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Importance of Distinct Water Environments in the Hydrolysis of Poly(DL-lactide-*co*-glycolide)

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ABSTRACT: A detailed examination of the role of water in the hydrolysis of poly(DL-lactide/glycolide) esters is reported. The hydrolysis rate of polyester pellets was independent of moderate changes in ionic strength, pH, and buffer concentration. Total water uptake by the polymer depended on ionic strength but not pH. The hydrolysis rates were independent of the total water content of the polymer. Solid-state ^{2}H-NMR qualitatively demonstrated the presence of two types of water (^2H_2O) environments within the polymer, bulk water with free rotation and bound water with hindered rotation. Differential scanning calorimetry also showed the presence of nonfreezing water, confirming different water environments within the polymers. Quantitative solid-state ^{2}H-NMR showed that the polymer contained a constant amount of water (^2H_2O) with hindered rotation. The molar quantity of ester groups in the polymer is 25-fold higher than the molar quantity of water with hindered rotation, suggesting that water's immobility and reactivity result from its selective hydrogen bonding to the oxygen of ester carbonyl groups. The increased hydrolysis rate, observed for polyesters with higher glycolide content, correlates with an observed increase in bound, reactive water.

Introduction

The polyesters of DL-lactide and glycolide have been investigated for the controlled release or delayed release of drugs for over 20 years.[1] An enormous amount of work has been reported on the application of these materials as biodegradable drug delivery matrices. Two products, Luepron Depot by TAP Pharmaceuticals and Zoladex by ICI have been approved by the Food and Drug Administration (FDA). Despite their successful application, the hydrolysis of DL-lactide/glycolide esters remains poorly understood. While recent reports by Vert and co-workers[2] have provided valuable insight into certain aspects of the hydrolysis of these polyesters, the role of water in the reaction is still unclear. For example, although it is known that increasing the DL-lactide content in amorphous DL-lactide-*co*-glycolide copolymers results in decreased hydrolysis rates, the underlying physicochemical reasons for this effect are unclear. Thus, a thorough understanding of the hydrolysis process is desirable so that the potential of DL-lactide-*co*-glycolide polymers as drug delivery matrices can be fully realized.

Previous studies in our laboratory comparing poly(DL-lactide-*co*-glycolide, 50:50) (PLG50:50) samples obtained from different commercial sources showed two regions of hydrolysis. The initial hydrolysis reaction, which followed pseudo-first-order kinetics, was followed by an accelerated rate of hydrolysis after the molecular weight (MW) reached ~10 000.[3] The simplest explanation for the initial hydrolysis behavior is that it is an uncatalyzed reaction between water and ester where the concentration of water remains essentially constant, resulting in the pseudo-first-order rate constants, $k' = k[H_2O]$. The subsequent accelerated hydrolysis rate could then be due to autocatalysis by the increased number of carboxylate groups and/or an increase in the concentration of reactive water in the polymer. The water uptake profile of the polymer, which was expected to play a critical role in the subsequent hydrolysis, showed that, although the water content of PLG50:50 increased over fivefold during degradation, constant pseudo-first-order kinetics were still observed. A possible explanation for the failure of the hydrolysis rate to increase with an increase in total water concentration within the polymer could be that only a portion of this water is reactive, resulting in polyester hydrolysis. The goal of this study was to characterize the effect of medium ionic strength, pH, and the role of water in polymer hydrolysis. Four different polymers—PLG50:50, poly(DL-lactide-*co*-glycolide, 65:35) (PLG65:35), poly(DL-lactide-*co*-glycolide, 85:15) (PLG85:15), and poly(DL-lactide) (PLA)—were studied. Water uptake and polymer MW were monitored as a function of time, and solid-state ^{2}H-NMR was used to characterize the water (^2H_2O) environment within the polymer.

Results and Discussion

Polyester pellets (4 mm × 3 mm) were prepared by melt-pressing spray-dried polymers in a thermostated punch and die set. These pellets were glasses that maintained their structural integrity throughout the time frame during which the water uptake studies were carried out.

