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Novel Self-Associating Poly(ethylene oxide)-*block*-poly(ϵ-caprolactone) Block Copolymers with Functional Side Groups on the Polyester Block for Drug Delivery

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ABSTRACT: The aim of this study was to develop micelle-forming poly(ethylene oxide)-*block*-poly(ϵ-caprolactone) (PEO-*b*-PCL)-based block copolymers bearing functional side groups on the PCL block. Substituted monomer, i.e, α-benzyl carboxylate-ϵ-caprolactone, was synthesized by anionic activation of ϵ-caprolactone and further treatment with benzyl chloroformate. Successful substitution of benzyl carboxylate on ϵ-caprolactone monomer was evidenced by ^{1}H NMR and mass spectroscopy. Ring-opening polymerization of α-benzyl carboxylate-ϵ-caprolactone with methoxy PEO (5000 g mol^{-1}) as initiator and stannous octoate as catalyst was used to prepare PEO-*b*-poly(α-benzyl carboxylate ϵ-caprolactone) (PEO-*b*-PBCL). Further catalytic debenzylation of PEO-*b*-PBCL produced PEO-*b*-poly(α-carboxyl-ϵ-caprolactone) (PEO-*b*-PCCL). Ring-opening polymerization of a ϵ-caprolactone/α-benzyl carboxylate-ϵ-caprolactone mixture and further reduction of the product were also used to prepare block copolymers with various degrees of benzyl carboxylate or carboxyl group substitution. The calculated molecular weights determined by ^{1}H NMR and gel permeation chromatography (GPC) for block copolymers were in good agreement with the theoretical values. The polydispersity of PEO-*b*-PBCL and PEO-*b*-PCCL block copolymers was 1.74 and 1.52, respectively. PEO-*b*-PBCL and PEO-*b*-PCCL block copolymers assembled to spherical micelles having average diameters of 62 and 20 nm based on dynamic light scattering (DLS) measurement, respectively. PEO-*b*-PBCL formed micelles at extremely low concentrations (cmc of 9.8×10^{-2} μM). The presence of carboxylic group on the PCCL block raised the cmc of PEO-*b*-PCCL to 1220×10^{-2} μM. For block copolymers with PCL-*co*-PCCL core structures, a decrease in cmc as well as an increase in size was observed as the level of PCL to PCCL was raised. Novel PEO-*b*-poly(ester) block copolymers with aromatic and reactive side groups on the polyester block have tremendous potential in the design of optimized carriers for the delivery of various therapeutic agents, as they can assemble to biodegradable nanoscopic micelles with chemically tailorable core structures.

Introduction

Amphiphilic block copolymers can self-assemble to nanoscopic, core/shell structures in which the hydrophobic core acts as a microreservoir for the encapsulation of drugs, proteins, or DNA, and the hydrophilic shell interfaces the media. Among different block copolymers designed for drug delivery, those with poly(ethylene oxide) (PEO), as the shell-forming block, and polyester or poly(amino acid)s (PLAA)s, as the core-forming block, are of increasing interest. This is due to the biocompatibility of PEO and potential biodegradability of polyester and PLAA which makes them safe for human administration. PLAA structures are advantageous over polyesters primarily due to a potential for the chemical attachment or electrostatic interaction of drugs, drug compatible moieties, genes, or intelligent vectors through free functional groups (e.g., amine or carboxylic acid) of the amino acid chain. In these structures, changes in the length of the hydrophobic/hydrophilic blocks, chemical structure of the side chain, and its substitution level may be used to achieve desired stability, biodegradation, drug loading, release, or activation properties. Through chemical engineering of the core structure in PEO-*b*-PLAA-based micelles, desired properties for the delivery of doxorubicin (DOX),[1,2] amphotericin B[3], methotrexate,[4] cisplatin,[5] and KRN-5500[6] are achieved.

Despite great potential for chemical modification, the long-term immunogenicity and biodegradability of different structures of the polyamide chain must still be established. In contrast, poly(ester)s have a history of safe application in human although less suitable for chemical engineering due to the lack of functional groups on the polymeric backbone. Introduction of functional groups to the polyester segment of PEO-*b*-polyester block copolymers such as PEO-*b*-poly(ϵ-caprolactone) (PEO-*b*-PCL) may result in the development of biodegradable self-assembling biomaterials with a potential for the attachment of different reactive compounds to the core-forming structure. PEO-*b*-PCL micelles have been used to encapsulate therapeutic agents with hydrophobic properties.[7−14] Polycaprolactone is a hydrophobic, semicrystalline polymer with a low glass transition temperature. Owing to these properties, PEO-*b*-PCL micelles have shown sufficient in-vivo stability and great promise in tumor targeted drug delivery by the enhanced permeability and retention (EPR) effect in several studies.[7,15] Attachment of reactive groups on the core-forming segment of the PEO-*b*-PCL block copolymers provides additional opportunities for modifications in the thermodynamic and kinetic stability, biodegradation, drug solubilization, and release properties of PEO-*b*-PCL micelles. Such structures have a capacity for the formation of hydrogen bonds and electrostatic interactions within the micellar core with drugs that bear hydrogen binding groups or charged moieties in their structure and potential for use as a pH-triggered drug release delivery system.

In this paper we report on the successful synthesis and self-assembly of benzyl carboxylate and carboxyl bearing PEO-*b*-PCL block copolymers, i.e., PEO-*b*-poly(α-benzyl carboxylate ϵ-caprolactone) (PEO-*b*-PBCL) and PEO-*b*-poly(α-carboxyl ϵ-caprolactone) (PEO-*b*-PCCL), respectively. The presence of

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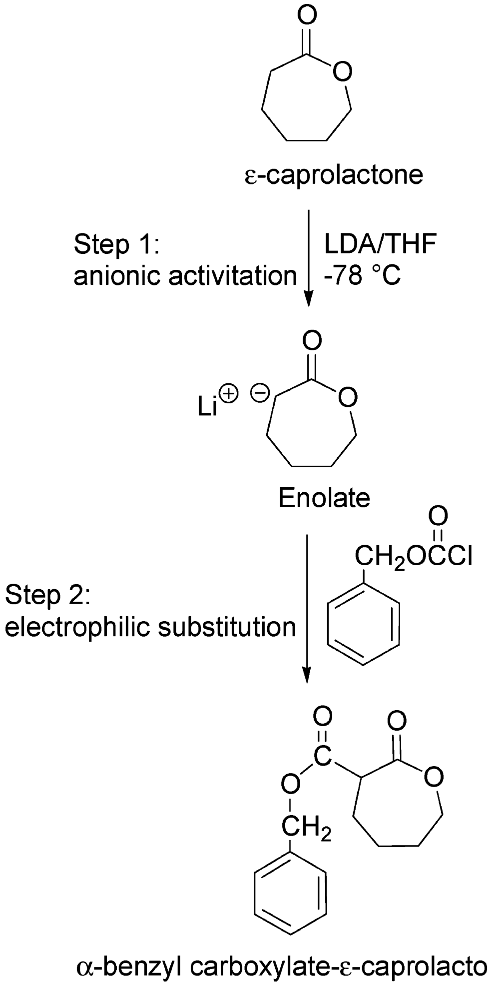
Scheme 1. Synthetic Scheme for the Preparation of α-Benzyl Carboxylate-ϵ-Caprolactone (α-Carbon-Substituted Monomer)



aromatic or carboxylic groups on the core-forming block may provide additional opportunities to modify polymer hydrophobicity, degradation, and crystallinity as well as thermodynamic and kinetic stability of PEO-*b*-PCL micelle. The core-functionalized micelles may also change the encapsulation and release properties for certain drugs through formation of $\pi-\pi$ interactions, hydrogen bonds, or electrostatic complexation between the core-forming block and drug and/or lead to the development of pH-sensitive micelles with triggered drug release at basic pHs.[16−18] Our results point to the self-association of prepared block copolymers to core/shell micellar structures with a great potential for chemical tailoring of the core to achieve optimal micellar size and thermodynamic and kinetic stability.

