Synthesis and Biodegradability Evaluation of 2-Methylene-1,3-dioxepane and Styrene Copolymers

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ABSTRACT: Biodegradable copolymers of 2-methylene-1,3-dioxepane (MDO) and styrene (ST) were synthesized by free-radical copolymerization using di-*t*-butyl peroxide (DTBP) as the initiator. The copolymers containing ester units were characterized by Fourier transform infrared (FTIR), ^{1}H-NMR, and ^{13}C-NMR spectroscopy. Their molecular weight and polydispersity index were determined by gel permeation chromatography (GPC). *In vitro* enzymatic degradation of poly(MDO-*co*-ST) was performed at 37°C in phosphate buffer solution (PBS, pH = 7.4) in the presence of *Pseudomonas* lipase or crude enzyme extracted from earthworm. The experiment showed that incorporating ester units into C—C backbone chain of polystyrene would result in a biodegradable copolymer. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 103: 1146–1151, 2007

Key words: 2-methylene-1,3-dioxepane; styrene; free-radical copolymerization; *Pseudomonas* lipase; earthworm; biodegradability

INTRODUCTION

Olefin polymers, such as polyethylene (PE), polypropylene (PP), and polystyrene (PS) have been found a wide variety of applications because of their good chemical, physical, and mechanical properties. Nevertheless, intensive application of polyolefin has brought about serious environmental pollution because of the nonbiodegradability of this class of polymers.[1] To overcome the drawback, it seems to be feasible and practical to introduce some biodegradable units into the polyolefin main chain.[2,3]

Cyclic ketene acetals, a class of special monomers, have gained considerable attention because they can copolymerize with vinyl monomers by conventional free-radical polymerization to give biodegradable copolymers with ester units in the backbone chain. Such a class of copolymers may have potential use as biodegradable materials. 2-Methylene-1,3-dioxepane (MDO) is one of the mostly used cyclic ketene acetal.[4–7] It was reported that MDO could undergo free-radical ring-opening polymerization completely to form poly(ε-caprolactone) (PCL). Di-*t*-butyl peroxide (DTBP) or 2,2′-azobisisobutyronitrile (AIBN) was usually used as the initiator for the above free-radical polymerization. Only a few works have been devoted to the synthesis and biodegradation studies of MDO copolymers with vinyl monomers.[8–11]

Earthworm is a widespread reptile living in the loose and moist soils. It attracts more and more interest in the area of waste biorecycling and biodegradation.[12] A variety of enzymes, such as catalase, cholinesterase, fibrinolytic enzyme, and superoxide dismutase[13] can be extracted from the earthworm bodies. It was reported that the crude enzymes extracted from earthworm could catalyze the degradation of polyolefin copolymers containing ester units in the backbone chain.[10]

In this study, we synthesized the copolymers of MDO and styrene (ST) which contain ester units in the backbone chain by free-radical copolymerization. The structure of the copolymers was characterized by Fourier transform infrared (FTIR), ^{1}H-NMR, and ^{13}C-NMR spectroscopy. Crude enzyme extracted from earthworm was used for the degradation of poly(MDO-*co*-ST). In comparison, we also investigated the degradation in the presence of *Pseudomonas* lipase, which can apparently accelerate the hydrolytic degradation of PCL chain.[14,15] The surface morphology of the copolymer before and after degradation was observed by SEM. The copolymer obtained may have potential use as biodegradable packaging materials because of its biodegradability.

EXPERIMENTAL

Materials

2-Methylene-1,3-dioxepane (MDO) (Shanghai Chemical Reagent Company, China) was prepared according

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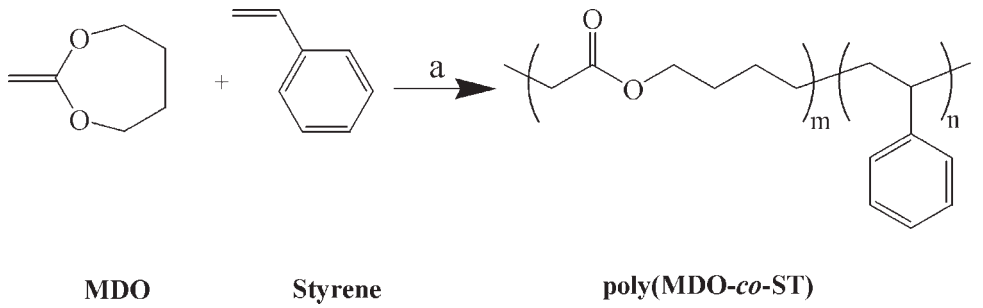

