Synthesis and Chemical Structural Analysis of Nitroxyl-Radical-Incorporated Poly(acrylic acid/lactide/ϵ-caprolactone) Copolymers

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ABSTRACT: The goal of this research is to synthesize biodegradable polymers that would have nitroxyl radical biological functions. Linear aliphatic polyesters were chosen as the starting materials. The hydroxyl-terminated polylactide/ϵ-caprolactones (PBLC-OHs) were first synthesized by melt ring-opening copolymerization in the presence of benzyl alcohol and stannous octoate. PBLC-OHs were used as the precursor for the synthesis of double bond-functionalized polylactide/ϵ-caprolactones (PBLC-Mas) by reacting the hydroxyl end groups of PBLC-OH with maleic anhydride in melt at 130 °C. Acrylic acid/lactide/ϵ-caprolactone graft copolymers (PBLCAs) were then successfully carried out by the radical copolymerization of acrylic acid and PBLC-Ma initiated by azobisisobutyronitrile. Finally, nitroxyl radicals [4-amino-2,2,6,6-tetramethylpiperidine-1-oxy (TAM)] were incorporated into the carboxylic acid sites of the acrylic acid/lactide/ϵ-caprolactone copolymer (TAM-PBLCA) by reacting TAM with PBLCA in the presence of *N*,*N*′-carbonyl diimidazole. A high content of TAM was incorporated into the PBLCA copolymer. The polymers synthesized were characterized by ^{1}H and ^{13}C NMR, Fourier transform infrared spectroscopy, and electron paramagnetic resonance spectra. © 2001 John Wiley & Sons, Inc. J Polym Sci Part A: Polym Chem 39: 4214–4226, 2001
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INTRODUCTION

Aliphatic polyesters such as polylactide (PLA), poly(ϵ-caprolactone), polyglycolide (PGA), and their copolymers are the group of biomaterials that have commercially successful applications because of their biodegradability[1,2] and biocompatibility.[3,4] Although these polymers have been used extensively as sutures, implant materials, and drug carriers, they do not have any inherent biological functions to actively participate in human body repair. These aliphatic polyesters are not "biologically active" and cannot exert biological activity directly. They only play a passive role in wound healing, tissue regeneration, and tissue engineering. It would be ideal to make these biomaterials biologically "alive" and perform some critical biological function, such as the ability to modulate inflammatory reactions to facilitate wound healing or to enhance host defenses against disease.[5]

One of the most recently discovered biological messengers is nitric oxide (NO ·). Nitric oxide is a very small but highly reactive free radical with expanding known biological functions. This molecule and its biological functions have recently become one of the most studied compounds in biochemistry and biology and the subject of several recent reviews. NO · acts both as an essential

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regulatory agent to normal physiological activities and as cytotoxic species in diseases and their treatments. For example, it was suggested that NO · could be used as a potent antiviral compound against poxvirus and herpes simplex virus type-1,[6] as a heart medicine against low oxygen supply (a condition know as myocardial ischemia),[7] and as an anti-inflammatory drug.[8] However, excessive introduction of NO · into the body may have adverse side effects like microvascular leakage, tissue damage in cystic fibrosis, septic shock, B-cell destruction, and possible mutagenic risk.[9–13] Therefore, it is very important to be able to develop a delivery vehicle to control the NO · concentration and its release.

One reported approach toward the research and development of such biologically active biomaterials was to chemically incorporate nitric oxide derivative (NOD) like tempamine nitroxyl radical (TAM) into the carboxylic chain ends of PGA macromolecules via amide linkage.[5] This TAM-incorporated PGA was able to retard the proliferation of smooth muscle cells as pure nitric oxide does. Because NOD was chemically incorporated into the chain ends of PGA or PLA, the NOD concentration was quite limited. It would be more beneficial if a wide range of NOD concentrations could be incorporated into its delivery vehicle for meeting a variety of specific biomedical needs.

In this article, we investigate the synthesis and characterization of the acrylic acid/lactide/ϵ-caprolactone graft copolymer that would have pendant carboxylic groups along the macromolecular backbone so that a wide range of NODs can be attached for achieving different extents of NO · biological activity. Our approach was to introduce unsaturated groups like double bonds into the chain ends of polylactide-*co*-ϵ-caprolactones by reacting them with maleic anhydride. These double bond-terminated copolymers were then used as the precursors to further copolymerize with acrylic acid monomer via free-radical mechanism. The resulting copolymer of acrylic acid, lactide, and ϵ-caprolactone was eventually chemically attached by TAM at the pendant carboxylic acid group according to our previously reported method.[5]

MATERIALS AND METHODS

Materials

Lactide (Aldrich Chemical Co., Milwaukee, WI) was purified by first dissolving it in toluene, heating up to 100 °C until the solid was dissolved, and then cooling down to room temperature to recrystallize; this process was repeated three times. The recrystallized lactide was then washed with dried ethyl ether and dried over P_2O_5 in vacuum as previously described.[14] ϵ-Caprolactone (Aldrich) was purified by drying with CaH_2 and distilled in vacuum at 100 °C. Maleic anhydride (99%), TAM (95%), 2-imidazolidinethione (98%), and 2,2′-azobisisobutyronitrile (AIBN, 98%) were all obtained from Aldrich and used without further purification. Stannous octoate (95%) was purchased from Sigma Chemical Co. (St. Louis, MO,) and used without further purification. Acrylic acid and benzyl alcohol were all purchased from Aldrich and purified by distilling in vacuum prior to use. Ethyl ether (anhydrous, Fisher Scientific, Fair Lawn, NJ), toluene (A. R. Mallinckrodt Baker, Inc., Paris, KY), and dioxane (99.8%, anhydrous, Aldrich) were dried by refluxing over benzophenone-Na complex and distilled in an atmosphere of dry argon. Chloroform (A. R. Mallinckrodt Baker) was extracted with water three times to remove residual alcohol, dried with anhydrous $MgSO_4$ overnight, and distilled in an atmosphere of dry argon. Petroleum ether (35–60 °C grade, A. R. Mallinckrodt Baker) was used without purification. Borosilicate (Pyrox) press reaction tubes (body 30 mm o.d. × 3 in long; neck 11 mm o.d. × 6 in long) were purchased from Scientific Group (Vineland, NJ) for melt polymerization.

Synthesis

The synthesis schemes involved four of the following basic tasks: (1) synthesis of polylactide, poly(ϵ-caprolactone), and polylactide-*co*-ϵ-caprolactone having one hydroxyl end group per macromolecule, (2) incorporation of unsaturated groups into the hydroxyl end of the polymers synthesized in (1), (3) copolymerization of the polymers synthesized in (2) with acrylic acid, and (4) chemical attachment of TAM onto the pendant carboxylic acid groups of the copolymers synthesized in (3).

Synthesis of Hydroxyl-Terminated Polylactide-co-ϵ-caprolactone (PBLC-OH), Polylactide (PBL-OH), and Poly(ϵ-caprolactone) (PBC-OH)

The synthesis of PBLC-OH that had its one end group capped by benzyl alcohol and the other end group remaining as a free —OH group was carried out by ring-opening polymerization of lactide

Table I. The Influences of Feed Ratio on the Molecular Weight of Polylactide/ε-Caprolactone Copolymer (PBLC-OH)

Feed Molar Ratio			Molecular Weight			
Benzyl Alcohol	ε-Caproyl (C unit)	α-Oxypropiony (L unit)	M_n[a] ($\times 10^3$)	M_w[b] ($\times 10^3$)	M_p[c] ($\times 10^3$)	Polydispersity (M_w/M_n)
1	3	15	1.76	3.05	2.85	1.73
1	7	35	3.28	4.73	4.80	1.44

[a] Number-average molecular weights (M_n's) determined by GPC with polystyrene standards.
[b] Weight-average molecular weights (M_w's) determined by GPC with polystyrene standards.
[c] Peak molecular weight (M_p).

and ϵ-caprolactone (2.5:1 molar ratio) in the presence of benzyl alcohol (trace amount) and stannous octoate (0.5% by weight) in a Pyrox polymerization tube. After repeatedly vacuum drying and argon refilling for several times, the polymerization tube was vacuum sealed and placed in an oil bath at 130 °C for 48 h to polymerize the monomers. After cooling to room temperature, the resulting product was dissolved in chloroform. The solution was poured into excess petroleum ether to precipitate the polymer. The precipitate was washed with distilled water for four times and dried over P_2O_5 under vacuum at room temperature until constant weight. By varying the molar ratio of lactide to ϵ-caprolactone monomers and benzyl alcohol, we could control the molecular weight of the polymer (Table I).

