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Synthesis of biodegradable poly(*trans*-4-hydroxy-*N*-benzyloxycarbonyl-L-proline)-*block*-poly(ε-caprolactone) copolymers and micellar characterizations

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Abstract

A series of novel types of diblock poly(*trans*-4-hydroxy-*N*-benzyloxycarbonyl-L-proline)-*block*-poly(ε-caprolactone) (PHpr10-*b*-PCL) copolymers were synthesized by ring-opening polymerization from macroinitiator poly(*trans*-4-hydroxy-*N*-benzyloxycarbonyl-L-proline) (PHpr10) and ε-caprolactone (ε-CL) in the presence of organocatalyst DL-lactic acid (DL-LA). The M_n of the copolymers increased from 3370 to 19,040 g mol^{-1} with the molar ratio (10–100) of ε-CL to PHpr10. These products were characterized by differential scanning calorimetry (DSC), ^{1}H NMR, and gel permeation chromatography. According to DSC, the glass-transition temperature (T_g) of the diblock copolymers depend on the molar ratio of monomer/initiator that were added. The hydrolytic degradation behavior of PHpr-*b*-PCLs was evaluated from weight-loss measurements and the change of M_n and M_w/M_n. With higher PCL contents resulted in a slower weight loss, while having a higher molecular weight loss percentage. Their micellar characteristics in an aqueous phase were investigated by fluorescence spectroscopy, transmission electron microscopy (TEM), and dynamic light scattering (DLS). The block copolymers formed micelles in the aqueous phase with critical micelle concentrations (CMCs) in the range of 1.33–4.22 mg L^{-1}. The micelles exhibited a spindly shape and showed a narrow monodisperse size distribution. The obtained micelles have a relatively high drug-loading of about 26% when the feed weight ratio of amitriptyline hydrochloride (AM) to polymer was 1/1. An increase of molecular weight and hydrophobic components in copolymers produced a higher CMC value and greater loading efficiencies were observed.

Keywords: Biodegradable; PHpr/PCL block copolymer; Critical micelle concentration

1. Introduction

During the past three decades, there has been a growing interest in preparing biodegradable and biocompatible polymers. Synthetic pseudo-poly(amino acid)s have the potential to be degraded in biological environments because of their polyester backbone. The main potential medical applications for synthetic pseudo-amino acid-based polymers are bioresorable sutures, screws or plates, and drug-delivery systems [1,2].

Recently, polymeric micelles derived from block copolymers in an aqueous phase have attracted much attention, not only because of their unique morphological behavior, but also because of their potential application in the area of drug delivery [3–9]. Block copolymer micelles have several characteristics such as nanosize and thermodynamic stability. In addition, their hydrophobic core is surrounded by a hydrophilic outer shell, so that the inner core can serve as a microcontainer for various substances. On the other hand, the solubility of micelles and the interactions of micelles with the external environment are determined by the chemical or physical nature of the hydrophilic outer shell. Therefore, it is important to systematically diversify the structure of amphiphilic block copolymers so that their micellar characteristics can be tailored for specific applications. However, the range of amphiphilic block copolymers which form micelles in an aqueous phase is limited [10,11]. Most micelle-forming amphiphilic block copolymers are based on hydrophilic poly(ethylene glycol) and the structural variations have been made mainly with hydrophobic blocks such as polyesters, polystyrene, poly(propylene oxide), and polyalkanes [12–17]. To our knowledge, no experimental work has so far reported on the studies of the micelles based on pseudo-poly(amino acid)

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block copolymers. In this study, we describe the synthesis and micellar characterization of the amphiphilic block copolymers based on hydrophilic pseudo-poly(4-hydroxy-L-proline) (PHpr). PHpr in aqueous phase has the capability to form hydrogen bonding with carboxyl or amino H-donor functionality. A biodegradable aliphatic polyester poly(ε-caprolactone) (PCL) was selected as a hydrophobic block. The micellar characteristics of these diblock copolymers in an aqueous phase were investigated by fluorescence spectroscopy, TEM, and DLS.

2. Experimental

2.1. Materials

N-CBz-Hpr (Fluka), pyrene (Aldrich), AM (Aldrich), and Stannous octoate ($SnOct_2$) (Strem) were used as received. ε-CL (Aldrich), and 1-hexanol (Fluka) were dried and vacuum distilled over calcium hydride. DL-LA (Aldrich) was dried over molecular sieves, 4 Å. Organic solvents such as tetrahydrofuran (THF), methanol, chloroform, and *n*-hexane were high pressure liquid chromatography (HPLC) grade and were used without further purification. Ultrapure water was used by purifying with a Milli-Q Plus (Waters).

2.2. Characterization

^{1}H NMR spectra were obtained on a Bruker WB/DMX-500 spectrometer at 500 MHz, with tetramethylsilane as an internal standard in chloroform-*d* ($CDCl_3$). A thermal analysis of the polymer was performed on a DuPont 9900 system that consisted of DSC. The heating rate was 20 °C min^{-1}. T_gs were read at the middle of the change in the heat capacity and were taken from the second heating scan after quick cooling. Number- and weight-average molecular weights (M_n and M_w, respectively) of the polymer were determining by a GPC system. It was carried out on a Jasco HPLC system equipped with a model PU-2031 refractive-index detector, and Jordi Gel DVB columns with pore sizes of 10^2, 500, and 10^3 Å. Chloroform was used as an eluent at a flow rate of 0.5 mL min^{-1}. Polystyrene standards with a low dispersity (Polymer Sciences) were used to generate a calibration curve. Data were recorded and manipulated with a Windows-based software package (Scientific Information Service Co.). UV–vis spectra were obtained using a Jasco V-550 spectrophotometer. The pyrene fluorescence spectra were recorded on a Hitachi F-4500 spectrofluorometer.