Materials and Methods

A. Materials. Methoxy-PEO (average molecular weight of 5000 g mol^{-1}), diisopropylamine (99%), benzyl chloroformate (tech 95%), sodium (in kerosin), butyllithium (Bu-Li) in hexane (2.5 M solution), palladium-coated charcoal, and pyrene were purchased from Sigma, St. Louis, MO. ϵ-Caprolactone was purchased from Lancaster Synthesis, UK. Stannous octoate was purchased from MP Biomedicals Inc., Germany. Fluorescent probe 1,3-(1,1′-dipyrenyl)propane was purchased from Molecular Probes. All other chemicals were reagent grade.

B. Methods. Synthesis of α-Benzyl Carboxylate-ϵ-Caprolactone. The method used for the synthesis of α-benzyl carboxylate-ϵ-caprolactone is shown in Scheme 1. Briefly, Bu-Li (24 mL) in hexane was slowly added to dry diisopropylamine (8.4 mL) in 60 mL of dry THF in a three-neck round-bottomed flask at −30 °C under vigorous stirring with continuous argon supply. The solution was cooled to −78 °C. ϵ-Caprolactone (3.42 g) was dissolved in 8 mL of dry THF and added to the above-mentioned mixture slowly, followed by the addition of benzyl chloroformate (5.1 g). The temperature was allowed to rise to 0 °C, and the reaction was quenched with 5 mL of saturated ammonium chloride solution.[19] The reaction mixture was diluted with water and extracted with ethyl acetate. The combined extracts were dried over Na_2SO_4 and evaporated. The yellowish oily crude mixture was purified twice over a silica gel column, and the purity of the compound was confirmed with thin-layer chromatography (TLC). The chemical structure was analyzed by ^{1}H NMR, ^{13}C NMR, IR (Figure 1), and mass spectroscopy. The reaction yield was calculated using the following equation:

$$\text{yield (\%)} = \frac{\text{amount of }\alpha\text{-benzyl carboxylate-}\epsilon\text{-caprolactone produced in the reaction}}{\text{predicted amount of }\alpha\text{-benzyl carboxylate-}\epsilon\text{-caprolactone to be produced in the reaction}} \times 100$$

Synthesis of α-Benzyl Carboxylate Bearing Block Copolymers. Block copolymers of PEO-*b*-PBCL were synthesized by ring-opening polymerization of α-benzyl carboxylate-ϵ-caprolactone using methoxy-PEO as initiator and stannous octoate as catalyst.[20,21] The synthetic method for the preparation of the block copolymer is shown in Scheme 2. Methoxy-PEO (MW: 5000 g mol^{-1}) (3.5 g), α-benzyl carboxylate-ϵ-caprolactone (3.5 g), and stannous octoate (0.002 equiv of monomer) were added to a 10 mL previously flamed ampule, nitrogen purged and sealed under vacuum. The polymerization reaction was allowed to proceed for 4 h at 140 °C in oven. The reaction was terminated by cooling the product to room temperature. The ^{1}H NMR spectrum of α-benzyl carboxylate-ϵ-caprolactone bearing block copolymer in $CDCl_3$ at 300 MHz (Figure 2A) was used to assess the conversion of α-benzyl carboxylate-ϵ-caprolactone monomer to PBCL comparing the intensity of $-O-CH_2-$ ($\delta = 4.25$ ppm) related peak for α-benzyl carboxylate-ϵ-caprolactone monomer to the intensity of the same protons for PBCL ($\delta = 4.05$ ppm). PEO-*b*-poly(ϵ-caprolactone)-*co*-poly(α-benzyl carboxylate-ϵ-caprolactone) (PEO-*b*-PCL-*co*-PBCL) block copolymer was also synthesized by ring-opening polymerization of a mixture of ϵ-caprolactone and α-benzyl carboxylate-ϵ-caprolactone at 50:50 and 75:25 molar ratios.

Reduction of α-Benzyl Carboxylate Bearing Block Copolymer to α-Carboxyl Bearing Block Copolymer. Carboxyl group bearing block copolymers, i.e, PEO-*b*-PCCL and PEO-*b*-PCL-*co*-PCCL, were obtained by the catalytic debenzylation of PEO-*b*-PBCL and PEO-*b*-PCL-*co*-PBCL in the presence of hydrogen gas, respectively (Scheme 2).[17,22] Briefly, a solution of PEO-*b*-PBCL or PEO-*b*-PCL-*co*-PBCL (1 g in 25 mL of THF) was placed into a 100 mL round-bottom flask. Charcoal coated with palladium (300 mg) was dispersed in this solution. The flask was sealed with a septum, and vacuum was applied through a needle for 10 min. The reaction flask was filled with hydrogen gas and maintained a continuous supply of hydrogen gas. The mixture was stirred vigorously with a magnetic stirrer and reacted with hydrogen for 24 h at room temperature. The reaction mixture was centrifuged at 3000 rpm to remove the catalyst. The supernatant was collected, condensed under reduced pressure, precipitated in diethyl ether, and washed repeatedly to remove impurities. The final product was collected and dried under vacuum at room temperature for 48 h. The ^{1}H NMR spectrum of reduced block copolymer in dimethyl-d_6 sulfoxide at 300 MHz was used to assess the conversion of benzyl carboxylate to carboxyl group following the disappearance of the characteristic aromatic peak at $\delta = 7.4$ ppm (Figure 2B).

Characterization of Synthesized Block Copolymers. The number-average molecular weight of PEO-*b*-PBCL and PEO-*b*-PCCL block copolymers was determined from the ^{1}H NMR spectrum comparing peak intensity of PEO ($-CH_2CH_2O-$, $\delta = 3.65$ ppm) to that of PBCL or PCCL ($-OCH_2-$, $\delta = 4.05$ ppm), respectively, considering a 5000 g mol^{-1} molecular weight for PEO (Figure 2A,B). The number-average molecular weight of PEO-*b*-PCL-*co*-PCCL block copolymers was determined by comparing the