The same method described previously was used to synthesize hydroxyl-terminated poly(ϵ-caprolactone) homopolymer (PBC-OH) and polylactide homopolymer (PBL-OH). The purpose of making PBC-OH and PBL-OH homopolymers was to provide controls for comparing and analyzing the chemical structure of PBLC-OH.

Synthesis of Double Bond-Terminated Polylactide-co-ϵ-caprolactone (PBLC-Ma)

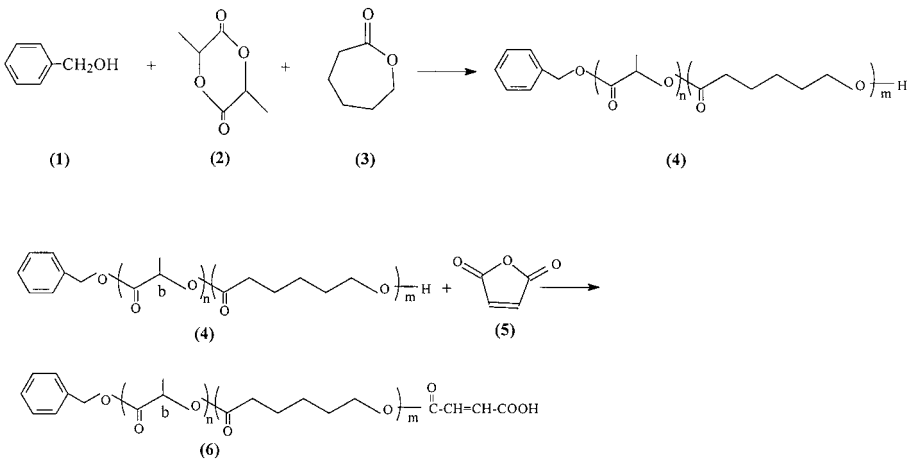
Maleic anhydride was used to introduce both unsaturated and carboxylic acid groups into PBLC-OH. The hydroxyl-terminated PBLC-OH and maleic anhydride (1:5 molar ratio) were placed in a three-necked flask under N_2 atmosphere at 130 °C for 24 h. After this reaction, the excessive maleic anhydride was distilled at 130 °C under vacuum, and the reaction mixture was dissolved in chloroform. The chloroform solution was extracted with water three times to remove the residual maleic anhydride and dried with anhydrous $MgSO_4$ overnight. The purified PBLC-Ma was obtained by precipitating the water-extracted chloroform solution in excess petroleum ether and dried in vacuum at room temperature.

Synthesis of Poly(acrylic acid/lactide/ϵ-caprolactone) Copolymer (PBLCA)

The double bond-terminated polylactide-*co*-ϵ-caprolactone (1.98 g), acrylic acid (3.0 g), and AIBN (0.0335 g) (1.1 wt % of acrylic acid) were dissolved in 20 mL of dioxane at room temperature in a three-necked flask under N_2. The mixture was then heated to 60 °C for 5 h. After removing most of the solvent by distillation at 120 °C, the reaction mixture was precipitated in cold water to remove the acrylic acid homopolymer byproduct. The precipitate, PBLCA, was filtered and washed with cold water three times and dried over P_2O_5 under vacuum at room temperature.

Synthesis of Nitroxyl-Radical-Incorporated Poly(acrylic acid/lactide/ϵ-caprolactone) Copolymer (TAM-PBLCA)

The chemical incorporation of TAM into the carboxylic acid sites of PBLCA was carried out according to our previously published procedure.[5] PBLCA (1.1392 g) was dissolved in 20 mL of dioxane at 50 °C, and 0.2851 g of *N*,*N*′-carbonyl diimidazole were then added. After 15 min, 0.3140 g of TAM dissolved in 5 mL of dioxane were added slowly to the reaction mixture at this temperature. The reaction mixture was vigorously stirred for several hours at 50 °C. The resulting solution mixture was added dropwise into petroleum ether to precipitate the TAM-PBLCA. This polymer was stirred in 100 mL of water for 3 h at room temperature to remove excess TAM, *N*,*N*′-carbonyl diimidazole, and imidazole produced during the reaction, filtered and washed with water four times, and then dried over P_2O_5 in vacuum at room temperature.



Scheme 1. Synthesis of the polylactide/ϵ-caprolactone copolymer (PBLC-OH, 4) from benzyl alcohol (1), L-lactide (2), and ϵ-caprolactone (3) and maleic acid end-capped poly(L-lactide/ϵ-caprolactone) copolymer (PBLC-Ma, 6) from the reaction of poly(L-lactide/ϵ-caprolactone) copolymer (PBLC-OH, 4) with maleic anhydride (5).

Characterization

^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity spectrometer operating at 300 MHz. The spectra were recorded in deuterated dimethyl sulfoxide (DMSO-d_6) (for PBLCA and TAM-PBLCA) or in deuterated chloroform (for PBC-OH, PBL-OH, and PBLC-OH and PBLC-Ma), and tetramethylsilane was used as an internal reference.

The molecular weight and molecular weight distribution of the synthesized polymers were measured by size exclusion chromatography and carried out with tetrahydrofuran as an eluent (1.0 mL/min) using a Waters 510 high-pressure liquid chromatographic pump, a Waters U6K injector, three PSS SDV columns (linear, 10^4, and 100 Å) in a series, and a Milton read-only memory differential refractomer detector. The columns were calibrated with polystyrene standards having a narrow molecular weight distribution.

Fourier transform infrared (FTIR) spectra were obtained from a PerkinElmer Magna-IR 560 spectrometer. The film for IR analysis was obtained by casting a DMSO solution (3 wt %/vol) of the polymer onto a KBr crystal. Omnic software was used for data acquisition and analysis.

The nitroxyl radical property of TAM-PBLCA was characterized by electron paramagnetic resonance (EPR) spectra at the X-band using a Bruker 200D SRC spectrometer operating at 9.6 GHz, using 100-KHz modulation.

Elemental analysis of nitrogen was conducted by Atlantic Microlab Inc., and the TAM content of TAM-PBLCA was calculated accordingly.

RESULTS AND DISCUSSION

Polylactide-*co*-ϵ-caprolactone (PBLC-OH)

As shown in Scheme 1, the synthesis of polylactide-*co*-ϵ-caprolactone (**4**) was performed via ring-opening polymerization of lactide and ϵ-caprolactone in the presence of benzyl alcohol and stannous octoate as previously described.[14] Although the structure **4** in Scheme 1 showed a ϵ-caprolactone unit-ending group, the PBLC-OH copolymer could also have a lactide unit as the ending group. There were many different catalysts used for the ring-opening polymerization of glycolide, lactide, and lactone. Among those catalysts reported, stannous octoate ($SnOct_2$) was the most frequently used for two main reasons. First, $SnOct_2$ is a highly efficient catalyst and allowed almost complete conversions even at very high monomer/catalyst ratios (e.g., 10^4:1).[15–18] Second, the risk of racemization was low, and 99% optically pure poly(L-lactide) could be prepared even at 150 °C when the reaction time was limited to a few hours.

