Some Insight into Hydrolytic Scission Mechanisms in Bioerodible Polyesters

Frank Alexis[1], Subbu Venkatraman[2], Santosh Kumar Rath[2], Leong-Huat Gan[3]

[1]*Institute of Bioengineering and Nanotechnology, Singapore 138669*
[2]*School of Materials Engineering, Nanyang Technological University, Singapore 639798*
[3]*Natural Sciences, National Institute of Education, Singapore 637616*

Received 5 October 2005; accepted 29 November 2005
DOI 10.1002/app.23888
Published online in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: PLLA, PDLLA, and PLGA copolymers have been studied to understand the details of their degradation behavior. All polymers exhibited a homogeneous mode of degradation, with uniform rates of degradation throughout the film. Crystallinity inhibited water absorption and hence retarded degradation. The degradation rate was increased by the presence of glycolic acid units in the PLGA copolymer; this effect overwhelms any decrease in degradation rate because of increased crystallinity due to the additional GA units. This effect is demonstrated quantitatively in this study. In PLGA polymers, there is evidence of unusual recrystallization behavior as degradation proceeds, due mainly to the higher rate of hydrolytic scission of the glycolide linkage compared with the lactide, as verified quantitatively with the use of ^{1}H NMR studies. Application of a Monte Carlo model to the degradation results, however, appears to show a random scission process. The details of the mechanistic study of different factors influencing the process of degradation, as reported here, may have important implications in terms of selecting the right material for specific biomedical applications. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 102: 3111–3117, 2006

Key words: biodegradable; biomaterials; Monte Carlo simulation; degradation

INTRODUCTION

The most important degradation mechanism of biodegradable polymers is chemical degradation via hydrolysis or enzyme-catalyzed hydrolysis. Applications in tissue engineering and drug delivery have primarily relied on materials that erode or degrade in body fluids so that the device ultimately disappears with no ill effects. In this context, aliphatic polyesters derived from lactic acid and glycolic acids are bioerodible polymers of growing interest in the fields of temporary surgical and pharmaceutical applications, in view of their biocompatibility and toxicological safety.[1]

Many factors[2–6] influence the degradation of poly(lactide-*co*-glycolide) polymers (PLGAs) including polymer composition[1,7] and molecular weight (M_w).[8,9] In general, polymer degradation is accelerated by greater hydrophilicity in the backbone or end groups, greater reactivity among hydrolytic groups in the backbone, less crystallinity,[10,11] and larger finished size[12,13] of the device. However, the relative magnitudes of the various effects are not clear. There is also some disagreement in the literature, in particular, about the effects of crystallinity and on the details of the scission process. To address some of these issues, we studied polyesters of different crystallinities and compositions by using weight changes, SEC, DSC, and NMR, as well as by applying Monte Carlo calculations to the weight loss data.

Polymer samples were carefully selected to present a variation in composition, crystallinity, and molecular weight. Poly(L-lactide) (PLLA; intrinsic viscosity IV = 2.04) is a semicrystalline homopolymer of L-lactide, while PDLLA (IV = 2.4), a random copolymer of D- and L-lactides, is completely amorphous. On the other hand, PLGA 80/20 (IV = 4.8) is a random copolymer composed of L-lactide and glycolide units, whereas PDLLGA 53/47 (IV = 0.84) is a random copolymer consisting of 53% of D- and L-lactide and 47% of glycolide.

The *in vitro* degradation studies have been done using phosphate buffer saline (pH 7.4). The polymers were characterized with respect to weight loss, water uptake, thermal behavior, morphological changes, average molecular weight changes (M_w), ^{1}H NMR and a Monte Carlo model so as to fully understand the degradation mechanisms.