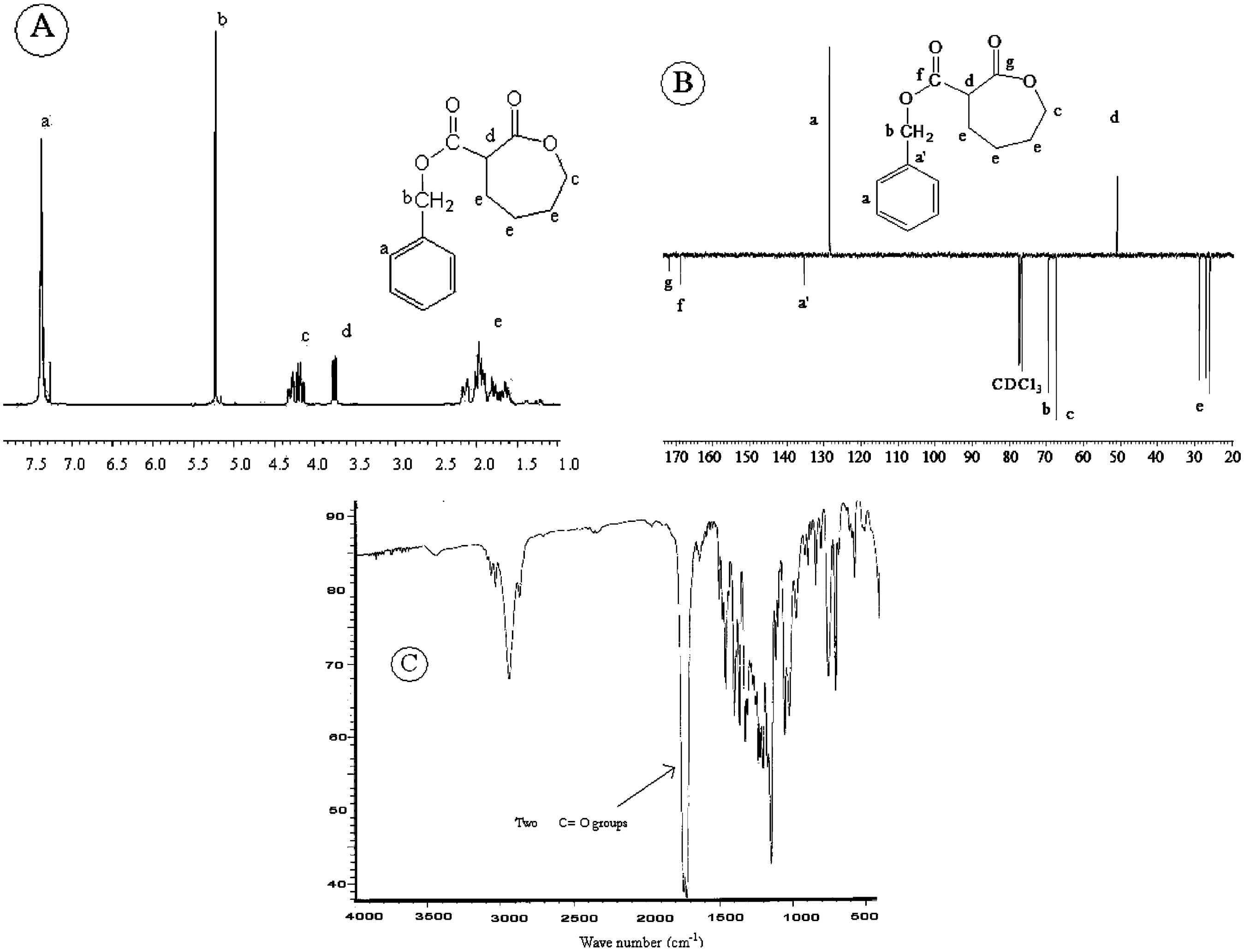


Figure 1. (A) ^{1}H NMR spectrum of α-benzyl carboxylate-ϵ-caprolactone (substituted monomer) in $CDCl_3$ and peak assignments. (B) ^{13}C NMR spectrum of α-benzyl carboxylate-ϵ-caprolactone and peak assignments. (C) IR spectrum of α-benzyl carboxylate-ϵ-caprolactone. Arrow indicates the presence of characteristic groups.

PEO peak intensity ($-CH_2CH_2O-$, $\delta = 3.65$ ppm) to that of PCL ($O{=}C-CH_2$, $\delta = 2.31$ ppm) and PCCL (CH_2 protons of $CH-CH_2$, $\delta = 1.90$ ppm) in its ^{1}H NMR spectrum (Figure 2C). The molar proportion of PCL and PCCL in PEO-*b*-PCL-*co*-PCCL was determined by comparing the peak intensity ratio of PCL ($O{=}C-CH_2$, $\delta = 2.31$ ppm) and PCCL (CH_2 protons of $CH-CH_2$, $\delta = 1.90$ ppm). The IR spectrum was obtained by dissolving the block copolymers in chloroform and preparing a thin film on NaCl disk.

The weight- and number-average molecular weight as well as polydispersity of prepared polymers were assessed by gel permeation chromatography (GPC). Briefly, 20 μL of polymer solution (20 mg/mL in THF) was manually injected into a 7.8 × 300 mm Styragel HMW 6E column (Waters Inc. Milford, MA) which was attached to an HP 1100 pump. The column was eluted with 1 mL/min THF. The elution pattern was detected by refractive index (model 410; Waters Inc.) and dynamic light scattering detectors (PD 2000 DLS; Precision Detectors, Franklin, MA) using polystyrene standard of two molecular weights ($M_w = 9580$ and 13700 g mol^{-1}).

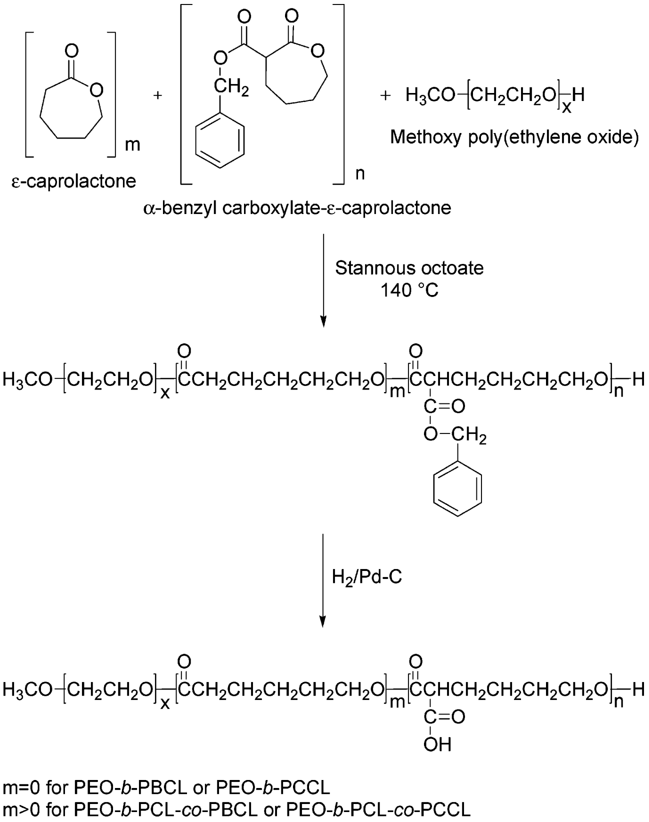
Assembly of Block Copolymers and Characterization of the Assembled Structures. Micellization was achieved by dissolving prepared block copolymers (30 mg) in acetone (0.5 mL) and dropwise addition (~1 drop/15 s) of polymer solutions to doubly distilled water (3 mL) under moderate stirring at 25 °C, followed by the evaporation of acetone under vacuum.[8]

Average diameter (intensity mean) and size distribution of prepared micelles were estimated by dynamic light scattering (DLS) using a Malvern Zetasizer 3000 at a polymer concentration of 10 mg/mL in water at 25 °C after filtration through 0.45 μm filter.

Morphology of self-assembled structures was investigated by transmission electron microscopy (TEM). An aqueous droplet of micellar solution (20 μL) with a polymer concentration of 1−1.5 mg/mL was placed on a copper-coated grid. The grid was held horizontally for 20 s to allow the colloidal aggregates to settle. A drop of 2% solution of phosphotungstic acid (PTA) in PBS (pH = 7.0) was then added to provide the negative stain. After 1 min, the excess fluid was removed by filter paper.[23] The samples were then air-dried and loaded into a Hitachi H 700 transmission electron microscope. Images were obtained at a magnification of 18 000× at 75 kV.

A change in the fluorescence excitation spectra of pyrene in the presence of varied concentrations of block copolymers was used to measure the critical micellar concentration (cmc) of prepared block copolymers. Pyrene was dissolved in acetone and added to 5 mL volumetric flasks to provide a concentration of 6×10^{-7} M in the final solutions. Acetone was then evaporated and replaced with aqueous polymeric micellar solutions with concentrations ranging from 0.05 to 1000 μg/mL. Samples were heated at 65 °C for an hour, cooled to room temperature overnight, and deoxygenated with nitrogen gas prior to fluorescence measurements. The excitation spectrum of pyrene for each sample was obtained at room temperature using a Varian Cary Eclipse fluorescence spectrophotometer (Victoria, Australia). Emission wavelength and excitation/emission slit were set at 390 and 5 nm, respectively. The intensity ratio of peaks at 339 nm to those at 334 nm was plotted against the logarithm of copolymer concentration. The cmc was measured from a sharp raise in intensity ratios (I_{339}/I_{334}) at the onset of micellization.

Scheme 2. General Synthesis Scheme for the Preparation of PEO-*b*-PBCL, PEO-*b*-PCCL, PEO-*b*-PCL-*co*-PBCL, and PEO-*b*-PCL-*co*-PCCL Block Copolymers



m=0 for PEO-*b*-PBCL or PEO-*b*-PCCL
m>0 for PEO-*b*-PCL-*co*-PBCL or PEO-*b*-PCL-*co*-PCCL

The viscosity of the micellar cores was estimated by measuring excimer to monomer intensity ratio (I_e/I_m) from the emission spectra of 1,3-(1,1′-dipyrenyl)propane at 373 and 480 nm, respectively. 1,3-(1,1′-Dipyrenyl)propane was dissolved in a known volume of chloroform to give a final concentration of 2×10^{-7} M. Chloroform was then evaporated and replaced with 5 mL of polymeric micellar solutions at a concentration of 1 mg/mL. Samples were heated at 65 °C for an hour and cooled to room temperature overnight. A stream of nitrogen gas was used to deoxygenate samples prior to fluorescence measurements. The emission spectrum of 1,3-(1,1′-dipyrenyl)propane was obtained at room temperature using an excitation wavelength of 333 nm. The excitation/emission slit was set at 5 nm.