Scheme 1 Synthesis of poly(MDO-*co*-ST). (a) DTBP, 120°C, chlorobenzene.

to the literatures.[6,12] ST was purchased from Shanghai Chemical Reagent Co., and washed with 10% NaOH and distilled under reduced pressure before use. Di-*t*-butyl peroxide (DTBP) was purchased from Shanghai Chemical Reagent Co. and used without further purification. Olive oil and poly(vinyl alcohol) (PVA, $M_n = 1750 \pm 50$, Shanghai Chemical Reagent Co.) were used as-received. Earthworms were obtained from local fishing shops and washed several times with distilled water before use. *Pseudomonas* lipase (EC 3.1.1.3, 40 U/mg) was purchased from Fluka.

Characterization

FTIR spectra were recorded on a Perkin–Elmer-2 spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were obtained on a Mercury VX-300 spectrometer. Deuterated chloroform ($CDCl_3$) was used as solvent and tetramethylsilane (TMS) was used as internal standard. Molecular weights and polydispersity index (M_w/M_n) of the copolymers 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 was used as eluent at a flow rate of 1.0 mL/min. Waters Millennium module software was used to calculate molecular weights on the basis of a universal calibration curve generated by narrow molecular weight distribution PS standard.

Copolymerization of MDO and ST

A 10-mL polymerization flask, charged with given amounts of MDO, ST, and 2 mol % DTBP in 2 mL dry chlorobenzene was sealed *in vacuo* at low temperature. The mixture was immersed in an oil bath and stirred with a magnetic stirring bar. After heating for a predetermined reaction time at given temperature, the system was quenched by immersing the flask in a cool water bath. The copolymer was dissolved in chloroform and precipitated with methanol, and the product was dried *in vacuo* for 24 h to a constant weight.

Extraction of crude enzyme from earthworm

About 500 g of earthworm was homogenized by tissue homogenizer in 1500 mL phosphate buffer solution (pH = 7.4, C = 20 m*M*, $C_{(NaN3)}$ = 1 m*M*). Sodium azide (NaN_3) was used as antimicrobial agent. The homogenate was centrifuged at 5000 rpm for 40 min at 4°C and the supernatant (crude enzyme buffer) was collected. The mensuration of enzymatic activity was carried out according to Yamada and Machida.[16] Emulsion of PVA and olive oil was used as the substrate, titrated by 0.05*M* NaOH solution. The activity of crude enzyme was found to be 65 U/mL.

In vitro enzymatic degradation of the copolymers

The copolymer films were prepared by solution casting and dried in vacuum until a constant weight was obtained. The size of polymer films was about 10 × 15 mm^2 and had a weight of 15–25 mg. The enzymatic degradation of poly(MDO-*co*-ST) was carried out in a GRANT OLS200 rotary shaker at

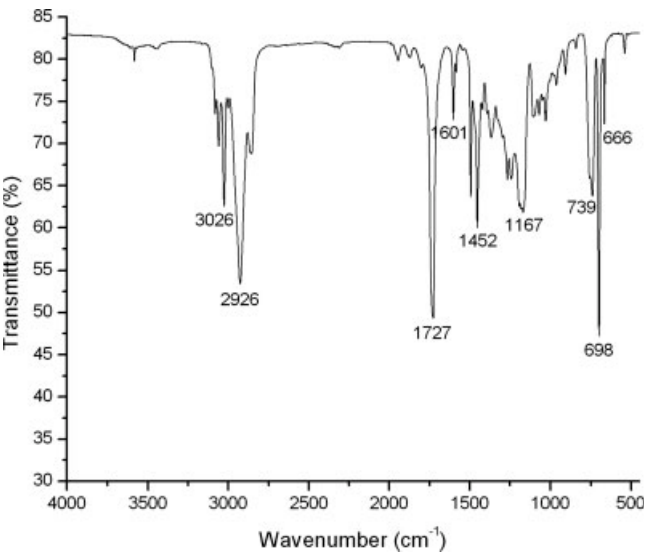

Figure 1 FTIR spectrum of poly(MDO-*co*-ST).