2.3. Preparation of hydroxyl end group poly(N-CBz-Hpr) macroinitiator (PHpr10)

Hydroxyl end group PHpr10 was prepared with 1-hexanol initiating the melt bulk polycondensation of *N*-CBz-Hpr. A total of 60 mg (0.59 mmol) of 1-hexanol and 1.56 g (5.89 mmol) of *N*-CBz-Hpr were homogenized in the melt. Then, 24.3 mg (1.5 wt%) of $SnOct_2$ was administered into a 50 mL two-neck round-bottomed flask equipped with a rubber septum and a magnetic stirring bar. The flask was purged with nitrogen and reacted at 140 °C for 48 h. The crude polymer was dissolved in $CHCl_3$, microfiltered, and then precipitated into excess CH_3OH with stirring. After purification, the macroinitiator PHpr10 was dried in vacuo for 24 h.

$M_n = 2770$; $M_w/M_n = 1.89$ (obtained from GPC analyses). ^{1}H NMR spectrum of PHpr10 is shown in Fig. 1(A).

2.4. Synthesis of PHpr-b-PCL diblock copolymers

An amount of PHpr10 with hydroxyl end group obtained above, various molar ratios of ε-CL, and a dry stirring bar were put into a two-neck round-bottomed flask. The polymerization was stared by the addition of DL-LA (0.1 equiv) as an organocatalyst and reacted at 140 °C for 12 h. The resulting product was dissolved in $CHCl_3$, and then precipitated into excess CH_3OH with stirring. The purified polymer was dried in vacuo for 24 h and analyzed. Representative ^{1}H and ^{13}C NMR spectra of the PHpr10-*b*-PCL are shown in Figs. 1(B), and 2, respectively.

2.5. Measurements of fluorescence spectroscopy

To prove the formation of the micelles, fluorescence measurements were carried out using pyrene as a probe. The fluorescence spectra of pyrene in aqueous solution were recorded at room temperature on a fluorescence spectrophotometer. The sample solutions were prepared by first adding known amounts of pyrene in acetone to a series of flasks. After the acetone had evaporated completely, measured amounts of micelle solutions with various concentrations of PHpr10-*b*-PCL were added to each of the flasks and mixed by vortexing. The concentration of pyrene in the final solutions was 6.1×10^{-7} M. The flasks were allowed to place overnight at room temperature to equilibrate the pyrene and the micelles. The emission wavelength was 390 nm for excitation spectra.

2.6. Measurements of size and size distribution

An average size and the size distribution of micelles were estimated by a dynamic light scattering (DLS) using a Particle-Size Analyzer (Coulter N4-Plus) at 20 °C. The intensity of a scattered light was detected at 90° to an incident beam. Measurements were made after the aqueous micellar solution was filtered with a microfilter having an average pore size of 0.2 (Advantec MFS, USA). An average size distribution of aqueous micellar solution was determined based on CONTIN programs of Provencher et al. [18].

2.7. Observation of transmission electron microscope

The morphology of the micelle was observed by TEM (JEM-1200-EXII). Drops of micelle solution were placed on a carbon film coated on a copper grid, and then were dried at room temperature. Observation was done at an accelerating voltage of 100 kV.