Ionic Strength and pH Effects on the Hydrolysis and Water Uptake of Poly(DL-lactide-*co*-glycolide, 50:50). PLG50:50 pellets were exposed to aqueous media under different ionic strengths and pH conditions; the MW and water uptake profiles were measured over time. The effect of ionic strength on the water uptake of PLG50:50 is shown in Figure 1a. Water uptake was determined to be proportional to the activity of water in solution, with increasing salt causing a decrease in the rate and extent of water uptake. As the ionic strength of the medium increased from distilled water to 0.154 and 0.604 M sodium chloride, the initial rate of water uptake decreased from 1.67 to 0.77 and 0.46%/day, respectively. The extent of water uptake after exposure to the different media for ~13 days was in the ratio of 1:2:3 for 0.604 M sodium chloride, 0.154 M sodium chloride, and water, respectively. An accelerated rate of water uptake was observed when the polymer MW reached ~10 000. The time of onset of this increased rate was independent of the ionic strength of the medium. This discontinuity indicates a change in bulk polymer hydration properties at a MW of ~10 000. The increased water in the polymer may be the result of its presence in the pores formed at the onset

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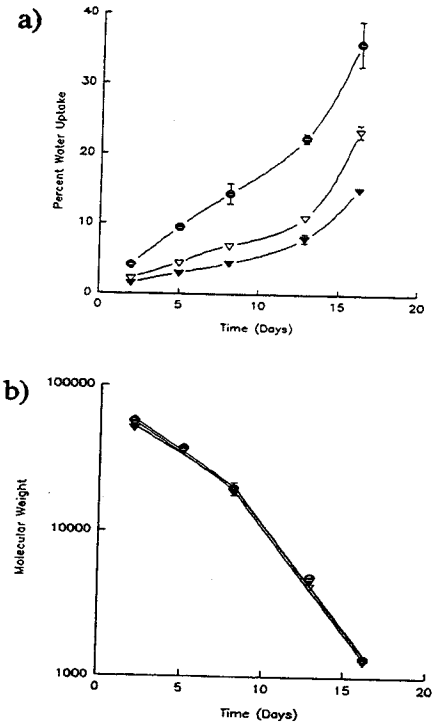

Figure 1. Water uptake (a) and molecular weight (b) of PLG50:50 in distilled H_2O (●), 0.154 M NaCl (▽), and 0.604 M NaCl (▼) as a function of time. Each point in a is the mean of three values and the standard deviation is shown.

Table 1. Pseudo-First-Order Rate Constants for the Hydrolysis of PLG50:50

degradation medium	k' (day^{-1}) region 1	k' (day^{-1}) region 2
distilled water	0.174 ± 0.017	0.329 ± 0.026
0.154 M NaCl	0.174 ± 0.020	0.330 ± 0.012
0.604 M NaCl	0.157 ± 0.020	0.330 ± 0.019
pH 7.2, PBS	0.164 ± 0.018	0.346 ± 0.021
pH 8.2, PBS	0.165 ± 0.024	0.329 ± 0.020
pH 7.2, 50 mM phosphate	0.170 ± 0.018	0.301 ± 0.019

of polymer degradation or through the increased hydration of the polymer as new carboxylate end groups are formed.

PLG50:50 hydrolysis in media of different ionic strengths (Figure 1b) shows that, although the water contents of the samples differed by as much as 3-fold, the hydrolysis profiles and rate constants were nearly identical (Table 1). Since the hydrolysis rates were independent of the total water content, it appears that total water content is not a rate-determining parameter in hydrolysis. This supports the hypothesis drawn from initial kinetic observations that suggested that only a portion of the water in the polymer is reactive. The similarity in these hydrolysis profiles also suggests that, although the total amount of water in the polymer differs, the reactive water present is constant and independent of the total water content of the polymer and the ionic strength of the degradation medium.

There has been some controversy regarding the effect of pH and buffer concentration on poly(lactide/glycolide) hydrolysis. Chu reported a faster hydrolysis rate of poly(glycolic acid) (PGA) in the presence of phosphate buffer and at high pH values.[4] Kenley *et al.*,[5] on the other hand, found the hydrolysis of PLG50:50 to be independent of pH. Reed and Gilding[6] also reported pH-independent hydrolysis of PGA, poly(L-lactide-*co*-glycolide, 50:50), and poly(L-lactide).