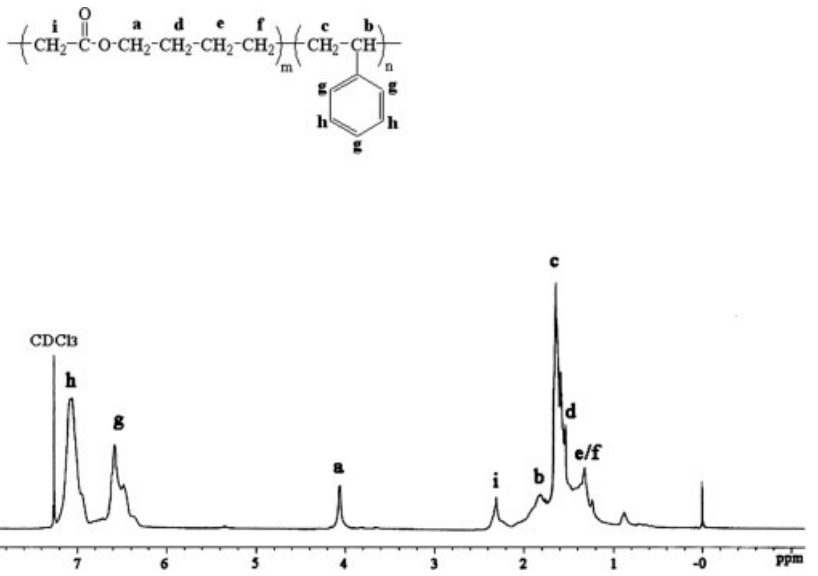

Figure 2 ^{1}H-NMR spectrum of poly(MDO-*co*-ST).

37°C by immersing polymer films in one of the two media: 5 mL phosphate buffer solution (pH = 7.4, 0.1*M*) containing 1 mg *Pseudomonas* lipase and 1 mg sodium azide (NaN_3); 5 mL phosphate buffer solution of crude earthworm enzyme. The solution containing enzyme was changed every 24 h to maintain enzyme activity. The samples were taken out of the solution over predetermined time intervals, washed with distilled water, and dried in vacuum at room temperature. The degradation rates were determined by weight loss. Weight loss is defined as Weight Loss (%) = $(W_i - W_d)/W_i \times 100\%$, where W_i is