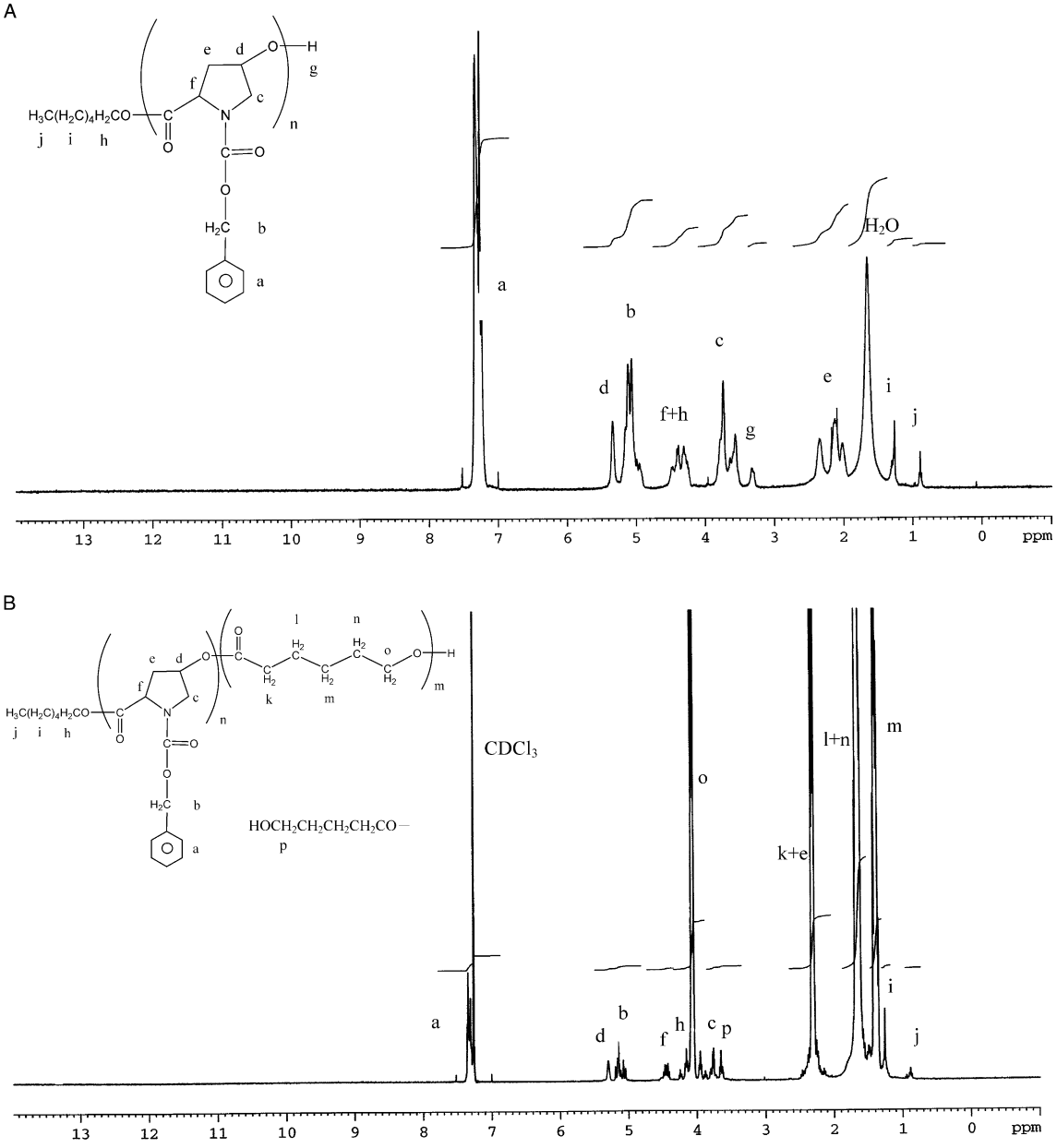


Fig. 1. Representative ^{1}H NMR spectra of (A) the PHpr10 macroinitiator (B) the PHpr10-*b*-PCL90 diblock copolymer in $CDCl_3$.

2.8. Determination of drug loading in micelles

Using oil-in-water solvent evaporation [19], PHpr10-*b*-PCL (20 mg) was dissolved in 6 mL methylene chloride followed by adding AM with various weight ratios to polymer (1/0.1–1/1) served as model drug. The solution was added dropwise to 150 mL distilled water containing 1 wt% poly(vinyl alcohol) under vigorous stirring. Droplet size was reduced by sonication. The emulsion was stirred at ambient temperature for overnight to evaporate solvent. The aggregated AM-loaded micelles were removed by centrifugation (3000 rpm × 30 min). Then, the micelles solution was lyophilized by concentration. The unloaded AM was eliminated by washed three times with distilled water and the micelles, which were obtained by vacuum-dried. A preweight amount of micelle was disrupted by an addition of acetonitrile (10 mL). Drug content was assayed spectrophotometrically at 240 nm using a Diode Array UV–vis Spectrophotometer. An AM content entrapped into the micelle was calculated from weight of the initial loaded drug and the amount of drug incorporated from the following equation. Drug loading efficiency (DLE) (percentage) = (weight of AM in micelle/weight of initial loaded AM) × 100.

2.9. In vitro degradation

In vitro degradation of about 50 mg of copolymer thin pellet was performed in 5 mL phosphate buffer solution (PBS, 0.067 M, pH 7.4) at 37 °C, and the buffer solution was changed every 2 days. At time intervals the specimen was removed, washed with distilled water, lyophilized, and weighed.

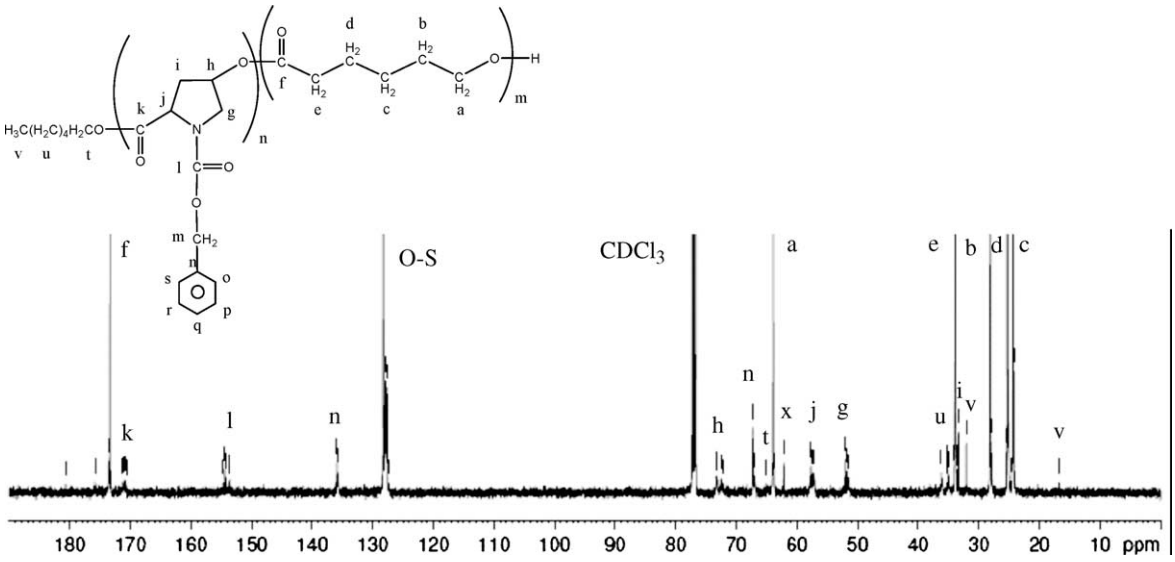


Fig. 2. ^{13}C NMR spectrum of PHpr10-*b*-PCL90 diblock copolymer in $CDCl_3$.

The degree of degradation (percentage) $= 100(D_0 - D)/D_0$, where D_0 is the weight of copolymer before degradation, and D is the weight of copolymer after degradation for a certain period.