EXPERIMENTAL

Materials

Poly(L-lactide) (PLLA; (intrinsic viscosity IV = 2.04 dL/g), poly(D,L-lactide) (PDLLA; IV = 2.4 dL/g),

Correspondence to: S. S.Venkatraman (assubbu@ntu.edu.sg).

poly(L-lactide-*co*-glycolide) 80:20 (PLGA 80/20; IV = 4.8 dL/g), and poly(D,L-lactide-*co*-glycolide) 53:47 (PDLLGA 53/47; IV = 0.84) were purchased from Purac Far East, Singapore. The polymers will be referred to in the following text as PLLA 2.04, PDLLA 2.4, PLGA 80/20, and PDLLGA 53/47, respectively. The numbers following the description of copolymers refer to the molar composition of lactide and glycolide units. Thus, PDLLGA 5347 0.84 is composed of 53% of D- and L-stereoisomers of lactide and 47% of glycolide, and the polymer has an intrinsic viscosity of 0.84 dL/g. The intrinsic viscosity data of the polymers were provided by the supplier, and the M_w was determined in our laboratory using size exclusion chromatography (SEC). Buffer solution (pH 7.4) was made from ready-made buffer salt (Hydrion™, Sigma–Aldrich, Singapore) by dissolving the powder from individual sachets into 500 mL of deionized water. $CDCl_3$ (99.8 atom % of deuterium), containing 0.03% (v/v) tetramethylsilane (TMS) and benzophenone (Sigma–Aldrich, Singapore) were used for quantitative ^1H NMR studies. Tetrahydrofuran and dichloromethane, both HPLC grade, were purchased from Tedia Chemical Company (Fairfield, CT).

Preparation of polymer films

Film casting was done from the resulting solution using a gravure rod. The concentration of polymer solution and the wet film thickness were adjusted so as to provide the final film thickness of 25 ± 5 μm after drying (measured using a digital micrometer). The wet film was allowed to dry at room temperature for 24 h. Subsequently, drying was continued in vacuum at 30°C for 20 more days. The above conditions achieved almost complete removal of solvent with minimal bubble formation. To eliminate the influence of sample size on the degradation process, all film samples had dimensions of 40 × 25 mm^2.

Degradation study

All films were put into individual glass vials (of 60 mL capacity each). The films were completely submerged at (37 ± 0.1)°C in 50 mL of the buffer solution (pH 7.4), forming a dilute solution (more than 100 times the sample weight) to ensure a constant pH of the solution. Films were removed at regular intervals and characterized for water uptake and weight loss as follows.

Water uptake

In a typical test, the film was wiped to get rid of excess buffer solution adhering on the surface, weighed (wet), and later dried to constant weight to determine the weight loss. Water uptake was calculated at each time point using the following equation:

$$\%\text{Water Uptake} = 100 \times \frac{W_{\text{wet}} - W_0}{W_0} \quad (1)$$

W_{wet} is the weight of the wet film at each time point, during the course of the study, and W_0 is the original dry weight of the film. Results presented are the average values obtained from the three samples for each film. All weights were measured to an accuracy of ±0.01 mg.

Weight loss

Weight loss of the films during the *in vitro* study period was measured from changes in dry weight of the original film and that at each time point. Once again, the values reported are the average of the three film samples. Following equation was used:

$$\%\text{Weight Loss} = 100 \times \frac{W_0 - W_t}{W_0} \quad (2)$$

where W_0 is starting dry weight, and W_t is dry weight at time t

Thermal analysis

TA Instruments' modulated™ differential scanning calorimeter (DSC 2920), equipped with refrigerated cooling system was used for measurement of the glass transition temperature (T_g), melting temperature (T_m), and crystallization temperature (T_c) of the samples before and during the *in vitro* study.

Samples were thoroughly dried to eliminate the effect of remaining moisture on thermal events. The degree of crystallization (DOC %) was calculated from the following relationship:

$$\%\text{DOC} = 100 \times \frac{\Delta H_m - \Delta H_c}{\Delta H^0} \quad (3)$$

ΔH^0 for PLLA = 93.1 J/g.[14,15]

Where, ΔH_m, ΔH_c, and ΔH^0, refer respectively to the enthalpy of melting, enthalpy of crystallization, and enthalpy of melting for the 100% crystalline sample of the same material. It was not possible to calculate the degree of crystallinity of PLGA 80/20 in the absence of the enthalpy data for a 100% crystalline material. Only the difference, $\Delta H_m - \Delta H_c$, representing the change in crystallinity has been plotted.