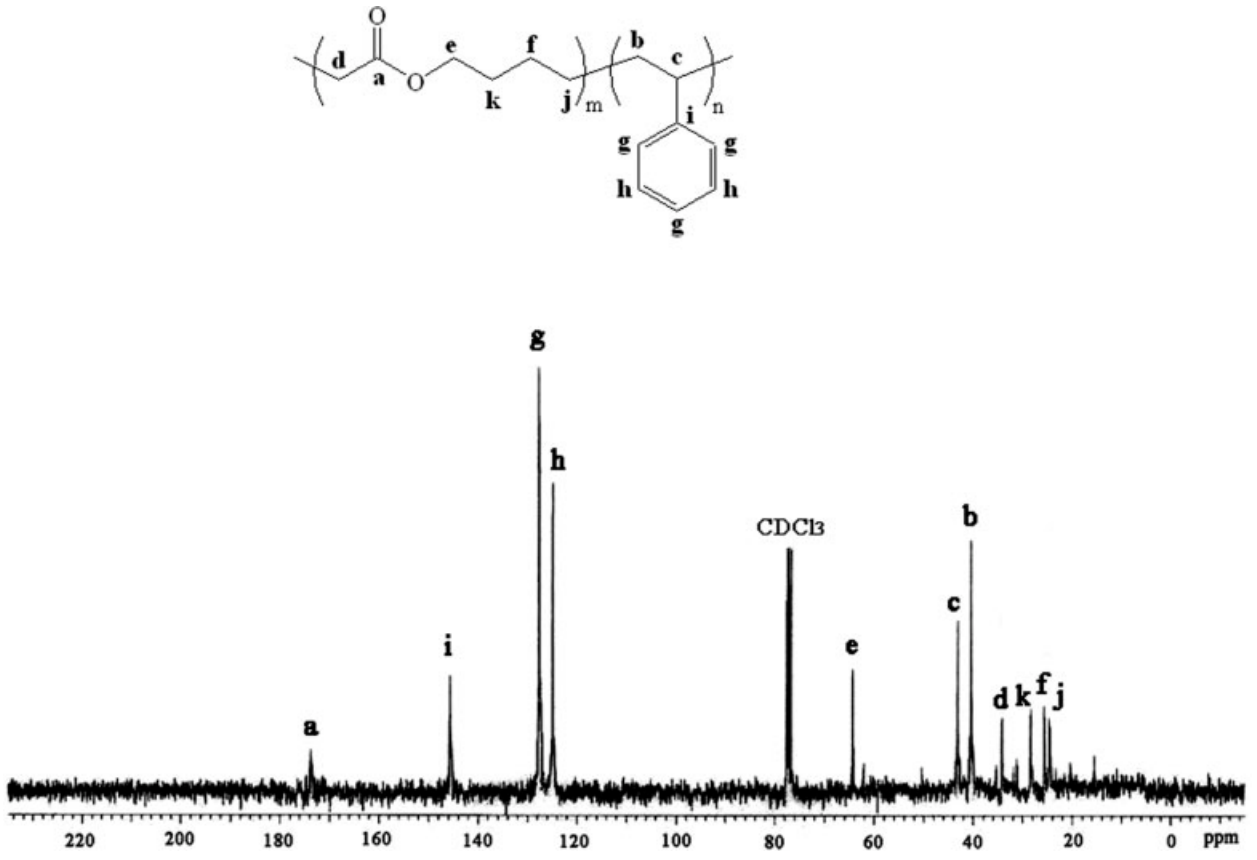

Figure 3 ^{13}C-NMR spectrum of poly(MDO-*co*-ST).

TABLE I
Copolymerization Data of MDO and Styrene

Entry	$F_{MDO/ST}$	f_{MDO}[a]	Time (h)	M_n	M_w/M_n	Yield (%)
1	90 : 10	21.7	24	12,800	1.70	54.6
2	80 : 20	14.3	24	18,600	2.53	39.1
3	70 : 30	6.5	24	36,900	2.11	47.6
4	80 : 20	10.2	12	17,700	1.83	29.7
5	80 : 20	8.5	36	23,500	2.42	32.4

[a] MDO, molar content determined by ^{1}H-NMR.

initial weight and W_d is weight after degradation at different time intervals.

Surface analysis

Surface changes of the copolymers before and after enzymatic degradation were observed by scanning electron microscope (Hitachi S-570). The samples were coated with gold–palladium for 70 s in an argon atmosphere before observation.

RESULTS AND DISCUSSIONS

Synthesis and characterization

Copolymerization of MDO and ST is illustrated in Scheme 1. The structure of the obtained copolymers was characterized by FTIR, ^{1}H-NMR, and ^{13}C-NMR. FTIR spectrum (Fig. 1, NaCl, film, cm^{-1}): 1727 (C=O); 3026, 2926 (C—H); 1601, 1452, 1167; 666, 698, 739 (phenyl C—H). Figure 2 shows the ^{1}H-NMR spectrum of poly(MDO-*co*-ST). ^{1}H-NMR ($CDCl_3$, ppm), δ: 1.18–1.58 [m (broad), 6H, —$CO_2CH_2CH_2$ CH_2CH_2—]; 1.62 [m (broad), 2H, —CH_2—CH—C_6H_5]; 1.82 [m (broad), 1H, —CH_2—CH—C_6H_5]; 2.33 [d (broad), 2H, —CH_2CO_2—]; 4.05 [t (broad), 2H, —$CO_2CH_2(CH_2)_3$—]; 6.30–7.25 [m (broad), 5H, —C_6H_5]. ^{13}C-NMR spectrum of poly(MDO-*co*-ST) is shown in Figure 3. ^{13}C-NMR ($CDCl_3$, ppm), δ: 24.48, 25.62, 28.10 (—$CO_2CH_2CH_2CH_2CH_2$—); 40.71 (—$CH_2CHC_6H_5$); 43.35 (—$CH_2CHC_6H_5$); 64.15 (—CO_2CH_2—); 125.22, 127.82, 145.68 (—C_6H_5); 173.78

