Synthesis and Characterization of Degradable Copoly(*trans*-4-hydroxy-L-proline/ϵ-caprolactone)

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ABSTRACT: The melt polycondensation reaction of *N*-protected *trans*-4-hydroxy-L-proline (*N*-Z-Hpr) and ϵ-caprolactone (ϵ-CL) over a wide range of molar fractions in the feed produced new and degradable poly(*N*-Z-Hpr-*co*-ϵ-CL)s with stannous octoate as a catalyst. The optimal reaction conditions for the synthesis of the copolymers were obtained with 1.5 wt % stannous octoate at 140°C for 24 h. The synthesized copolymers were characterized by IR spectrophotometry, ^{1}H NMR, differential scanning calorimetry, and Ubbelohde viscometry. The values of the inherent viscosity (η_{inh}) and glass-transition temperature (T_g) of the copolymers depended on the molar fractions of *N*-Z-Hpr. With an increase in the *trans*-4-hydroxy-*N*-benzyloxycarbonyl-L-proline (*N*-CBz-Hpr) feed from 10 to 90 mol %, a decrease in η_{inh} from 2.47 to 1.05 dL/g, and an increase in T_g from −48 to 49°C were observed. The *in vitro* degradation of these poly(*N*-CBz-Hpr-*co*-ϵ-CL)s was evaluated from weight-loss measurements. © 2003 Wiley Periodicals, Inc. J Appl Polym Sci 88: 3176–3182, 2003

INTRODUCTION

Biodegradable polymers are widely used as drug carries for controlled drug release and as operation-repairing materials in medical surgery.[1,2] Polycaprolactone (PCL) is one of the biodegradable polymers that can be degraded by ester-bond hydrolysis. Because of its good drug transportability, PCL is a potential biodegradable polymer for the field of biomedicine. However, the degradation rate of PCL is not fast because its crystallinity is too strong to be hydrolyzed and its hydrophobic nature causes trouble in the microencapsulation processes used for drug delivery systems.[3,4]

To improve the hydrophilicity and control the degradability of PCL, one may introduce comonomer and hydrophilic pendant functional groups into the polymer chain.[5–17] Feijen et al.[11] reported the copolymerization of ϵ-caprolactone (ϵ-CL or **2**) and morpholine-2,5-dione with a pendant hydrophilic functional group (MDP) with stannous octoate [Sn(II) Oct] as a catalyst. However, they failed to achieve copolymers with contents of MDP exceeding 20%. Wang and Feng[13] reported the copolymerization of ϵ-CL with (3*S*)-3-[(benzyloxycarbonyl)methyl]morpholine-3,5-dione (BMD). The conversion of BMD was higher (>80%), and the number-average molecular weight of the resulting copolymers ranged from 5800 to 43,000. However, the reaction process is very complicated. Recently, Jérôme and coworkers[14–18] reported that the ring-opening polymerization of functional lactones, such as γ-ethylene ketal-ϵ-caprolactone, γ-(triethylsilyloxy)-ϵ-caprolactone, and γ-bromo-ϵ-caprolactone, yielded polyesters containing ketal, ketone, alcohol, and bromide functional groups.

Pseudopoly(amino acid)s belong to the newest class of biodegradable polymers, having the advantages of being nontoxic, biodegradable, biocompatible, and fitted with pendant functional groups on the backbone. In previous articles,[19–22] we reported the synthesis of the pseudopoly(amino acid)s of *trans*-4-hydroxy-L-proline (Hpr). The objective of this study was to investigate the polycondensability of Hpr with ϵ-CL. These new copolymers were identified by ^{1}H-NMR, IR, and differential scanning calorimetry (DSC). The effects of the comonomers on the inherent viscosity (η_{inh}), glass-transition temperature (T_g), and rate of degradation were also examined.

