Synthesis and *In Vitro* Degradation of Copolymers of Glycolide and 6(*R*,*S*)-Methylmorpholine-2,5-dione

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ABSTRACT: Copolymerization of glycolide (GA) and 6(*R*,*S*)-methylmorpholine-2,5-dione (MMD) was carried out in bulk using stannous octoate as an initiator. The effects of temperature, time, concentration of stannous octoate, and the feed composition on the copolymerization were investigated. DSC analysis showed that glass transition temperature (T_g) of the resulting copolymers (PGM) increased with increase of MMD content in the copolymers, while melting temperature (T_m) and heat of fusion (ΔH) decreased. *In vitro* degradation was performed in two media: PBS buffer (pH 7.4) and distilled water (pH 6.8). It showed that the erosion rates increased with increasing MMD content in the original copolymers, and the degradation proceeded more rapidly in PBS buffer than in distilled water. Based on the changes of composition and crystallinity of the samples during the degradation, it is suggested that the degradation proceeded through two main stages: first, mainly in amorphous regions and then in crystalline ones. © 1997 John Wiley & Sons, Inc. J Appl Polym Sci **63**: 643–650, 1997

Key words: glycolide; 6(*R*,*S*)-methylmorpholine-2,5-dione; bulk ring-opening copolymerization; biodegradable; *in vitro* degradation

INTRODUCTION

Poly(glycolic acid) (PGA) is the simplest biodegradable poly(α-hydroxy ester), which was used for surgical suture as early as in the 1960s.[1] But its high crystallinity and poor solubility in most solvents make it difficult to process, limiting its applications, especially for drug-delivery systems. To improve its crystallinity, solubility, and degradation behavior, copolymers of glycolide with other cyclic monomers, such as lactide (LA)[2–6] and ε-caprolactone (ε-CL),[5,7] were prepared and investigated extensively.

6(*R*,*S*)-Methylmorpholine-2,5-dione (MMD) is a cyclic monomer with both an ester bond and an amide bond in one molecule, which can be converted into alternating polyesteramide by ring-opening polymerization.[8] This polymer is nontoxic, intrinsically amorphous, and fast degradable *in vitro* or *in vivo*. The synthesis and degradation of random copolymers of DL-LA or ε-CL with MMD were studied by Helder et al.[9] and Veld et al.[10] Their results indicated that the copolymers of LA or ε-CL with MMD degraded faster than did their individual homopolymers, PLA and PCL. Similarly, the crystallinity, degradability, and some other properties of poly(glycolic acid) may be changed by the incorporation of MMD units into poly(glycolic acid) chains. In our recent research, copolymers of glycolide and MMD were synthesized and their degradation behavior was investigated to develop more practical biodegradable polymers.

EXPERIMENTAL

Materials

6(*R*,*S*)-Methylmorpholine-2,5-dione (MMD) was prepared as described in the literature.[8] The

crude product was purified by recrystallization from CHCl$_3$, followed by subsequent sublimation at reduced pressure. The melting point of the purified product is 97–99°C (literature,[8] mp 98.5–100°C). Glycolide and stannous octoate were purchased from Sigma Co. Toluene was dried over CaH$_2$ and distilled just before use.

Polymerization

Ring-opening polymerization was carried out in bulk using stannous octoate as an initiator in a silanized tube (5 mL), which was sealed in a vacuum ($P = 0.1$ mbar) as described in the literature.[8] GA and MMD were purified by sublimation three times before polymerization. The crude polymers were dissolved in hexafluoroisopropanol and precipitated from a 10-fold excess of ethanol to remove the unreacted monomers and oligomers.

Polymer Characterization

The composition of the purified copolymer was determined by ^{1}H-NMR spectra recorded on a Bruker 300 MHz spectrometer using trifluoroacetic acid as a solvent and TMS as an internal reference. A DuPont 1090 differential scanning calorimeter was used to measure the T_g, T_m, and heat of fusion of the homo- and copolymers, using standard indium for calibration. Intrinsic viscosities of the copolymers were measured using an Ubbelohde viscometer and hexafluoroisopropanol (HFIP) as a solvent at 25°C, while for the degraded samples, viscosity measurements were carried out in trifluoroacetic acid.