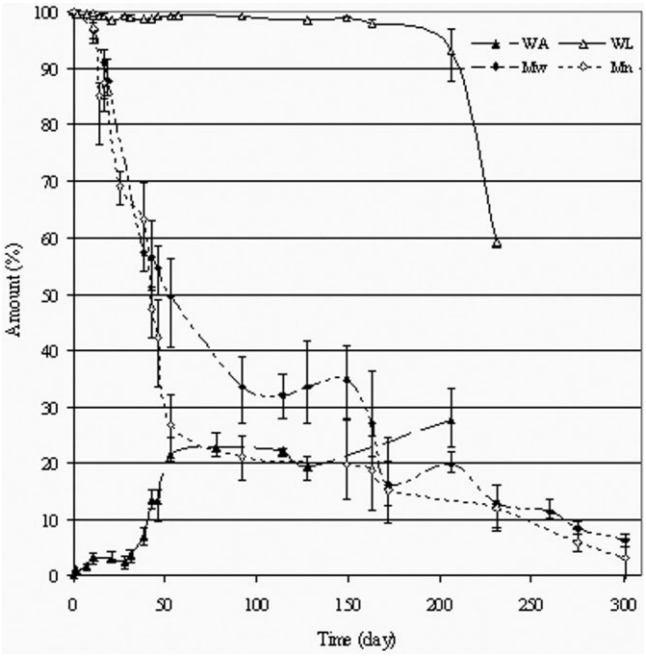

Figure 1 Overlay of water absorption, weight loss, M_w and M_n of PDLLA 2.4 versus immersion time.

Size exclusion chromatography

Weight and number average molecular weight (M_w and M_n) and molecular weight distribution of the polymers were determined using an Agilent series 1100 liquid chromatography system. Molecular weights of samples were obtained relative to polystyrene standards.

^{1}H NMR studies

The copolymer composition of PDLLGA 53/47 0.84, before and after the *in vitro* degradation were determined using ^{1}H NMR (Bruker Advance 400 MHz spectrometer). All spectra were recorded using $CDCl_3$ as the solvent and TMS as internal reference. The sample solutions were prepared in appropriate concentrations in $CDCl_3$, containing 0.1% (w/w) benzophenone as internal standard to eliminate possible differences during NMR quantifications. The composition of the copolymer remained after hydrolytic degradation was calculated from integration of peak areas of lactide unit (δ = 5.2 ppm) and glycolide unit (δ = 4.8 ppm) protons, with reference to the benzophenone aromatic protons (7.5–8.0 ppm) as internal standards.

Monte Carlo calculations

Mechanistic details of polymer materials undergoing degradation have been studied using different models. In particular, the randomness of the chain scission is one of the details considered. A Monte Carlo procedure, which attempts a comparison of simulation results with experimentally determined molecular weights (as a function of the degree of degradation (or conversion)), allows some insight into scission paths.[16] We have tried to use this model to assess the randomness of the hydrolytic scission. This model has been used to study the randomness of depolymerization reactions following chain scission with and without volatilization of products formed (monomer and/or oligomers), and may be applicable for our study. In the case of degradation occurring with chain scission with subsequent volatilization, polymer samples undergo changes in degree of polymerization as well as weight loss. The degradation can be followed as a function of the degree of conversion, c, representing the weight loss:

$$c = 1 - \frac{w}{w_0} \tag{4}$$

where, w_0 is the weight of the original sample, and w is the weight of the degraded sample, corresponding to any degree of conversion, c.