In view of these contrasting results, the hydrolysis and water uptake profiles of PLG 50:50 were studied in three

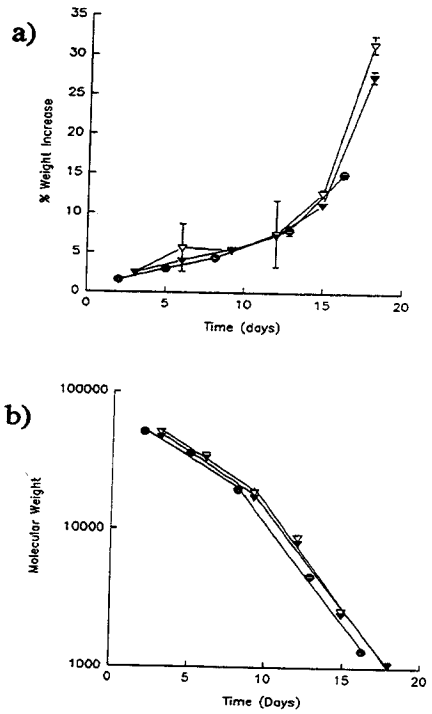

Figure 2. Water uptake (a) and molecular weight (b) of PLG50:50 after exposure to unbuffered media (●) and after exposure to pH 7.2 (▽) and pH 8.2 (▼) sodium phosphate buffer, each with μ = 0.604 M. Each point in a is the mean of three values and the standard deviation is shown.

media: unbuffered 0.604 M sodium chloride, 150 mM sodium phosphate (pH 7.2), and 150 mM sodium phosphate (pH 8.2), each with μ = 0.604 M. The water uptake and hydrolysis profiles are presented in Figure 2. Pseudo-first-order rate constants for hydrolysis are given in Table 1. Both the water uptake profiles and the hydrolysis profiles were very similar in all three media. The independence of hydrolysis rates with modest pH changes corroborates the results of Keneley *et al.*[5] and Reed and Gilding,[6] indicating that at these pH values neither hydroxyl ions nor hydronium ions participate in the hydrolysis reaction. Nearly identical hydrolysis profiles were obtained in 50 and 150 mM pH 7.2 sodium phosphate, suggesting no general-acid or general-base catalysis by buffer species (Table 1).

One reason for the absence of acid-base catalysis may be that the polymer is impermeable to ionized species. Charged dyes, amaranth and malachite green, are exluded from the polymer, and urea, a small polar molecule, does not diffuse through the polymer.[7] In addition, pellets exposed to salt solutions do not show an increased mass after drying as would be expected if salt had diffused into the polymer. Although substantial variation in the water uptake was observed after 7 and 12 days at pH 7.2 (Figure 2a), the same samples did not reflect this variation in the MW measurement (Figure 2b). This variation might result from water trapped in pores or other nonreactive environments.

Investigation of the Water Environment by Solid-State ^{2}H-NMR. The hydrolysis kinetics of PLG50:50 in various media suggested the presence of multiple types of water within the polymer. To investigate the water environment within the polymer more directly, PLG50:50 pellets containing known amounts of ^2H_2O were examined by solid-state ^{2}H-NMR spectroscopy. The use

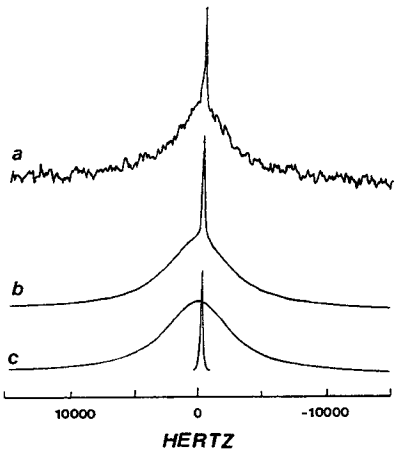

Figure 3. Experimental (a), curve fitted (b), and deconvoluted (c) solid-state ^{2}H-NMR spectra of PLG50:50 containing 1.17% ^2H_2O.