Compounds having free hydroxyl groups, such as alcohols, have frequently been used as coinitiating agents in a ring-opening polymerization. In this study, benzyl alcohol was selected as a coinitiator because its incorporation as a benzylester end group could be easily detected by both ^{1}H and ^{13}C NMR. Thus, the resulting PBLC-OH macromolecule had one of its two end groups capped by benzyl alcohol, and the other end group as a hydroxyl group. The molecular weight of PBLC-OH could be varied by the molar ratio of monomers to

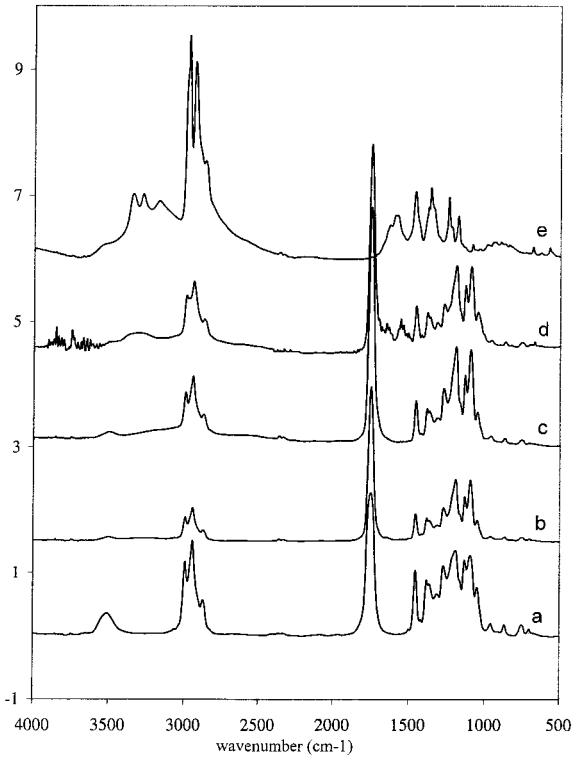


Figure 1. FTIR spectra of the copolymers synthesized: (a) benzyl alcohol end-capped poly(L-lactide/ϵ-caprolactone) copolymer (PBLC-OH), (b) maleic acid end-capped poly(L-lactide/ϵ-caprolactone) copolymer (PBLC-Ma), (c) poly(L-lactide/ϵ-caprolactone/acrylic acid) copolymer (PBLCA), (d) nitric oxide derivative-attached poly(L-lactide/ϵ-caprolactone/acrylic acid) copolymer (TAM-PBLCA), and (e) nitric oxide derivative, 4-amino-2,2,6,6-tetramethylpiperidine-1-oxy (TAM).

benzyl alcohol as shown in Table I. The gel permeation chromatographic trace of PBLC-OH indicated the absence of low molecular weight polymers and/or byproducts. The purified PBLC-OH was a very sticky colorless solid.

The FTIR spectrum of PBLC-OH is shown in Figure 1 (spectrum a). The main absorption bands of PBLC-OH were assigned as the following: 3560 cm^{-1} for the O—H absorption, 3000 cm^{-1} for CH_3 stretching, 2970 and 2920 cm^{-1} for CH_2 stretching, 1780 cm^{-1} for C═O stretching, 1470 cm^{-1} for the benzene ring, and two bands at 1280–1230 cm^{-1} and 1170–1130 cm^{-1} for the stretching of C—O.

The ^{1}H NMR spectra of PBL-OH, PBC-OH, and PBLC-OH are depicted in Figure 2. Because the synthesis of PBLC-Ma and PBLCA from PBLC-OH involved the reaction limiting to the chain ends of PBLC-OH, the backbone sequence of PBLC-OH remained intact. Therefore, the results of the ^{1}H NMR structure analysis of PBLC-OH could also be used to describe the structure of PBLC-Ma and PBLCA. The detailed analysis of the NMR spectra is given subsequently.

By comparison with the ^{1}H NMR spectra of the PBC-OH and PBL-OH homopolymers, it was not difficult to assign peaks in the ^{1}H NMR spectrum (300 MHz NMR) of the PBLC-OH copolymer (Fig. 2). Each group was assigned as the following: δ (ppm) = 7.409–7.294 (H_1), 5.297–5.000 ($H_{2,3}$), 4.440–4.306 (H_2, end group), 4.398–4.225 (H_4), 2.513–2.219 (H_5), and 1.933–1.235 ($H_{6,7,8,9}$). The H_4 end group was not found.

Among those peaks in Figure 2, both H_4 and H_5 showed diad sensitivity, whereas H_6, H_7, H_8, and H_9 were insensitive to the sequence effect. H_2 showed a multiplet. H_5 appeared to be sensitive to the α-oxypropionyl (L) unit coupled to the carbonyl group of the ϵ-oxycaproyl (C) unit but gave little response to the α-oxypropionyl (L) unit attached to the ϵ-oxygen atom on the other side of the ϵ-oxycaproyl unit (C). Peak 5 was assigned to the α-methylene proton that was connected to the C unit [C-C sequence or poly(ϵ-caprolactone) ho-

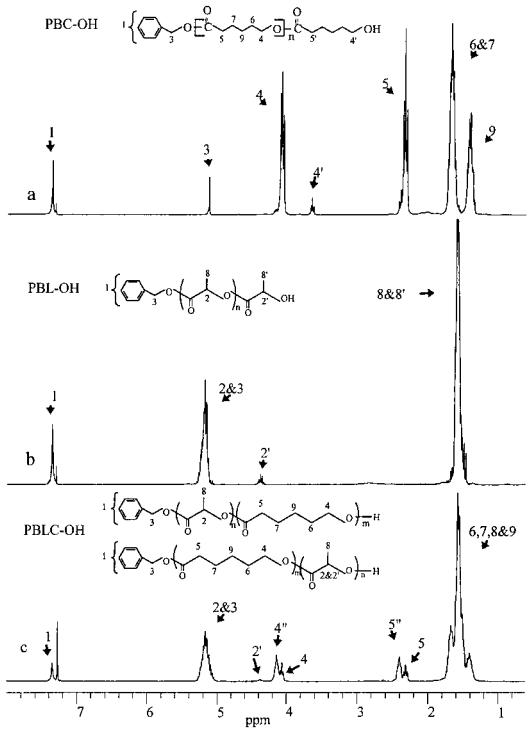


Figure 2. ^{1}H NMR spectra of the copolymers synthesized: (a) poly(ϵ-caprolactone) homopolymer (PBC-OH), (b) poly(L-lactide) homopolymer (PBL-OH), and (c) poly(L-lactide/ϵ-caprolactone) copolymer (PBLC-OH).

mopolymer]. Peak 5″ was assigned to those coupled with the L unit in the C-L sequence. H_4 was also sensitive to the L unit coupled to the ϵ-oxygen atom. Peaks 4 and 4″ were assigned to the —OCH_2— proton in the C-C sequence and the C-L sequence, respectively.

The H_4 end group (CH_2—OH) from the C unit could be found at 3.70–3.50 ppm in the 500-MHz ^{1}H NMR spectra. The molar ratio of the H_4 end groups CH_2—OH to H_2 end group —$CH(CH)_3$—OH in PBLC-OH was 1:19.62 and much lower than the composition ratio of the corresponding copolymer. The compositional ratio of the ϵ-oxycaproyl unit (C) to the α-oxypropionyl unit (L) in PBLC-OH was 1:4.53; however, the feed molar ratio of C/L in polymerization crude was 1:5. This difference between the actual composition ratio of the copolymer and the feed molar ratio qualitatively demonstrated that the polymerization rate of ϵ-caprolactone monomer was higher than the rate of lactide monomer in this polymerization condition.