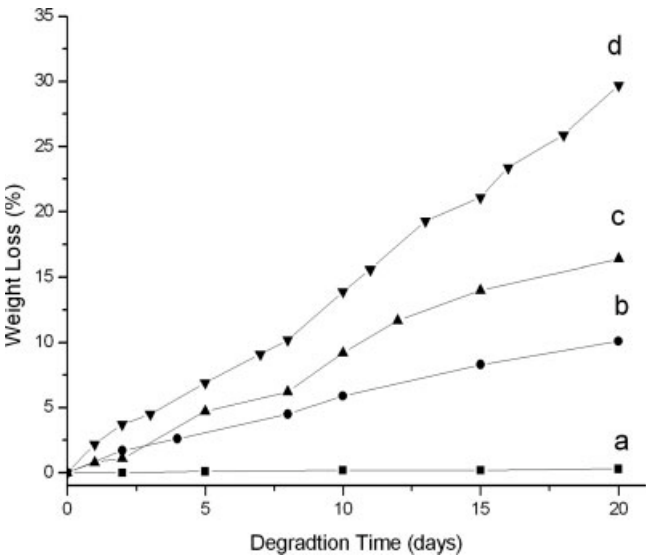

Figure 4 Enzymatic degradation of poly(MDO-*co*-ST) in phosphate buffer solution (pH = 7.4, 37°C) and in the presence of *Pseudomonas* lipase. (a) Polystyrene; (b) poly($MDO_{6.5}$-*co*-ST); (c) poly($MDO_{14.3}$-*co*-ST); and (d) poly($MDO_{21.7}$-*co*-ST).

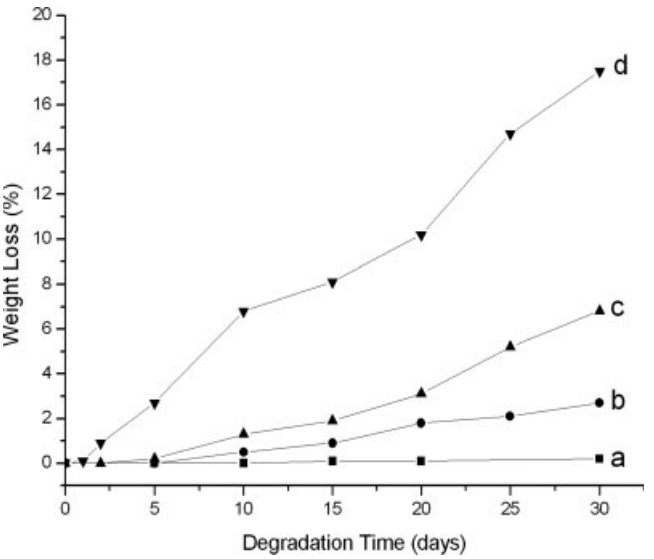

Figure 5 Degradation of poly(MDO-*co*-ST) in phosphate buffer solution (pH = 7.4, 37°C) in the presence of crude enzyme extracted from earthworm. (a) Polystyrene; (b) poly($MDO_{6.5}$-*co*-ST); (c) poly($MDO_{14.3}$-*co*-ST); and (d) poly($MDO_{21.7}$-*co*-ST).