EXPERIMENTAL

Materials

Hpr, benzyloxychloroformate, acetic anhydride, acetic acid, and ϵ-CL were purchased from Aldrich Chemical Co. (Milwaukee, WI) Sn(II) Oct was purchased from Strem Chemical Co. (Newburyport, MA) Organic solvents (tetrahydrofuran, methanol, chloroform, *N*,*N*-dimethylformamide, and ethyl acetate) and inorganic compounds (sodium sulfate and sodium bicarbonate)

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were reagent-grade and were purchased from Merck Chemical Co. (Darmstadt, Germany).

Characterization

IR spectra were measured on a Jasco IR Report-100 IR spectrophotometer (Tokyo, Japan). Samples were either neat on NaCl plates or pressed into KBr pellets. ^{1}H-NMR spectra were recorded at 500 MHz (with a Brucker WB/DMX-500 spectrometer, Ettlingen, Germany) with tetramethylsilane as an internal standard in chloroform-*d*. Elemental analyses were run on a PerkinElmer model 2400 CHN analyzer (Wellesley, MA). The η_{inh} values were measured with an Ubbelohde viscometer at 30°C. A thermal analysis of the polymers was performed on a DuPont 9900 system that consisted of a differential scanning calorimeter (Newcastle, DE). The heating rate was 20°C/min. T_g's were read at the middle of the change in the heat capacity and were taken from the second heating scan after quick cooling.

Synthesis of the *N*-protected *trans*-4-hydroxy-L-proline monomers (*N*-Z-hpr or 1)

trans-4-Hydroxy-*N*-(1-oxoethyl)-L-proline (*N*-Ace-Hpr or **1a**) and *trans*-4-hydroxy-*N*-benzyloxycarbonyl-L-proline (*N*-CBz-Hpr or **1b**) were prepared according to the method described in our previous article.[17]

Synthesis of copolymer 3

The polymerization was conducted in a round flask with a sidearm. The purified monomer **1** (10 mmol) and **2** (10 mmol) as a comonomer were added to the flask. Then, the catalyst Sn(II) Oct (1.5 wt %) was added. The flask was purged with nitrogen, and the reaction was carried out at 140°C (or 200°C). The reaction was carried out initially *in vacuo* (160 mmHg) for 21 h and subsequently *in vacuo* (40 mmHg) for 3 h. The crude polymer was dissolved in tetrahydrofuran and then precipitated into *n*-hexane with stirring. After purification, the polymer was dried *in vacuo* for 24 h and analyzed. Representative ^{1}H-NMR and IR spectra of **3** are shown in Figures 1 and 2, respectively. Elemental analyses of the representative **3** copolymers indicated that the experimental and calculated elements were approximately matched.

Copolymer **3A**

Elem. Anal. Found: C, 57.07%; H, 6.83%; N, 5.37%. Calcd.: C, 57.85%; H, 7.07%; N, 5.33%.

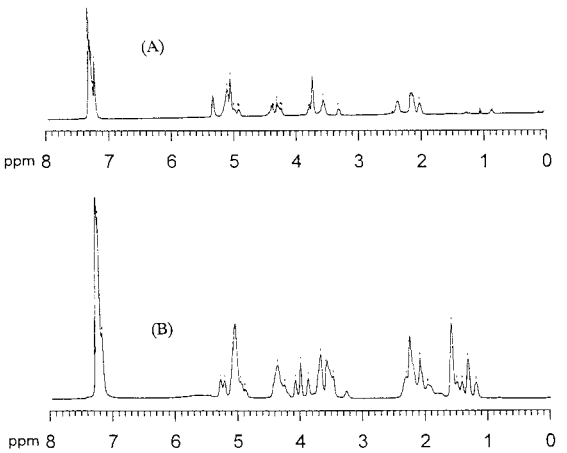


Figure 1 Representative ^{1}H-NMR spectra of (A) the poly(*N*-CBz-Hpr) homopolymer and (B) the copoly(*N*-CBz-Hpr-*co*-ϵ-CL) copolymer (**3G**; with a monomer composition of 70/30 mol %).