Results and Discussion

Preparation of PEO-*b*-PCL Block Copolymers with Aromatic Side Groups on the PCL Block. Attachment of aromatic side groups to PEO-*b*-PCL block copolymer was carried out through conjugation of benzyl chloroformate with ϵ-caprolactone monomer producing α-benzyl carboxylate-ϵ-caprolactone monomer (Scheme 1)[19,24] and further ring-opening polymerization of this new monomer by methoxy-PEO (Scheme 2). In preliminary studies, direct conjugation of benzyl carboxylate group to PEO-*b*-PCL block copolymers through generation of lithium carbanion on the PCL block following treatment with lithium diisopropylamine (LDA) was tried, as suggested by Gimenez et al. and Ponsart et al. for homopolymers of PCL.[24,25] This approach led to a loss in the intensity of peak at $\delta = 3.65$ ppm in the ^{1}H NMR spectrum of product reflecting the loss of PEO chain during the formation of polycarbanion (data not shown). The decrease in molar mass of substituted polymer was also evident when Ponsart et al. carried a similar reaction with PCL homopolymer. Cleavage of the ester bonds in the block copolymer backbone during the formation of the polycarbanion is a side reaction occurring in parallel to the proton extraction by LDA. Transesterification by inter- or intramolecular autocondensation in the homopolymer or block copolymer backbone is a possible explanation for this effect.

Anionic activation of ϵ-caprolactone monomer was performed using freshly prepared nonnucleophilic strong base LDA to extract a methylene proton from α-position ($-CH_2-C{=}O$). The generated lithium carbanion was then quenched with benzyl chloroformate to obtain α-benzyl carboxylate-ϵ-caprolactone (Scheme 1). After column chromatography α-benzyl carboxylate-ϵ-caprolactone was isolated as a clear thick oily liquid. The product produced a single spot at R_f value of 0.3 in TLC. The yield of reaction was 53.8%. The structure was confirmed by combined analysis of ^{1}H NMR, ^{13}C NMR, IR, and mass spectroscopy (Figure 1). In 300 MHz ^{1}H NMR spectroscopy in $CDCl_3$ corresponding proton peaks were observed at δ: 1.60−2.20 (m, 6H); δ: 3.75 (dd, 1H); δ: 4.13−4.35 (m, 2H); δ: 5.23 (s, 2H); δ: 7.40 (m, 5H) (Figure 1A). The peak at 3.75 ppm (Figure 1A) for α-benzyl carboxylate-ϵ-caprolactone, which corresponds to a single proton instead of two protons of ϵ-caprolactone monomer, indicates the successful substitution of the benzyl carboxylate on ϵ-caprolactone monomer at the α-position. The presence of two negative peaks for carbonyl at 168.70 and 171.67 ppm and the generation of a new characteristic positive peak at 50.86 ppm in the ^{13}C NMR spectrum also confirm the chemical structure of the reaction product (Figure 1B). The presence of two sharp carbonyl peaks in the IR spectrum at 1725 and 1760 cm^{-1} corresponds to the carbonyl groups in lactone and benzyl carboxylate, respectively (Figure 1C). Finally, mass spectroscopy resulted in the formation of peaks at *m*/*z* 248.99, *m*/*z* 230.95, *m*/*z* 164.82 and *m*/*z* 132.84 (data not shown). The presence of molecular ion (M^+) peak at *m*/*z* 248.99, $M^+ + Na$ peak at *m*/*z*, 271.00 and $M^+ + K$ peak at *m*/*z* 287.00 in the mass spectrum provided additional evidence for the chemical structure of the synthesized monomer.

PEO-*b*-PBCL block copolymer was synthesized by ring-opening polymerization of α-benzyl carboxylate-ϵ-caprolactone using methoxy-PEO as initiator and stannous octoate as catalyst according to the procedure reported previously for the polymerization of ϵ-caprolactone.[8] Synthesis of homopolymers of ϵ-caprolactone bearing carboxyl, benzyl carboxylate, or benzyloxy group on the methylene group in the γ-position of ϵ-caprolactone by ring-opening polymerization of the functionalized ϵ-caprolactone monomer using stannous octoate or Al(O^{i}Pr)$_3$ has also been reported, recently.[26,27] The percent of conversion of PEO-*b*-PBCL block copolymer from α-benzyl carboxylate-ϵ-caprolactone was found to be 91%. In 300 MHz ^{1}H NMR spectroscopy in $CDCl_3$ corresponding proton peaks for the product were observed at δ: 1.25−1.90 (m, 6H); δ: 3.30−3.45 (s, 3H; t, 1H); δ: 3.65 (s, 4H); δ: 4.05 (t, 2H); δ: 5.15 (s, 2H); δ: 7.35 (s, 3H) (Figure 2A). The presence of peaks at $\delta = 7.35$ and 5.15 ppm, which are due to the aromatic and methylene protons of the benzyl carboxylate group, respectively, confirms the polymerization of α-benzyl carboxylate-ϵ-caprolactone and the presence of aromatic groups in the structure of block copolymer. Furthermore, the characteristic downfield shift of the methylene protons ($-OCH_2-$ of α-benzyl carboxylate-ϵ-caprolactone) from $\delta = 4.25$ to 4.05 and $O{=}C-CH-$ proton from $\delta = 3.75$ to 3.28 in the ^{1}H NMR spectra (Figures 1A and 2A) strongly indicates the ring-opening polymerization of the monomer and formation of block copolymers. The IR spectrum of PEO-*b*-PBCL block copolymer is shown in Figure 3A. The characteristic peaks observed at 1725 and 1735 cm^{-1} ($C{=}O$ stretching) compared to one peak at 1725 cm^{-1} for PEO-*b*-

