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Prediction of Microclimate pH in Poly(lactic-*co*-glycolic Acid) Films

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Abstract: An equilibrium mathematical model that accurately predicts microclimate pH (μpH) in thin biodegradable polymer films of poly(lactic-*co*-glycolic acid) (PLGA) is described. μpH kinetics was shown to be primarily a function of: (i) kinetics of water-soluble acid content and composition in the polymer matrix and (ii) polymer/water partition coefficient of water-soluble degradation products (P_i). Polymers were coated on standard pH glass electrodes, and μpH was measured potentiometrically. Water-soluble acid distribution and content in PLGA films were determined by pre-derivatization HPLC. Polymer degradation products partitioned favorably in the polymer phase relative to water (P_i range: ~6−100), and P_i increased with increasing hydrophobicity of the acidic species according to a linear free energy law related to reversed phase HPLC retention time for the corresponding derivatized bromophenacyl esters. The μpH predicted by the model was in excellent agreement with experimental μpH for several PLGAs as a function of time and PLGA lactic/glycolic acid ratio. These data may be useful to slowly release pH-sensitive PLGA-encapsulated bioactive substances and provide a general framework for predicting partitioning behavior of degradation products in biodegradable polymers.

Introduction

Poly(lactic-*co*-glycolic acid) (PLGA) is one of only a few biodegradable and biocompatible polymers used in pharmaceutical products or medical devices approved by U.S. Food and Drug Administration.[1] PLGAs have shown great potential as controlled delivery carriers for peptides, proteins, vaccine antigens, and poly(nucleic acids), as polymer scaffolds for tissue engineering and cell-based therapies,[2−7] and more recently, as coatings in drug-eluting stents.[8] However, insufficient stability of encapsulated bioactive substances, especially proteins and nucleic acids, has been a principal obstacle for development of polymer delivery systems.[9−13] Characterizing the deleterious polymer microenvironment, in which labile encapsulated substances are exposed for weeks to months during PLGA bioerosion in vivo, is crucial to develop stabilization approaches.

Upon their hydrolysis PLGA polyesters and related bioerodible polymers, which possess acidic monomers (e.g., lactic and glycolic acids) in their backbones, become acid producers. It has long been recognized that during incubation under physiological conditions, the build-up of acidic PLGA degradation products may result in a lowering of microclimate pH (μpH), viz. the pH in aqueous pores of the polymer.[1,14−17] Indeed, some commercial PLGA delivery systems for peptides require excellent peptide stability in acidic media.[18,19] However, the acidic μpH has been implicated definitively only recently as a principal stress for the instability of encapsulated acid-labile proteins, and incomplete protein release from PLGAs.[1,9,20] Improved drug stability has been achieved by μpH control via the incorporation of poorly water-soluble bases, which has stabilized small anticancer agents and several therapeutic proteins such as encapsulated vincristine,[21] basic fibroblast growth factor,[20] and tetanus

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toxoid.[22] Such bases, also known as antacids, remain as solids in the polymer matrix and dissolve in response to decreased μpH. Moreover, μpH control has been suggested to inhibit acid-triggered tissue irritation during formulation administration[23] and prevent cell damage from polymer scaffolds commonly used in tissue engineering.[24]

For optimization of μpH control in PLGA, several techniques to directly monitor μpH have been developed, including ^{31}P nuclear magnetic resonance,[25] electron paramagnetic resonance,[26] confocal fluorescence microscope imaging,[27] and potentiometry.[28] Measured μpH is commonly acidic (pH $<$ 5) and dependent on numerous factors including PLGA lactic/glycolic acid ratio, matrix size, thickness, and porosity.

Despite these important developments, little attempt to understand the physical chemical basis of μpH has been reported. Therefore, we describe here the development of an equilibrium model to attempt for the first time quantitative prediction of PLGA μpH, which is simultaneously monitored by using simple PLGA films coating pH electrodes. Concomitantly, this analysis revealed the role of the partitioning behavior of biodegradative water-soluble acids, for which we provide a unique methodology for its determination, based on linear free energy theory.

Theoretical Section

Physical-Chemical Description and Basic Assumptions. The μpH can be mathematically related to several major processes, as shown in Figure 1A. Upon immersing a PLGA film within a physiological buffer, water penetrates the polymer phase with high speed (diffusivity $\approx 5 \times 10^{-8}$ cm^2 s^{-1})[29] and rapidly fills in the occlusions formed within the polymer matrix caused by removal of organic solvents and water as well as by other factors during processing. After brief hydration, degradation of polymer occurs throughout the matrix, and water pores begin to grow in size and coalesce.[29] Accordingly, two separate phases coexist in the microenvironment of polymer matrix:[30] the polymer phase and aqueous pores, and water-soluble biomacromolecules insoluble in the polymer such as proteins reside in the aqueous phase (or to a certain extent at the polymer/water interface). The degradation products, from both random chain scission and end scission of polyester hydrolysis,[29] can be either water soluble or water insoluble (depending on their chain length) and water-soluble acids are released into degradation medium, for example, by diffusion through the polymer matrix.[31] A rapid equilibrium is assumed to exist between the polymer and the aqueous pore liquid at sufficiently high polymer-molecular weight necessary to maintain the two separate phases. Low-molecular weight acidic oligomers, which are water soluble, partition into the pore liquid, where dissociation takes place, producing a low μpH.