The Monte Carlo parameter R_m is calculated from the ratio of the number average degree of polymerization during degradation (X_n), at the degree of conversion c, and the degree of polymerization (X_{n0}) of original material, using the relationship in eq. (5).

$$R_m = (1 - c)\frac{X_{n0}}{X_n} \tag{5}$$

with $0 \leq c \leq 1$

The parameter R_m when plotted against degree of conversion c gives an indication of the characteristic degradation path followed by the system under the conditions of study. Random chain scission is characterized as a hyperbolic profile whereas nonrandom

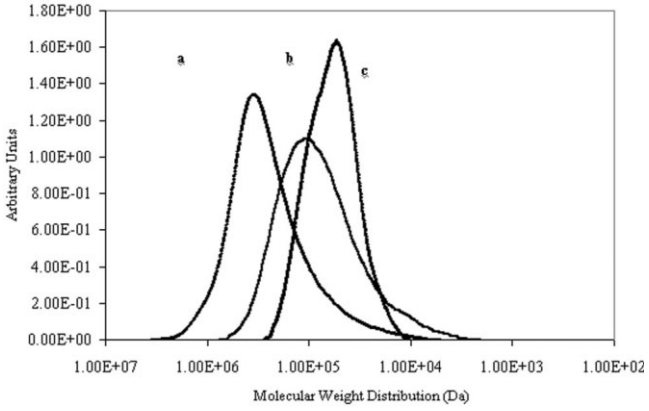

Figure 2 SEC chromatograms of PDLLA, showing changes in molecular weight distribution with immersion time (a) reference sample, (b) 128 days, and (c) 275 days.

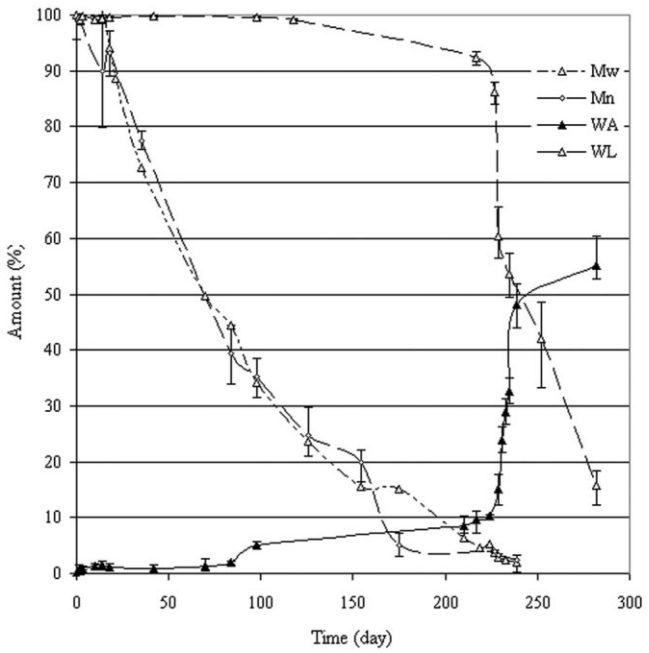

Figure 3 Overlay of water absorption, weight loss, M_w and M_n of PLGA 80/20 4.8 versus immersion time.

chain scission is defined as linear profile.[16] Monte Carlo calculations for PLGA 80/20 4.8 and PDLLGA 53/47 0.84 were carried out from number average molecular weights M_n and M_{n0} (obtained from SEC, and corresponding to X_n and X_{n0}) and the degree of conversion, c (calculated from weight loss during the *in vitro* study, and the original weight of samples).

RESULTS AND DISCUSSION

Water absorption, weight loss, and change in m_w

As expected, the semicrystalline PLLA polymer does not absorb much water while decreasing in molecular weight by about 20–50% (Figure not shown). In sharp contrast, the amorphous PDLLA (Fig. 1) follows a three-step water absorption behavior, with initial water absorption of less than 10% for 25 days. This is followed by a second phase characterized by a high increase in water absorption to form a plateau for 100 more days, and a final phase with another increase in water absorption due to onset of oligomer production and leaching out of the polymer film. The second rise in water uptake parallels the beginning of substantial weight loss. The decrease in weight average molecular weight, M_w, parallels the water absorption data to a large degree. The interesting observation here is that the onset of measurable weight loss occurs when the molecular weights have decreased to about 15% of the initial value (roughly 50 kDa). On examining the M_w and M_n changes for each polymer, we find that both the averages decrease by the same amount during the initial stages of degradation, and that the SEC chromatograms are unimodal (Fig. 2). This is true of all polymers, and is indicative of homogeneous degradation. These results agree with Grizzi et al., who concluded that the critical thickness below which the hydrolytic degradation of amorphous PLGA polymer is homogeneous is in the range of 0.2–0.3 mm. The PLLA and PDLLA polymers that we have studied here appear to undergo homogeneous degradation, also in the thickness range of about 0.02–0.03 mm.

