Synthesis and *In Vitro* Degradation of Novel Copolymers of Cyclic Carbonate and D,L-Lactide

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ABSTRACT: Cyclic carbonate 9-phenyl-2,4,8,10-tetraoxaspiro-[5,5]undcane-3-one is a six-membered carbonate monomer with a rigid side group. Novel biodegradable poly(carbonate-ester), poly(9-phenyl-2,4,8,10-tetraoxaspiro-[5,5]undecan-3-one-*co*-D,L-lactide) P(CC-*co*-DLLA), was synthesized by ring-opening polymerization in bulk, using stannous octanoate catalyst. Effects of polymerization conditions such as catalyst concentration, reaction time, and reaction temperature on the polymerization were investigated. The structure of the copolymers was characterized by FTIR, ^{1}H NMR, and ^{13}C NMR. Their molecular weights and polydispersity index were determined by gel permeation chromatography. The protecting benzyl ketal group was removed subsequently by catalytic hydrogenation to give a poly(carbonate-ester) containing pendant hydroxyl groups. The *in vitro* degradation of the copolymers was performed at (37 ± 0.5)°C in two media: phosphate buffer solution (pH 7.4) or Tris-HCl buffer solution (pH 8.6) in the presence of proteinase K. The degradation rate can be regulated by adjusting the composition of two monomers. The pendant hydroxyl groups in the copolymers resulted in a prominent enhancement of the degradation rate in PBS. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 101: 1988–1994, 2006

Key words: poly(carbonate-ester); biodegradable; ring-opening polymerization; enzymatic degradation; proteinase K

INTRODUCTION

Aliphatic polycarbonates have attracted much attention in biomedical and environmental fields because of their biocompatibility, biodegradability, and low toxicity.[1–3] They have been extensively studied as bioabsorbable suture, bone fixing materials, and drug-controlled-release carriers.[4,5] During the research of biomedical polymer materials, introduction of various pendent functional groups into polymer side chain is of particular importance and can readily accommodate to the need of further modifications. Some previous studies include the attachment of drugs, improvement of surface hydrophilicity, and promotion of bioadhesion.[6–8] Ring-opening polymerization is an effective way to get polycarbonates with high molecular weights. Among the cyclic carbonates, six-membered cyclic carbonates attract most attention because they are easier to prepare, more suitable for ring-opening polymerization, and without decarboxylation during the polymerization process.[9]

Polylactide (PLA) is the most prevalent synthetic biodegradable polyester. As one of the promising biomaterials for pharmaceutical and medical application, its functionalization and improvement of hydrophilicity remain attractive to many research groups. For drug delivery systems, a suitable degradation rate is the most key requirement for matrix materials. The hydrolytic degradation characteristics of PLA homo- and copolymers have been widely studied in recent years.[10–13] Because of the internal autocatalysis, which results in carboxyl end groups concentration difference between the surface and the center, thick samples of PLA degraded more rapidly inside than at the surface.[14,15] The enzymatic degradation of PLA has been extensively investigated during the past two decades, and among numerous selected enzymes, proteinase K, pronase, and bromelain have apparent acceleration effect on the degradation of PLLA.[16] Further studies showed that proteinase K degraded *l*-lactyl units preferentially while *d*-lactyl ones are biostable.[13]

Surface biodegradable polymers include polycarbonates,[17] polyanhydrides,[18,19] and polyorthoesters.[20,21] Their matrices were highly hydrophobic, and unstable chemical bonds exist in the polymer main chains. For example, poly(1,3-trimethylene carbonate) is a widely used biodegradable aliphatic polycarbonate and it shows surface erosion phenomenon in long-term process of *in vivo* degradation.[22] It is important to maintain

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bioactivity of protein and peptide drugs effectively. Surface erosive polymers ensured that the protein of the surface released simultaneously while the protein inside maintain their bioactivities. Through copolymerization of the aliphatic carbonates and LA, we can obtain various desired biodegradable materials for drug delivery.

To obtain synthetic biopolymer with adjustable degradation rate that can be further modified, in this study, we focus our interest on novel random copolymers of D,L-lactide and aliphatic cyclic carbonate with functional pendent groups, 9-phenyl-2,4,8,10-tetraoxaspiro-[5,5]undcane-3-one (CC). The poly(carbonate-*co*-ester)s with different compositions were prepared by ring-opening copolymerization with $Sn(Oct)_2$ as catalyst. To evaluate the biodegradability of obtained copolymers, *in vitro* hydrolysis and enzymatic degradation behaviors were also investigated. Subsequent deketalization of the copolymer by catalytic hydrogenation gave a hydroxyl-containing poly(carbonate-ester). The presence of hydroxyl groups leads to an increased degradation rate.