In Vitro Degradation

The samples for degradation were fabricated as round sheets by compression under 6 ton/cm^2 of pressure at room temperature (diameter: 13 ± 0.2 mm; thickness: 0.75 ± 0.10 mm; weight: 110 ± 5 mg). Degradation experiments were conducted in two media: distilled water (pH 6.8) and 0.1M PBS buffer (pH 7.4). The accurately weighted sheets were immersed in each medium and kept at 37 $\pm$ 0.5°C in a shaking air-bath operating at a frequency of 60 rpm. At the end of each immersion period, the sheets were taken out, washed with distilled water three times, and dried at room temperature under reduced pressure. Then, the weight loss, water absorption, intrinsic viscosity, composition, glass transition temperature, melting temperature, heat of fusion of the residual
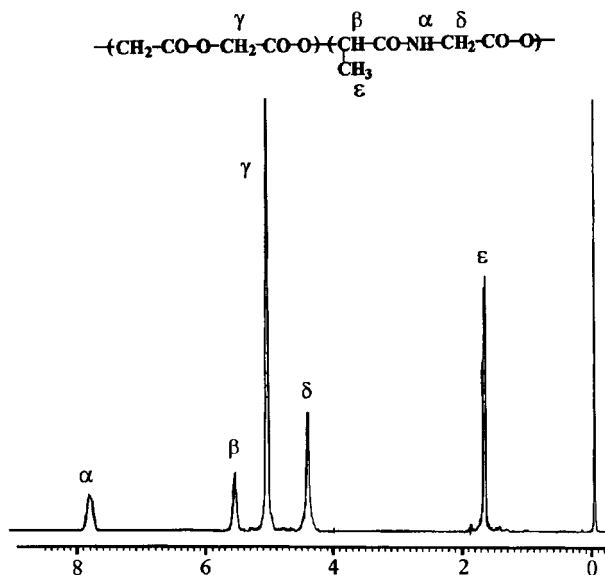

Figure 1 ^{1}H-NMR spectrum of PGM with 44.6% molar content of MMD units (solvent: trifluoroacetic acid).

sheets, and pH value of the degradation media were measured.

RESULTS AND DISCUSSION

Copolymerization of Glycolide and MMD

Both glycolide and MMD are six-membered cyclic monomers and can be homopolymerized in the bulk in the presence of stannous octoate. Our results indicated that the copolymerization of glycolide and MMD can be also conducted using similar conditions. Figure 1 represents the ^{1}H-NMR spectrum of a PGM copolymer. The composition of PGM copolymer was obtained by the following formula:

MMD molar content (%)

$$= 2 \cdot I\delta \cdot 100/(I\gamma + 2 \cdot I\delta)$$

where $I\delta$ and $I\gamma$ are the intensities of peak δ and peak γ, respectively. DMF is a nonsolvent for PGA, while PMMD is soluble in it. For a simply physical mixture of PGA and PMMD homopolymers, PMMD can be extracted from the mixture by DMF, leaving PGA undissolved. Table I shows the extraction results of PGM copolymers with different compositions by DMF. It was observed that the soluble fraction increased as the MMD content increased in the original copolymers and the copolymers with more than 66% molar content of MMD were completely dissolved in DMF. More-

Table I   Changes in Composition and Weight of PGM Copolymers Prior and Postextraction by DMF[a]

After Extraction	Original Composition (MMD Molar Content)							
	9.4	18.9	26.1	34.9	44.6	66.0	84.8	100
Composition[b]	10.1	18.1	16.7	22.2	20.1	—	—	—
Percent weight[c]	86	68	58.4	35.7	31.5	0	0	0

[a] Polymer/DMF: 1/100 (g/mL); T: 60°C; extraction time: 10 h.
[b] MMD molar content in the unsoluble residues.
[c] Percent weight ratio of the residues to original polymers.

over, the remaining undissolved fraction was not pure PGA homopolymer, but contained MMD units. These results indicated that the copolymerization indeed resulted in the formation of copolymers.