of ^2H_2O as a probe for investigating the water environment in solids has been described by McCall *et al.*[8a] and more recently by Chan and Harrison.[8b] Similar applications of proton and deuterium NMR spectroscopy include studies of the water environments in polymeric membranes,[9] hydrogels,[10] and proteins.[11] The uptake profiles of water and ^2H_2O were somewhat different: 0.781 ± 0.029 μmol/mg-day for H_2O *versus* 0.560 ± 0.021 μmol/mg-day for ^2H_2O. Much of this difference is probably due to the slower hydrolysis rate in ^2H_2O as compared to H_2O. Hydrolysis affords hydroxyl and carboxylate end groups, leading to an increase in the hydrophilic properties of the polymer. The region 1 pseudo-first-order rate constants in H_2O and ^2H_2O were 0.174 ± 0.017 and 0.035 ± 0.001 day^{-1}, respectively. The magnitude of the rate difference between H_2O and ^2H_2O ($k_H/k_D \approx 5$) is within the range expected for a primary deuterium isotope effect, indicating an O–H or O–^{2}H bond of the solvent is broken in the rate-limiting step of the reaction.[12] Although there are significant differences in the chemical behavior of H_2O and ^2H_2O, for the purposes of these studies it is assumed that the physical behavior of the two molecules within the solid polymer is the same.

Using NMR spectroscopy for probing the environment of water in solids is based on the line broadening observed when molecular rotation is hindered. When water (or ^2H_2O) molecules are free, as in a nonviscous liquid, Brownian motion causes intermolecular interactions to time average, and narrow resonance lines result. When the water molecules are interacting with a solid, the molecular rotation may be hindered or nonexistent on the NMR time scale, which results in broad resonance lines. More detailed information on NMR line shapes in solids and the NMR of solids can be found elsewhere.[13]

A typical spectrum obtained from a PLG50:50 pellet (~100 mg) containing ~1 mg of ^2H_2O is presented in Figure 3. This spectrum is clearly the sum of a broad resonance line and a narrow resonance line. The broad resonance line results from ^2H_2O molecules (bound ^2H_2O) interacting with the polymer and having hindered molecular rotation. The narrow resonance results from ^2H_2O that, although present in the polymer, does not appear to be associated with the polymer and behaves similarly to bulk ^2H_2O (free ^2H_2O).

It was possible that some or all of the broad resonance line could be due to ^{1}H–^{2}H exchange at carboxyl and hydroxyl groups of the polymer. To test this possibility, a polymer pellet was allowed to absorb ^2H_2O and then dried, and the spectrum was measured; then the sample was dried further, and the spectrum was measured again. The spectra of the dried samples are presented in Figure 4 with a sample containing ~1 mg of ^2H_2O shown for comparison. The signal disappeared with vacuum-drying, indicating that any signal arising from exchangeable protons on the polymer is negligible.

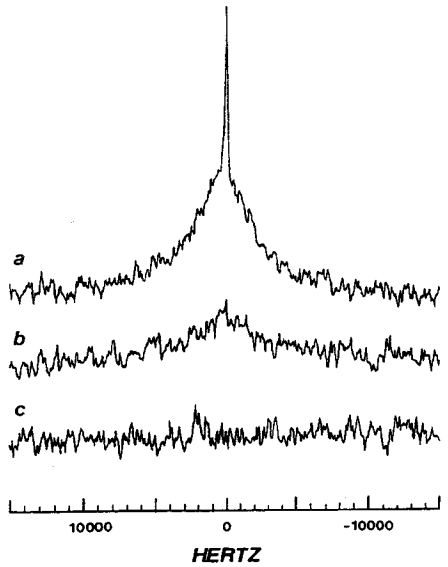

Figure 4. Solid-state ^{2}H-NMR spectra of PLG50:50 containing 1.17% ^2H_2O (a), the sample dried under vacuum (b), and the sample dried further under high vacuum (c).