The results for the copolymer PLGA 80/20 4.8 are presented in Figure 3. The material degrades almost completely at the end of 235 days of immersion in buffer. Water uptake is rapid, reaching up to 55% after about 280 days. Percent weight loss closely follows the trend in water uptake. The M_w at the onset of rapid weight loss (when 90% weight loss is observed, or the critical M_w) is about 45 kDa, about 5% of original value. These data show that the integrity of the matrix remains intact until about 95% of the original molecular weight has been lost.

The molecular weight (M_w and M_n) changes faster for PDLLGA 53/47 with immersion time (figure not shown). PDLLGA 53/47 films start to lose substantial mass after about 35 days of immersion and completely degrade after 3 months.

Hydrolysis kinetics

Using a semilog plot of the M_w versus time of PLLA 2.04 and PDLLA 2.4 (data not shown), as well as for PLGA 80/20 4.8 and PLGA 53/47, the apparent first-order rate constants for degradation were calculated from the slopes. These values are summarized in Table I with the respective coefficients of correlation for all the polymers.

TABLE I
Apparent First-Order Degradation Constants for the Polymers Used

First-order degradation constants	PLLA (IV = 2.04)	PDLLA (IV = 2.4)		PLGA 80/20 (IV = 4.8)	PDLLGA 53/47 (IV = 0.84)
Period (day)	0–300	0–150	150–250	0–239	1–60
k_{deg} (day^{-1})	0.0009	0.0087	0.0088	0.0127	0.0506
R^2	0.982	0.9752	0.9642	0.9827	0.989
Initial DOC (%)	35	0	0	4.5	0

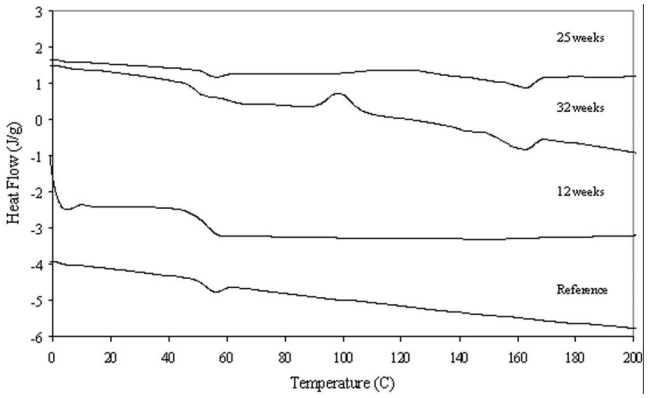

Figure 4 Traces of DSC thermograms for PLGA 80/20 4.8 showing the appearance of the crystallization peak with immersion time.

The relative order of the rate constants reflects the relative influence of crystallinity (compare PDLLA 2.4 and PLLA 2.04) and of chemistry (glycolide versus lactide). This is further elaborated in the Conclusions.

Unusual thermal behavior of PLGA 80/20

The thermal behavior of PLGA 80/20 has some unusual features that are not seen with any of the homopolymers, or with the copolymer PLGA 53/47. Figure 4 presents the traces of DSC thermograms for PLGA 80/20 4.8 showing the appearance of a crystallization peak with immersion time. This peak appears because of one of two possible reasons: (a) the lower-M_w molecules, formerly part of the amorphous phase, have reorganized into crystalline domains, or (b) the preferential generation of lactide-rich segments during degradation leads to crystallization of these segments upon heating above their T_gs. For reasons be described later, in particular based on the ^{1}H NMR results, we favor the latter explanation.