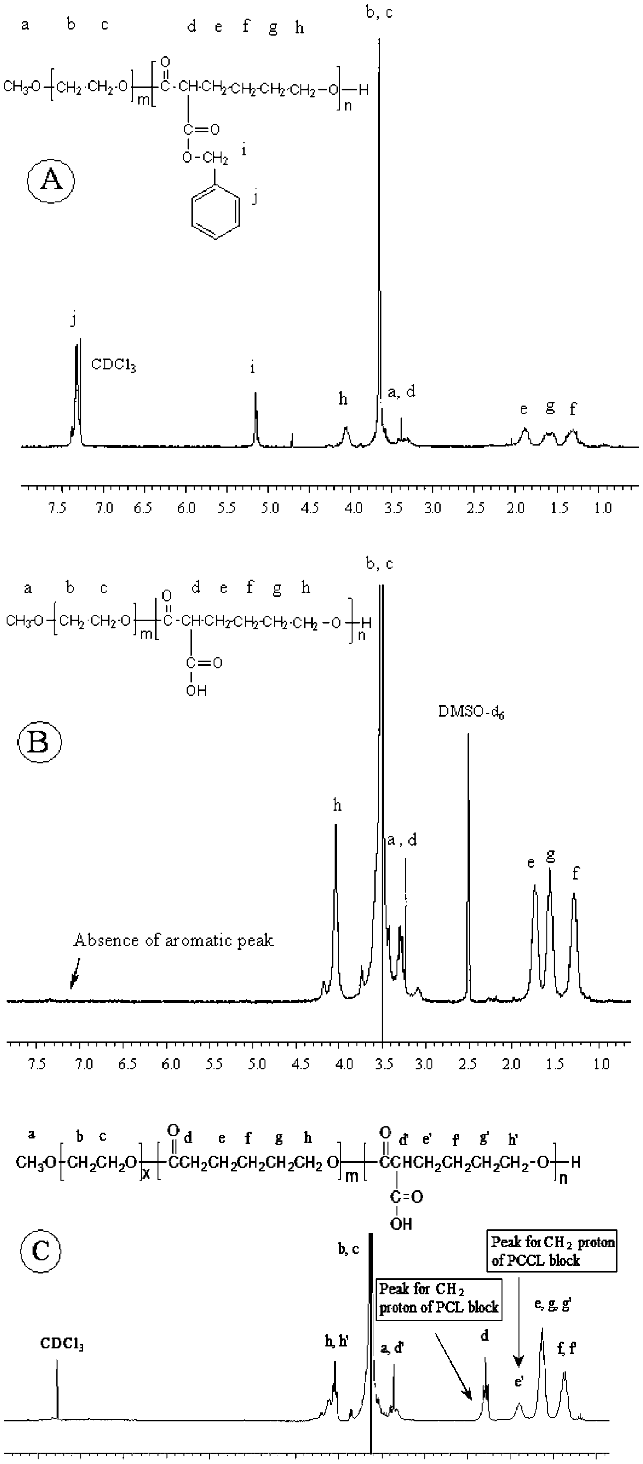


Figure 2. ^{1}H NMR spectrum and peak assignments for (A) PEO-*b*-PBCL in $CDCl_3$, (B) PEO-*b*-PCCL in DMSO-d_6, and (C) PEO-*b*-PCL_{16}-*co*-$PCCL_{10}$ in $CDCl_3$.

PCL (data not shown) indicate the presence of two different positions for carbonyl groups present in the structure of PEO-*b*-PBCL. Besides, characteristic peaks related to the C−H stretching aromatic appeared in the fingerprint region of the IR spectrum for PEO-*b*-PBCL at 690−750 cm^{-1} (Figure 3A).

The characteristics of prepared PEO-*b*-PBCL block copolymer are summarized in Table 1. The molecular weight of prepared PEO-*b*-PBCL block copolymer, measured by comparing the peak intensities of four methylene protons of PEO (δ = 3.65) and two methylene protons of PBCL (δ = 4.05) in the ^{1}H NMR spectrum, was calculated to be 9700 g mol^{-1} (equal to a degree of α-benzyl carboxylate-ε-caprolactone polymerization of 19). A good agreement between the measured and theoretical molecular weights was observed (Table 1). Besides, the number-average molecular weight measured by ^{1}H NMR was close to the one measured by GPC (Table 1). The unimodal distribution of the block copolymer from GPC analysis (Figure 4) is particularly significant since it lends further support to the view that the final product is indeed a block copolymer rather than a blend of homopolymers. The resulting copolymer showed a broad polydispersity (M_w/M_n = 1.74) compared to the unfunctionalized PEO-*b*-PCL block copolymer (M_w/M_n = 1.04) and tailing in its GPC chromatogram (Figure 4), which may be attributed to the presence of traces of PBCL homopolymer in the reaction product.

Synthesis and Characterization of PEO-*b*-PCCL Block Copolymers. Synthesis of PEO-*b*-PCL block copolymers bearing carboxyl groups on the PCL block was accomplished through reduction of benzyl carboxylate on the PEO-*b*-PBCL block copolymer. The pendant benzyl carboxylate was readily removed by the catalytic hydrogenolysis to yield a carboxyl-functionalized PEO-*b*-PCL block copolymer. The ^{1}H NMR spectrum of synthesized block copolymer in dimethyl-d_6 sulfoxide at 300 MHz showed peaks at δ: 1.20−1.90 (m, 6H); δ: 3.22−3.38 (s,3H; t, 1H); δ: 3.50 (s,4H); δ: 4.03 (t, 2H). No peak was observed at δ = 7.40 and δ = 5.15 (Figure 2B). The latter peaks correspond to the aromatic and the methylene protons of the benzyl carboxylate group, respectively. The IR spectrum of PEO-*b*-PCCL block copolymer showed a large broad peak from 2500 to 3500 cm^{-1}, which indicates the presence of hydrogen-bonded carboxyl groups. This broad peak was absent in the IR spectrum of PEO-*b*-PBCL block copolymer (Figure 3A,B). The characteristic peaks related to the aromatic C−H stretching (at 690−750 cm^{-1}) were also absent here.

The characteristic properties of PEO-*b*-PCCL block copolymer are summarized in Table 1. The molecular weight of prepared PEO-*b*-PCCL block copolymer measured by comparing the peak intensity of four methylene protons of PEO (δ = 3.65 ppm) and two methylene protons of PCCL (δ = 4.05 ppm) in the ^{1}H NMR spectrum was calculated to be 7530 g mol^{-1} (corresponding to a degree of polymerization of 16 for α-carboxyl-ε-caprolactone). The molecular weight measured by GPC was in good agreement with the ^{1}H NMR results. The polymer population was unimodal (Figure 4), and the molecular weight distribution was also broad (M_w/M_n = 1.52) like PEO-*b*-PBCL block copolymer. The broad molecular weight distribution of PEO-*b*-PCCL may be attributed to the presence of trace amounts of low molecular weight PCCL homopolymer.

Synthesis and Characterization of PEO-*b*-PCL-*co*-PCCL Block Copolymer. Two block copolymers PEO_{114}-*b*-PCL_{16}-*co*-$PCCL_{10}$ (equivalent to 60% and 40% PCL and PCCL in molar basis, respectively) and PEO_{114}-*b*-PCL_{25}-*co*-$PCCL_5$ (equivalent to 83% and 17% PCL and PCCL in molar basis, respectively) were synthesized from copolymerization of ε-caprolactone and α-benzyl carboxylate-ε-caprolactone and further reduction of the product. Thin-layer chromatography using hexane:ethyl acetate (3:1 ratio) as the mobile phase and cerium molybdate solution (Hanessian's Stain) as indicator confirmed the purity of block copolymers from free ε-caprolactone and α-benzyl carboxylate-ε-caprolactone (data not shown). ^{1}H NMR spectra of these block copolymers clearly show the characteristic peaks for O=CH$_2$− protons of PCL (at δ = 2.31 ppm) and −CH$_2$ protons of PCCL (at δ = 1.90 ppm) and complete