Table 2. Peak Widths and Area Fractions of Broad and Narrow ^{2}H-NMR Signals from PLG50:50 Pellets Containing ~1 to ~5% ^2H_2O

	peak width (Hz)		% area	
% ^2H_2O	broad	narrow	broad	narrow
1.17	5843	200	95.2	4.8
2.66	3810	152	44.2	55.8
3.24	2763	138	31.2	68.8
5.24	2706	126	32.8	67.2

The ^{2}H-NMR spectra of PLG50:50 pellets containing ~1 to ~5% ^2H_2O were measured, the peaks were deconvoluted, and the area under each peak was determined (Table 2). Line widths of the broad peaks decreased from ~5800 to ~3800 Hz when the ^2H_2O content increased from 1.17 to 2.66%. Increasing the ^2H_2O content to 3.24% resulted in continued narrowing to ~2700 Hz, but a further increase to 5.24% gave no additional change in peak width. The width of the narrow peaks decreased from ~200 to ~125 Hz for samples containing from 1.17 to 5.26% ^2H_2O, respectively. These observations indicate that the rotational freedom of bound and free water is related to the total amount of water present. As total water content increased, the mobility of the bound and free water apparently also increased. This could result, in part, from increased plasticization of the polymer by absorbed water.

To quantitate the amount of bound and free ^2H_2O present, a linear relationship between total peak area and amount of ^2H_2O was first established. The amount of ^2H_2O responsible for each peak was then calculated by multiplying the area fraction from Table 2 by the total amount of ^2H_2O in the sample, determined by mass difference. A plot of the amount of ^2H_2O in the bound and free states as a function of total ^2H_2O content (Figure 5) shows that, as the amount of ^2H_2O increased from ~1 to ~5 mg, the amount present in the bound state remained nearly constant while the free ^2H_2O increased from ~0 to ~4 mg. Since the bound water remained nearly constant,

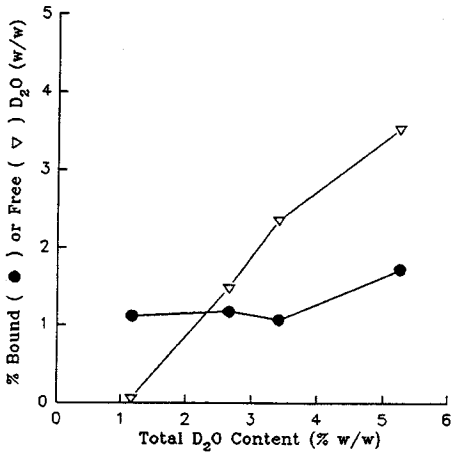

Figure 5. Percent ^2H_2O present in the free and bound states as a function of total ^2H_2O content of PLG50:50.

pseudo-first-order hydrolysis kinetics would be expected from the uncatalyzed hydrolysis reaction if the bound water corresponded to the reactive water responsible for hydrolysis. The molar quantity of ester groups in the polymer is 25-fold higher than the molar quantity of water with hindered rotation, suggesting that water's immobility and reactivity result from its selective hydrogen bonding to the oxygen of ester carbonyl groups. The free water probably occupies free volume between the polymer chains since at the early stages of hydrolysis no mass loss has occurred and no interconnecting network of pores can be measured within the polymer pellet.

Since PLG50:50 polymers absorb water and the NMR data indicate that most of the water will be free, it is expected that this water will interact with incorporated agents such as those used in drug delivery applications.[1] This could have profound effects on the stability of incorporated proteins since it has been demonstrated that small amounts of water can induce the formation of insoluble protein aggregates in the solid state.[14]

Investigation of Water Environment by Differential Scanning Calorimetry. Differential scanning calorimetry (DSC) provided additional support for the presence of different water environments in PLG50:50. PLG50:50 disks (~20 mg) were exposed to water at 37°C, the amount of water absorbed was determined by mass difference, and freezing of the imbibed water was observed by DSC. Polymer containing 3.4% water showed a very small freezing exotherm for water with ΔH_f = 2.25 J/g. Comparing this value with that for distilled water (ΔH_f = −230.6 J/g) suggests that none of the water present in the polymer was able to freeze. As water content was increased from 7.1 to 13.2%, the freezing exotherms also increased from −41.99 to −61.24 J/g, respectively. Comparing these values with ΔH_f for pure water suggests that while a portion of the water within the polymer behaves as bulk liquid water, another portion is influenced by the polymer and is inhibited from freezing.