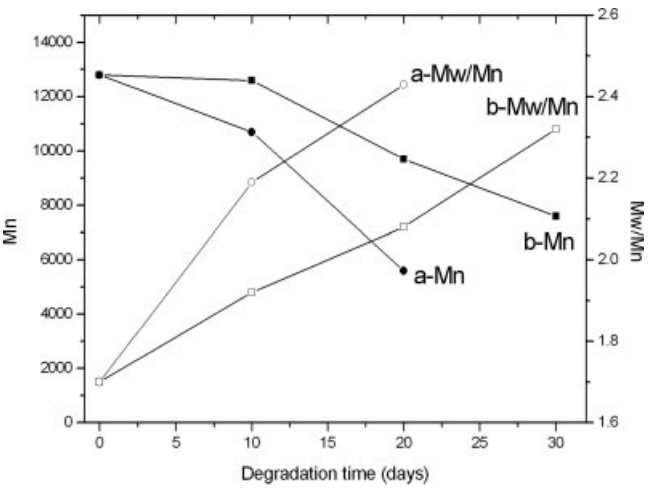

Figure 6 Molecular weight and polydispersity index changes of poly($MDO_{21.7}$-*co*-ST) in the degradation process in the presence of (a) *Pseudomonas* lipase and (b) crude enzyme extracted from earthworm.