Copolymer **3B**

Elem. Anal. Found: C, 53.88%; H, 6.20%; N, 6.95%. Calcd.: C, 56.02%; H, 6.46%; N, 7.18%.

Copolymer **3E**

Elem. Anal. Found: C, 62.52%; H, 5.58%; N, 4.79%. Calcd.: C, 63.15%; H, 5.52%; N, 5.30%.

Copolymer **3F**

Elem. Anal. Found: C, 62.41%; H, 5.67%; N, 4.56%. Calcd.: C, 63.15%; H, 5.66%; N, 5.09%.

Copolymer **3G**

Elem. Anal. Found: C, 62.31%; H, 6.18%; N, 4.38%. Calcd.: C, 63.15%; H, 5.92%; N, 4.67%.

Copolymer **3I**

Elem. Anal. Found: C, 62.58%; H, 7.02%; N, 2.92%. Calcd.: C, 63.14%; H, 6.96%; N, 3.00%.

Copolymer **3J**

Elem. Anal. Found: C, 62.82%; H, 7.61%; N, 1.95%. Calcd.: C, 63.14%; H, 7.62%; N, 1.95%.

Copolymer **3K**

Elem. Anal. Found: C, 62.82%; H, 8.08%; N, 1.09%. Calcd.: C, 63.14%; H, 8.12%; N, 1.13%.