Factors Affecting Copolymerization

The effects of polymerization temperature, time, concentration of stannous octoate, and the monomer ratios in the feed were investigated to seek optimal conditions for the bulk copolymerization of GA and MMD. Table II shows the effect of polymerization temperature and time. The higher yield, intrinsic viscosity, and MMD content of the copolymer were obtained at 130°C. At temperature 115°C, all the above parameters were lower than those at 130°C, which may be due to the incomplete polymerization. The lowest molecular weight of copolymers was observed at 160°C, which probably resulted from an increase rate of depolymerization and side reactions at higher temperature. It was reported that the bulk homopolymerization of glycolide at 170°C produced a white, brittle polymer with high yield,[11] while only a dark brown oligomer with low molecular weight was obtained with a very low yield for MMD at similar temperature. For homopolymer-

ization of MMD, the optimal reaction temperature was reported to be about 130°C.[9] Our result showed that the appropriate temperature for the copolymerization of GA and MMD was also about 130°C. This may be due to that the incorporation of MMD units into PGA decreased its thermostability and increased side reactions during the copolymerization, which was supported by the change in color of the reaction products from white to brown with increasing MMD content in the feeds. After 22 h, there was no remarkable change in yield and intrinsic viscosity with prolonged polymerization time while the MMD content of the copolymer obtained at 22 h was somewhat lower.

Table III shows the effect on the copolymerization of the concentration of stannous octoate. The yield, intrinsic viscosity, and MMD content in the copolymers increased with increasing the concentration of stannous octoate up to 0.08% (I/Ms, mol), and then leveled off. Compared with bulk polymerization of L-L, for which the appropriate ratio of stannous octoate to monomer is about 0.01% (mol), the optimal ratio of initiator to monomers in the copolymerization of MMD and glycolide is much greater. This phenomenon may be caused by the loss of initiator activity by side reactions due to the incorporation of MMD units, which contain amide bonds. Some authors re-

Table II   Effects of Temperature and Time on the Copolymerization[a]

T (°C)	t (h)	Yield (%)	MMD[b]	[η]
115	45	85.6	14.0	0.38
160	45	91.4	19.0	0.33
130	45	91.7	19.0	0.45
130	22	93.6	15.0	0.40
130	64	92.7	18.0	0.42

[a] Molar ratio of initiator to monomers I/Ms (%): 0.06; MMD molar content in the feed: 20%.
[b] MMD molar content (%) in copolymers.

Table III   Effect of the Concentration of Stannous Octoate on the Copolymerization[a]

I/Ms (%)	Yield (%)	MMD[b]	[η]
0.02	83.5	12.0	0.39
0.06	92.7	18.0	0.42
0.08	94.7	17.0	0.45
0.20	93.4	17.0	0.45
0.38	94.9	18.0	0.47

[a] MMD molar content in the feed: 20%; T: 130°C; t: 64 h.
[b] MMD molar content (%) in copolymers.

Table IV   Copolymerization of glycolide and MMD at Different Monomer Ratios in the Feed[a]

MMD[b]	MMD[c]	Yield (%)	[η]	T_g (°C)	T_m (°C)	ΔH (J/g)
0	0	98.0	—	42.5	222.9	83.9
9.9	9.4	99.0	0.43	53.8	214.7	71.5
20.1	19.5	98.0	0.42	62.5	209.3	58.5
30.1	26.1	94.1	0.40	67.5	205.8	46.0
40.0	34.9	90.7	0.40	72.1	201.2	34.9
49.9	44.6	88.6	0.39	74.8	196.6	19.9
74.9	66.0	79.2	0.37	75.2		
90.1	84.9	78.5	0.36	86.2		
100	100	62.0	0.32	90.5		

[a] T: 130°C, t: 48 h, I/Ms (mol): 0.06/100.
[b,c] MMD molar content: (b) in the feed; (c) in the copolymer.

ported that the carbonyl oxygen of the amide bond of the polymer or monomer can coordinate to the tin atom, which would decline the initiator activity.[12–14]

The results of copolymerization of GA and MMD at different monomer ratios in the feed are shown in Table IV. It can be seen that both yield and intrinsic viscosity of the polymers gradually decreased with an increasing molar ratio of MMD in the feed. The MMD molar content in all copolymers were slightly lower than those in their monomer feed. These results may reflect the difference of reactivity between MMD and glycolide. According to the literature, the polymerization reactivity of glycolide is higher than that of DL-LA,[15] and the latter is more active than is MMD.[9]