EXPERIMENTAL

Materials

Lactic acid (AR) was purchased from Hubei university chemical factory and used as received. All the solvents were distilled before use. Stannous octoate [$Sn(Oct)_2$] was purchased from Shanghai Chemical Reagent Co. and purified by distillation under reduced pressure and dissolved in freshly dried toluene prior to use. Pentaerythritol and benzylaldehyde are of AR grade and were used without further purification. Proteinase K (EC 3.4.21.14, 30 mAnsonU/mg) was supplied by Fluka.

Measurements

Infrared (IR) spectra were recorded on a PerkinElmer-2 spectrometer. The ^1H NMR and ^{13}C NMR were recorded with a Mercury VX-300 spectrometer, using $CDCl_3$ or DMSO-d_6 as solvent and tetramethylsilane as an internal standard. Molecular weights and molecular weight distributions were determined by gel permeation chromatography (GPC) using a Waters 2690-D liquid chromatography equipped with Shodex K803 and K805 gel columns and an internal Waters 2410 refractive-index detector. Chloroform or dimethylformamide was used as the eluent at a flow rate of 1.0 mL min^{-1}. Calibrations were fulfilled with narrow-molecular-weight distributed polystyrene standards.

Preparation of D,L-lactide (DLLA)

Lactic acid (180 g, 2.0 mol) was mixed with 1.2 g zinc powder in a 250 mL flask. The mixture was stirred at 90°C under reduced pressure for 4 h. Since the water was not distilled out, the temperature was increased to 160°C. The dehydration reaction proceeded for about 6 h, and then the temperature was increased to 250°C rapidly. The yellow crude product was distilled out gradually. The crude product was dissolved in 200 mL $CHCl_3$ and washed by $NaHCO_3$ saturated solution for three times. The organic layer was dried by anhydrous $MgSO_4$ and the filtrate obtained was concentrated under reduced pressure. The white product was recrystallized from ethyl acetate for five times (yield, 65%; mp, 125–127°C).

2-Phenyl-5,5-bis(hydroxymethyl)-1,3-dioxane

Pentaerythritol (68 g, 0.50 mol) was dissolved in 500 mL warm distilled water. Benzylaldehyde (53 g, 0.50 mol) and 2.5 mL concentrated hydrochloric acid were added to the solution in turn. After rapid stirring for 6 h at room temperature, the mixture was placed undisturbed for 24 h. The crude product was washed three times with 200 mL distilled water and recrystallized from Na_2CO_3 solution and toluene (yield, 83%).

Cyclic carbonate 9-phenyl-2,4,8,10-tetraoxaspiro-[5,5]undcane-3-one (CC)