Hydrolysis and Water Uptake of Poly(DL-lactide-*co*-glycolide) of Different Compositions. Water uptake profiles of PLG50:50, PLG65:35, PLG85:15, and PLA are presented in Figure 6a. The solid lines show that the initial rates of water uptake were similar among the different polymers. The slight lag time observed for PLG85:15 and PLA could be due to the somewhat higher T_g or the slower hydrolysis of these polymers. Both PLG50:50 and PLG65:35 showed accelerated water uptake after about 10 and 20 days, respectively. The accelerated rate of water uptake began when the MW reached ~10 000

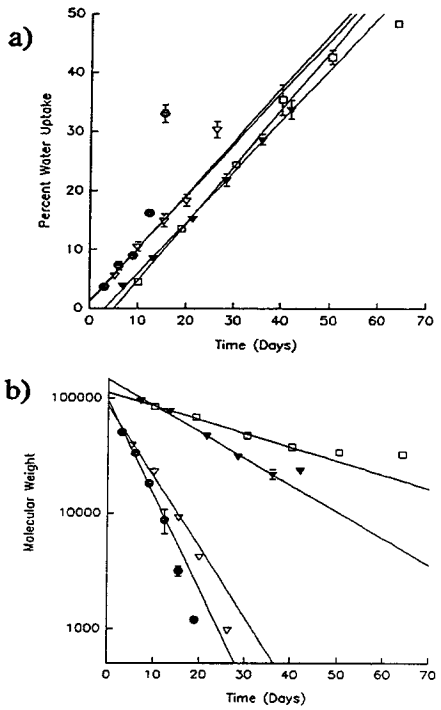

Figure 6. Water uptake (a) and molecular weight (b) of PLG50:50 (●), PLG65:35 (▽), PLG85:15 (▼), and PLA (□) exposed to pH 7.2 isotonic 50 mM sodium phosphate buffer at 37 °C.

and ~4000 for PLG50:50 and PLG65:35, respectively. Two conclusions can be drawn from the different MW's at the onset of accelerated water uptake depending on the environment of the water being absorbed. If the water present is water of hydration, this result suggests that increasing the lactic acid (LA) content leads to more hydrophobic oligomers that must be cleaved into smaller units before sufficient carboxyl and hydroxyl end groups are present to show increased hydrophilicity and accelerate water uptake. Alternatively, if the water is present in pores, the differences in water uptake as a function of MW might be caused by the differences in the mechanical properties of the two polymers. These differences might result in the formation of pores in PLG50:50 matrices at a higher MW than in PLG65:35 matrices.

The MW profiles of PLG50:50, PLG65:35, PLG85:15, and PLA are presented in Figure 6b. Pseudo-first-order rate constants for region 1 hydrolysis were 0.188 ± 0.013, 0.141 ± 0.017, 0.0533 ± 0.0024, and 0.0277 ± 0.0014 day^{-1} for PLG50:50, PLG65:35, PLG85:15, and PLA, respectively. Examination of the GPC chromatograms over time revealed changes in the MW distribution with hydrolysis. PLG50:50 and PLG65:35 showed increased polydispersity with a skewing to larger retention volumes (lower MW) after 12 and 15 days, respectively. Visual inspection of these pellets revealed a rigid white exterior surrounding a viscous liquid interior. Thus, the skewing to low MW is probably due to the surface-to-center segregation reported by Li and Vert.[2] PLG85:15 and PLA showed some increased polydispersity after 42 and 64 days, respectively; however, the peak molecular weights remained about the same.