DSC Analysis

The samples for DSC analysis were treated at 130°C for 30 min and then gradually cooled to room temperature in about 3 h just before measurement. The DSC thermograms of PGM copolymers with different compositions are shown in Figure 2. The results show that the glass transition temperatures (T_g's) of the copolymers increase with MMD contents while the melting points (T_m's) gradually decrease. Only one glass transition temperature was observed for each of the copolymers, which revealed a good compatibility of the two monomeric repeating units. The heats of fusion (ΔH_s) of the copolymers as listed in Table IV also decreased with an increased MMD content. Figure 3 shows the relationship of ΔH_s and the weight ratios of MMD units. Extrapolating the curve to $\Delta H = 0$ gives the composition of the copolymer (59.9% molar content of MMD),

at which the copolymers start becoming amorphous. The DSC thermograms, as presented in Figure 2, also showed that the copolymers with more than 66% molar content of MMD are completely amorphous.

For a copolymer with a two-component, one-phase system (the repeating units are compatible), the glass transition temperature can be described empirically by Fox[16] as

$$1/T_g = w_1/T_g^1 + w_2/T_g^2 \qquad (1)$$

where w_1 and w_2 are the mass fractions of components 1 and 2, respectively. Figure 4 shows the comparison between the T_g's measured by DSC and the values calculated by the Fox equation. The results indicate that the measured T_g's are higher than the calculated values. This result may be explained by the crystallization tendency of GA repeating units. P(MMD), as illustrated above, is an intrinsically amorphous polymer, but PGA is a crystallizable one. In the amorphous regions, being the dominative factor for the T_g, there would be more MMD units than in the crystalline regions, which could give rise to a higher T_g. Furthermore, the presence of crystalline particles also increases T_g.

As described above, copolymerization of GA with increasing amounts of MMD caused a decrease in T_m values. The melting point depression for an AB copolymer with A units capable of crystallizing and B units which cannot crystallize and are rejected from the crystal can be estimated by the following equation[17]:

$$1/T_m - 1/T_m^0 = -(R/\Delta H) \cdot \ln \rho \qquad (2)$$

where T_m is the melting temperature of a copolymer with an X_A molar fraction of A units; T_m^0, the equilibrium melting temperature of an ideal crystal of the corresponding homopolymer; R, is the gas constant; ΔH, the heat of fusion per mol of A repeating units; and ρ, the probability of the occurrence of an A unit next to other A units. For a random copolymer, ρ is equal to X_A, but for a block copolymer, ρ is approximately equal to 1, meaning that the T_m of the copolymer is only slightly lower than the T_m^0. Shown in Figure 5 are the reciprocals of T_m measured by DSC and estimated by eq. (2) (where $\rho = X_A$) at different compositions. It can be seen that the T_m values determined by DSC are higher than are the values estimated by eq. (2), which implied that ρ was larger than X_A. This result might be caused by that the
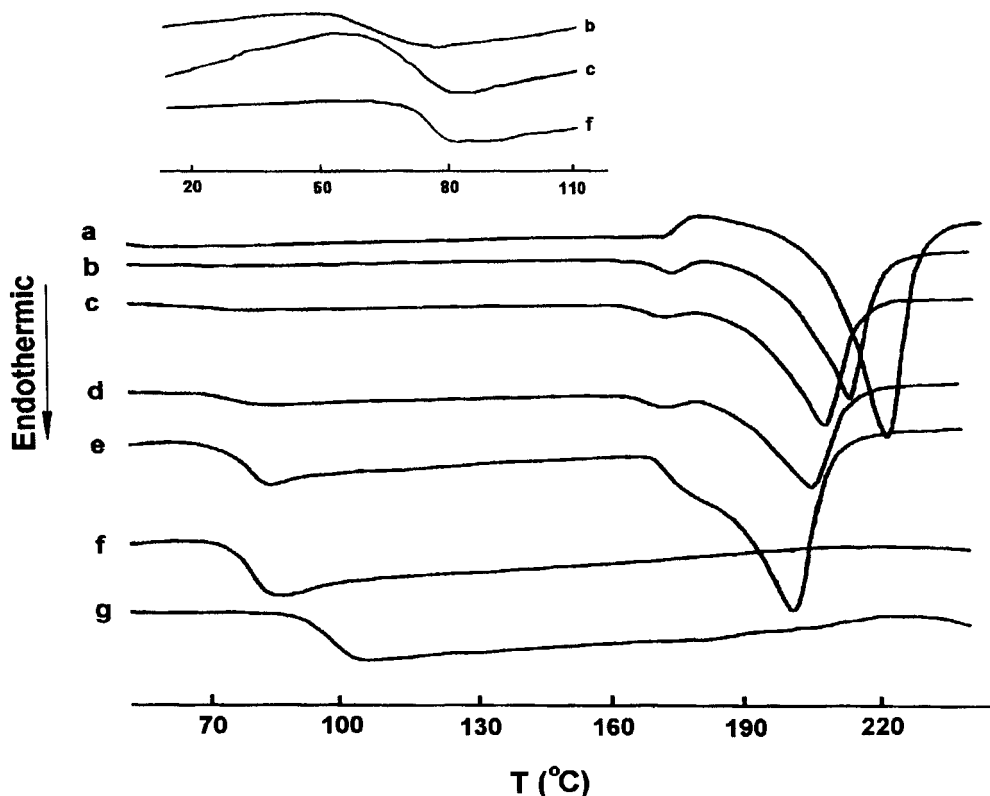
Figure 2 DSC thermograms of PGM with different compositions. MMD molar content (%): (a) 0; (b) 9.4; (c) 19.5; (d) 26.1; (e) 34.9; (f) 66.0; (g) 100.