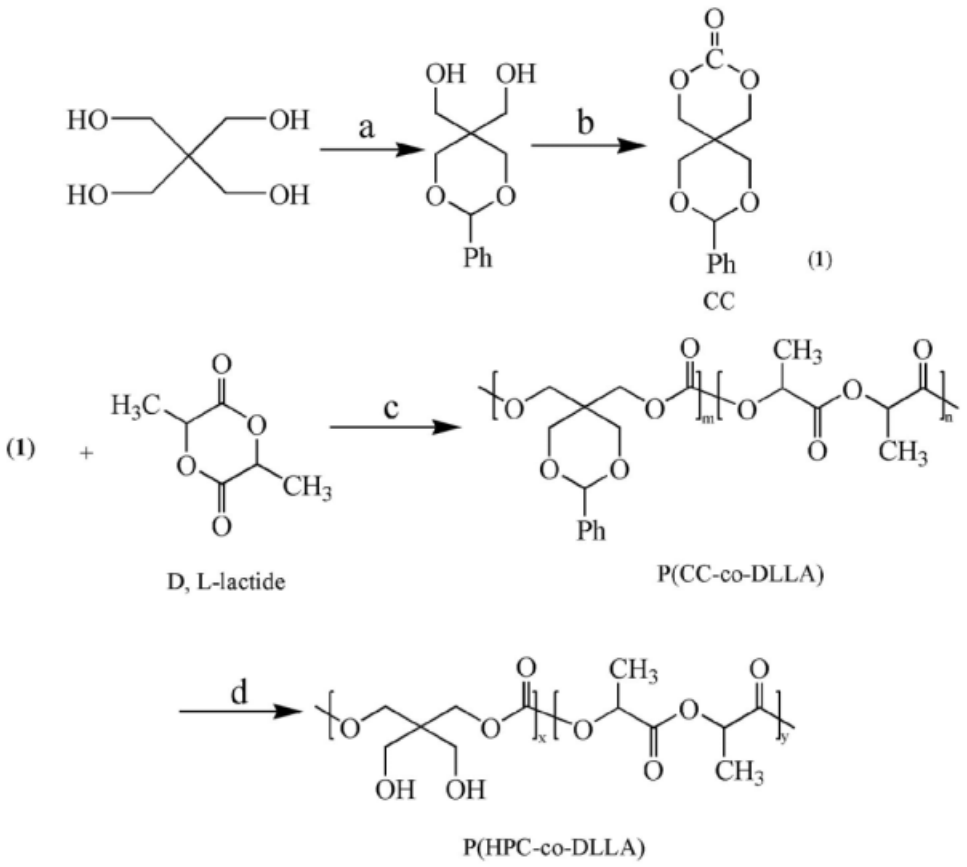
Triethylamine (35.8 mL, 0.25 mol) was added dropwise to a mixture of 2-phenyl-5,5-bis(hydroxymethyl)-1,3-dioxane (25.4 g, 0.11 mol) and ethyl chloroformate (23.5 mL, 0.24 mol) dissolved in 600 mL THF at 0°C. The reaction mixture was stirred for 2 h at room temperature and the byproduct triethylammonium chloride was filtered out. The filtrate was concentrated under reduced pressure below 50°C. The residue was recrystallized from THF at least four times (yield, 87%; mp, 169–170°C).

Copolymerization of cyclic carbonate and DLLA

Ring-opening copolymerization of CC cyclic carbonate and D,L-lactide was carried out in bulk using $Sn(Oct)_2$ as an initiator in a thoroughly dried single-necked round-bottom flask, which was sealed under reduced pressure. The mixture was immersed in an oil bath and stirred with a magnetic stirring bar. After predetermined reaction time at a predesigned temperature, the crude copolymers were dissolved in dichloromethane and precipitated with methanol. The product was dried in vacuum for over 24 h. FTIR (KBr, cm^{-1}): 1748 (C═O in CC and D,L-lactide).

In vitro degradation of poly (CC-*co*-DLLA)

The *in vitro* degradation experiments were conducted in two media: 0.1*M* phosphate buffer solution (PBS, pH 7.4); Tris-HCl buffer solution (pH 8.6) containing



Scheme 1. Copolymerization of 9-phenyl-2,4,8,10-tetraoxaspiro-[5,5]undcane-3-one cyclic carbonate and D,L-lactide. (a) PhCHO, HCl; (b) $ClCO_2Et$, Et_3N, THF, 0 °C; (c) $Sn(Oct)_2$, in bulk; and (d) Pd/C(10%), DMF/CH_3OH, 3.5 MPa, 40°C

1.0 mg proteinase K and 1.0 mg sodium azide (NaN_3, preventing bacteria development) per 5 mL solution. Caky polymer sheets were prepared by compress molding at 200°C and rapid cooling at room temperature (diameter, 8 ± 0.5 mm; thickness, 0.6 ± 0.1 mm). The accurately weighed samples were immersed in each medium and kept at (37 ± 0.5)°C in a shaking water-bath. The buffer/enzyme system was changed every 24 h to restore the original level of enzymatic activity. The samples were taken out at the end of each immersion period, washed with distilled water, and dried in vacuum at room temperature. The degradation rate was determined by the weight loss. Weight loss is defined as Weight Loss (%) = $(W_i - W_d)/W_i \times 100\%$, where W_i is initial weight and W_d is weight after degradation at different time intervals.

Removal of the protecting benzyl ketal groups and *in vitro* degradation of poly(HPC-*co*-DLLA)

Pd/C (0.12 g, 10%) was added to a solution of poly(CC-*co*-DLLA) (50/50) (0.8 g) in a 1 : 1 mixed solvent of DMF/CH_3OH. Hydrogenation was carried out at 40°C under 3.5 MPa. The mixture was stirred for 32 h and the Pd/C catalyst was removed by filtration. The filtrate was evaporated to give poly(HPC-*co*-DLLA). The product was washed with water and dried in a vacuum at room temperature overnight (yield, 51%).