The ^{13}C NMR spectra of PBL-OH, PBC-OH, and PBLC-OH are shown in Figure 3. By comparison with the published ^{13}C NMR spectra of lactide homopolymer initiated by 2-propanol[19] and high molecular weight ϵ-caprolactone homopolymer,[14] and ϵ-caprolactone homopolymer initiated by alcohol,[20,21] each group of peaks in the ^{13}C NMR spectra of PBC-OH [Fig. 3(a)] and PBL-OH [Fig. 3(b)] was assigned as follows. For PBC-OH, δ 173.516 ($C_{10'}$), 173.338 (C_{10}), 173.079 ($C_{10''}$); 135.958, 128.404, and 128.032 (C_1); 65.936 (C_3); 63.963 (C_4), 62.831 ($C_{4''}$), 62.200 ($C_{4'}$); 34.088 ($C_{5''}$), 33.958 (C_5), 32,212 ($C_{5'}$); 28.200 (C_6); 25.386 (C_9), 25.240 ($C_{9'}$); 24.698 ($C_{7'}$), 24.431 (C_7). In the ^{13}C NMR spectra of PBL-OH [Fig. 3(b)], each group of peaks was assigned as the following: 175.053, 174.859, and 174.664 ($C_{11'}$); 170.038, 169.964, and 169.877 ($C_{11''}$); 169.715, 169.569, and 169.456 (C_{11}); 135.359, 128.760, 128.647, and 128.388 (C_1); 69.576 and 69.462 ($C_{2''}$); 69.155 (C_2); 67.263 (C_3); 66.745 and 66.405 ($C_{2'}$); 20.565 ($C_{8''}$); 20.193 ($C_{8'}$); 16.813 (C_8).

The ^{13}C NMR spectra of PBLC-OH copolymer [Fig. 3(c)] were so complex because of the sequence effects of the α-oxypropionyl (L) and ϵ-oxycaproyl (C) units that it was very difficult to assign each peak definitely. Fortunately, by comparison the ^{13}C NMR spectrum of PBLC-OH with the ^{13}C NMR spectra of PBC-OH and PBL-OH and the NMR peaks of PBLC-OH still could be assigned region by region as follows. There were four regions: the benzyloxy region (designated as the "B" region); the carbonyl region; the carbon 2, 3, and 4 region; and the carbon 5, 6, 7, 8, and 9 region. To simplify the notation, we used * that meant that the ^{13}C NMR data came from the unit designed by *. For example, C_{10}, C-C*-L means that the carbon #10 data came from the C* unit (ϵ-oxycaproyl) in the C-C*-L sequence.

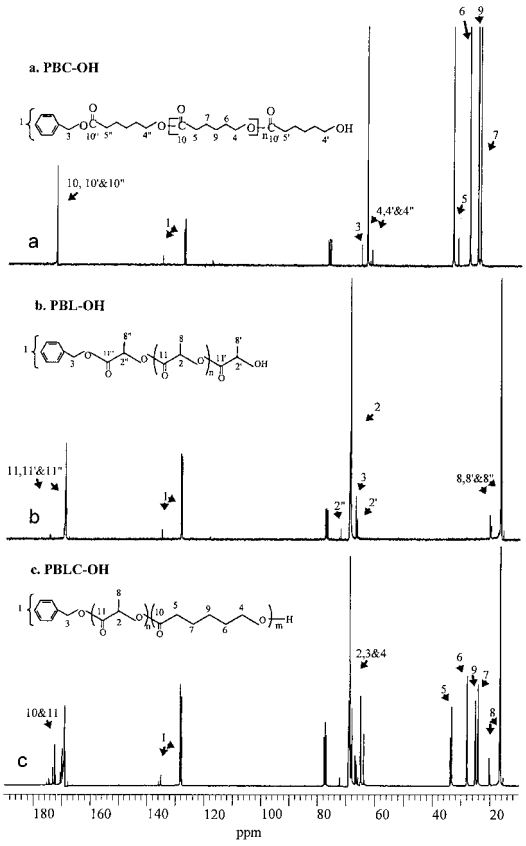


Figure 3. ^{13}C NMR spectra of the copolymers synthesized: (a) poly(ϵ-caprolactone) homopolymer (PBC-OH), (b) poly(L-lactide) homopolymer (PBL-OH), and (c) poly(L-lactide/ϵ-caprolactone) copolymer (PBLC-OH).

In the carbonyl region (Fig. 4), each group of peaks was assigned as follows. δ 175.360, 174.713, 174.503, and 174.325 (C_{11}, L*-OH); 173.274 (C_{10}, C-C*-C); 172.627 (C_{10}, C-C*-L); 172.400 (C_{10}, L-C*-L); 173.015 (C_{10}, B-C*); 170.702 and 170.524 (C_{11}, C-L*-C); 170.168, 170.039, 169.941, and 169.764 (C_{11}, L-L*-C and B-L*); 169.509, 169.440, 169.311, 169.197, and 169.084 (C_{11}, L-L*-L); 167.920 (residual lactide monomer).

In the B region, δ 136.087, 135.408, 135.198, 128.517, 128.388, 128.129, and 128.016 were assigned as carbon in the benzyl ring.

In the carbon 2, 3, and 4 region (Fig. 5), each group of peaks was assigned as follows. δ 69.317,

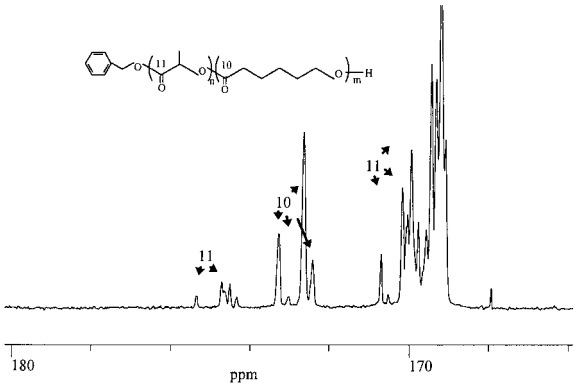


Figure 4. Carbonyl region of the ^{13}C NMR spectrum of poly(L-lactide/ϵ-caprolactone) copolymer (PBLC-OH).

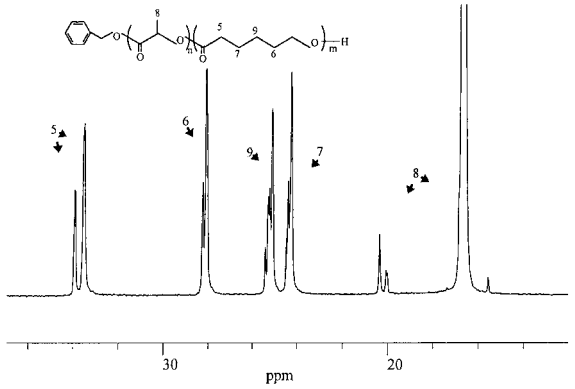


Figure 6. Carbon 5, 6, 7, 8, and 9 regions of the ^{13}C NMR spectrum of poly(L-lactide/ϵ-caprolactone) copolymer (PBLC-OH).

69.220, 69.042, 68.896, and 68.718 (C_2, L*-L); 68.411, 68.282, and 68.088 (C_2, L*-C); 66.988 and 66.745 (C_3, B*-L); 65.936 (C_3, B*-C); 66.616 and 66.502 (C_2, L*-OH); 65.111 and 64.885 (C_4, C*-L); 63.963 (C_4, C*-C).

In the carbon 5, 6, 7, 8, and 9 region (Fig. 6), each group of peaks was assigned as follows. δ 33.878 (C_5, C*-C); 33.457 (C_5, C*-L); 28.216 (C_6, C*-C); 28.055 (C_6, C*-L); 25.418, 25.272, and 25.208 (C_9, C*-C); 25.078 (C_9, C*-L); 24.464 and 24.382 (C_7, C*-C); 24.221 (C_7, C*-L); 20.339 (C_8, L*-B); 20.023 and 19.977 (C_8, L*-OH); 16.570 (C_8); 15.535 (residual lactide monomer).

Similar to the ^1H NMR spectra, the CH(CH_3)—H end group from the α-oxypropionyl unit (L) was detectable at 66.502 ppm in the ^{13}C NMR spectra besides the benzyl ester end group. However, the CH_2—OH end group from the ϵ-oxycaproyl unit (C) was too little to be detected.