3. Results and discussion

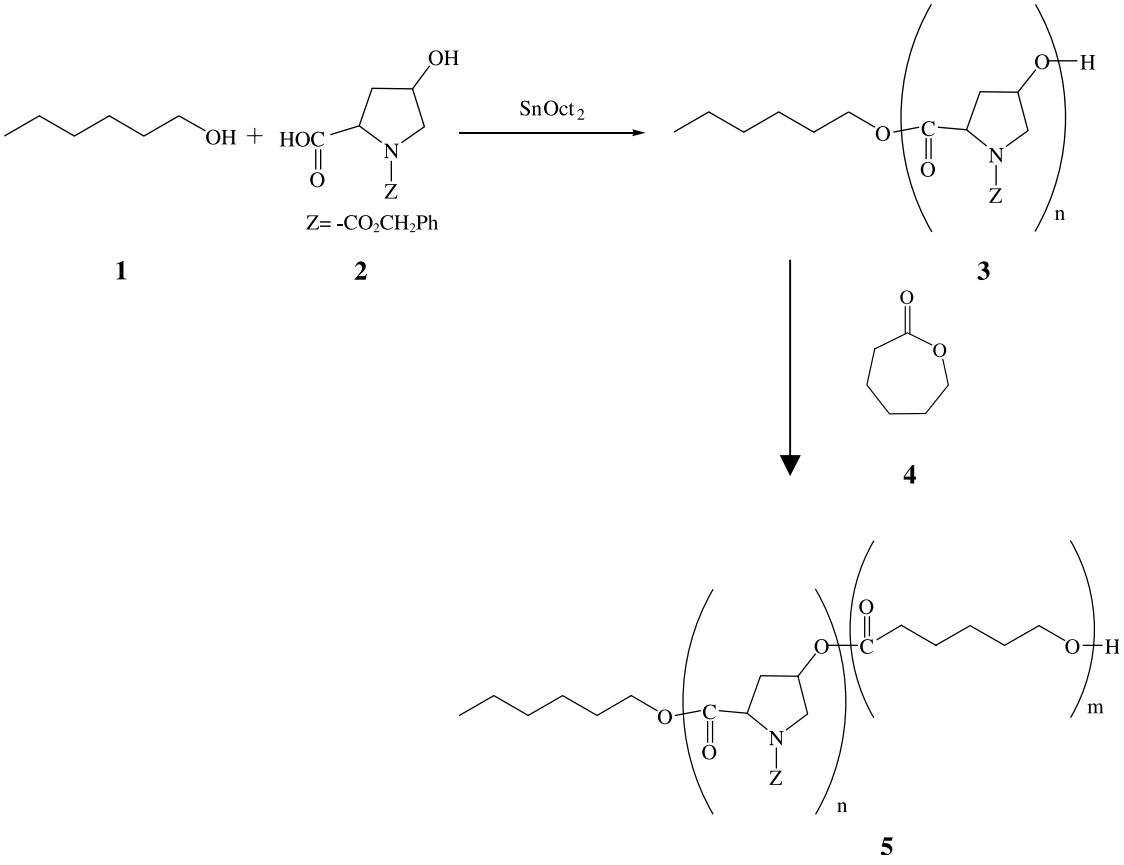
3.1. Synthesis and characterization of PHpr10-b-PCL copolymers

Various PHpr10-*b*-PCL diblock copolymers were obtained via the ring-opening polymerization of ε-CL with hydroxyl-terminated macroinitiator PHpr10. The synthesis of amphiphilic PHpr-*b*-PCL block copolymers is illustrated in Scheme 1. First, the hydroxyl-terminated PHpr3 was prepared by the homopolycondensation of *N*-CBz-Hpr and initiator 1-hexanol (with the molar ratio 10/1) in the presence of $SnOct_2$ (1.5 wt%) as catalyst in bulk at 140 °C for 48 h. The primary hydroxyl group of 1-hexanol is more reactive than the secondary hydroxyl group of Hpr in the polymerization. The prepared PHpr macroinitiator (PHpr10) was characterized by GPC ($M_n = 2770$; $M_w/M_n = 1.89$) and ^{1}H NMR spectroscopy (Fig. 1(A)).

The hydroxyl group of PHpr10 was used as the initiation sites for the ring-opening polymerization of ε-CL with an organocatalyst [20] to produce the block copolymers, PHpr10-*b*-PCL. To obtain the optimal reaction condition for the preparation of the copolymer, several parameters (i.e. types and amounts of acid catalysts and reaction time) were examined in the study. The data are summarized in Table 1. The effect of the type of acid catalyst on the ring-opening polymerization of ε-CL with PHpr10 was carried out at 140 °C for 24 h. The catalytic effect of lactic acid ($M_n = 18{,}060$, entry 3) appeared significantly than acetic acid ($M_n = 2920$, entry 2) or without catalyst ($M_n = 5290$, entry 1). Lactic acid catalyzed efficient the polymerization of ε-CL with PHpr10 in reasonable yield (79%). As the amount of the lactic acid was reduced from 1 to 0.1 equiv, the M_n of the copolymer was increased from 18,060 to 26,290 (entries 3, 4). However, when the reaction time was decreased from 24 to 1.5 h, a decreased M_n from 26,290 to 6180 was observed (entries 4–9). In the optimal reaction time 12 h, the compositions in the block copolymer is nearly equivalent to the feed ratio and the $M_{n,GPC}$ close to the $M_{n,calcd}$ (entry 5). Based on these finding, we performed all the subsequent polymerization reactions with DL-LA (0.1 equiv) as the catalyst at 140 °C for 12 h. The advantage of DL-LA as catalyst that is nontoxic with no necessity to remove after the completed reaction.

With the fixed PHpr10 macroinitiator, copolymers with different compositions were prepared by changing the comonomer ε-CL feed ratio for DL-LA catalyzed polymerization at 140 °C for 12 h. The results of the polymerization are compiled in Table 2. The yields were moderate. The M_ns of the block copolymers obtained from the copolymerization of ε-CL and PHpr increased with the increase of the molar ratios of ε-CL to PHpr in feed. The M_ns of the copolymer increase from 3370 to 19,040 g mol^{-1} with M_w/M_n between 1.54 and 2.74 observed; the molar ratios of ε-CL to PHpr in feed increase from 10 to 100. The molar ratio of the compositions in the block copolymers were analyzed by ^{1}H NMR. The amounts of comonomer incorporated into the copolymer could be calculated from a comparison of the integral area of the absorption peaks $\delta = 5.05$–5.21 ppm of the benzylic protons of PHpr10 with the absorption peaks $\delta = 1.64$ ppm of the C_3 and C_5 methylene protons of PCL. The conversion of copolymerization of the monomers was slight lower than the corresponding feeds. However, there is good agreement between the assumed molar masses ($M_{n,calcd}$) and determined by GPC ($M_{n,GPC}$).