The powder-like sample was compressed to a compact sheet under 30 MPa. The *in vitro* hydrolysis of the copolymer was measured in phosphate buffer solution (PBS, pH 7.4) at (37 ± 0.5)°C.

RESULTS AND DISCUSSION

Synthesis and characterization of poly(CC-*co*-DLLA)

Ring-opening polymerization of cyclic carbonates provides an effective approach to obtain high molecular weight aliphatic polycarbonates. Poly(CC-*co*-DLLA) copolymers were synthesized by copolymerization of 9-phenyl-2,4,8,10-tetraoxaspiro-[5,5]undcane-3-one cyclic carbonate and D,L-lactide in bulk using $Sn(Oct)_2$ as the catalyst. The carbonate monomer 9-phenyl-2,4,8,10-tetraoxaspiro-[5,5]undcane-3-one was prepared as described in the literature.[23] The route to get the copolymers is illustrated in Scheme 1.

The data from NMR spectroscopy of the polymers are fully consistent with the anticipated chemical

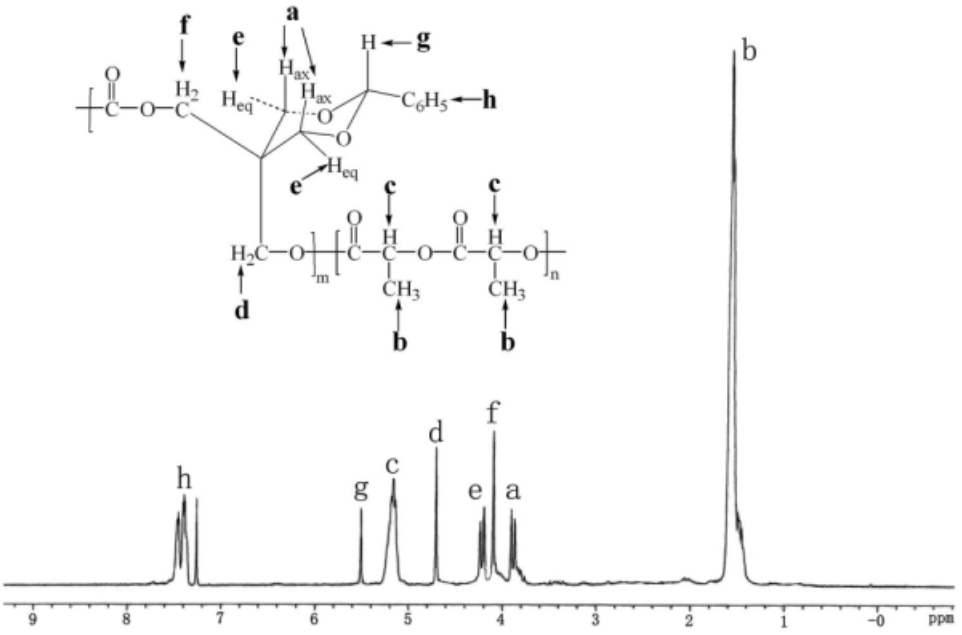


Figure 1 ^{1}H NMR spectrum of P(CC-*co*-DLLA) (30/70).

structure. Figure 1 represents the ^{1}H NMR spectrum of a copolymer containing 30 mol % CC. ^{1}H NMR ($CDCl_3$, ppm), δ: 1.50–1.56 (s, 3H, —CH_3), 5.16–5.18 (q, 1H, —COCH), 5.50 (s, 1H, CH—Ph), 4.09(s, 2H, O—CH_2—C), 4.70 (s, 2H, C—CH_2—O), 7.39–7.45 (m, 5H, —C_6H_5). Because of inner proton coupling effect of cyclic side group, double peaks appeared at both the chemical shifts δ3.86 and δ4.19. δ: 3.86–3.90 (d, 2H, CH_2—C—CH_2, axial H), 4.19–4.23 (d, 2H, CH_2—C—CH_2, equatorial H).