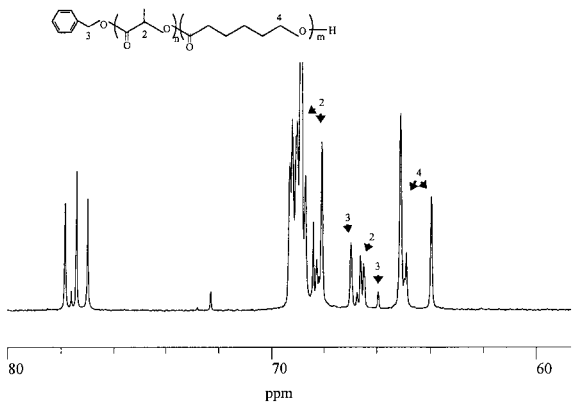


Figure 5. Carbon 2, 3, and 4 regions of the ^{13}C NMR spectrum of poly(L-lactide/ϵ-caprolactone) copolymer (PBLC-OH).

In contrast with the proton data, all carbon NMR data were sensitive to the sequence effect. When the α-oxycaproyl unit was considered as the * unit (C*), the carbonyl carbon C_{10} showed a triad (Fig. 4). The peak at 173.247 ppm could be assigned to the signals of C_{10} in the C-C*-C sequence by comparison with the corresponding carbonyl carbon signals in the PBC-OH homopolymer [Fig. 3(a)]. The PBLC-OH copolymer that had a high molar fraction of α-oxypropionyl unit (X_L = 0.82) showed a strong signal at 172.627 ppm for C_{10} in the L-C*-C sequence and a weak signal at 172.400 ppm for the L-C*-L sequence. This observation indicated that only a small fraction of C unit appeared in the C-C*-C sequence; most of the C unit existed in the C*-L sequence.

When the α-oxypropionyl unit was considered as the * unit (L*), a more complicated but similar phenomenon was observed. The carbonyl carbon C_{11} revealed a tetrad (Fig. 4). By comparison with the corresponding carbonyl carbon signals in PBL-OH homopolymer [Fig. 3(b)], the peaks from 175.360 to 174.325 ppm in the PBLC-OH copolymer were assigned to C_{11} of the end α-oxypropionyl unit L*-OH, and the peaks from 169.569 to 169.084 ppm were assigned to the L-L*-L sequence. Other carbonyl carbon signals, 170.702–170.524 ppm were assigned to the C-L*-C sequence, and 170.168–169.764 ppm were assigned to the L-L*-C sequence. The strongest peaks appeared at 169.440–169.197 ppm, demonstrating that most L units preferred to connect to the L unit when PBLC-OH copolymer had a high molar fraction of the α-oxypropionyl unit (X_L = 0.82).

The more complicated but more informative signals appeared at the region from 69.317 to

Table II. Molecular Weights[a] of Polymers Synthesized

Polymers	M_n ($\times 10^3$)	M_w ($\times 10^3$)	M_p ($\times 10^3$)	Polydispersity (M_w/M_n)
PBLC-OH	3.28	4.73	4.80	1.44
PBLC-Ma	2.93	7.05	6.53	2.41
PBLCA	4.61	7.17	9.21	1.56
TAM-PBLCA	1.61	4.55	4.05	2.83

[a] Determined by gel permeation chromatography (GPC).
PBLC-OH: benzyl alcohol end-capped poly(L-lactide/ε-caprolactone) copolymer.
PBLC-Ma: maleic acid end-capped poly(L-lactide/ε-caprolactone) copolymer.
PBLCA: poly(L-lactide/ε-caprolactone/acrylic acid) copolymer.
TAM-PBLCA: nitric oxide derivative attached poly(L-lactide/ε-caprolactone/acrylic acid) copolymer, where TAM is nitric oxide derivative, 4-amino-2,2,6,6-tetramethylpiperidine-1-oxy.

63.963 ppm (Fig. 5). In this area, there were three kinds of carbons—methine carbon C_2 of the α-oxypropionyl unit, ϵ-methylene carbon C_4 of the ϵ-oxycapronyl unit, and methylene carbon C_3 of the benzyloxy unit. By comparing the ^{13}C NMR data of PBLC-OH copolymer in Figure 5 with the corresponding ^{13}C NMR spectra of PBC-OH homopolymer and PBL-OH homopolymers [Figs. 3(a,b)], the peaks at 69.317–68.411 ppm in Figure 5 were assigned to C_2 in the L*-L sequence (including the B-L*-L and L-L*-L sequences). δ 66.988 and 66.745 ppm were assigned to C_3 in the B*-L sequence, δ 65.936 ppm to C_3 in the B*-C sequence, and 63.963 ppm to C_4 in the C-C*-C sequence. The other peaks, δ 68.411–68.088 ppm were assigned to C_2 in the L-L*-C and B-L*-C sequences. δ 66.616 and 66.936 ppm were assigned to C_2 of the end L unit (L*-OH), δ 65.111 and 64.885 ppm to C_4 in the C-C*-L and L-C*-L sequences.

Both the benzyl ester end group and the —$CH(CH)_3$—OH end group of the L unit were found in both the ^1H NMR and ^{13}C NMR spectra of PBLC-OH. This observation suggested that the polymerization mechanism was consistent with the one Kricheldorf et al.[19] suggested. The coordination between $SnOct_2$ and benzyl alcohol and the subsequent binding of this complex alcohol with lactone monomer would catalyze the nucleophilic addition reaction between the complex alcohol and the lactone monomer. It was the characteristic of this mechanism that the propagation steps involved exclusively the free orbital of the catalyst, and neither covalent nor ionic bonds were involved.

Double Bond-Terminated Polylactide-*co*-ϵ-caprolactone (PBLC-Ma)

PBLC-Ma was synthesized by reacting hydroxyl-terminated PBLC-OH copolymer with maleic anhydride in the melt for 24 h at 130 °C (Scheme 1). As a result, the hydroxyl functionality in PBLC-OH was converted to a maleic monoester acid. It appeared that slight chain fragmentation on PBLC-OH also occurred as evidence in a wider molecular weight distribution D (D from 1.44 of PBLC-OH to 2.41 of PBLC-Ma) and the reduction in number-average molecular weight (M_n, 11% reduction) (Table II). The weight-average molecular weight (M_w), however, showed 49% increase. It is unclear why M_n and M_w showed opposite dependence on the maleic anhydride reaction.

The ^1H NMR spectra of PBLC-Ma are illustrated in Figure 7. These ^1H NMR data showed several distinctive peaks at 6.546–6.312 ppm that were the characteristics of double bonds in the maleic acid, which were absent in PBLC-OH, an indication that the maleic acid segment had been successfully attached onto the PBLC-OH chain ends. However, there were some unexpected peaks at the δ 7.052–7.031 ppm and 6.947–6.883 ppm regions. These unexpected peaks of PBLC-Ma at 6.947–7.052 ppm could be assigned

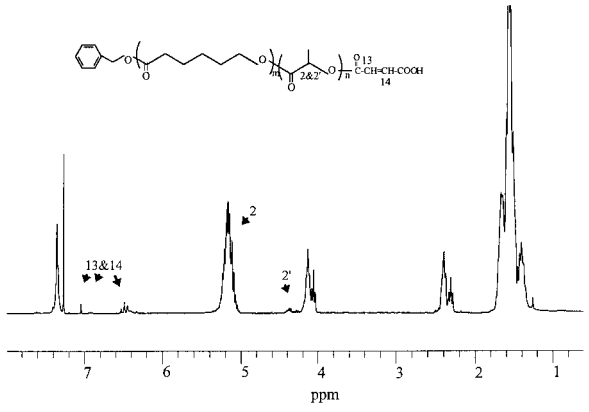


Figure 7. ^1H NMR spectrum of maleic acid end-capped poly(L-lactide/ϵ-caprolactone) copolymer (PBLC-Ma).

to the double bond segments existing in the fumaric monoester acid resulting from the rearrangement reaction of maleic monoester acid (Scheme 4). It is well known that maleic acid can be converted in part into fumaric acid by a rearrangement reaction when heated to a temperature slightly above its melting point.[22] Furthermore, the δ's of these unexpected peaks in PBLC-OH were within the ^{1}H NMR signals of the double bond region of the fumaric monoester acid (between 7.1 and 6.6 ppm).[23] The ratio of the peak integral at 6.947–7.052 ppm (for fumaric monoester acid) to 6.546–6.312 ppm (for maleic monoester acid) was used to calculate the content of fumaric monoester acid, and the calculation indicated that 13% of the double bond in PBLC-Ma had been rearranged from maleic monoester acid to fumaric monoester acid.

