles prepared by using all diblock copolymers at 1% concentration. In general, micelles of spheres, rods, lamellae form have been reported [16]. In the present study, most of the micelles exhibited a spherical sphere, nonspherical micelle was observed only rarely at AFM. Figure 2 shows AFM image of the micelle prepared with $E_{45}C_8$ diblock copolymer. The AFM images revealed that polymeric micelle formed with MPEG-PCL diblock copolymers is spherical in shape. The diameters of the polymeric micelles observed by AFM were in good agreement with the results of the DLS observations. It can be suggested that most of the micelles formed by using MPEG-PCL diblock copolymers have a core-shell structure, rather than a multicore structure of several hundreds of nanometers, which is formed by the association of individual micelles.

Table 2. CMC and mean hydrodynamic diameters of micelles formed by MPEG-PCL diblock copolymers

No.	$M_{n,\ NMR}$	CMC x 10^{3} [a] (mg/mL)	Diameter [b] (nm)
$E_{13}C_{20}$	550-2040	2.13	212
$E_{17}C_{20}$	750-2060	2.09	202
$E_{23}C_{20}$	1000-2130	2.01	243
$E_{45}C_{8}$	2000-780	20.7	199
$E_{45}C_{10}$	2000-1060	3.16	102
$E_{45}C_{20}$	2000-1980	2.31	130
$E_{45}C_{32}$	2000-3200	2.26	125
$E_{45}C_{47}$	2000-4760	1.50	111
$E_{45}C_{60}$	2000-6000	1.16	133

[a] Measured by the fluorescence technique.
[b] Measured by dynamiclight scattering (the micelles prepared with 1% concentration of diblock copolymers).

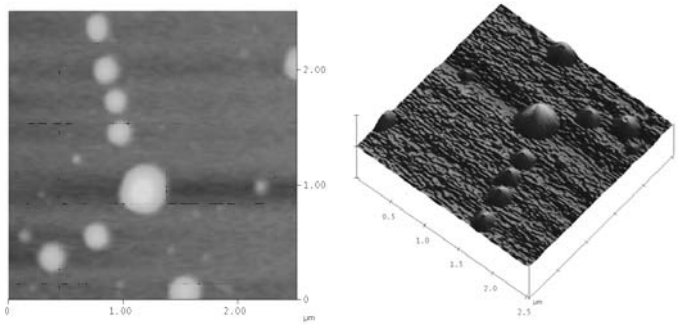


Figure 2. AFM image of micelle prepared with MPEG-PCL diblock copolymer ($E_{45}C_{8}$).

Next, a fluorescence measurement using pyrene as a probe was carried out to determine critical micelle concentration (CMC). Pyrene, a hydrophobic molecule, was preferentially distributed in the micelle core (Scheme 2), causing changes in the photophysical properties. 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 change compared to pyrene molecules in water. The characteristic shift feature of pyrene excitation spectra from 335 to 338 nm is observed as shown in Figure 3, indicating partitioning of pyrene into the hydrophobic micellar core. This shift was utilized to determine the CMC values. Figure 4 shows the fluorescence intensity ratio (I_{338}/I_{335}) of pyrene excitation spectra vs logarithm of various MPEG-PCL diblock copolymer concentrations. A substantial increase of the intensity ratio begins above a certain concentration, indicating the onset of micelle formation. Therefore, the interception of two straight lines in the low concentration range is determined as CMC (Figure 4). The CMCs of MPEG-PCL diblock copolymers with different PCL and MPEG blocks were examined. The obtained CMC values are listed in Table 2. The CMC values are in the range of 2.07 x 10^{-2} - 1.16 x 10^{-3} mg/mL. As the CMCs of MPEG-PCL diblock

copolymers with different MPEG blocks and constant PCL block were examined (Figure 4A), no large difference of CMC observed as the length of hydrophilic MPEG block changed. Meanwhile the CMC value decreased according to increasing the length of hydrophobic PCL block as MPEG-PCL diblock copolymers with different PCL length in the same MPEG length were examined (Figure 4B). This indicates that the hydrophobic PCL blocks mainly affect the CMC.