Typical ^{1}H NMR spectrum of PHpr10 with a molar ratio of [*N*-CBz-Hpr]/[1-hexanol] = 10 and the block copolymer PHpr10-*b*-PCL90 with a molar ratio of [ε-CL]/[PHpr10] = 90 were shown in Fig. 1. The typical signals of the main-chain of the PHpr blocks are seed at $\delta = 1.99$–2.42 (H^e, C_3 methylene



Scheme 1. Synthesis of poly(*N*-CBz-Hpr)-*b*-poly(ε-CL) diblock copolymers.

protons), 3.50–3.84 (H^c, C_5 methylene protons), 4.28–4.55 (H^f, C_2 methine proton), 5.05–5.21 (H^b, benzylic protons of the protecting group), 5.35 (H^d, C_4 methine proton), and 7.30 (H^a, phenyl protons of the protecting group) ppm, respectively, as reported for linear poly(*N*-CBz-Hpr) [21]. The signal of H^b presents two or double peaks, not a single. This may be due to the effect of the chirality in proline. The same phenomenon has also been observed in reports of Park [22] and Langer et al. [23]. There are several additional peaks, more or less overlapped, resulting from the incorporation of 1-hexanol at $\delta = 0.89$ (protons of methyl), 1.25 (protons of methylene), and 4.28 ppm (–OCH_2–, methylene protons). For the block copolymer PHpr10-*b*-PCL90, besides the typical signals of the PHpr10 and 1-hexanol, the absorption peaks of PCL blocks were shown at $\delta = 1.39$ (H^m, C_4 methylene protons), 1.64 (H^{1+n}, C_3 and C_5 methylene protons), 2.30 (H^k, C_2 methylene protons), and 4.07 (H^o, C_6 methylene protons) ppm, respectively. There is additional signal of the end group of the block copolymer, that is, the signal assigned to the $HOCH_2$-methylene protons (H^p, $\delta =$ 3.65 ppm), which is the end group of the PCL block. The secondary hydroxyl proton signal at $\delta = 3.31$ ppm for the original PHpr macroinitiator disappeared. This demonstrated that the PHpr macroinitiator completely initiated ε-CL polymerization to form the block copolymer within the detection limits of NMR.

Table 1
The reaction conditions of the block copolymerization of ε-caprolactone with hydroxyl-terminated PHpr10

Entry	Organocatalyst (equiv)	Time (h)	Molar ratio of ε-CL over PHpr10 determined by ^{1}H NMR [ε-CL]/[PHpr10]	Yield (%)	$M_{n,GPC}$	$M_{n,NMR}$[a]	M_w/M_n[b]
1	–	24	40/1	17	5290	7020	2.40
2	CH_3CO_2H(1)	24	73/1	57	2920	10,830	2.15
3	DL-LA (1)	24	88/1	79	18,060	10,950	1.58
4	DL-LA (0.1)	24	96/1	75	26,290	13,860	1.55
5	DL-LA (0.1)	12	99/1	64	19,040	13,690	1.54
6	DL-LA (0.1)	9	81/1	67	10,500	12,290	1.84
7	DL-LA (0.1)	6	65/1	44	7280	10,460	1.61
8	DL-LA (0.1)	3	62/1	71	6260	10,040	2.50
9	DL-LA (0.1)	1.5	77/1	70	6180	11,750	2.04

The reaction was performed with the feed molar ratio (100/1) of [ε-CL]/[PHpr10] at 140 °C.
[a] $M_{n,NMR}$ is determined by ^{1}H NMR spectroscopy of diblock PHpr10-*b*-PCL.
[b] Number-average molecular weight (M_n) and weight-average molecular weight (M_w) are determined by GPC.

Table 2
Results of the block copolymerization of ε-caprolactone initiated with hydroxyl-terminated PHpr10 as a macroinitiator in bulk at 140 °C with DL-LA (0.1 equiv) as catalyst for 12 h

Entry	Molar ratio of ε-CL over PHpr10 in feed [ε-CL]/[PHpr10]	Molar ratio of ε-CL over PHpr10 determined by ^{1}H NMR [ε-CL]/[PHpr10]	Yield (%)	$M_{n,GPC}$	$M_{n,calcd}$ [a]	$M_{n,NMR}$ [b]	M_w/M_n [c]	T_g [d] (°C)	T_m [d] (°C)	T_c [d] (°C)
1	100/1	99/1	64	19,040	14,170	13,030	1.54	−56	55	
2	90/1	73/1	59	10,680	13,030	11,090	1.64	−50	56	−11
3	80/1	60/1	50	9860	11,890	9610	1.63	−50	54	−9
4	70/1	48/1	57	10,300	10,750	8240	1.81	−47	55	−3
5	60/1	41/1	59	10,250	9610	7440	1.98	−46	54	2
6	50/1	39/1	61	9130	8140	6890	1.87	−37	53	33
7	40/1	23/1	57	7440	7000	5060	2.13	−29	52	36
8	30/1	16/1	48	7060	5860	4270	2.22	−18		
9	20/1	12/1	46	5710	4720	3810	2.19	9		
10	10/1	4/1	44	3370	3580	2900	2.74	36		

M_n of PHpr10: 2770.
[a] $M_{n,calcd} = M_{n,PHpr10} + [\varepsilon\text{-CL}]/[PHpr10] \times 114$.
[b] $M_{n,NMR}$ is determined by ^{1}H NMR spectroscopy of PHpr10-*b*-PCL.
[c] Number-average molecular weight (M_n) and weight-average molecular weight (M_w) are determined by GPC.
[d] Determined from DSC thermograms at 20 °C min^{-1}.

The ^{13}C NMR spectrum of PHpr10-*b*-PCL90 diblock copolymer is shown in Fig. 2. The typical signals of the PCL blocks are seed at $\delta = 24.5$, 25.3, 28.1 and 33.7 (C^{b-e}, the methylene carbons), 63.9 (C^a, the methylene carbon), and 173.4 (C^f, the carbonyl group) ppm, respectively. The adsorption peaks of PHpr blocks are shown at $\delta = 33.4$ (C^i, the methylene carbon), 51.7 (C^g, the methylene carbon), 57.6 (C^j, the methine carbon), 67.1 (C^m, the methyl carbon of protecting group), 72.8 (C^h, the methine carbon), 127.9 (C^{o-s}, 3° aromatic carbons), 135.9 (C^n, 4° aromatic carbon), 154.3 (C^l, carbamate carbonyl), and 171.0 (C^k, ester carbonyl) ppm. Some additional weak peaks at $\delta = 16.8$, 32.1, 62.4, and 65.0 ppm are also found in Fig. 2. They are assigned to the end groups.