It was also observed that not all of the hydroxyl end groups of PBLC-OH had been esterified by maleic anhydride. In the ^{1}H NMR spectrum of PBLC-Ma, the intensity of the methine group adjacent to the terminal hydroxyl group $H_{2'}$ was reduced significantly when compared with the corresponding NMR spectrum of PBLC-OH [Fig. 2(c)], but these peaks did not disappear completely. Only 54% of the hydroxyl end groups in the PBLC-OH copolymer reacted with maleic anhydride and converted to double bond functionality.

The ^{13}C NMR spectra of PBLC-Ma are given in Figure 8. The characteristic peaks for the double bond carbons (C_{13} and C_{14}) appeared at δ 136.395, 134.195–134.065, and 129.342–129.116 ppm. The peaks for the carbonyl C_{12} and C_{15} were at 165.541 and 165.072 ppm, respectively. As expected, the signals at 66.502–66.616 ppm for the methine $C_{2'}$ of the L unit adjacent to the terminal hydroxyl functionality were reduced significantly, but these peaks did not disappear completely. The peaks at 70.077–69.705 ppm were assigned to the terminal methine carbon 2″ in the C unit (ϵ-oxycaproyl unit) adjacent to the maleic monoester acid.

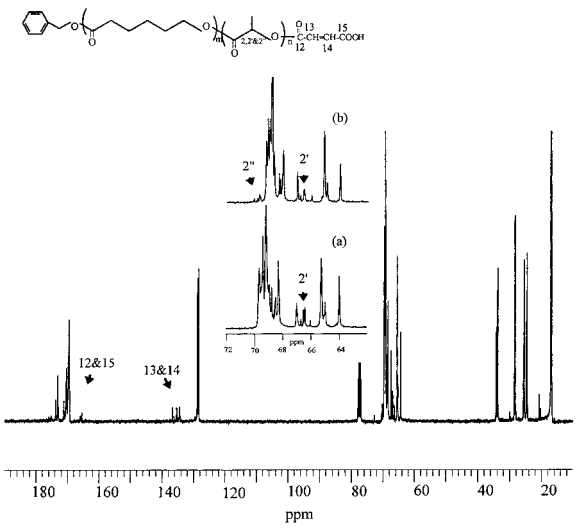
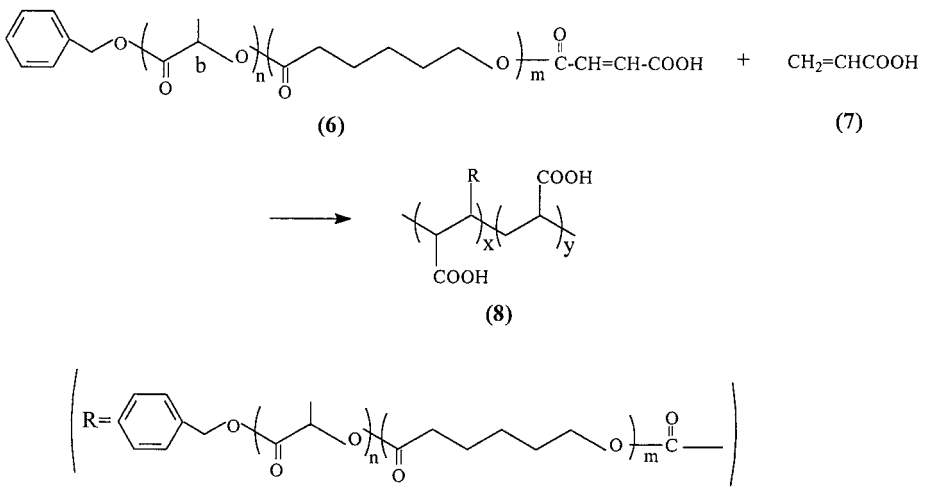


Figure 8. ^{13}C NMR spectrum of maleic acid end-capped poly(L-lactide/ϵ-caprolactone) copolymer (PBLC-Ma).

When compared with the reaction of oilgo(ϵ-caprolactone)s with maleic anhydride,[24] PBLC-OH copolymer was found to be more difficult to react with maleic anhydride. Under the same reaction condition, the hydroxyl functionality of the oilgo(ϵ-caprolactone)s was completely converted to double bond functionality. On the contrary, only 54% hydroxyl functionality of PBLC-OH had been reacted. Because 95% of PBLC-OH copolymer had the α-oxypropionyl end group (L unit), it could be considered that the α-oxypropionyl unit end group [—COCH(CH_3)—OH] was more difficult than the ϵ-oxycaproyl unit end group [—CO(CH_2)$_4$$CH_2$—OH] to react with maleic anhydride. In other words, the hydroxyl functionality adjacent to the L unit had a much lower chemical reactivity than that adjacent to the C unit. This difference in reactivity may be mainly attributed to the different reactivity between primary and second alcohols with maleic anhydride. In addition, the —OCO— group that was adjacent to the α-oxypropionyl end group was a stronger electron-withdrawing group, whereas the —OCO— group in the C unit was four methylene groups away, and hence, its electron-withdrawing effect was significantly smaller as a result of the distance. The —CH_3 group in the L unit, however, was a weak electron donor and could counteract somehow this electron-withdrawing effect.

Poly(acrylic acid/lactide/ϵ-caprolactone) Copolymer (PBLCA)

PBLCA copolymer was synthesized by free-radical polymerization of acrylic acid (Aa) with double bond-functionalized PBLC-Ma (Scheme 2). Because PBLC-Ma has only one double bond functionality to take part in the polymerization, the resulting PBLCA could be considered as a graft copolymer with poly(acrylic acid) as the main backbone and PBLC as the graft segment. The variation of molecular weight from PBLC-Ma to PBLCA is found in Table II. M_n increased after



Scheme 2. Synthesis of poly(L-lactide/ϵ-caprolactone/acrylic acid) copolymer (PBLCA, 8) by radical polymerization of the maleic acid end-capped polylactide/ϵ-caprolactone copolymer (PBLC-Ma, 6) and acrylic acid (7).

the copolymerization of PBLC-Ma with acrylic acid, but the increment of M_n was less than one time; thus, the average graft number of PBLC was no more than two. It should be possible to control the content of carboxyl group and the molecular weight of PBLCA by varying the reaction condition.

The ^{1}H NMR spectrum of PBLCA in DMSO-d_6 is shown in Figure 9. By comparison these NMR data (Fig. 9) with the ^{1}H NMR spectrum of PBLC-Ma (Fig. 7), the characteristic signals for the double bond group at 7.052–7.031 and 6.546–6.312 ppm that were originally existed in PBLC-Ma disappeared in PBLCA, and new signals for the acrylic acid segments appeared. The peak at 1.745 ppm was assigned to the methylene hydrogen H_{16}, and 12.245 ppm was assigned to the carboxyl hydrogen H_{18}. The signals for the methine hydrogen of the acrylic acid segment H_{17} were overlapped with the peaks of α-methylene hydrogen of the ϵ-oxycaproyl unit at 2.513–2.219 ppm. The peaks at 3.401 and 2.486 ppm were the solvent signals of the residual hydrogen of DMSO-d_6.