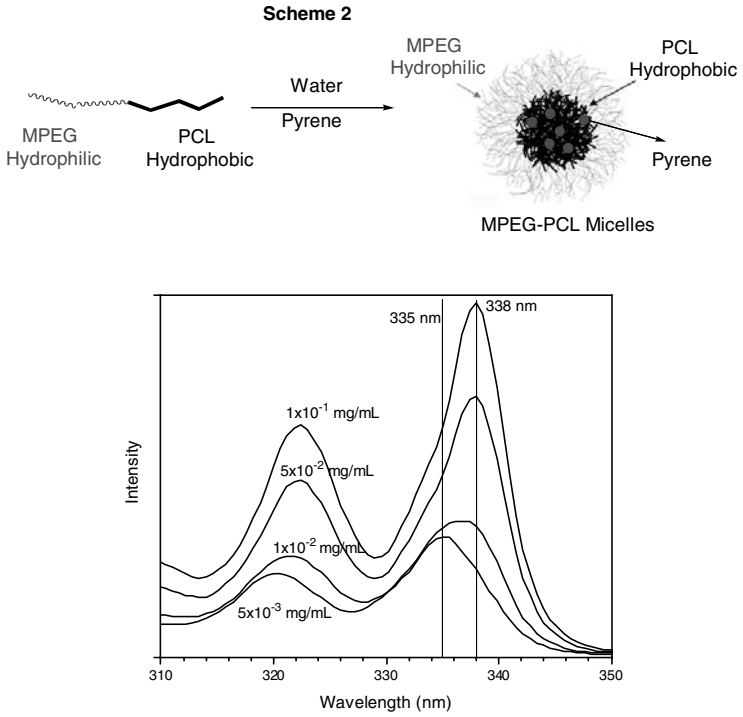


Figure 3. Excitation spectra of pyrene as a function of MPEG-PCL diblock copolymer concentration in water at room temperature.

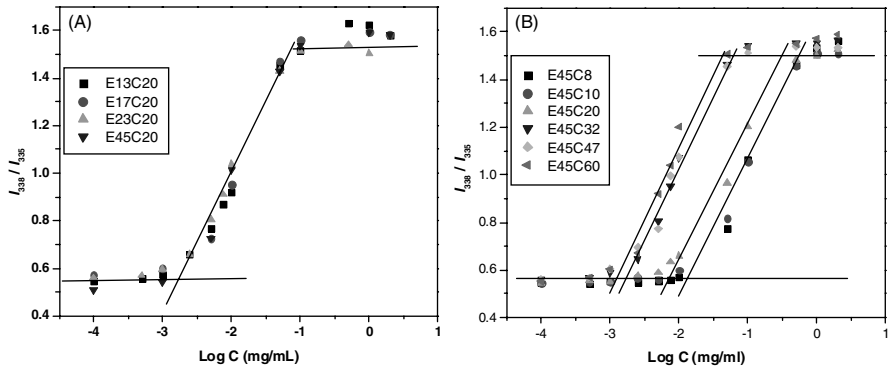


Figure 4. Plot of I_{338}/I_{335} (from pyrene excitation spectra) vs log C for concentration (C) of MPEG-PCL diblock copolymers.

In conclusion, MPEG–PCL diblock copolymers were successfully prepared by the polymerization of CL using terminal alcohol of MPEG as an initiator via an activated monomer mechanism, which offered new biomedical materials with well-defined structures. The micelle formation of MPEG-PCL diblock copolymers in an aqueous phase was confirmed by NMR, dynamic light scattering, AFM and fluorescence techniques. CMCs of the diblock copolymers were in range of 2.07×10^{-2} - 1.16×10^{-3} mg/mL depended on the block lengths of diblock copolymers. The diameters of micelles, measured by DLS, were 100-250 nm. Micelles of a spherical shape were observed by AFM, although nonspherical micelle such as rod-like micelle was observed rarely. We confirmed that the micelles formed with MPEG-PCL diblock copolymers have possibility as a potential hydrophobic drug delivery vehicle because a hydrophobic drug could be preferentially distributed in the micelle core.

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