distribution of MMD units or GA units in the copolymer chains is not completely random due to the different polymerization reactivities of the monomers. This suggestion is supported by the data listed in Table I. If the two kinds of units were exclusively random in the copolymer chains, compositions of the residues after extraction should be equal to that of the original copolymers. However, the extraction experiments showed a

dramatic decrease of MMD contents in the residues.

In Vitro Degradation

A series of PGMs with various amounts of MMD units was prepared to study the effect of MMD units on the *in vitro* degradation of the copolymers. The percentage of MMD molar content and
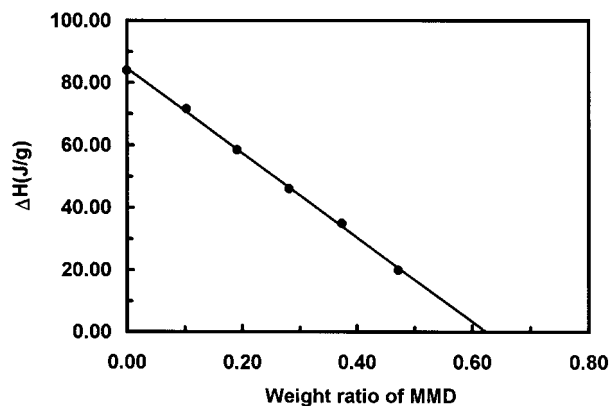

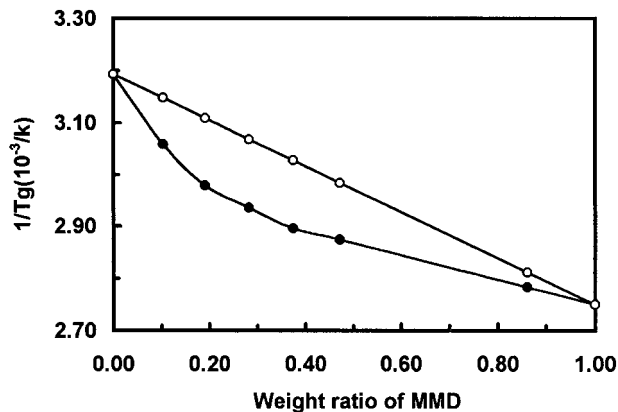**Figure 3** Relationship of ΔH_s and the weight ratios of MMD units.