It is well established that increasing the LA content of PLG polymers results in slower hydrolysis rates;[15] however, the reasons for these differences are not known. Poly(DL-lactide) and copolymers of DL-lactide and glycolide are all completely amorphous; thus the different hydrolysis rates with these polymers cannot arise from morphological

Table 3. Peak Widths and Area Fractions of Broad and Narrow ^{2}H-NMR Signals from PLG50:50, PLG65:35, PLG85:15, and PLA Pellets Containing ~1% ^2H_2O

	peak width (Hz)		% area	
polymer	broad	narrow	broad	narrow
PLG50:50	5843	200	95.2	4.8
PLG65:35	3986	150	88.3	11.7
PLG85:15	2089	91	83.8	16.2
PLA	1962	88	83.4	16.6

differences. Differences in total water content cannot explain the differences in hydrolysis rates. Two additional explanations can be proposed: (1) lactide–lactide differences in the concentrations of reactive water could result in differences in hydrolysis rates or (2) intrinsic differences in the hydrolytic stability of lactide–lactide ester linkages *versus* lactide–glycolide or glycolide–glycolide ester linkages could be responsible for the observed behavior. Previous studies by Li and Vert[2] did not show compositional changes with hydrolysis as would be expected with reactivity differences, so it is unlikely that there are intrinsic differences in the stability of different ester linkages.

To examine the second possibility—that differences in the concentration of reactive water within the different polymers correlate to differences in hydrolysis rates—the water environment was investigated by ^{2}H-NMR. Table 3 summarizes the results from the deconvolution of the ^2H_2O peaks and compares these with the data obtained for PLG50:50. The increased lactide content has two effects on the water environment within the polymers. As the lactide content of the copolymers increased, the width of both the broad and the narrow peaks decreased and the fraction of ^2H_2O in the bound state also decreased. The narrowing of peaks indicates that bound water has greater rotational freedom with increased lactide content. This could mean that the methyl group present in the lactide residues increases the hydrophobicity enough to hinder the hydrogen bonding of water molecules with the ester linkages, thus resulting in a more mobile water environment. This study suggests that the hydrolysis rate of copolymers of DL-lactide and glycolide is directly related to the hydrogen-bonding ability of the ester groups.

In conclusion, the hydrolysis rates of PLG esters are independent of moderate changes in degradation medium ionic strength, pH, and buffer concentration. The total water uptake of polymer depends on ionic strength but not pH. Bound water remains nearly constant with changes in total water from 1 to 5%. Pseudo-first-order hydrolysis kinetics suggest that bound water is the reactive species in polyester hydrolysis. The hydrolysis rates but not the water uptake rates are dependent on the DL-lactide content of DL-lactide/glycolide polyesters. The hydrolysis is related to the bound water corresponding to the reactive water content of these polymers.

Experimental Section

Polymers and Their Characterization. Poly(DL-lactide-*co*-glycolide, 50:50) (lot no. 051-68-1), poly(DL-lactide-*co*-glycolide, 65:35) (lot no. 051-11-1), poly(DL-lactide-*co*-glycolide, 85:15) (lot no. 101-36-1), and poly(DL-lactide) (lot no. 101-64-2) were obtained from Birmingham Polymers, Inc. (Birmingham, AL). Polymer molecular weights were determined by GPC at a flow rate of 1.0 mL/min in chloroform at 35 °C using a 7.8 mm (i.d.) × 30 cm Ultrastyragel linear column (Waters, Milford, MA) and a refractive index detector (Shimadzu RID-6A, Columbia, MD). Polystyrene molecular weight standards (600–104 000) at 0.25% (w/v) in chloroform were used to calibrate the column. Sample concentrations were ~0.5% (w/v) in chloroform. The MW's determined in this work correspond to the peak of the GPC chromatogram as previously described in detail by Vert *et al.* and Spenlehauer *et al.*[16]

Fabrication of Polymer Pellets. PLG50:50, PLG65:35, PLG85:15, and PLA were dissolved in methylene chloride (3% (w/v)) and spray-dried using a Yamato Pulvis Mini-Spray GA-32 spray drier (Yamato Scientific Co., Chicago, IL). The spray-drying conditions were as follows: inlet temperature = 37–41 °C, outlet temperature = 26–30 °C, drying air = 0.48–0.50 m^3/min, atomization air = 0.5 kgf/cm^2, and a solution flow rate 6 mL/min. Approximately 25 mg samples of the spray-dried polymers were then compressed with a hydraulic press using a 4-mm-diameter standard concave punch-and-die set contained in a thermostated holder at 70 °C. The PLG50:50 and PLG65:35 pellets were clear yellow glasses, while the PLG85:15 and PLA pellets were clear, colorless glasses.