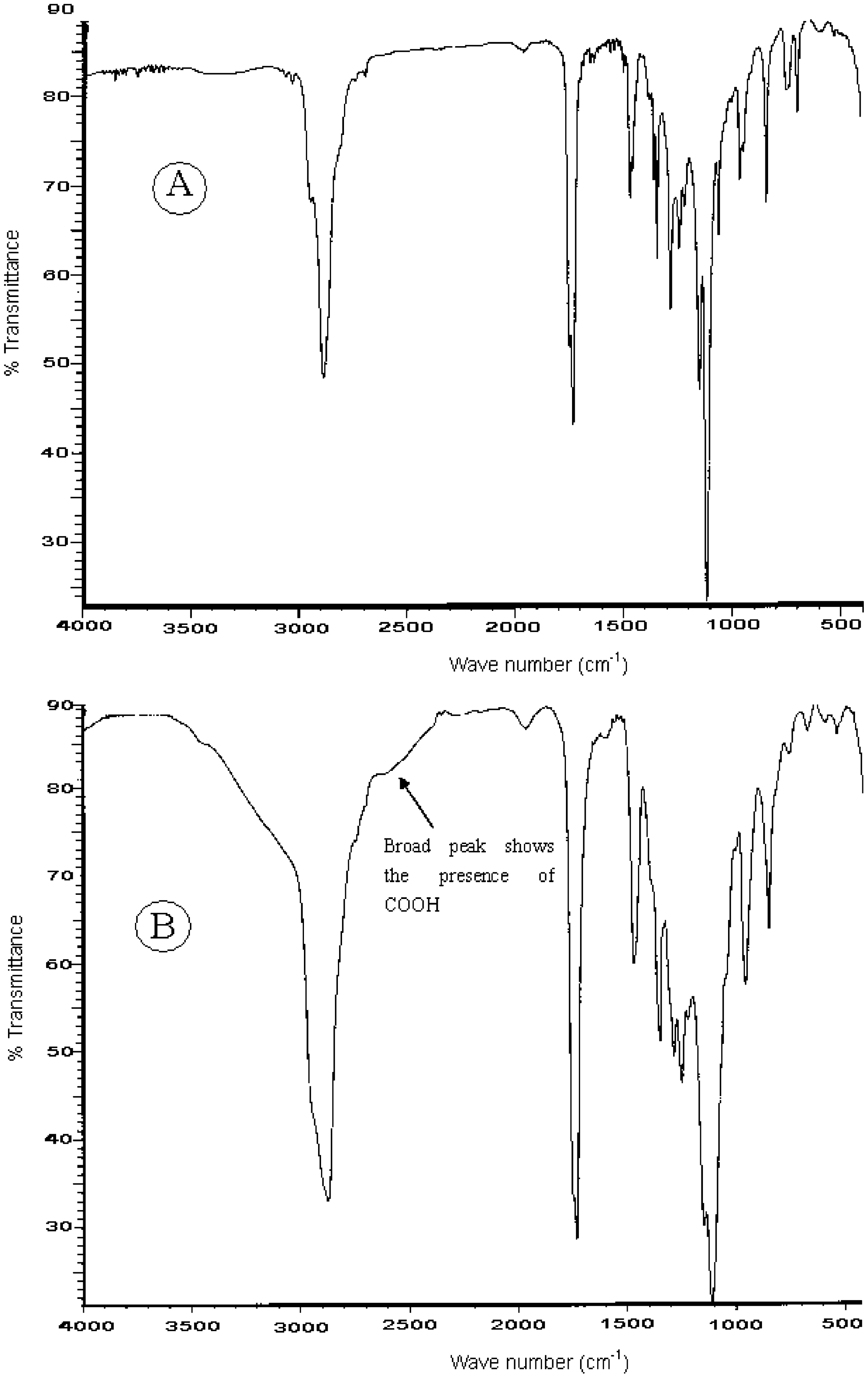


Figure 3. IR spectrum of (A) PEO-*b*-PBCL and (B) PEO-*b*-PCCL. Arrow indicates the presence of broad peak caused by OH of pendant COOH group. The IR samples were prepared using the sodium chloride disk method.

Table 1. Characteristics of Prepared Block Copolymers

block copolymer[a]	theor mol wt (g mol^{-1})	M_n (g mol^{-1})[b]	M_n (g mol^{-1})[c]	polydispersity index[d]
PEO$_{114}$-*b*-PCL$_{42}$	10000	9800	11500	1.04
PEO$_{114}$-*b*-PBCL$_{19}$	10000	9700	9200	1.74
PEO$_{114}$-*b*-PCCL$_{16}$	8000	7530	7200	1.52
PEO$_{114}$-*b*-PCL$_{16}$-*co*-PCCL$_{10}$	8800	8400	9600	1.47
PEO$_{114}$-*b*-PCL$_{25}$ -*co*-PCCL$_{5}$	8750	8650	15600	1.53

[a] The number shown as a subscript indicates the polymerization degree of each block determined from ^{1}H NMR spectroscopy. [b] Number-average molecular weight measured by ^{1}H NMR. [c] Number-average molecular weight measured by GPC. [d] Polydispersity index = M_w/M_n measured by GPC.

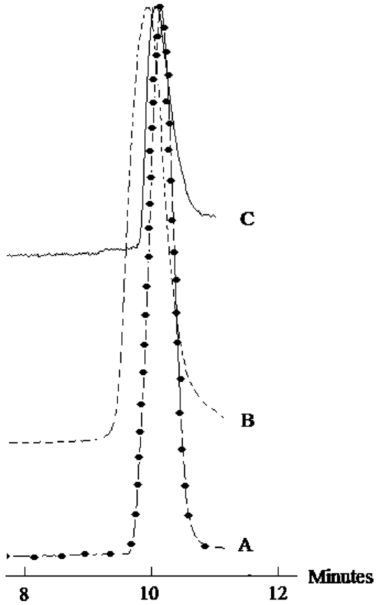


Figure 4. GPC chromatogram for (A) MePEO, M_n = 5000 g mol^{-1}, M_w/M_n = 1.62; (B) MePEO-*b*-PBCL, M_n = 9700 g mol^{-1}, M_w/M_n = 1.74; and (C) MePEO-*b*-PCCL, M_n = 7530 g mol^{-1}, M_w/M_n = 1.52.

reduction of benzyl carboxylate group to COOH (Figure 2C). The degree of polymerization for the hydrophobic block (unsubstituted + substituted ϵ-caprolactone) determined by comparing the peak intensities of four methylene protons of PEO (δ = 3.65 ppm) and two methylene protons of PCL ($-CH_2-C{=}O$, δ = 2.31 ppm) plus two methylene protons of PCCL (CH_2-CH-, δ = 1.90 ppm) in the ^{1}H NMR spectrum was calculated to be 26 and 30 for PEO$_{114}$-*b*-PCL$_{16}$-*co*-PCCL$_{10}$ and PEO$_{114}$-*b*-PCL$_{25}$-*co*-PCCL$_5$, respectively (Table 1). The molecular weight of the PCL and PCCL block was determined by comparing the peak intensities of the methylene protons of the PCL unit (at δ = 2.31 ppm) to the methylene protons of the PCCL unit (at δ = 1.90 ppm). Both copolymers gave unimodal molecular weight distributions but produced broad peaks in GPC. The polydispersity values for PEO$_{114}$-*b*-PCL$_{16}$-*co*-PCCL$_{10}$ and PEO$_{114}$-*b*-PCL$_{25}$-*co*-PCCL$_5$ were 1.47 and 1.53, respectively.

Assembly of Block Copolymers and Characterization of Self-Assembled Structures. Synthesized block copolymers were assembled to polymeric micelles by a cosolvent evaporation method as described in a previous publication.[8] The average diameter for PEO-*b*-PBCL micelle determined by the DLS technique was 61.9 ± 2.9 nm. Micellar population showed a relatively broad distribution (PI = 0.39) compared to the unfunctionalized PEO-*b*-PCL (PI = 0.20) block copolymer micelles. The results of size measurements by DLS for three carboxyl bearing block copolymers at different levels of COOH substitution revealed a relatively small average diameter (19.9–38.3 nm) for the micellar peak, but secondary peaks at larger diameters were also present. A relatively high degree of polydispersity for the self-assembled structures in these polymers (0.5–0.9) may be a reflection of the secondary association of PCCL bearing micelles (Table 2). The size of micelles for all PCCL containing block copolymers decreased with an increase in the number of carboxyl groups on the polymeric backbone (Table 2). The composition and the molecular weight of the block copolymer are known to significantly influence the size and polydispersity of the resultant micelles.[28] In this case, association of the exposed COOH groups, localized in the core/shell interface during synthesis or by folding of the hydrophobic block, through hydrogen bonding is speculated to be a possible reason for the aggregation of micellar structures.

The TEM picture of PEO-*b*-PBCL shows the formation of a true spherical-shaped micelle having a clear boundary, and the average diameter was 31.5 nm in the dry state (Figure 5A). While micelles formed from PEO-*b*-PCCL block copolymers were found to be much smaller (10.3 nm based on TEM images) and the shapes of the micelles are not clearly defined (Figure 5B). A similar trend was observed in the size measurement obtained from the DLS technique. The difference in size measured by these two methods (61.9 vs 31.5 nm for PEO-*b*-PBCL and 19.9 vs 10.3 nm for PEO-*b*-PCCL) is due to the hydration of the PEO chain in the aqueous medium used in DLS measurements.