The ^{13}C NMR spectrum of poly(CC-*co*-DLLA) (50/50) is shown in Figure 2. Signals from CC and DLLA monomer units can be clearly observed. Two different carbonyl C═O peaks appeared at 154.3 and 169.8 ppm, respectively. It can be seen that the carbonyl carbon resonance of the CC units in the copolymer

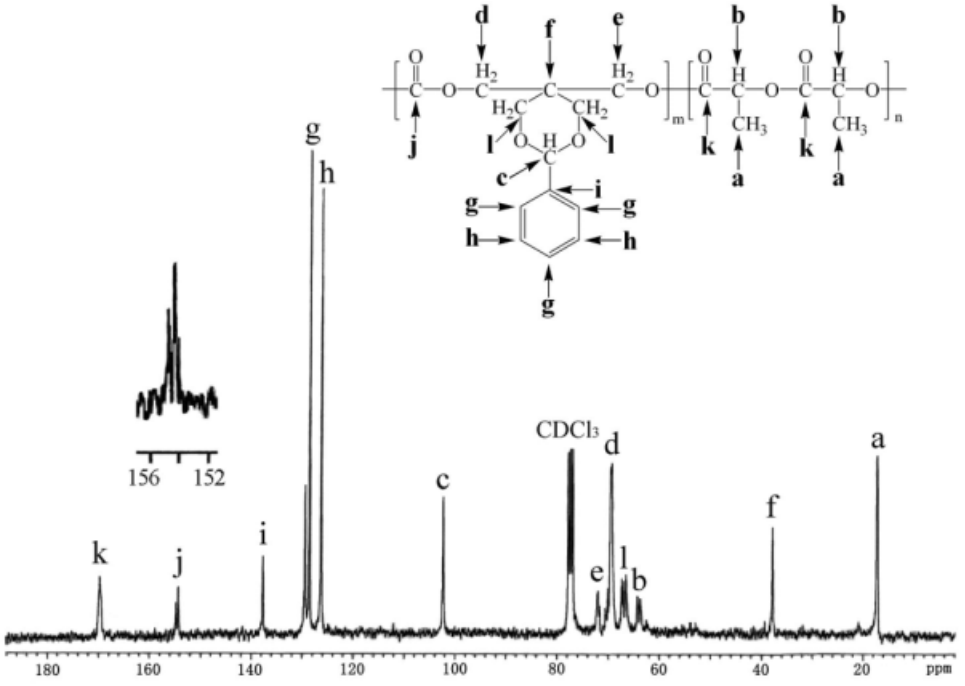


Figure 2 ^{13}C NMR spectrum of P(CC-*co*-DLLA) (50/50).

TABLE I
Partial Copolymerization Data of Cyclic Carbonate 9-phenyl-2,4,8,10-tetraoxaspiro-[5,5]undcane-3-one (CC) and D,L-lactide (DLLA)[a]

Entry	$F_{CC/DLLA}$[b]	$f_{CC/DLLA}$[c]	M_n	M_w/M_n	Yield (%)
1	100:0	100:0	22,400	1.83	86.6
2	90:10	87:13	26,800	1.68	82.1
3	70:30	65:35	37,500	1.80	79.3
4	50:50	43:57	48,900	1.85	76.7
5	30:70	22:78	57,200	1.73	78.5
6	10:90	5:95	69,100	1.62	73.7
7	0:100	0:100	82,500	1.74	81.4

[a] $Sn(Oct)_2$ as catalyst, molar ratio of comonomer to catalyst $[M]/[I] = 1000$, 180°C, 24 h, in bulk.
[b] Comonomer feed ratio in mol/mol.
[c] The molar ratio of two repeating units in the copolymer determined by ^1H NMR.

splits into several peaks in the range of 154.0–154.6 ppm, which is because of the different chemical environments caused by the different sequences in the copolymer chain. Therefore, from the ^{13}C NMR spectra, we can further confirm that the two comonomers are copolymerized by random copolymerization. In addition, the NMR spectra of poly(carbonate-ester)s offered no evidence for decarboxylation occurring during the copolymerization.

The effects of reaction conditions on copolymerization was also investigated, including comonomer feed ratio, reaction time, reaction temperature, and initiator concentration. As shown in Table I, the copolymerization of CC and DLLA was carried out using a variety of comonomer feed ratios with $[M]/[I]$ of 1000 at 180°C for 24 h, and the molar ratio of CC in feed was varied from 100 to 0%. The molecular weights of two homopolymers and poly(CC-*co*-DLLA) copolymers with different compositions are listed in Table I. The practical chemical composition of the poly(CC-*co*-DLLA) was determined by ^1H NMR spectrometry. As

TABLE II
Effects of Various Reaction Conditions on the Copolymerization[a]

Entry	Reaction time (h)	Temperature (°C)	M_n	M_w/M_n	Yield (%)
1	4	180	10,200	1.67	69.4
2	8	180	23,600	1.88	72.8
3	16	180	31,400	1.75	81.9
4	24	180	39,200	1.87	78.2
5	32	180	32,200	2.02	70.3
6	16	160	24,300	1.84	65.5
7	24	160	31,600	2.06	66.2
8	16	220	16,500	1.77	69.9
9	24	220	21,100	1.95	68.3

[a] The feed molar ratio of CC/ DLLA was 50:50, $Sn(Oct)_2$ as catalyst, molar ratio of monomer to catalyst $[M]/[I] = 500$, in bulk.