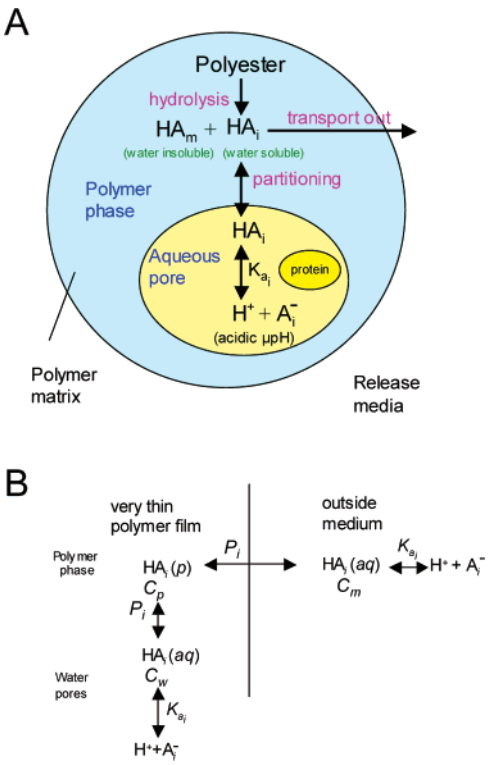

Figure 1. Equilibrium models for prediction of microclimate pH (A) and for determination of PLGA/water partition coefficients for small hydroxy acids (B).

To reduce the complexity of the approach in the developed model, μpH was directly related to the water-soluble acids existing in the polymer matrix, and the following principal assumptions were made. First, any acid concentration gradient in the polymer matrix occurs over a negligible distance relative to the thickness of matrix.[27] This homogeneous pH, therefore, is the average pH inside the matrix, which is determined by the pore water-soluble acid content and pore-specific volume. Second, external buffer ions do not extensively diffuse in the polymer matrix, prohibiting their significant participation in the pore acid−base equilibrium. Third, virtually all water uptake in the polymer matrix is localized in aqueous pores, and water content in polymer phase is negligible. Finally, inside water pores, activity coefficients for all oligomeric species are unity, and ionic strength is low.

Quantitative Treatment. The moles of the *i*th water-soluble acid in the polymer matrix (n_{HA_i}) recoverable from polymer at any time includes the sum of moles of HA$_i$ in the polymer phase, moles of HA$_i$ and conjugate base A$_i^-$ in the aqueous pores, which can be written as a function of the corresponding molar concentrations ($C^p_{HA_i}$,$C^w_{HA_i}$,$C^w_{A_i}$) and volume of the polymer (V_p) and aqueous pore (V_w) phases, respectively:

$$n_{HA_i} = C^p_{HA_i}V_p + (C^w_{HA_i} + C^w_{A_i})V_w \quad (1)$$

According to the assumption of negligible water uptake by the polymer phase, the aqueous and polymer volumes may be related to the mass of the dry (M_{film}) and hydrated

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$[(\phi_w + 1)\, M_{film}]$ polymer matrix:

$$V_w = \frac{M_{film}\phi_w}{\rho_w} \quad (2)$$

$$V_p = \frac{M_{film}}{\rho_p} \quad (3)$$

where ϕ_w is water uptake (i.e., ratio of water to dry film masses) and ρ_p and ρ_w are the densities of the polymer phase and pore water, respectively.

We note the definition of the polymer/water partition coefficient of the ith water-soluble acid as follows:

$$P_i \equiv \frac{C^p_{HA_i}}{C^w_{HA_i}} \quad (4)$$

Therefore, insertion of eqs 2−4 into eq 1, and normalizing for M_{film} defines the water-soluble acid content of HA_i in the polymer matrix ($\hat{n}_{HA_i}$) in eq 5 as follows:

$$\hat{n}_{HA_i} \equiv \frac{n_{HA_i}}{M_{film}} = \frac{1}{\rho_p} C^w_{HA_i} P_i + (C^w_{HA_i} + C^w_{A_i}) \frac{\phi_w}{\rho_w} \quad (5)$$