The ^{13}C NMR spectrum of PBLCA (Fig. 10) was more informative with respect to the chemical structure of PBLCA. The characteristic peaks of the double bond carbons at 136.395, 134.195–134.065, and 129.342–129.116 ppm disappeared completely in PBLCA (Fig. 10 vs Fig. 8). The corresponding signals at 165.541 and 165.072 ppm for

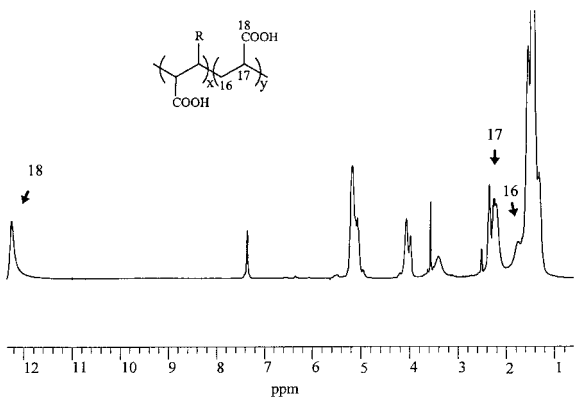


Figure 9. ^{1}H NMR spectrum of poly(L-lactide/ϵ-caprolactone/acrylic acid) copolymer (PBLCA).

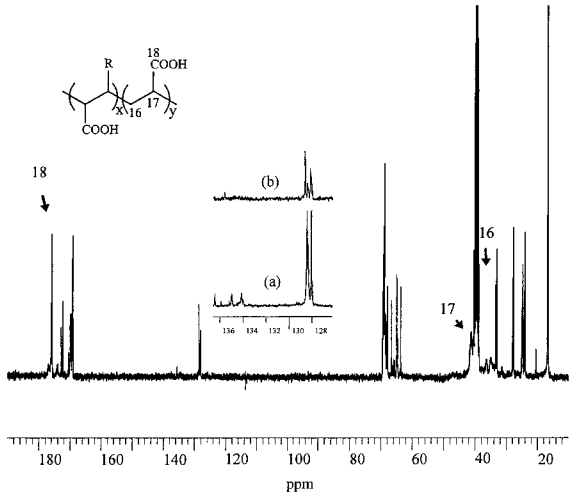


Figure 10. ^{13}C NMR spectrum of poly(L-lactide/ϵ-caprolactone/acrylic acid) copolymer (PBLCA).

the carbonyl C_{12} and C_{15} disappeared in PBLCA as compared with PBLC-Ma (Fig. 8). This indicated that all the double bond groups in PBLC-Ma had polymerized. The new signals for the poly(acrylic acid) appeared as follows. The multipeaks at δ 177.268–176.686 and 176.346–175.651 ppm were assigned to the carboxyl carbons. The peaks at δ 41.852–40.460 ppm were assigned to the methine C_{17} adjacent to the carboxyl group; δ 36.546–35.899, 35.252–34.460, and 31.532–31.079 ppm were assigned to methylene C_{16}.

Nitroxyl-Radical-Incorporated Poly(acrylic acid/lactide/ϵ-caprolactone) (TAM-PBLC)

TAM-PBLCA was synthesized by reacting TAM with PBLCA. Tempamine nitroxyl radicals were chemically incorporated into the carboxyl groups via an amide linkage. The content of TAM was as high as 8.32 wt % calculated by nitrogen elemental analysis.

Because of the free-radical characteristic of TAM nitroxyl radicals in TAM-PBLCA, TAM-PBLCA was expected and found to exhibit an EPR spectrum that had the characteristic of nitroxyl radicals (Fig. 11). This EPR spectrum showed two types of signals. One (peak a) had a relatively broad and short line width, whereas the other (peak b) showed a sharp, narrower and taller line width that was the characteristic EPR spectrum of the nitroxyl radicals. Peak b was believed to come from those TAMs attached to the —COOH in the *y* block, that is, the acrylic acid block (Scheme 3), whereas peak a was from the TAM that attached to —COOH in the *x* block that has a large PBLC chain segment as a pendant group. Hence, the broadening of an EPR spectrum shown in peak a was expected to be attributed to the restricted tumbling motion of TAM because the steric influence of the large PBLC segment in the *x* block would greatly limit the free-radical motion.

The FTIR spectrum of TAM-PBLCA is depicted in Figure 1(d). The peak at 1640 cm^{-1} for the absorption of N—H in the amine group of TAM [Fig. 1(e)] moved to 1580 cm^{-1} for the N—H absorption in the amide group of TAM-PBLCA. The other new peak at 1690 cm^{-1} was attributed to the absorption of the amide carbonyl group.

The NMR spectra gave more information about the TAM attachment reaction. In the ^1H NMR spectrum of TAM-PBLCA (Fig. 12), the characteristic peak at 12.248 ppm for the carboxyl group disappeared completely, whereas the new peaks

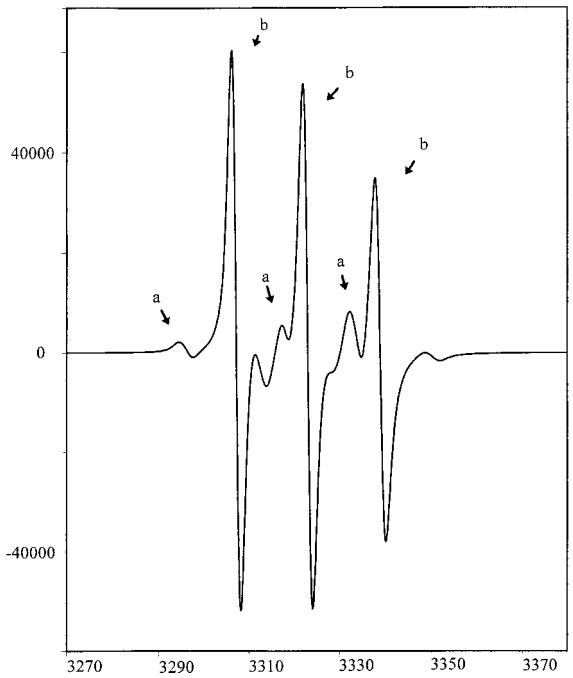


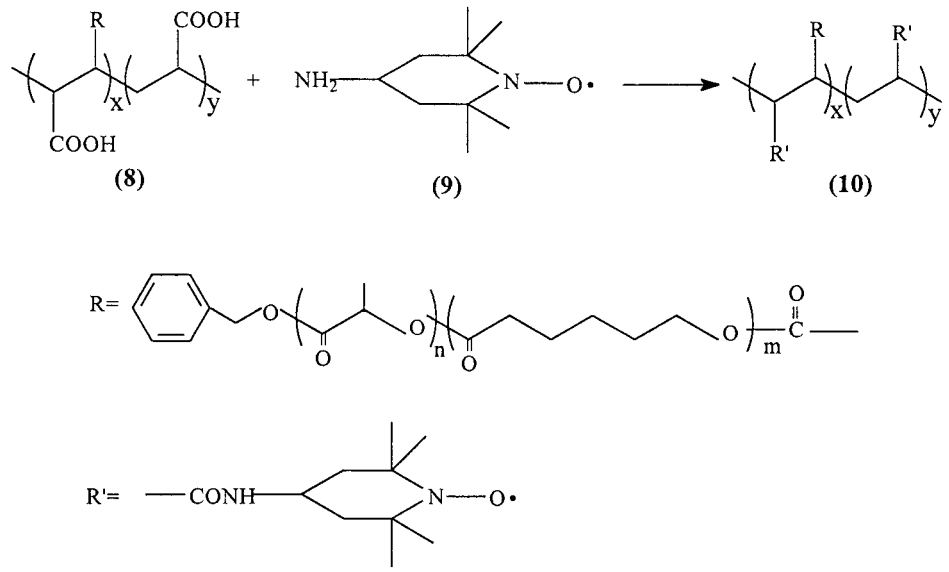
Figure 11. ESR spectra of nitric oxide derivative-attached poly(L-lactide/ϵ-caprolactone/acrylic acid) copolymer (TAM-PBLCA). Peak a: TAM attached on the maleic acid segment in the *x* block and peak b: TAM attached on the poly(acrylic acid) segment in the *y* block (refer to Scheme 2 for *x* and *y* blocks in PBLCA).

at 8.320 to 8.048 ppm for the amide group appeared. In agreement with the results of the EPR test, the ^1H NMR spectrum showed two kinds of amides—one was the product from the reaction between the carboxyl group of the acrylic acid unit and TAM in the *y* block, and the other was obtained from the reaction between the carboxyl group of maleic acid and TAM in the *x* block. Other new signals at 4.226 ppm were assigned to the methine hydrogen of the TAM group—2.082 ppm for the TAM methylene hydrogen and 0.961–0.707 ppm for the methyl hydrogen of TAM. The weak peaks at 2.980 and 1.067 ppm were assigned to the residual free TAM.