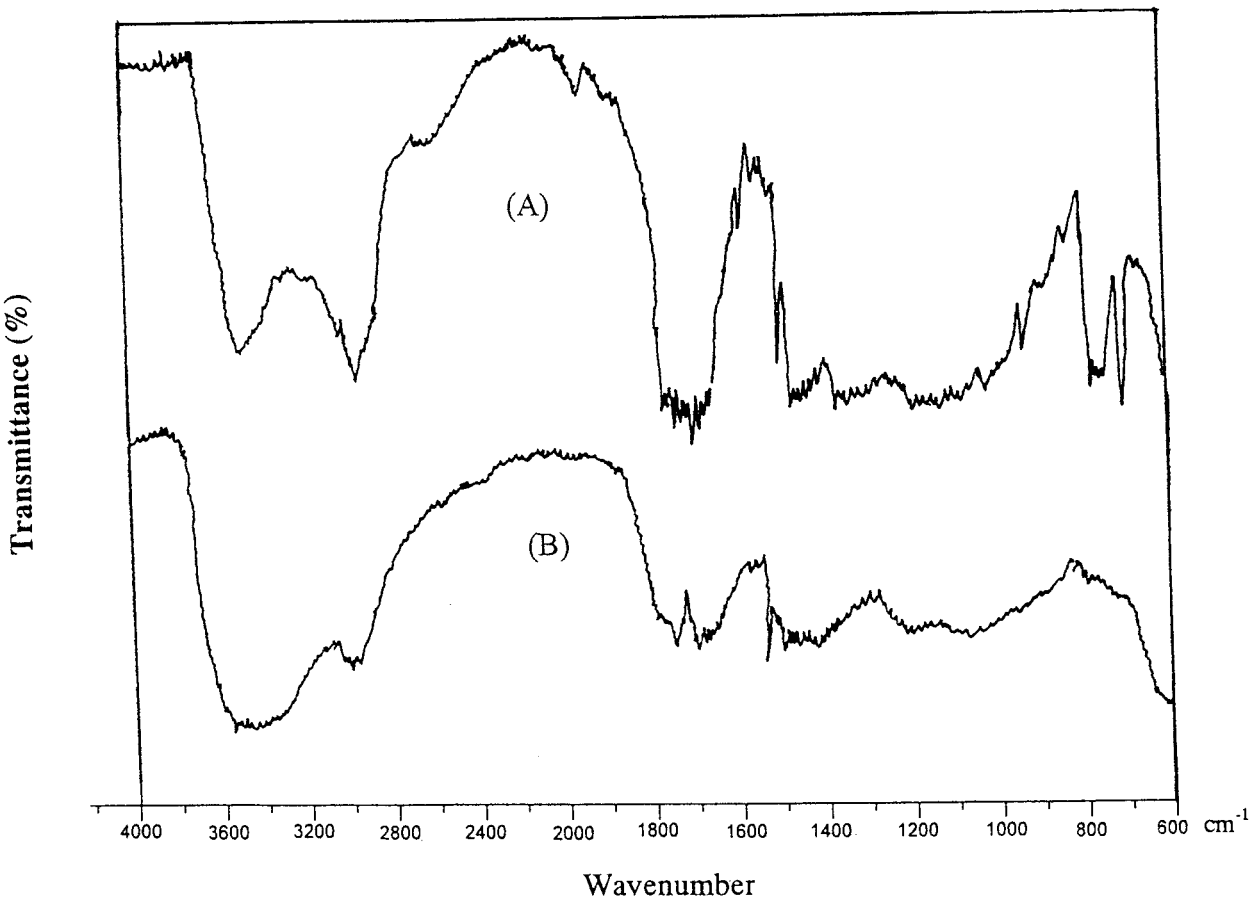


Figure 2 Representative IR spectra of **3G**: (A) the *N*-CBz protected copolymer and (B) the deprotected copolymer.

Deprotection of the amino-protecting group of the new copolymer

A 10 wt % palladium-on-charcoal catalyst (1 g) was added to a solution of poly(*trans*-4-hydroxy-*N*-benzyloxycarbonyl-L-proline-*co*-ϵ-caprolactone) (**3G**) in tetrahydrofuran (10 mL). With vigorous stirring, 1,4-cyclohexadiene was slowly added to the mixture. Stirring was continued at room temperature for 48 h, and then the palladium catalyst was removed by filtration. The solution was concentrated to a total volume of 3 mL by partial evaporation under reduced pressure. The concentrated solution was poured into *n*-hexane to precipitate, and the deprotected polymer poly(Hpr-*co*-ϵ-CL) (**4**) was obtained and then analyzed by IR [Fig. 2(B)].

Degradation of the copolymer

The N-protected copolymer films were prepared by a pressing technique. That is, 40 mg of copolymer powder was pressed into a solid pellet *in vacuo* for 5 min. For the degradation study, each film was placed in a small bottle containing 5 mL of M/15 phosphate buffer solutions (pH 7.4). The bottle was then incubated at 37°C. At time intervals, the specimen was removed, washed with distilled water, lyophilized, and weighed. The degree of degradation was estimated as follows:

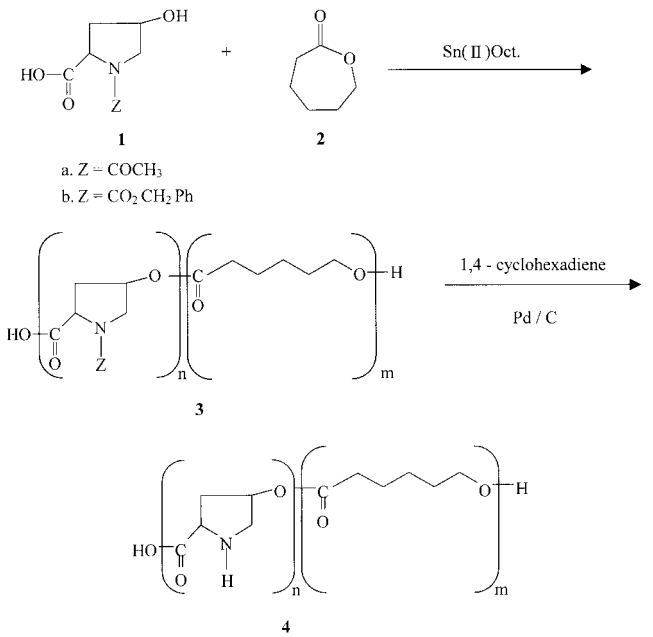
$$\text{Degree of degradation (\%)} = 100(D_0 - D)/D_0$$

where D_0 is the weight of the copolymer before degradation and D is the weight of the copolymer after degradation for a certain period.