Figure 4 $1/T_g$ vs. weight ratio of MMD units: (●) measured by DSC; (○) calculated by the Fox equation.
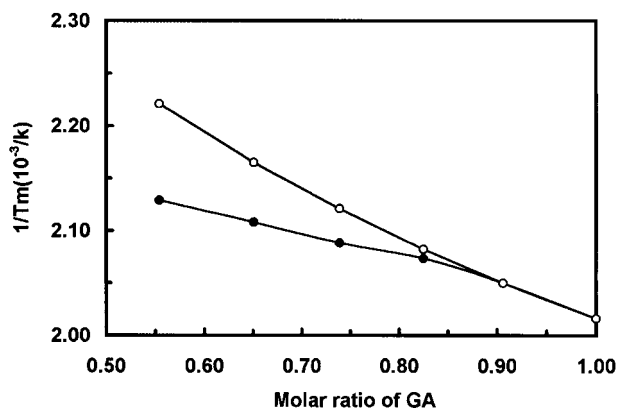
Figure 5 $1/T_m$ vs. molar ratio of GA units: (●) measured by DSC; (○) calculated by eq. (2).

values of intrinsic viscosity of PGM10, PGM30, and PGM50 are 11.6, 28.5, and 45.6 and 0.253, 0.343, and 0.323, respectively. Figure 6(a) shows the weight loss profiles of the PGM sheets at dif-

ferent immersion times. The apparent weight loss was observed from the onset of the experiment, especially for PGM30 and PGM50, which may be due to the presence of oligomers. The PGM sheet with a higher MMD content showed a more rapid weight loss. The degradation proceeded faster in the PBS buffer than in distilled water. Figure 6(b)–(d) show the changes of water absorption, intrinsic viscosity, and pH values of the degradation media (distilled water). Water absorption was detectable just after immersion and then increased gradually. The rates of water absorption increased significantly with increase of MMD content in the copolymers. The changes in the intrinsic viscosity of the remaining polymers and pH of the degradation media are in good agreement with the weight loss and water-absorption profiles. These results may be accounted for by the changes in crystallinity and hydrophilicity of the (co)polymers. As mentioned above, the crystallinity of PGM copolymers decreased with increased
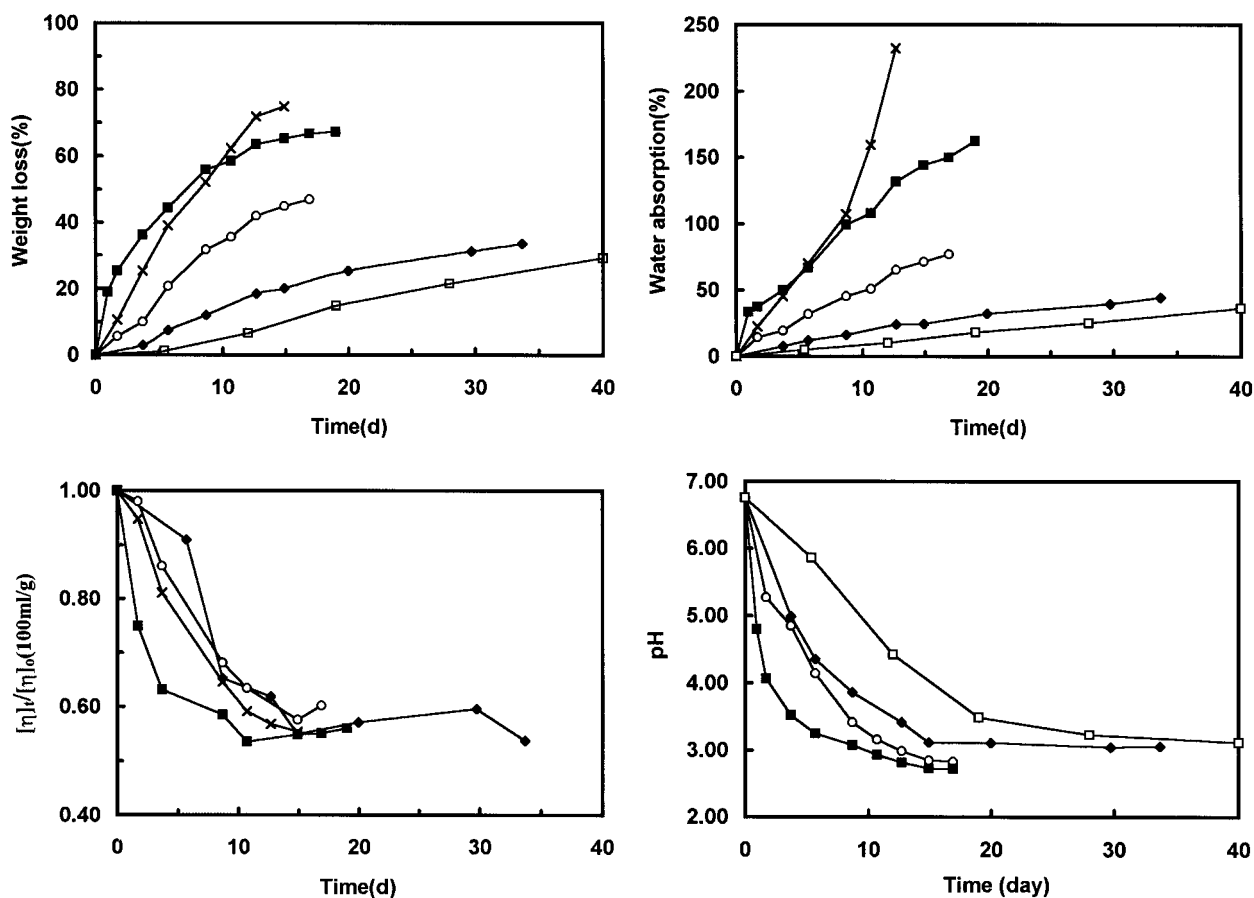