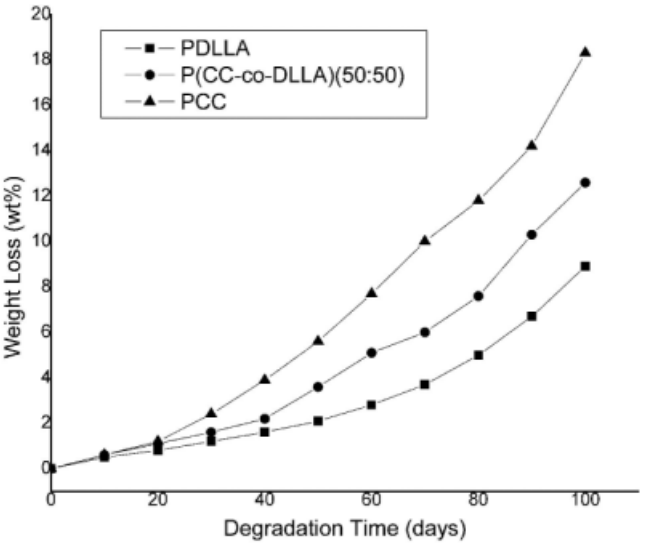


Figure 3 *In vitro* degradation of PCC, PDLLA, and P(CC-*co*-DLLA) (50 : 50) in phosphate buffer solution (0.1*M*, pH 7.4, (37 ± 0.5)°C).

can be seen, the ratio of CC repeating units in the copolymer is lower than that in the corresponding CC monomer feed ratio, indicating that D,L-lactide monomer has a higher reactivity in comparison with CC monomer under the reaction conditions we investigated. As the molar ratio of CC was varied from 100 to 0%, molecular weights of copolymers increased correspondingly, and this result confirms the above conclusion about reactivity ratio. GPC results showed that the poly(carbonate-ester)s had unimodal distributions and the polydispersities were in the range of 1.62–1.85.

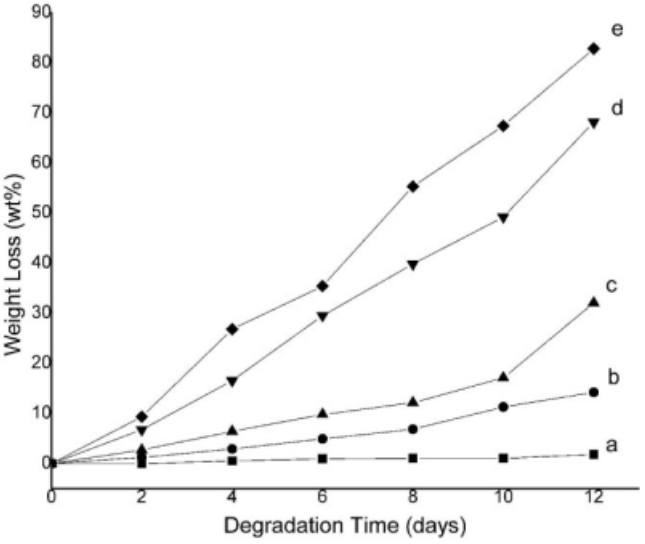


Figure 4 Enzymatic degradation of poly(CC-*co*-DLLA) by proteinase K in Tris-HCl buffer solution (0.1*M*, pH 8.6, (37 ± 0.5)°C). CC/DLLA: (a) 100/0, (b) 70/30, (c) 50/50, (d) 30/70, and (e) 10/90.