RESULTS AND DISCUSSION

Copolymerization

The copolymerizations of **1** and **2** were investigated over a wide range of compositions via the ring-opening mechanism, in which the active hydroxy group of *N*-Z-Hpr induced a selective acyl-oxygen cleavage of the lactone ring, thereby forming an external ester block (Scheme 1). The polymerization was performed in bulk with Sn(II) Oct as a catalyst, and the results of the copolymerization are listed in Table I. For the determination of the optimum copolymerization conditions, the optimal catalyst level was determined by the copolymerization of **1a** and **2** with equivalent mo-



Scheme 1 Condensation copolymerization of **1** and **2**.

lar ratios at different catalyst concentrations ranging from 0 to 3 wt % at 200°C for 24 h. The results are shown in Figure 3. The ^{1}H-NMR compositions in the copolymers were about the same (52/48 mol %) in the four experiments. However, the η_{inh} values of the copolymers were greater in the presence of the catalyst than in the absence of the catalyst. The highest η_{inh} value of the copolymer (η_{inh} = 1.40 dL/g) was obtained with 1.5 wt % Sn(II) Oct. This was due to the degree of copolymerization of the comonomers being higher than the others when 1.5 wt % Sn(II) Oct was used as a catalyst. However, the yields of the copolymers did not show significant differences for all copolymerizations.

As the reaction temperature decreased from 200 to 170°C, η_{inh} decreased from 1.30 to 0.95 dL/g. However, T_g increased from 76 to 89°C (copolymers **3B** and **3D**). This may be due to the molar fraction of the hard segment *N*-Ace-Hpr in copolymer **3D** being higher than that in copolymer **3B** and the intermolecular interaction in copolymer **3D** being stronger than that in copolymer **3B** because of the larger fraction of the terminal carboxylic acid group in copolymer **3D**. However, in the poly(*N*-CBz-Hpr-*co*-ϵ-CL) system, the resulting copolymer turned brown at 200°C. This may be due to a decomposition of *N*-CBz-Hpr partially occurring during the polymerization. Therefore, the copolymerizations of *N*-CBz-Hpr with ϵ-CL were performed with 1.5 wt % Sn(II) Oct as a catalyst at 140°C for 24 h.

The effects of the monomer compositions in the feed on the copolymerization of **1** and **2** were investigated over a wide range of compositions. With an increase in the contents of *N*-Z-Hpr incorporated into the copolymers, a decrease in η_{inh} of the copolymers was observed. For the poly(*N*-Ace-Hpr-*co*-ϵ-CL) system, with an increase in the *N*-Ace-Hpr feed from 50 to 80 mol %, a decrease in η_{inh} from 1.42 to 1.30 dL/g (in methanol) was observed (copolymers **3A** and **3B**). A similar effect was seen for the poly(*N*-CBz-Hpr-*co*-ϵ-CL) system, with an increase in the *N*-CBz-Hpr feed from 10 to 90 mol % and a decrease in η_{inh} from 2.47 to 1.05 dL/g (in chloroform) (copolymers **3E**, **3F**, **3G**, **3H**, **3I**, **3J**, and **3K**). When the initiator 1,6-hexanediol (2 mol %) was added to the copolymerization, an increase in η_{inh} from 1.29 to 1.49 dL/g (in chloroform) and a

TABLE I
Results of the Melt Copolymerization of 1 and 2 with Variations of the Molar Fraction in the Feed[a]