Figure 5 presents the difference in enthalpy of melting and recrystallization peaks ($\Delta H_m - \Delta H_c$), as a function of immersion time. There is an initial increase in DOC (up to about 200 days) followed by a decrease. The differential DOC ($H_m - H_c$) increases during the 0–200 day period because the amorphous region is slowly being depleted by preferential attack of the amorphous domains. Subsequent decrease in the differential DOC is clearly due to attack on the crystalline domains.

NMR results

To shed further light on the details of the hydrolytic mechanism, we studied PDLLGA 53/47 copolymer using proton NMR. Fig. 6 presents the ^{1}H NMR spectra of original PDLLGA 53/47 0.84, and PDLLGA 53/47 0.84 after 50 days of immersion. These results

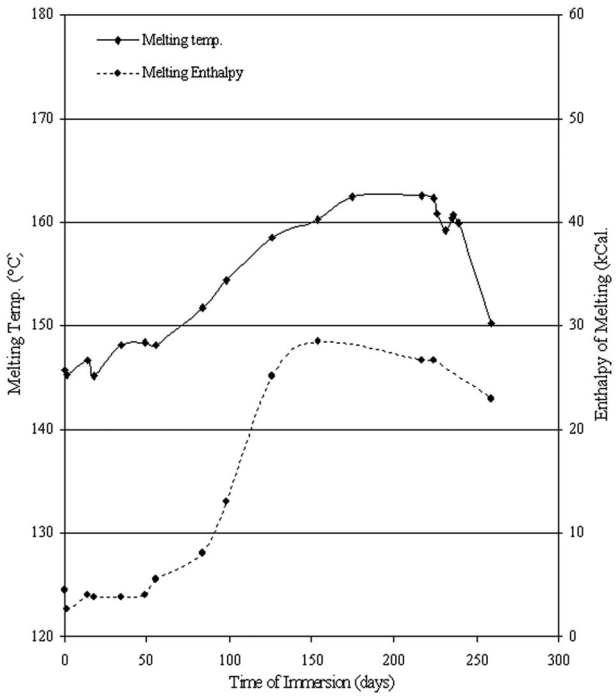

Figure 5 Differential crystallinity ($\Delta H_m - \Delta H_c$) for PLGA 80/20 4.8.

show the evidence of a preferential degradation of the glycolide when compared with the lactide linkages. This is independent of the faster degradation rate of amorphous compared to crystalline regions shown earlier (PLLA and PDLLA) because PDLLGA 53/47 is totally amorphous. Figure 7 presents the residual contents of the lactide and glycolide of the copolymer remaining after degradation as determined by ^{1}H NMR. It can be clearly seen that the glycolide fragments/units degraded faster than the lactide using benzophenone as a reference. The lactide content, which was shown to be 55.4% in the original sample

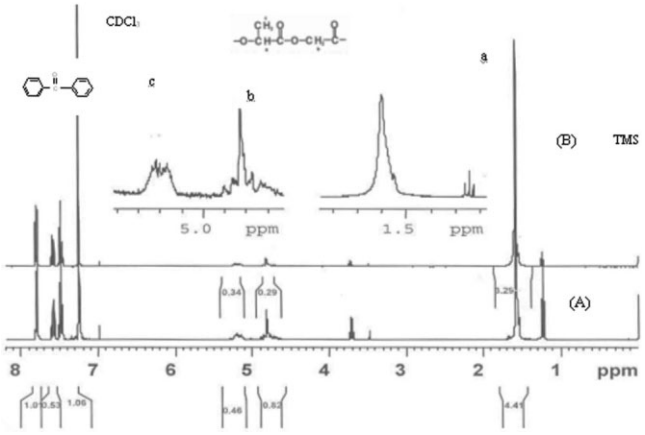

Figure 6 ^{1}H NMR spectra of PDLLGA 53/47 0.84 before immersion (A) and after 50 days immersion (B).