The thermal behaviors of the block copolymers are shown in Table 2. According to DSC, as the composition ratio of [ε-CL]/[PHpr10] drops lower than 16, the PHpr10-*b*-PCLs exhibited only T_g. Therefore, the copolymers were amorphous (entries 8–10). With an increase in the contents of ε-CL incorporated into the copolymers, a decrease in T_g of the copolymers was observed. The values of T_g decreased from 36 to −56 °C when the molar ratio of [ε-CL]/[PHpr10] increased from 4 to 99. This is due to the fact that ε-CL is a soft component, when a larger amount of flexible linkages was incorporated into the macromolecular backbone, there was a decrease in T_g. However, when the composition ratio of [ε-CL]/[PHpr10] beyond 23, the block copolymers also exhibited a T_m in the range 52–56 °C and a T_c decreased from 36 to −11 °C with increasing the length of PCL block. The T_m of the copolymers were slightly lower than the PCL ($T_m = 60$ °C) [24], indicating that the presence of PHpr blocks decreased the crystallinity of the copolymer with respect to the PCL homopolymer.

Fig. 3 shows the typical GPC curves of diblock copolymers as compared with those of the original PHpr10 macroinitiator. The GPC traces show a unimodal distribution of the block copolymer and do not show the presence of any possible homopolymerized PCL. In each block copolymer, the peak shifted toward a higher molecular weight region in comparison with the peak of the original PHpr10 macroinitiator, with little change in the molecular weight distribution. In all, these preliminary results shown that the block copolymerization of

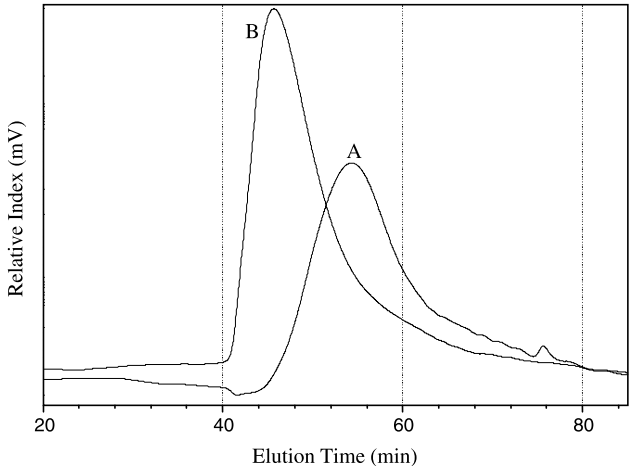


Fig. 3. GPC curves of (A) the PHpr10 macroinitiator and (B) the PHpr10-*b*-PCL90 diblock copolymer.

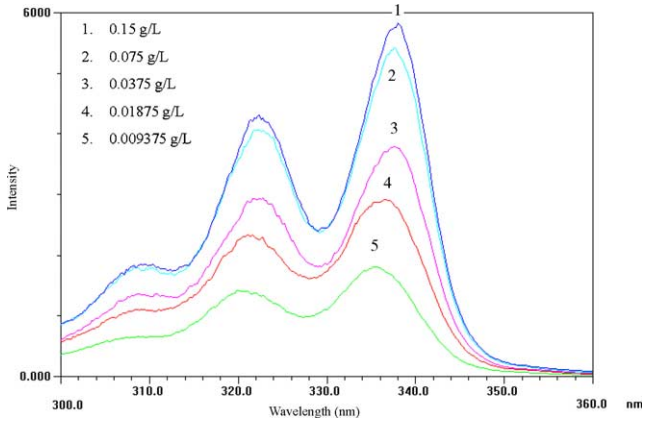


Fig. 4. Excitation spectra of pyrene as a function of PHpr10-*b*-PCL90 concentrations in deionized water, $\lambda_{em} = 390$ nm.

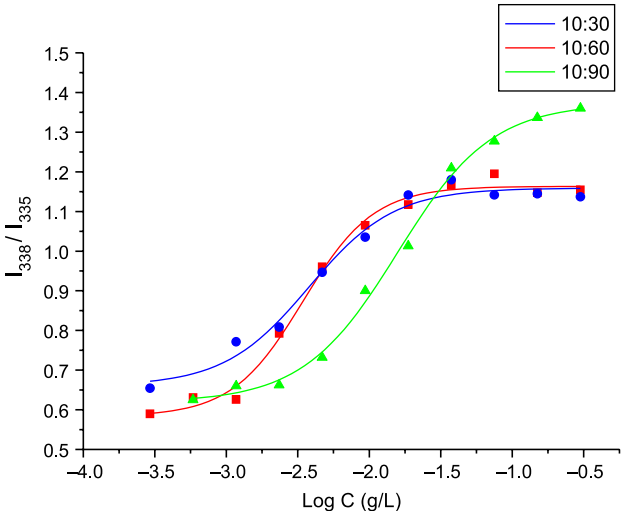


Fig. 5. Plot of the intensity ratio I_{338}/I_{335} (from pyrene excitation spectra, [Pyrene] $= 6.1 \times 10^{-7}$ M) vs. log C for block copolymers (▲, 10/90; ■, 10/60; ●, 10/30), $\lambda_{em} = 390$ nm.

the hydroxyl-group terminated PHpr10 macroinitiator and ε-CL using DL-LA catalyst was successful under the experimental conditions used.

3.2. Micelles of block copolymers

The amphiphilic nature of the block copolymers, consisting of hydrophilic PHpr and hydrophobic PCL blocks, provides an opportunity to form micelles in water. The characteristics of the block copolymer micelles in an aqueous phase were investigated by fluorescence techniques. The critical micelle concentrations (CMCs) of the block copolymers in an aqueous phase were determined by a fluorescence technique using pyrene as a probe [25].