The cmc of all diblock copolymers were determined by fluorescence spectroscopy using pyrene as the fluorescent probe. Pyrene has a strong hydrophobic character and very low water solubility. At block copolymer concentrations above cmc, pyrene preferentially partitions into hydrophobic microdomains of the micellar core, resulting in the concurrent change in the molecule's photophysical properties. As a result, with a rise in the level of block copolymers a red shift is observed in the excitation spectrum of pyrene. This property is used to measure the cmc of block copolymers. A sharp rise in intensity ratio of peaks at 339 nm to those at 334 nm from the excitation spectra of pyrene indicates the onset of micellization (Figure 6). Using this method, low cmc values (in the micromolar range) were revealed for PEO-*b*-PCL and PEO-*b*-PBCL block copolymers. The average cmc for PEO$_{114}$-*b*-PCL$_{42}$ and PEO$_{114}$-*b*-PBCL$_{19}$ was calculated at 18.2 × 10^{-2} μM and 9.8 × 10^{-2} μM, respectively. The cmc for PEO-*b*-PBCL with a hydrophobic block degree of polymerization of 19 is even lower than PEO-*b*-PCL having a hydrophobic block degree of polymerization of 42. The lower cmc values for PEO-*b*-PBCL clearly shows that introduction of hydrophobic benzyl carboxylate group to the poly(ϵ-caprolactone) makes self-association of block copolymers, thermodynamically, more favorable. Whereas, copolymers having large number of COOH groups in their hydrophobic block have a lower tendency for self-association.[17] More importantly, the presence of an aromatic group on the PCL block seems to be even more effective than elongating the PCL block in pushing the cmc to lower concentrations. It is noteworthy to mention that the cmc value for PEO-*b*-PBCL is 5 times lower than the reported cmc for PEO-*b*-poly(β-benzyl L-aspartate) (PEO$_{110}$-*b*-PBLA$_{19}$). The cmc value for PEO-*b*-PBLA measured by an identical method is 55 × 10^{-2} μM. The

Table 2. Characteristics of Prepared Block Copolymer Micelles (n = 3)

block copolymer	av micellar size ± SD (nm)[a]	av size of secondary peaks (nm)	PDI	cmc[c] ± SD (μM)	I_e/I_m[d] ± SD
PEO_{114}-*b*-PCL_{42}	40.0 ± 2.0		0.20	$18.2 \times 10^{-2} \pm 0.01$	0.055 ± 0.007
PEO_{114}-*b*-$PBCL_{19}$	61.9 ± 2.9		0.39	$9.8 \times 10^{-2} \pm 0.01$	0.028 ± 0.002
PEO_{114}-*b*-$PCCL_{16}$	19.9 ± 2.3	368 (60%)[b]	0.90	$1220 \times 10^{-2} \pm 0.42$	0.025 ± 0.002
PEO_{114}-*b*-PCL_{16}-*co*-$PCCL_{10}$	24.4 ± 2.4	256 (45%)[b]	0.58	$130 \times 10^{-2} \pm 0.05$	0.027 ± 0.003
PEO_{114}-*b*-PCL_{25}-*co*-$PCCL_{5}$	38.3 ± 4.5	290 (40%)[b]	0.53	$44.5 \times 10^{-2} \pm 0.02$	0.035 ± 0.002

[a] Intensity mean estimated by the DLS technique. [b] Numbers in parentheses indicate the frequency of secondary peak in micellar population in percentage. [c] Measured from the onset of a rise in the intensity ratio of peaks at 339 nm to peaks at 334 nm in the fluorescence excitation spectra of pyrene plotted vs logarithm of polymer concentration. [d] Intensity ratio (excimer/monomer) from emission spectrum of 1,3-(1,1′-dipyrenyl)propane in the presence of polymeric micelle.

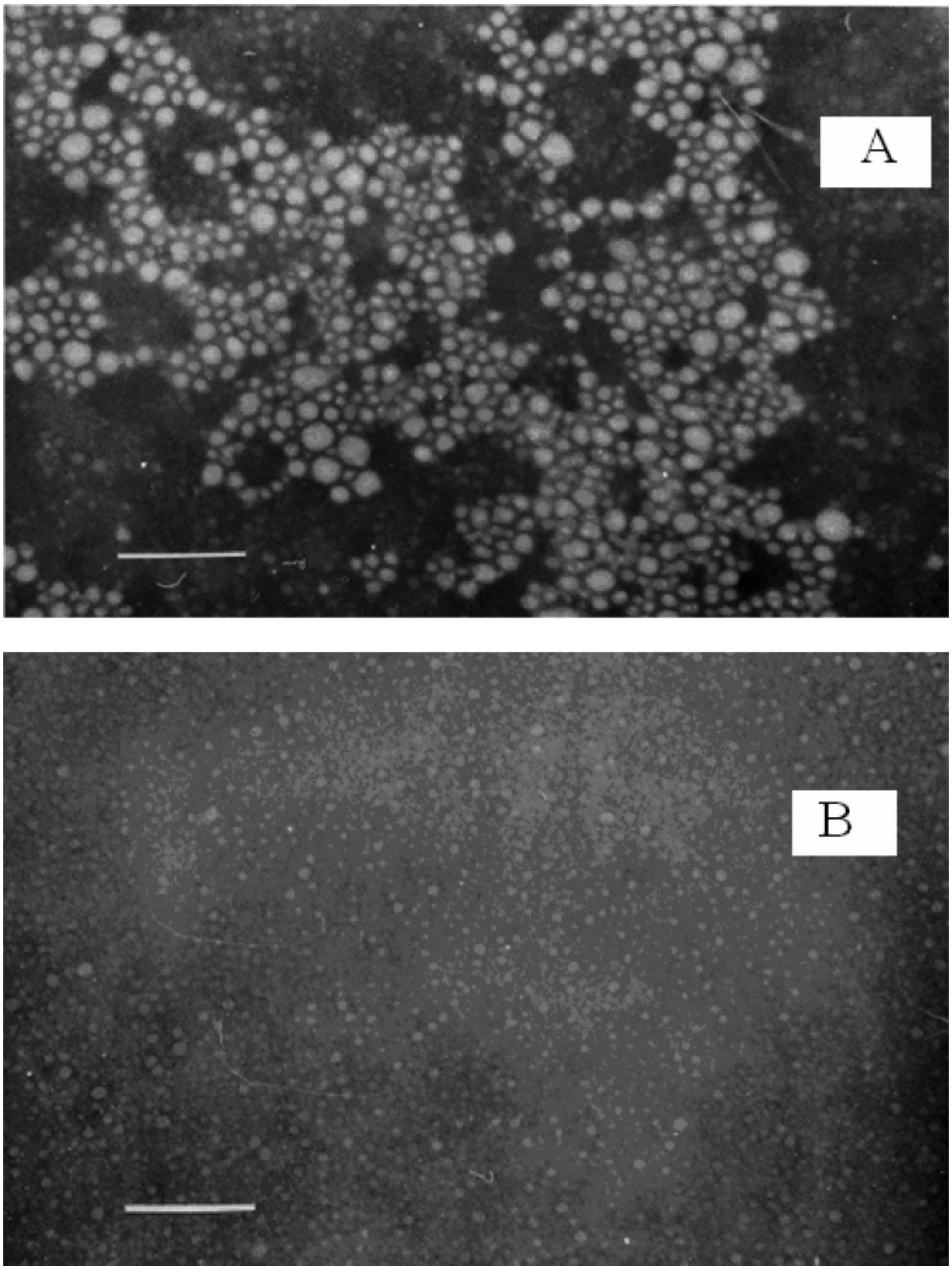


Figure 5. TEM picture of micelles prepared from (A) PEO-*b*-PBCL and (B) PEO-*b*-PCCL block copolymers (magnification 18000 × 6.1). The bar on the images represents 200 nm.

lower cmc of PEO-*b*-PBCL reflects a better thermodynamic stability of this micellar structure compared to PEO-*b*-PBLA in the biological system.

The cmc for PEO_{114}-*b*-$PCCL_{16}$ (with 100% COOH substitution on the PCL block) was 1220×10^{-2} μM, i.e., 67-fold higher than the cmc value for PEO_{114}-*b*-PCL_{42} (Table 2). The significant increase in the cmc of PEO_{114}-*b*-$PCCL_{16}$ compared to PEO_{114}-*b*-PCL_{42} is attributed to the substitution of a hydrophilic group, i.e., COOH, as well as lower degree of polymerization in the core-forming block. Compared to PEO_{114}-*b*-$PCCL_{16}$, a

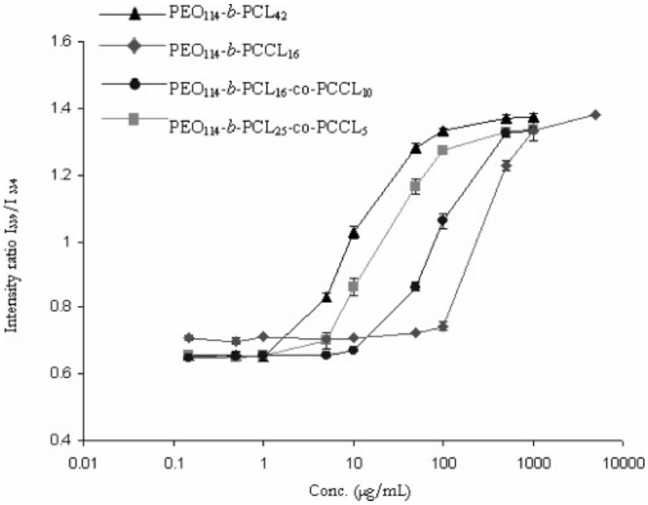


Figure 6. Intensity ratio (339 nm/334 nm) of pyrene (6×10^{-7} M) from excitation spectrum as a function of block copolymer concentration. Each point represent average ± SD ($n = 3$).