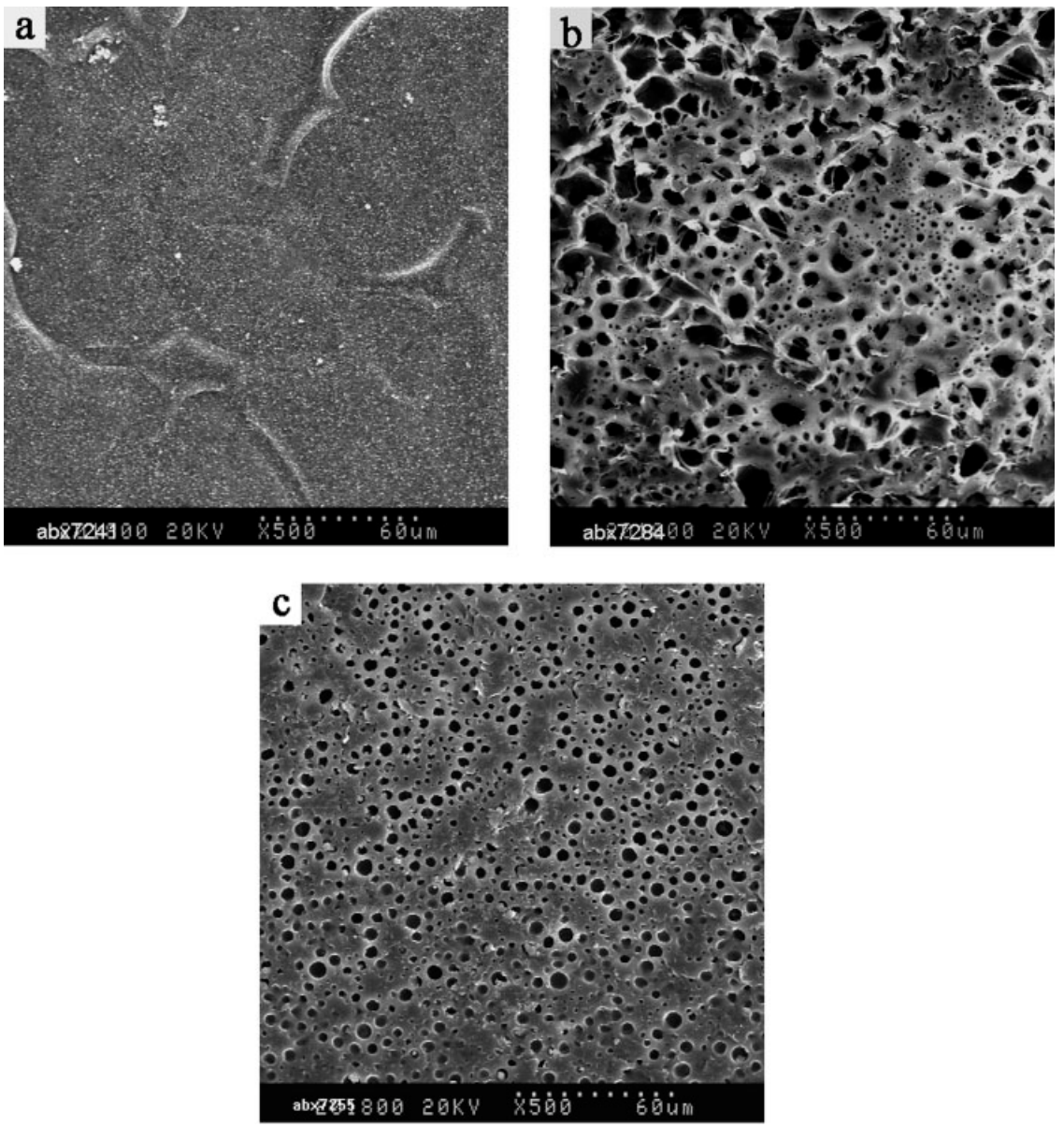

Figure 7 SEM micrographs of poly($MDO_{21.7}$-*co*-ST) surface forms (magnification: ×500). (a) Before degradation; (b) after degradation for 20 days at 37°C in PBS containing *Pseudomonas* lipase; and (c) after degradation for 30 days at 37°C in PBS containing crude enzyme extracted from earthworm.

(—CO_2—). The appearance of several small peaks between 20 and 40 ppm was probably because of the branch structure of copolymer in the process of the chain propagation.[7,10]

The molecular weights and polydispersity index (M_w/M_n) of the copolymers are listed in Table I. The values of the reactivity ratios r_{MDO} and r_{ST} were 0.021 and 22.6.[4,5] In fact, MDO shows rather low reactivity and tendency to copolymerize with most common vinyl monomers. It can be seen from Table I that ST units were the main composition of the copolymer even at a high feed molar ratio of MDO to ST. The polydispersity index was in the range of 1.70–2.53.

In vitro enzymatic degradation of poly(MDO-*co*-ST)

Enzymatic degradation results of the polymer films in the presence of *Pseudomonas* lipase are shown in Figure 4. After 20 days, there was not any obvious weight loss in the degradation of homopolystyrene because C—C main chain could not be enzymatically degraded. On the other hand, there was a 29.7% weight loss of poly($MDO_{21.7}$-*co*-ST) under the same condition. Figure 5 demonstrates the degradation process of the copolymers in the presence of crude enzyme from earthworm. It could be seen that there was a same tendency during the degradation of copolymers; the higher content of MDO units in the

copolymer would cause a faster degradation rate. The copolymer degradation catalyzed by crude enzyme extracted from earthworm could be an interactional process because there are a variety of enzymes in earthworm bodies. However, *Pseudomonas* lipase only accelerates the hydrolytic degradation of ester units at the copolymer main chain because of its high selectivity to PCL chain.

Figure 6 shows the changes of molecular weights and polydispersity index during the enzymatic degradation process of poly($MDO_{21.7}$-*co*-ST). The molecular weight decreased in the presence of either *Pseudomonas* lipase or crude enzyme from earthworm, and at the same time, the polydispersity index increased gradually, which may resulted from the formation of oligomers in the degradation process of backbone chain.

Figure 7 displays SEM images of poly($MDO_{21.7}$-*co*-ST) before and after enzymatic degradation at predetermined time intervals. Before degradation, the copolymer film showed a relatively smooth and compact surface without any holes on it. Under the action of *Pseudomonas* lipase or crude enzyme from earthworm for 20 or 30 days, many irregular cavities [Fig. 7(b), from 2.0 to 20 μm] or small holes [Fig. 7(c), from 1.0 to 10 μm] appeared on the sample surface. The SEM images further confirmed that both *Pseudomonas* lipase and crude enzyme from earthworm have similar effect to accelerate the degradation rate of the copolymer.

CONCLUSIONS

Ester units were incorporated into the backbone chain of nonbiodegradable PS by free-radical copolymerization of cyclic ketene acetal MDO and ST. The structure, composition, and molecular weights of these copolymers were investigated. As expected, the obtained copolymers could enzymatically be degraded in the presence of *Pseudomonas* lipase or crude enzyme from earthworm. The degradation rate of the copolymer increased with increasing the molar content of MDO units. The SEM results indicated the surface erosion changes of the copolymers before and after enzymatic degradation. It is a feasible route to synthesize biodegradable polyolefins.

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