Water Uptake and Hydrolysis Profiles. PLG50:50 pellets were degraded at 37 °C in (1) distilled water, (2) 0.154 M sodium chloride, (3) 0.604 M sodium chloride, (4) pH 7.2 50 mM sodium phosphate adjusted to μ = 0.154 M with sodium chloride, (5) pH 7.2 150 mM phosphate buffered saline (PBS), (6) pH 8.2 150 mM PBS, and (7) 99.9 atom % ^2H_2O (lot nos. 89F3656 and 50H3689, Sigma Chemical Co., St. Louis, MO). The degradation of PLG65:35, PLG85:15, and PLA was conducted in pH 7.2 50 mM sodium phosphate adjusted to μ = 0.154 M with sodium chloride. Preweighed pellets were placed in screw cap test tubes containing medium and agitated at 37 °C in an incubator–shaker (Lab-line Instruments, Inc., Melrose Park, IL). At specific sampling times, the tubes were removed and the water uptake, molecular weight, and residual polymer mass were determined. The amount of imbibed water was measured by blotting the pellets to remove any surface water and weighing them using a Sartorius microbalance (Model M3P-000V001, McGaw Park, IL). The water content was then calculated as follows: % water uptake = 100 × (hydrated weight − initial weight)/initial weight. Studies showed that the results from Karl Fischer titration and weight gain were comparable. The pellets were then placed in a vacuum desiccator to remove all water and weighed again to detect any mass loss. Measurements were performed in triplicate, and the variability observed in Figures 1 and 2 includes both the blotting and weighing steps. The dried polymers were dissolved in chloroform and the molecular weights determined by GPC as described above.

^{2}H-NMR Studies. The water environment within degrading polymer specimens was investigated by ^{2}H-NMR of polymer pellets solvated in ^2H_2O. The water environment could not be studied directly by ^{1}H-NMR because of interference by hydrogen nuclei present in the polymers; thus ^2H_2O was used as a model solvent and observations were extended to water.

Pellets weighing ~100 mg were fabricated from PLG50:50, PLG65:35, PLG85:15, and PLA obtained from Birmingham Polymers, Inc. The pellets were weighed and then exposed to deuterium oxide (99.9 atom %) (lot no. 50H3689, Sigma Chemical Co., St. Louis, MO) at 37 °C. The ^2H_2O content was determined from the mass difference between the solvated and unsolvated states. The ^{2}H-NMR spectra of the solvated pellets were then collected by signal averaging 1600 scans using a Bruker MSL-300 spectrometer (Billerica, MA). The spectrometer, operating at 47.073 MHz, was equipped with a high-power probe fitted with a 5-mm plug-in coil tuned to ^{2}H. The transmitter power was adjusted to obtain a 3.5-μs 90° pulse. A quadrapole echo pulse sequence was used with a 3-s recycle time. The sweep width was set at 100 000 Hz.

The background signal due to exchangeable protons in the polymer was determined by solvating a PLG50:50 pellet, drying it under house vacuum, and collecting the spectrum. The pellet was then dried further at ca. 50 °C under high vacuum (~10/μTorr) and the spectrum measured again.

Sample spectra obtained were deconvoluted into component signals using software available on the spectrometer (Linesim, version 881117.1, Bruker, Billerica, MA).

Differential Scanning Calorimetry. PLG50:50 samples (~20 mg) containing known amounts of water and distilled water were sealed in aluminum pans. Freezing exotherms were obtained using a Perkin-Elmer DSC7 (Wilton, CT) by cooling at 10 °C/min from +25 to −30 °C.

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