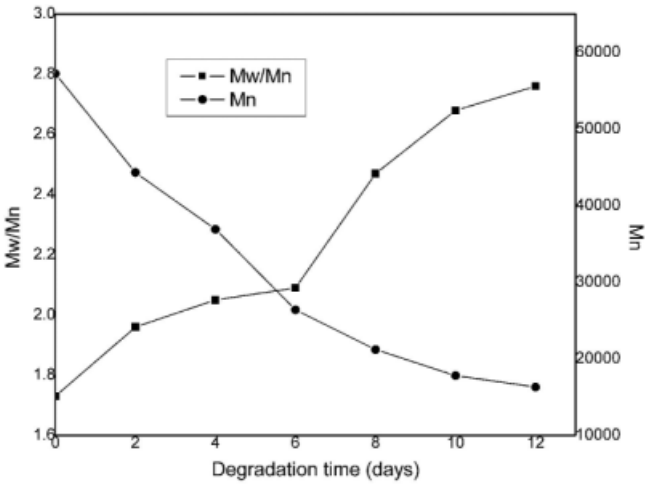


Figure 5 Molecular weight and polydispersity index change of poly(CC-*co*-DLLA) (30/70) in Tris-HCl buffer solution (0.1*M*, pH 8.6, (37 ± 0.5)°C) with proteinase K.

Table II shows the effects of reaction time and temperature on polymerization when molar ratio of monomer to initiator was 500. Because of the co-melting effect, the mixture of CC and DLLA (molar ratio 50/50) can be copolymerized in bulk at 160°C, although the melting point of CC is above 160°C. For copolymerization reaction at 180°C, the largest M_n was observed when the reaction time is 24 h. With the increase of reaction time or temperature, molecular weight decreased gradually because of decomposition of the copolymer.

Degradation behavior of homo and copolymers

The *in vitro* hydrolytic degradation of homo and copolymers without enzyme was carried out in phosphate buffer solution (PBS, pH 7.4, 0.1*M*) at (37 ± 0.5)°C. As shown in Figure 3, homo PCC has a higher weight loss rate than PDLLA. Thus, introducing that calculated proportion of DLLA units can regulate the degradation rate of PCC.

Enzymatic degradation of copolymers with various composition were investigated in a Tris-HCl buffer solution (pH 8.6, 0.1*M*) containing 1.0 mg proteinase K and 1.0 mg sodium azide per 5 mL at (37 ± 0.5)°C and the results were presented in Figure 4. The results showed that the more the molar composition of CC, the less the extent of weight loss of copolymer, because proteinase K accelerates the degradation of PLLA specifically. Therefore, poly(CC-*co*-DLLA) (10/90) degraded faster and about 85% weight loss was observed after immersing the sample in enzyme solution for 12 days, because it contains most of the DLLA units among the samples tested. The degradation of poly(CC-*co*-DLLA) (30/70) in Tris-HCl buffer solution with proteinase K (pH 8.6, 37.5°C) was evaluated by the molecular weight change at different time intervals. As shown in Figure 5, the copolymer molecular weights decreased with the increase in degradation time. At the same time, the polydispersity index (M_w/M_n) increased from 1.73 to 2.76 gradually.

Deprotection and *in vitro* degradation of poly (HPC-*co*-DLLA)

Deprotection of the copolymer by hydrogenolysis using Pd/C as a catalyst has been successfully performed under 3.5 MPa in a DMF/CH_3OH (1 : 1) medium. The ^1H NMR spectrum (Fig. 6) of the deprotected sample was obtained in DMSO-d_6. δ: 1.48–1.55 (s, 3H, —CH_3), 5.18–5.22(q, 1H, —COCH), 3.32–3.38(d, 4H, HO—CH_2—C), 4.03(s, 4H,