Figure 6 Evolution of some parameters during the degradation of PGM copolymers: (□) PGA; (♦) PGM10; (○) PGM30; (■) PGM50 in distilled water; (x) PGM30 in PBS buffer. (a) Weight loss; (b) water absorption; (c) intrinsic viscosity; (d) pH of the media (distilled water).
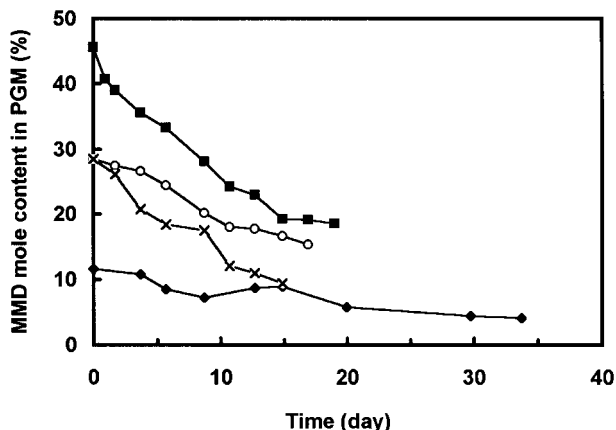
Figure 7 Changes in composition of PGM copolymers during degradation: (◆) PGM10; (○) PGM30; (■) PGM50 in distilled water; (x) PGM30 in PBS buffer.

Figure 8 Changes in heat of fusion for (○) PGM30 and (■) PGM50 in distilled water and (x) PGM30 in PBS buffer.

MMD content. The crystalline regions are more resistant to degradation and, therefore, the copolymers rich in MMD units degraded more rapidly. The more hydrophilic character of the degraded fractions enriched in MMD units may also be a factor that accelerates the degradation. Figure 7 shows the changes of chemical composition of the copolymers, which indicates that the MMD content in the copolymers decreases with time and the declining rate is larger for samples with a higher MMD content in the original copolymers.