Copolymer **3**	Polymerization temperature (°C)	Monomer composition in the feed (mol %)	^{1}H-NMR composition in the copolymer (mol %)	η_{inh} (dL/g)	T_g (°C)
N-Ace-Hpr/ε-CL[b]					
3A	200	50/50	54/46	1.42	70
3B	200	80/20	74/26	1.30	76
3C	200	20/80	24/76	0.71	−22
3D	170	80/20	79/21	0.95	89
N-CBz-Hpr/ε-CL[c]					
3E	140	90/10	87/13	1.05	49
3F	140	80/20	80/20	1.17	36
3G	140	70/30	68/32	1.21	24
3H	140	50/50	46/54	1.29	4
3I	140	30/70	34/66	1.91	2
3J	140	20/80	20/80	2.23	−34
3K	140	10/90	10/90	2.47	−48
N-CBz-Hpr/ε-CL/1,6-hexanediol[c]					
3L	140	49/49/2	44/54/2	1.49	1

[a] The reaction was performed with 1.5 wt % Sn(II) Oct as a catalyst for 24 h.
[b] η_{inh} was measured at a concentration of 0.1 g/dL in CH_3OH at 30°C.
[c] η_{inh} was measured at a concentration of 0.1 g/dL in $CHCl_3$ at 30°C.

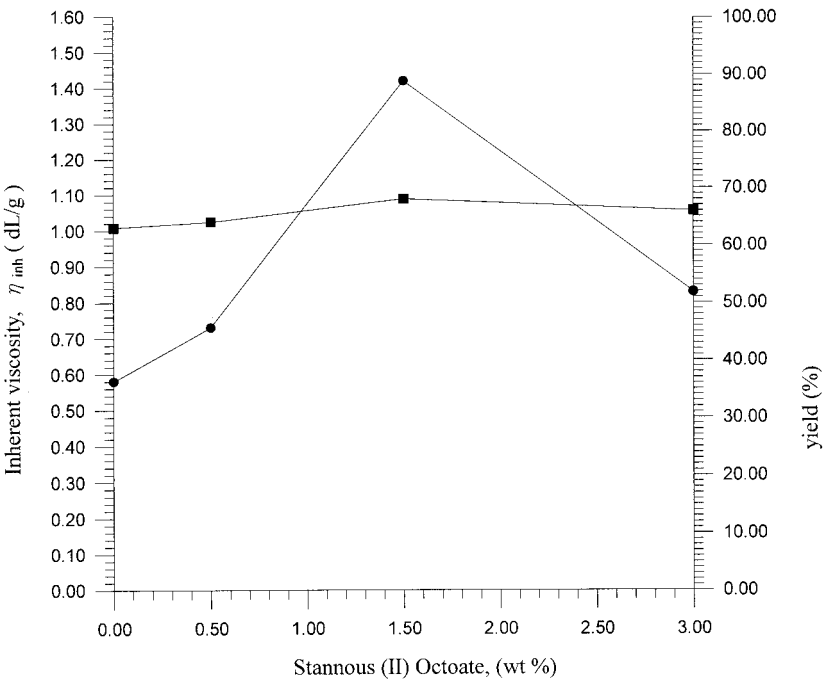


Figure 3 Effect of the amount (wt %) of the stannous(II) octoate catalyst on (●) η_{inh} and (■) the yield of copolymer **3** (with monomer compositions of around 52/48 mol %) prepared by the polycondensation of **1a** and **2** at 200°C for 24 h.

decrease in T_g from 4 to 1°C were observed (copolymers **3H** and **3L**).

The compositions in the copolymers were analyzed by ^{1}H-NMR. The amounts of the comonomer incorporated into the copolymer could be calculated from a comparison of the integral areas of the resonance peaks (δ = 5.15 ppm) of the methine proton (C4—H) of the proline with the absorption peaks (δ = 0.4–0.8 ppm) of the methylene protons (C3, C4, and C5) of ϵ-CL. The molar ratio percentages of the comonomers incorporated into the copolymers are shown in Table I. These results show that a degree of conversion of the comonomers close to the corresponding feeds.