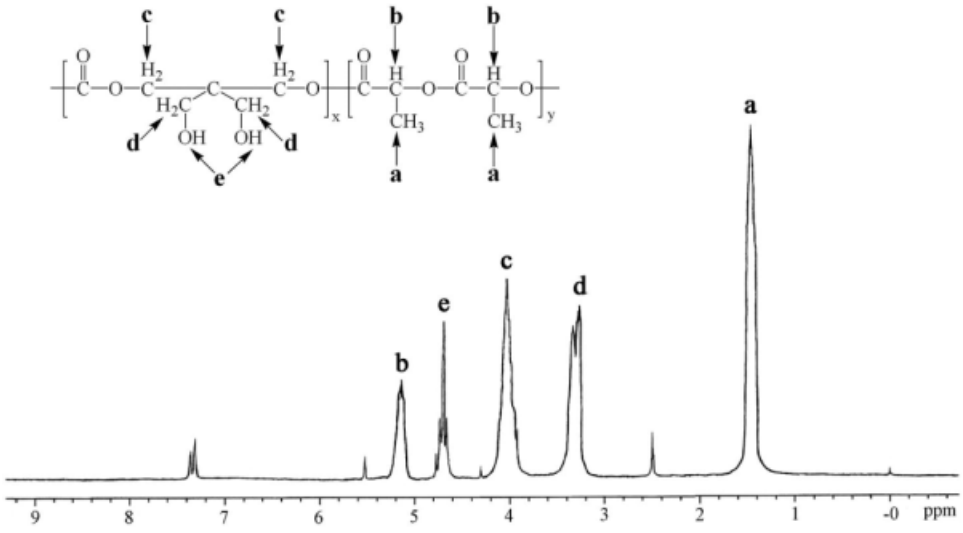


Figure 6 ^1H NMR spectrum of P(HPC-*co*-DLLA) (50/50).

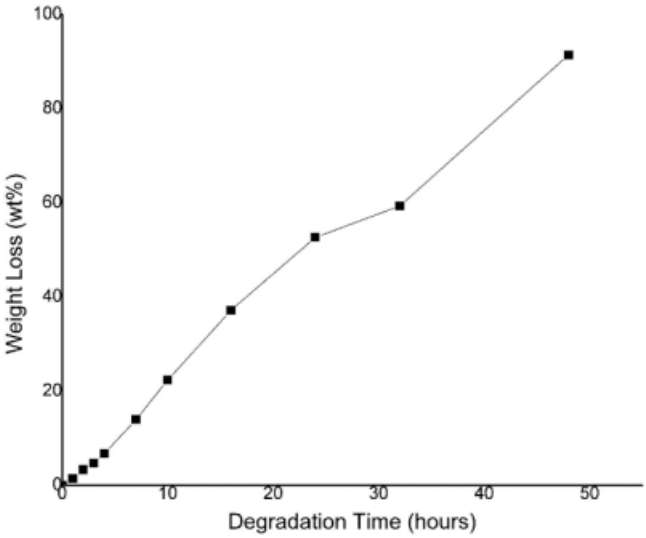


Figure 7 *In vitro* degradation of P(HPC-*co*-DLLA) (50:50) in phosphate buffer solution (0.1*M*, pH 7.4, (37 ± 0.5)°C).

O—CH_2—C), 4.71–4.75(t, 2H, —OH). It was found that the degree of debenzylation was achieved near 91% under the optimum condition according to the intensity ratio of residual benzyl and hydroxyl protons. Increasing the reaction temperature could lead to degradation of the polymer backbone chain. The obtained poly (HPC-*co*-DLLA) (50/50) is soluble in DMF and DMSO, and its solubility in CH_2Cl_2, CH_3OH, toluene, or ether is poor.

The *in vitro* hydrolytic degradation of poly(HPC-*co*-DLLA) (50/50) was carried out in phosphate buffer solution. Compared with the unprotected sample (Fig. 7), the degradation rate of poly(HPC-*co*-DLLA) increased rapidly. Its weight loss was 90.4% after 48 h and the M_n decreased from 36,700 to 4600. This result confirmed that pendant hydroxyl groups improved the hydrophilicity of poly(carbonate-ester) chains and enhanced its hydrolytic degradability.

CONCLUSIONS

A new poly(carbonate-ester) poly(CC-*co*-DLLA) was prepared by ring-opening polymerization of 9-phenyl-2,4,8,10-tetraoxaspiro-[5,5]undecan-3-one cyclic carbonate and D,L-lactide in bulk using stannous octoate as catalyst. Temperature, reaction time, and initiator concentration affected the copolymerization. ^{1}H NMR analysis of copolymer showed that DLLA has a higher reactivity than that of CC monomer at 180°C. The *in vitro* hydrolytic and enzymatic degradation was investigated using poly(CC-*co*-DLLA) copolymers with different compositions. The copolymers with a higher DLLA content enzymatically degraded more rapidly, and the degradation proceeded much faster in the presence of proteinase K than in the presence of PBS buffer. The results suggest that the degradation rate of copolymer poly(CC-*co*-DLLA) can be controlled by adjusting the composition of the two monomers. A new hydroxyl-containing poly(carbonate-ester) was achieved after the catalytic hydrogenation to poly(CC-*co*-DLLA). Its degradability improved greatly and introduction of pendant hydroxyl groups could facilitate further functionalizations.

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