The weight loss behavior as presented in Figure 6(a) revealed that the weight loss rates of the sheets of PGM30 and PGM50 gradually slowed down after 10 days. The changes of MMD content with degradation time also showed a similar tendency. It suggested that the degradation proceeded through two stages: mainly in amorphous regions at first and then in crystalline ones. The changes in the heat of fusion of PGM copolymers as presented in Figure 8 supplied additional evidence. The results show that the crystallinity of PGM 30 (both in distilled water and in PBS buffer) gradually increased with degradation time within the test period, while there was a maximum for PGM 50. It is in good agreement with the results on the degradation of PGA,[18,19] PLGA,[6,20] PLLA,[21] and PCL.[22] Generally, as reported in the literature,[19,21] the disordered amorphous regions are easy to be attacked by degradation media and degrade faster than do the crystalline ones, making the crystalline fraction increase. Meanwhile, during degradation, short chains composed of high numbers of GA units are more mobile and more susceptible to recrystallization. This would also cause an increase in crystallinity. Figure 9
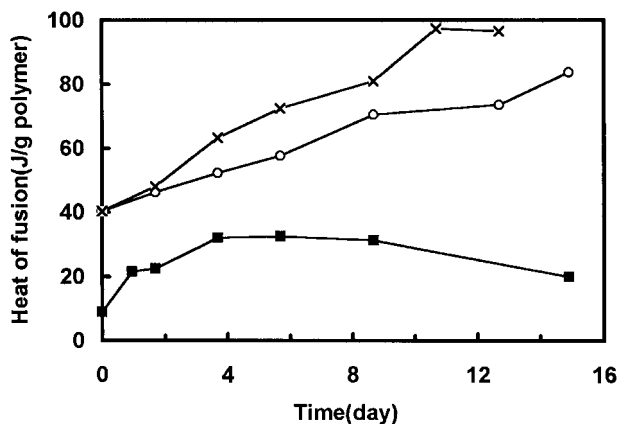
presents the DSC thermograms of PGM 30 samples at different degradation times in distilled water. There was a general trend that the T_m's decreased with the degradation time, which may be due to the degradation in the crystalline regions that made the molecular weight of the polymers
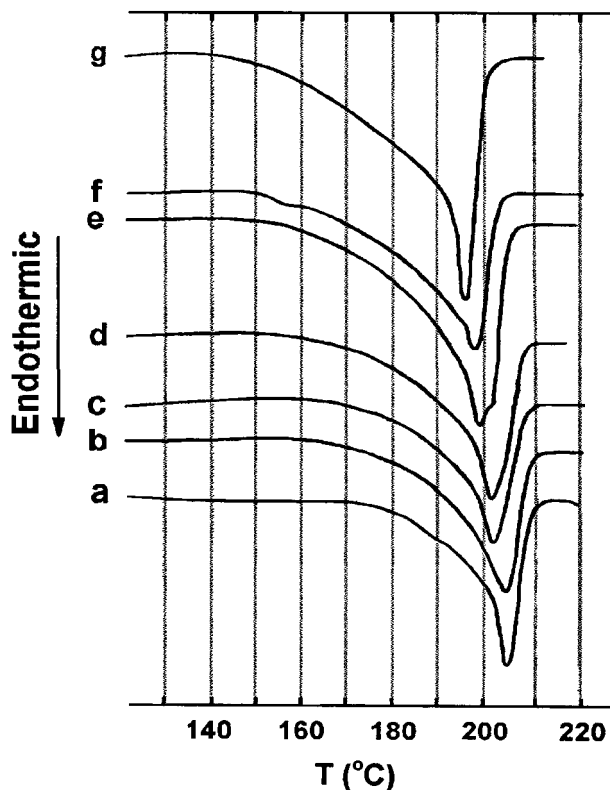

Figure 9 DSC thermograms of PGM30 at different degradation times in distilled water. t (day): (a) 0; (b) 1.7; (c) 3.7; (d) 5.7; (e) 8.7; (f) 12.7; (g) 14.9.

decrease and the amounts of crystalline defects increase.

CONCLUSION

Bulk copolymerization of GA and MMD was performed using stannous octoate as an initiator. Both temperature and initiator concentration affected the copolymerization. The optimal temperature was found to be about 130°C, at which the yield, intrinsic viscosity, and MMD content of the copolymers were higher, while higher temperature led to the copolymer with the lowest molecular weight. In comparison with the homopolymerization of LA, a higher concentration of stannous octoate is needed to increase the yield and MMD content of the copolymer. The incorporation of MMD units into PGA chains remarkably changed its crystallinity. T_g increased with MMD content, while T_m and ΔH decreased.

In vitro degradation was carried out using PGM copolymers with different compositions. The copolymers with a higher MMD content degraded more rapidly, while the degradation proceeded faster in the PBS buffer than in distilled water. It was found that the degradation proceeded through two stages. The variety of crystallinity and hydrophilicity are suggested to be a possible explanation for the above degradation results.

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