Thermal analysis

Thermoanalytic measurements were made with DSC equipment. The T_g's of the copolymers are shown in Table I. According to DSC, *N*-Z-Hpr/ϵ-CL copolymers exhibited only T_g. Therefore, all the copolymers were amorphous. With an increase in the contents of *N*-Z-Hpr incorporated into the copolymers, an increase in T_g of the copolymers was observed. For *N*-Ace-Hpr/ϵ-CL copolymers, the values of T_g increased from −22 to 76°C when the molar ratio percentage of *N*-Ace-Hpr increased from 24 to 74 mol % (copolymers **3A**, **3B**, and **3C**). A similar result was observed for *N*-CBz-Hpr/ϵ-CL copolymers: the T_g's increased from −48 to 49°C when the molar ratio percentages of *N*-CBz-Hpr increased from 10 to 87 mol % (copolymers **3E**, **3F**, **3G**, **3H**, **3I**, **3J**, and **3K**). This is due to the fact that *N*-Z-Hpr is a hard component in comparison with ϵ-CL. Therefore, when more rigid linkages, such as cyclic proline groups, were incorporated into the macromolecular backbone, this restricted the rotation of the copolymer chain and caused an increase in T_g.

Deprotection of *N*-protected poly(*N*-CBz-Hpr-*co*-ϵ-CL)

The benzyloxycarbonyl (CBz) protecting group of the copolymer is usually removed by solvolysis with HBr/HOAc. However, the strong acid could lead to the chain scission of the polymer because of acid-catalyzed ester hydrolysis. To eliminate this problem of acid-catalyzed chain fragmentation, we adopted a different deprotection method: catalytic transfer hydrogenation. This method is generally used in peptide chemistry to yield free amine without degradation of the polymer chains. 1,4-Cyclohexadiene was used as an effective hydrogen donor under mild conditions. Palladium over activated carbon (10 wt %) was used as the catalyst for the hydrogenolysis of the aromatic CBz protecting group.

In a comparison with the ^{1}H-NMR spectrum of the *N*-protected copolymer, we found that the peaks around δ = 5.05 and 7.29 ppm, which were assigned to the hydrogen atoms of the benzyl protecting group, decreased in the spectrum of the deprotected copolymer after 68 h of hydrogenation. However, the data from the ^{1}H-NMR spectrum also revealed that about 32% of the benzyl group still remained unremoved. This was mainly due to the higher steric effect, which might have

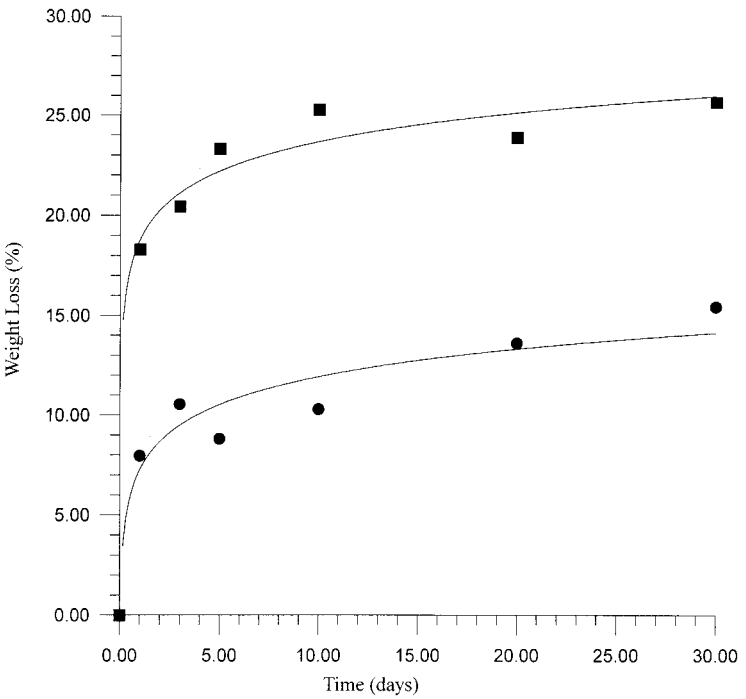


Figure 4 Weight losses of (●) homopoly(*N*-CBz-Hpr) and (■) copoly(*N*-CBz-Hpr-*co*-ϵ-CL) (**3E**; with a monomer composition of 90/10 mol %) treated in M/15 phosphate buffer solutions (pH 7.4) at 37°C.