Excitation spectra of pyrene in the PHpr10-*b*-PCL90 solution with various concentrations are shown in Fig. 4. As it can be seen, the fluorescence intensity increases with increasing the concentration of PHpr10-*b*-PCL90. The characteristic feature of pyrene excitation spectra, a red shift of the (0,0) band from 335 to 338 nm upon pyrene partition into micellar hydrophobic core, was utilized to determine the CMC values of PHpr-*b*-PCL block copolymers. Fig. 5 shows the intensity ratios (I_{338}/I_{335}) of pyrene excitation spectra versus the logarithm of PHpr10-*b*-PCL90, PHpr10-*b*-PCL60, and PHpr10-*b*-PCL30 block copolymers concentration. The CMC was determined from the intersection of straight line segments, drawn through the points at the lowest polymer concentrations, which lie on a nearly horizontal line, with that going through the points on the rapidly rising part of the plot. The CMC values of the block copolymers were 4.22 mg L^{-1} (PHpr10-*b*-PCL90), 1.78 mg L^{-1} (PHpr10-*b*-PCL60), and 1.33 mg L^{-1} (PHpr10-*b*-PCL30), respectively, depending on the block composition, and increase with increasing of PCL chain length. The CMC values were much lower than those of low molecular weight surfactants, e.g. 2.3 g L^{-1} for sodium dodecyl sulfate (SDS) in water, and were comparable with those of other polymeric amphiphiles.

From the dynamic light scattering measurements, the average size of the micelle formed by PHpr10-*b*-PCL90 was 136.5 ± 35.4 nm, and size distribution showed a narrow and monodisperse unimodal pattern as shown in Fig. 6. Also, the morphology of the micelle formed by PHpr10-*b*-PCL90 was shown in Fig. 7. The spindly shaped micelles of various length predominated, and the spherical micelles were observed only occasionally.

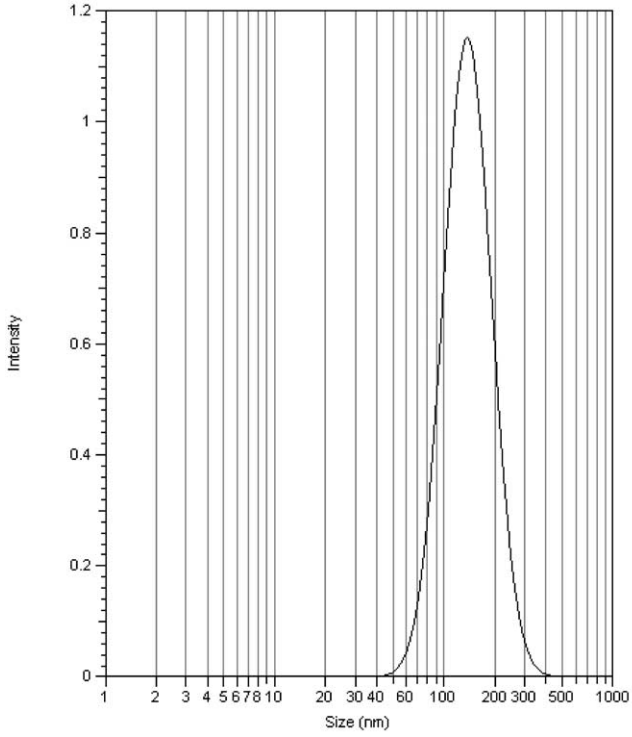


Fig. 6. Typical size distribution profile of PHpr10-*b*-PCL 90 copolymeric micelle by dynamic light scattering measurement.

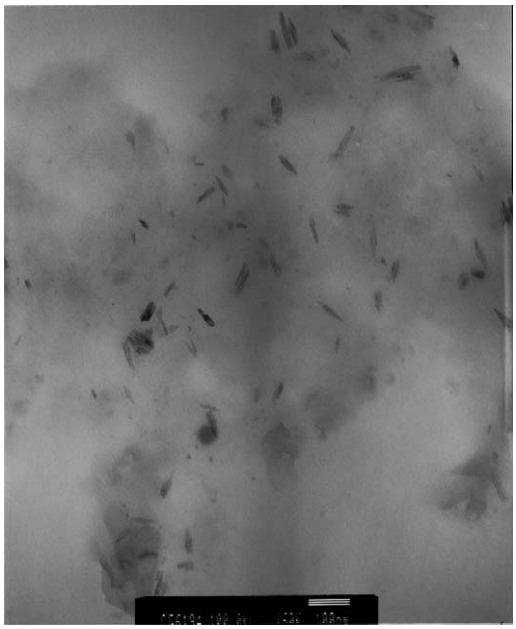


Fig. 7. TEM photograph of the micelles formed by PHpr10-*b*-PCL90 (scale bar = 100 nm).

3.3. Drug loading efficiency

An amount of AM incorporated into PHpr10-*b*-PCL micelles was calculated by the difference in weight ratio of AM in nanosphere to the preweighed AM-loaded micelles, which was calculated by UV absorbance after removing free AM and AM-bounded on the surface of PHpr10-*b*-PCL micelles by sonication with distillated water. The amount of AM introduced into the micelle by controlling the weight ratio between polymer and drug is shown in Table 3. The drug loading efficiency increased with the increasing weight ratio of drug to polymer. For example, in the case of PHpr10-*b*-PCL90, the feed weight ratio of AM to polymer increase from 0.1 to 1, the DLE increase from 4.0 to 25.8% (entries 1–4). We could obtain micelles having a relatively high DLE of about 26% when the feed weight ratio of AM to polymer was 1/1. Also, the drug loading efficiencies depending on composition of block polymer were described. As the molar composition ratio of [PCL]/[PHpr10] increase from 30 to 90, the DLE increase from 9 to 25.8% (entries 4–6). It coincides well with that reported the loading amount of drug increases with the molecular weight of block copolymer [5].