(as against 53% reported by the supplier), steadily increases to about 62%, over a period of 41 days of degradation. The glycolide content, on the other hand, declines from about 45.6% of the original value to about 38%, over the same period. This finding is in general qualitative agreement with the results of Hakkarainen et al.[6] and Kamei et al.[17] using different techniques, both of which suffered from some limitations in quantitation.

Chemically, steric crowding and reduced electrophilicity of the carbonyl carbon inhibit the rate of hydrolytic degradation of lactide link in comparison with the glycolide.

Monte Carlo calculations

For the two PLGA copolymers, it was also possible to perform Monte Carlo calculations in an attempt to confirm the randomness of scission events. Figure 8 shows the overlaid Monte Carlo plots for PLGA 80/20 4.8 and PDLLGA 53/47 0.84, the two polymers that exhibited measurably high weight loss. Using the SEC values for M_n and the weight loss values, we computed R_m and other parameters in eq. (5). As seen from the Figure 8, both the copolymers showed patterns of random chain degradation. The hyperbolic profile was defined by three different parts, an increase, a dome, and a decrease. The first part corresponds to the degradation of the polymer without weight loss; the dome corresponds to the "stabilization region" and the beginning of the domination of the weight loss factor; and the last part shows the decrease corresponding to the leaching out of oligomers. The maximum of the Monte Carlo curve applied to PLGA 80/20 4.8 was much higher than PDLLGA 53/47 0.84 because of a domination of weight loss factor caused by a slower degradation rate. PLGA 80.20 shows almost no weight loss for about 300 days in contrast to PDLLGA 53/47, leading to a larger effect on the R_m and lowering its

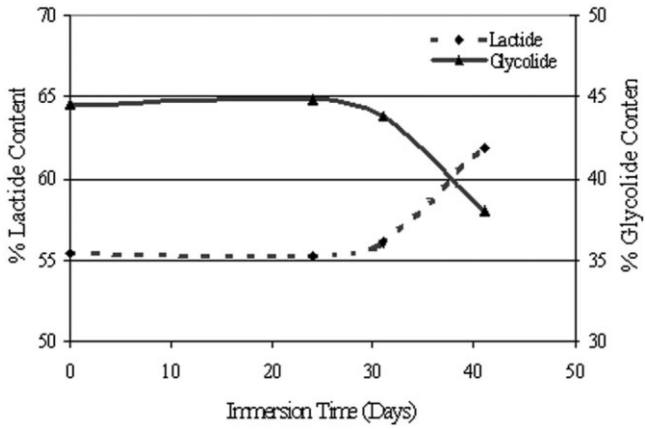

Figure 7 Relative percentages of lactide and glycolide content in PDLLGA 53/47, as calculated from ^{1}H NMR.

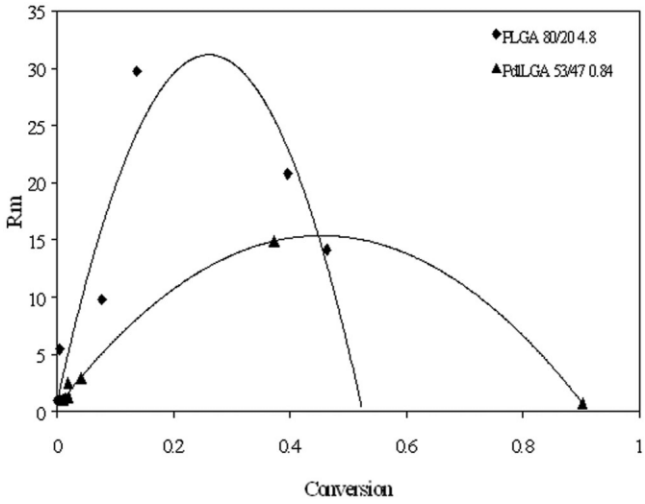

Figure 8 Monte Carlo plots for PLGA 80/20 4.8 and PDLLGA 53/47 0.84.

value compared with that of PLGA 80/20. Again, the conclusion is that the overall hydrolytic scission process is random in nature (in the sense that no end-linking is preferred against backbone scission), in spite of the higher reactivity of the glycolide unit. This probably also reflects the fact that the two polymers are random copolymers with very few blocks.