prevented it from coming into contact with Pd/C powder.

Structural characterization

Representative ^{1}H-NMR spectra of the copolymer *N*-CBz-Hpr-*co*-ϵ-CL (**3G**) (with a monomer composition of 70/30 mol %) and the homopolymer of *N*-CBz-Hpr are shown in Figure 1. Characteristic resonance peaks at δ = 7.40–7.25 ppm (due to the protons of aromatic rings of the CBz protecting group), δ = 5.28–4.90 ppm (due to the C4 methine proton of Hpr and the benzylic protons of the CBz protecting group), δ = 4.41–4.15 ppm (due to the C2 methine proton of Hpr), δ = 4.09–3.89 ppm (due to the C6 methylene protons of ϵ-CL), δ = 3.71–3.40 ppm (due to the C5 methylene protons of Hpr), δ = 3.25 ppm (due to the hydroxy proton), δ = 2.39–1.80 ppm (due to the C3 methylene protons of Hpr and the C2 methylene protons of ϵ-CL), and δ = 1.58–1.20 ppm (due to the C3, C4, and C5 methylene protons of ϵ-CL) can be seen.

The representative IR spectra of the *N*-CBz protected copolymer **3G** and the deprotected polymer **4** exhibit strong ester carbonyl bands at 1738 and 1740 cm^{-1}, respectively. The most distinctive features of **4** were the almost complete absence of aromatic C—H (out-of-plane bending) absorption at 699 and 745 cm^{-1} from the CBz protecting group and the presence of a broad amino band (—NH) at 3400 cm^{-1}. This indicated the almost complete removal of the CBz group and the formation of the pendant amino group. Also, an elemental analysis of the copolymers indicated that the experimental and calculated values were close to each other.

Preliminary *in vitro* degradation study

The *in vitro* degradation of *N*-protected poly(*N*-CBz-Hpr-*co*-ϵ-CL) was evaluated from the weight loss of the sample. The degradation profiles of *N*-protected poly(*N*-CBz-Hpr-*co*-ϵ-CL) (**3E**) with a monomer composition of 90/10 mol % and homopoly(*N*-CBz-Hpr) at 37°C under physiological conditions (pH 7.4) are shown in Figure 4. The results indicate that the degradation rate was affected by the composition of the polymers. The degree of degradation showed a gradual increase with time after a rapid increase in the initial stage, that is, a parabola-type degradation pattern. The degradability of copoly(*N*-CBz-*co*-ϵ-CL) was higher than that of homopoly(*N*-CBz-Hpr). This may be due to the structure of homopoly(*N*-CBz-Hpr) being more rigid than that of copoly(*N*-CBz-Hpr-*co*-ϵ-CL). Therefore, the hydrolysis of the acyl oxygen of the copolymer was easier than that of the homopolymer.

CONCLUSIONS

A series of copoly(*N*-Z-Hpr-*co*-ϵ-CL)s with various compositions were synthesized from **1** and **2**. The η_{inh} and T_g values of the resulting copolymers were controlled by the amounts of the comonomers added. As the molar fraction of *N*-CBz-Hpr decreased, η_{inh} of the

copolymer increased. However, T_g of the copolymer decreased. These copolymers degraded under physiological conditions. The degradability of copoly(*N*-CBz-Hpr-*co*-ϵ-CL) was higher than that of homopoly(*N*-CBz-Hpr).

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