Table 3
Drug loading efficiency of AM-loaded PHpr10/PCL diblock copolymeric micelles

Entry	Molar composition of copolymers PHpr/PCL	Feed weight ratio polymer/AM	DLE (%)
1	10/90	1/0.1	4.0
2	10/90	1/0.25	5.6
3	10/90	1/0.5	23.0
4	10/90	1/1	25.8
5	10/60	1/1	21.0
6	10/30	1/1	9.0

DLE (%) = (weight of AM in micelle/weight of initial loaded drug) × 100.

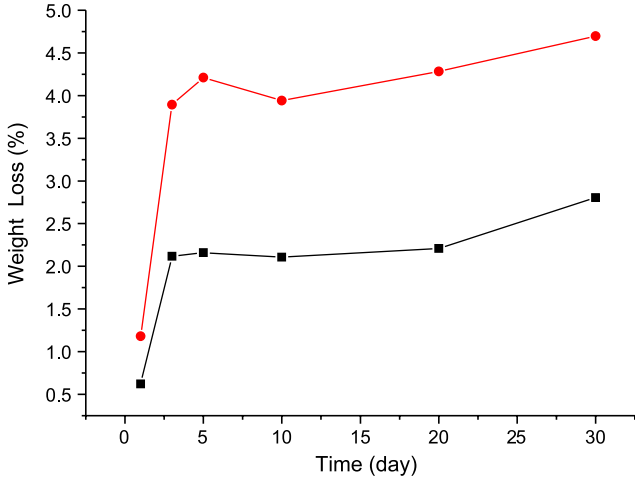


Fig. 8. Weight loss of diblock copolymers PHpr-*b*-PCLs with monomer compositions of 10/30 mol (●), and 10/60 mol (■) in which it was treated in 0.067 M phosphate buffer solution (pH 7.4) at 37 °C.

3.4. Preliminary in vitro degradation study

As a model of biodegradation, the in vitro degradation of block PHpr10-*b*-PCLs was evaluated from weight loss of the sample. The degradation profiles of PHpr10-*b*-PCLs with monomer compositions of 10/60 mol, and 10/30 mol at 37 °C under physiological conditions (pH 7.4) are portrayed in Fig. 8. The results indicated that the degradation were slow; the weight loss showed only 4.7 wt% for PHpr10-*b*-PCL30, and 2.8 wt% for PHpr10-*b*-PCL60, respectively, after immersion for 30 days. The weight loss of PHpr10-*b*-PCL was higher than the starred PHpr-*b*-PCL, but lower than the PHpr-*ran*-PCL [26]. The degradation of PHpr10-*b*-PCL30 was faster than PHpr10-*b*-PCL60. This was due to the molecular weight of the PHpr10-*b*-PCL60 being higher than the PHpr10-*b*-PCL30, and the molar fraction of hydrophobic PCL block in the PHpr10-*b*-PCL60 being larger than the PHpr10-*b*-PCL30. The weight loss profile presented an initial burst that may be due to the lower molecular weight segments in the pellet more rapidly degraded to water-soluble oligomers within the first

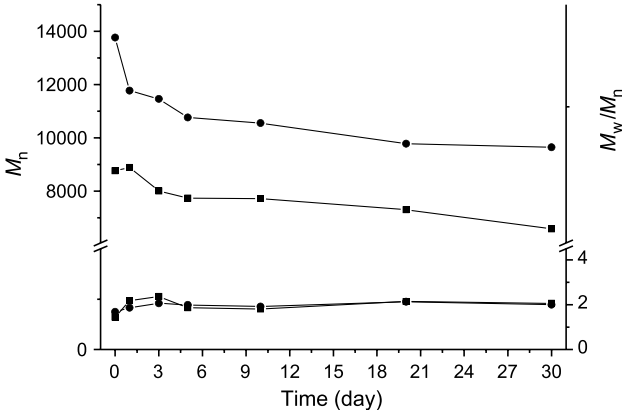


Fig. 9. Plot of M_n and M_w/M_n change of degradation of diblock PHpr-*b*-PCLs with monomer compositions of 10/30 mol (■), and 10/60 mol (●) in which it was treated in 0.067 M phosphate buffer solution (pH 7.4) at 37 °C.

5 day. Also, the changes in the molecular weight for these copolymers as a function of immersion time were investigated. The results are depicted in Fig. 9. After immersion for 30 days, the M_n decreased from 13,770 to 9640 g mol^{-1} for PHpr10-*b*-PCL60 and from 8760 to 6580 g mol^{-1} for PHpr10-*b*-PCL30, respectively. The polydispersity index ($I_p = M_w/M_n$) increased from initial 1.45–1.69 to 2.00–2.07 after immersion for 30 days. In contrast to the weight loss percentage, the molecular weight loss percentages of the PHpr10-*b*-PCL60 (30%) higher than the PHpr10-*b*-PCL30 (25%). These results indicate that polymer degradation is believed to occur predominantly via random chain scission by simple hydrolysis of the ester bond linkage. Dissolution of material starts only when the molecular weight has decreased to a certain level and, as a consequence, soluble degradation products are formed.

4. Conclusion

This work showed that the biodegradable diblock copolymers PHpr10-*b*-PCLs were successfully synthesized from macroinitiator PHpr10 and ε-CL in the presence of organocatalyst DL-LA. The molecular weight and unit compositions of the diblock copolymers were controlled by the molar ratios of ε-CL to the macroinitiator. The hydrolysis of PHpr10-*b*-PCLs varied as a function of the copolymer composition. Those copolymers with higher PCL contents showed a slower weight loss, while having a higher molecular weight loss percentage. CMC values were detected by fluorescence probe technique. An increase of molecular weight and hydrophobic components in an amphiphilic diblock copolymer produced a higher CMC value and greater loading efficiencies. Dynamic light scattering experiments showed that the average size of micelles was about 136.5 ± 35.4 nm. The morphology of the micelles exhibited a spindly shape. The drug release measurements are under way in our laboratory.

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