CONCLUSIONS

Tsuji and Ikada[11] have reported that the decrease in PLLA molecular weight was more rapid for PLLA films with higher initial crystallinity. They have explained the findings in terms of the crystalline domains introducing defects into the amorphous matrix, thus facilitating the penetration of water. We find more complex effects.

Using Table I, we find that when comparing PLLA with PLGA 80/20, the measured rate does increase with increase in glycolide units; and the completely amorphous PDLLA shows a slower rate of M_w decrease compared with semicrystalline PLGA 80/20. Taken together, these observations clearly demonstrate the following:

a. crystallinity decreases the rate, and
b. the effect of adding glycolide units overwhelms the effect of crystallinity

In other words, though both amorphous content and glycolide content increase the degradation rate, the glycolide unit addition has a much more dramatic effect on the rate.

The results presented show some new features of the degradation process for this type of polyesters:

a. Copolymers of L-lactide and glycolide undergo cleavage preferentially at the glycolide linkages;

TABLE II
Critical Molecular Weights for Polymers for Onset of Weight Loss

Critical molecular weight	PDLLA	PLGA 80/20	PLGA 53/47
M_w (kDa)	41	45	17
M_n (kDa)	22	20	9

because of this, unusual recrystallization of the L-lactide-rich units occurs during degradation. This effect is absent in DL-lactide copolymers.

b. Adding 20% of glycolide units increases the rate much more than reducing the crystallinity by 35%.

c. Measurable weight starts to occur when samples attain a certain M_w range (when the M_w reaches 90% of initial value; see Table II).

References

1. Fukuzaki, H.; Yoshida, M.; Asano, M.; Kumakura, M.; Mashimo, T.; Yuasa, H.; Imai, K.; Yamanaka, H. Biomaterials 1990, 11, 441.
2. Kenley, R. A.; Lee, M. O.; Mahoney, T. R.; Sanders, L. M. Macromolecules 1987, 20, 2398.
3. Makino, K.; Oshima, H.; Kondo, T. J. Microencapsulation 1996, 3, 293.
4. Chu, C. C. J Biomed Mater Res 1981, 15, 19.
5. Lee, K. H.; Won, C. Y.; Chu, C. C.; Gitsov, I. J. Polym Sci Part A: Polym Chem 1999, 37, 3558.
6. Hakkarainen, M.; Albertsson, A. C.; Karlsson, S. Polym Degrad Stab 1996, 52, 283.
7. Park, T. G. Biomaterials 1995, 16, 1123.
8. Park, T. G. J Controlled Release 1994, 30, 161.
9. Wang, N.; Qiu, J. S.; Wu, X. S. In Tailored Polymeric Materials for Controlled Delivery Systems; Mc Culloch, I.; Shalaby, S. W., Eds.; ACS Symposium Series 709; American Chemical Society: Washington, DC, 1998; p 243.
10. Nakamura, T.; Hitomi, S.; Watanabe, S.; Shimizu, Y.; Jamshidi, K.; Hyon, S. H.; Ikada, I. J Biomed Mater Res 1989, 23, 1115.
11. Tsuji, H.; Ikada, Y. Polym Degrad Stab 2000, 67, 179.
12. Witt, C.; Kissel, T. Eur J Pharma Biopharma 2001, 51, 171.
13. Grizzi, I.; Garreau, H.; Li, S.; Vert, M. Biomaterials 1995, 16, 305.
14. Weiler, W.; Gogolewski, S. Biomaterials 1996, 17, 529.
15. Mainil-Varlet, P.; Curtis, R.; Gogolewski, S. J Biomed Mater Res 1997, 36, 360.
16. Chiantore, O.; Guaita, M. J Appl Polym Sci: Appl Polym Symp 1993, 52, 1.
17. Kamei, S.; Inoue, Y.; Okada, H.; Yamada, M.; Ogawa, Y.; Toguchi, H. Biomaterials 1992, 13, 953.