A characteristic carbonyl region of the ^{13}C NMR spectra is shown in Figure 13. The signals of the carbonyl carbon at 175.984–175.838 ppm in PBLCA [Fig. 13(b)] moved to 176.443 ppm in TAM-PBLCA [Fig. 13(a)] when the carboxylic acid group in PBLCA was converted into the amide group via the TAM attachment.

CONCLUSION

Polylactide/ϵ-caprolactone with benzyl ester and terminal hydroxyl end groups were successfully

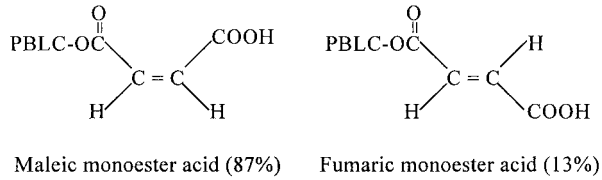


Scheme 3. Synthesis of nitric acid derivative-attached poly(L-lactide/ϵ-caprolactone/acrylic acid) copolymer (TAM-PBLCA, 10) from the reaction of poly(L-lactide/ϵ-caprolactone/acrylic acid) copolymer (PBLCA, 8) with 4-amino-2,2,6,6-tetramethylpiperidine-1-oxy (TAM, 9).

synthesized by bulk polymerization in the presence of benzyl alcohol using stannous octoate as a catalyst. ^{1}H NMR spectroscopy revealed that the molar fraction of the ϵ-oxycaproyl unit (C) was significantly lower at the chain end than other locations of the PBLC copolymer. It was proposed that the polymerization rate of ϵ-caprolactone was higher than that of lactide at 130 °C.

The hydroxyl-functionalized ϵ-caprolactone homopolymer could be completely converted to the maleic monoester acid via its reaction with maleic anhydride. However, it is more difficult for the PBLC-OH copolymer (with 95% α-oxypropionyl end group and 5% ϵ-oxycaproyl end group) to react with maleic anhydride because of the electronic influence and the steric influence. However, 3% maleic monoester acids were converted into fumaric monoester acid during the reaction between the hydroxyl functionality of PBLC-OH and PBL-OH with maleic anhydride at 130 °C for 24 h.

The double bond of PBLC-Ma was highly reactive, and it was not difficult to carry out the free-radical polymerization of acrylic acid and PBLC-Ma to obtain PBLCA. TAM was chemically incorporated into the carboxylic acid sites of PBLCA in the mild condition. In this study, the pendant carboxylic acid groups in PBLCA were quantitatively converted to amide linkages via TAM, and the TAM content in TAM-PBLCA was as high as 8.32 wt %.



Scheme 4. Two kinds of double bond-terminated poly(ϵ-caprolactone)s.

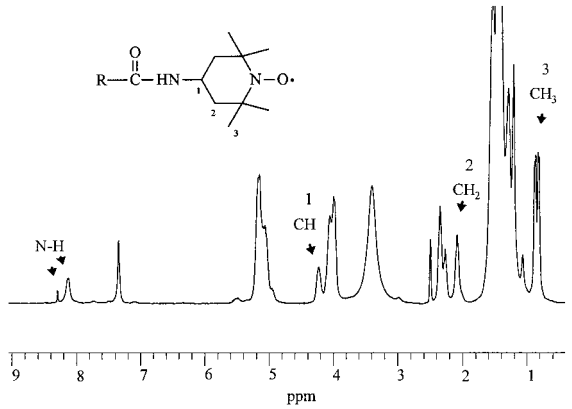


Figure 12. ^{1}H NMR spectrum of nitric oxide derivative-attached poly(L-lactide/ϵ-caprolactone/acrylic acid) copolymer (TAM-PBLCA).

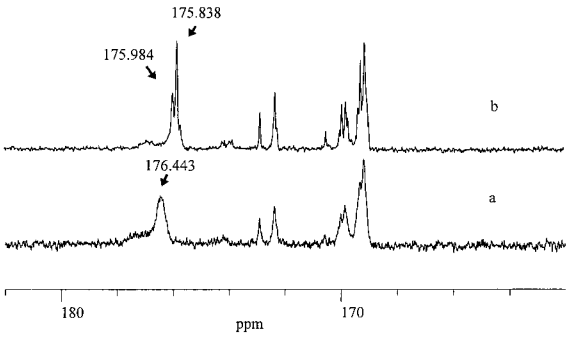


Figure 13. Comparison of the carbonyl carbon region of ^{13}C NMR spectra between (a) nitric oxide derivative-attached poly(L-lactide/ϵ-caprolactone/acrylic acid) copolymer (TAM-PBLCA) and (b) poly(L-lactide/ϵ-caprolactone/acrylic acid) copolymer (PBLCA).

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REFERENCES AND NOTES

1. Schindler, A.; Jeffcoat, R.; Kinmel, G. L.; Pitt, C. G.; Zweidinger, R. Contemp Top Polym Sci 1979, 2, 25.
2. Alexander, H.; Parsons, J. R.; Strauchler, I. D.; Corcoran, S. F.; Gona, O.; Mayott, C.; Weis, A. B. Orthop Rev 1981, 10, 41.
3. Barrows, T. H. Clin Mater 1986, 1, 233.
4. Jedlinki, Z.; Kurcok, P.; Walach, W.; Janeczek, H.; Radecka, I. Makromol Chem 1993, 194, 1081–1089.
5. Lee, K.; Chu, C.; Quimby, F.; Klaessig, S. Macromol Symp 1998, 130, 71–80.
6. Karupiah, G.; Xie, Q. W.; Buller, R.; Nathan, C.; Duarte, C.; Macmicking, J. D. Science 1993, 261(5127), 1445.
7. Park, K. H.; Rubin, L. E.; Gross, S. S.; Levi, R. Circ Res 1992, 71(4), 992.
8. Wallace, J. L., et al. Eur J Pharmacol 1995, 280(1), 63.
9. Moilamant, E., et al. Ann Med 1995, 27(3), 359.
10. Liu, R. H., et al. Mutat Res 1995, 339(2), 73.
11. Dusting, G. J., et al. J Vasc Res 1995, 32(3), 143.
12. Schmidt, H. H., et al. Cell 1994, 78, 919.
13. Francoeur, C., et al. Inflammation 1995, 19(5), 587.
14. Lang, M.; Bei, J.; Wang, S. J Biomater Sci Polym Ed 1999, 10(4), 501–512.
15. Kricheldorf, H. R.; Meier-Haack, J. Makromol Chem 1993, 194, 715.
16. Zhu, K. J.; Lin, X.; Yang, S. J Appl Polym Sci 1990, 39, 1.
17. Sawhney, A.; Pathak, C. P.; Hubbell, J. A. Macromolecules 1993, 26(581), 1.
18. Nijenhuis, A. J.; Grijpma, D. W.; Pennings, A. J. Macromolecules 1992, 25, 6419.
19. Kricheldorf, H. R.; Kreiser-Saunders, I.; Boettcher, C. Polymer 1995, 36(6), 1253–1259.
20. Stevels, W. M.; Ankone, M. J. K.; Dijkstra, P. J.; Feijen, J. Macromolecules 1996, 29, 6132–6138.
21. Stevels, W. M.; Pintat, S.; Slaghek, T. M. Polym Bull 1999, 42, 257–264.
22. Windholz, M. The Merck Index, 9th ed.; Merck & Co., Inc., 1976; p 740.
23. Standard Proton NMR Spectra Collection, Sadtler Research Laboratories, pp 44481–44490.
24. Lang, M.; Chu, C. C. Synthesis and Structural Analysis of Three-Arm Poly(ϵ-caprolactone)s and Their Cross-Linked Network, in preparation.