10-fold decrease in the cmc of block copolymer was observed for PEO$_{114}$-*b*-PCL$_{16}$-*co*-PCCL$_{10}$. This may be due to an increase in the degree of polymerization in the core-forming block from 16 to 26, the introduction of hydrophobic PCL, and/or a reduction in the number of free COOH groups on the PCL backbone. At a similar degree of polymerization for the core-forming block (26−30), a decrease in the ratio of COOH substituted ϵ-caprolactone to ϵ-caprolcatone in the core-forming block from 10 to 5 led to a 2.9-fold decrease in cmc (Table 2). For PEO$_{114}$-*b*-PCL$_{16}$-*co*-PCCL$_{10}$ and PEO-*b*-PCL$_{25}$co-PCCL$_5$ block copolymers, cmc was calculated to be 130×10^{-2} and 44.5×10^{-2} μM, respectively (Table 2). These results are consistent with previous findings on the effect of core hydrophobicity on cmc value.[23,29] Therefore, controlled introduction of aromatic or carboxylic group in the PEO-*b*-PCL backbone may be used to adjust the cmc and stability of resulted polymeric micelles.

Evidence for the limited motion in the core of PEO-*b*-PCL-based micelles prepared in this study was obtained from the fluorescence emission spectra of 1,3-(1,1′ dipyrenyl)propane in the presence of block copolymers at concentration above cmc (~1000 μg/mL).[23,30] Like pyrene, 1,3-(1,1′ dipyrenyl)propane is a hydrophobic fluorescent probe that preferentially partitions into hydrophobic microdomain at polymeric concentrations above cmc. In this case, formation of excimers that emit light at 480 nm may become restricted due to a high rigidity of its environment. Very low excimer to monomer (I_e/I_m) intensity ratios in the emission spectrum of the dipyrene probe for the prepared micelles (0.025−0.055) reflect a high viscosity for the hydrophobic core. The rigidity of the micellar core is believed to be the reason for slow dissociation of polymeric micelles and controlled rate of drug release.[31] As stated in Table 2, I_e/I_m ratios of dipyrene probe in the presence of PEO-*b*-PBCL and PEO-*b*-PCCL micelles was significantly lower than unfunctionalized PEO-*b*-PCL micelles ($p < 0.05$, unpaired Student's *t* test) reflecting the presence of more rigid core in the aromatic or carboxylic group bearing micelles. No significant change in I_e/I_m ratios of dipyrene probe was detected between PEO-*b*-PBCL and PEO-*b*-PCCL micelles ($p > 0.05$, unpaired Student's *t* test). This may be due to the formation of strong intramicellar $\pi-\pi$ interactions between the aromatic rings of PBCL or hydrogen bonding between the carboxylic groups of the PCCL core. PEO-*b*-PCL$_{25}$-*co*-PCCL$_5$ block copolymer had a significantly higher I_e/I_m ratio (0.035) than PEO-*b*-PCL$_{16}$-*co*-PCCL$_{10}$ (0.027) and PEO-*b*-PCCL$_{16}$ (0.025) block polymers ($p < 0.05$, unpaired Student's *t* test). The lower rigidity of the micellar core in this particular structure may be due to the presence of less carboxyl substituents where intramicellar hydrogen bonding between carboxyl groups of the core is less significant than the block copolymers containing higher carboxyl substitution.

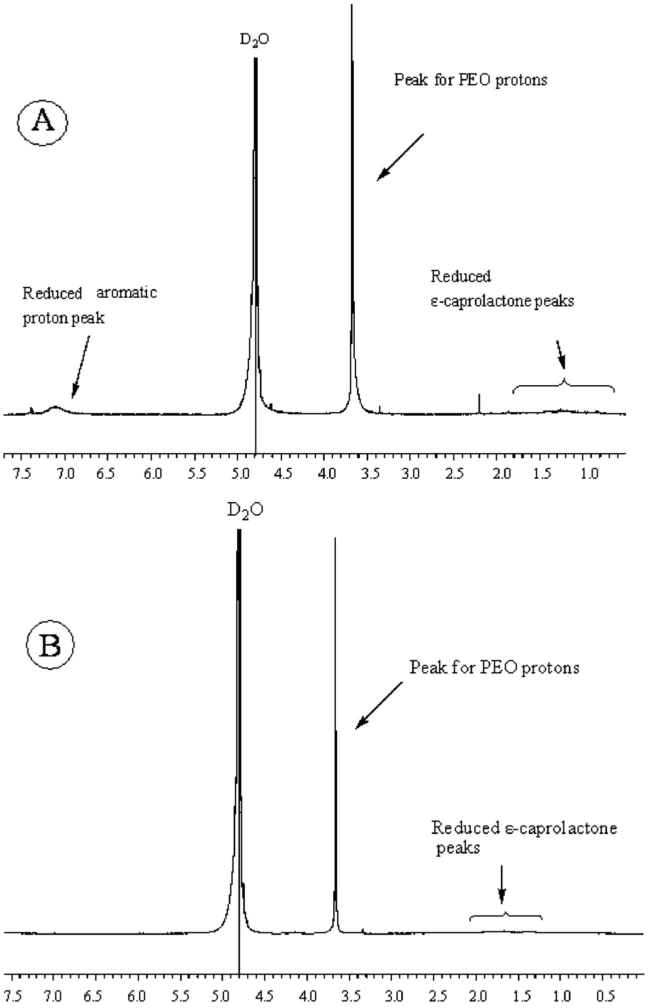


Figure 7. ^{1}H NMR spectrum of (A) PEO-*b*-PBCL and (B) PEO-*b*-PCCL block copolymer micelle in D_2O.

Further proof for the formation of core/shell structures from prepared block copolymers in an aqueous environment and limited mobility of the inner core of PEO-*b*-PCL-based micelles was obtained by ^{1}H NMR spectroscopy in D_2O (Figure 7). Because of the limited mobility of the inner core of polymeric micelles in D_2O, the intensity of proton peaks originated from the core-forming block reduced dramatically compared to the one in $CDCl_3$ or DMSO-d_6 where the formation of micelle is not expected. Small broad peaks at 7.4 ppm (Figure 7A) and 1.2−2.0 ppm (Figure 7A,B) characteristic of the protons of the aromatic and ϵ-caprolactone of PBCL and PCCL segment show restricted motions of these protons compared to the ^{1}H NMR spectrum of the same block copolymer in $CDCl_3$ or DMSO-d_6 (Figure 2A,B). Studies on the biocompatibility and biodegradation rate of this novel class of functionalized PEO-*b*-poly(ester) block copolymers are currently underway.

Conclusion

A family of novel biodegradable PEO-*b*-poly(ester) block copolymers having functional pendant α-benzyl carboxylate or carboxyl group attached with the core forming block was successfully synthesized. PEO-*b*-PCL-based block copolymers with modified hydrophobic blocks were able to form spherical micelles of nanoscopic dimension in water that had rigid cores. Micelles with core structures containing aromatic groups (PEO-*b*-PBCL) were more thermodynamically stable than the parent PEO-*b*-PCL micelle. Biodegradable PEO-*b*-poly(ester) micelles with benzyl carboxylate and carboxyl groups in the micellar core may serve as new polymeric micellar delivery systems for the chemical conjugation, optimized solubilization, and controlled delivery of several therapeutic agents.

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