Under highly acidic conditions, since $P_i/\rho_p \gg \phi_w/\rho_w$ for PLGA (see measured P_i values below),

$$\underset{\text{(term 1)}}{\frac{P_i}{\rho_p} C^w_{HA_i}} \gg \underset{\text{(term 2)}}{\frac{\phi_w}{\rho_w} C^w_{HA_i}} \geq \underset{\text{(term 3)}}{\frac{\phi_w}{\rho_w} C^w_{A_i^-}} \quad (6)$$

and the third term in eq 5 may be neglected (see below for a non-acidic case when this assumption is not used). Accordingly, eq 5 may be rearranged to yield the acid concentration in the pores as a function of acid content in the polymer matrix, independent of pH as follows:

$$C^w_{HA_i} = \frac{\hat{n}_{HA_i}}{\frac{P_i}{\rho_p} + \frac{\phi_w}{\rho_w}} \quad (7)$$

To obtain μpH as a $f(C^w_{HA_i})$, i.e., mildly acidic to neutral pH case, we need to consider the charge balance and the dissociation constant as follows:

$$C_{H^+} = \sum_i C^w_{A_i^-} \quad (8)$$

$$C^w_{A_i^-} = \frac{C^w_{HA_i} K_{a_i}}{C_{H^+}} \quad (9)$$

Inserting eq 9 into eq 8 gives

$$C_{H^+} = \sqrt{\sum_i C^w_{HA_i} K_{a_i}} \quad (10)$$

Inserting eq 7 into eq 10, and noting the definitions of pH and the mole fraction of water-soluble acid i in the polymer matrix ($X_{HA_i} \equiv \hat{n}_{HA_i}/\hat{n}^T_{HA}$) gives μpH:

$$\mu\text{pH} \equiv -\log C_{H^+} = -\frac{1}{2}\log\left[\hat{n}^T_{HA} \sum_i \frac{X_{HA_i} K_{a_i}}{\left(\frac{P_i}{\rho_p} + \frac{\phi_w}{\rho_w}\right)}\right] \quad (11)$$

where $\hat{n}^T_{HA}$ is the total water-soluble acid content in the polymer matrix (i.e., $\hat{n}^T_{HA} \equiv \sum_i \hat{n}_{HA_i}$).

In instances where μpH is not highly acidic, the inequality eq 6 is no longer valid and $C^w_{HA_i}$ not only depends on $\hat{n}^T_{HA}$, but also on pH. In this instance, eq 9 is inserted into eq 5 as follows:

$$\hat{n}_{HA_i} = \frac{1}{\rho_p} C^w_{HA_i} P_i + \left(C^w_{HA_i} + \frac{C^w_{HA_i} K_{a_i}}{C_{H^+}}\right)\frac{\phi_w}{\rho_p} \quad (12)$$

Noting the definition of $pK_{a_i}(\equiv -\log K_{a_i})$, we can rearrange eq 12 to get the similar form as eq 7, but now considering ionization:

$$C^w_{HA_i} = \frac{\hat{n}_{HA_i}}{\frac{P_i}{\rho_p} + \frac{\phi_w}{\rho_w}(1 + 10^{\mu\text{pH} - pK_{a,i}})} \quad (13)$$

and inserting eq 13 into eq 10 and noting eq 11 gives the transcendental solution to μpH:

$$F(\mu\text{pH}_R) = \mu\text{pH}_R + \frac{1}{2}\log\left[\hat{n}^T_{HA} \sum_i \frac{X_{HA_i} K_{a_i}}{\frac{P_i}{\rho_p} + \frac{\phi_w}{\rho_w}(1 + 10^{\mu\text{pH}_R - \text{p}K_{a,i}})}\right] = 0 \quad (14)$$

where μpH_R is the root when $F = 0$.

Predicting Partition Coefficients in the PLGA/Water System from the Adjusted Retention Time (t'_R) of Derivatized Acids in HPLC. According to linear free energy theory, for reverse phase chromatography of a homologous series, t'_R can be related to free energy as follows:[32]

$$\log\frac{P^{LC}_{n+1}}{P^{LC}_n} = \frac{2.303(\Delta G^\circ_{n+1}{}' - \Delta G^{\circ\prime}_n)}{RT} = \log\frac{t'_{R(n+1)}}{t'_{R(n)}} \quad (15)$$

where n is the number of repeated chain length, P^{LC}_n is the partition coefficient between stationary phase and mobile phase for the species with n chain length, and $(\Delta G^\circ_{n+1}{}' - \Delta G^{\circ\prime}_n)$ is the free energy of partitioning per monomer group between phases. Because $P_{(n+1)/n}$ can also be related with free energy $(\Delta G^\circ_{(n+1)/n})$ as in eq 15 for the PLGA/water system, a system adjusting factor k can be added to relate the two free energy differences:

$$(\Delta G^\circ_{n+1}{}' - \Delta G^{\circ\prime}_n) = k(\Delta G^\circ_{n+1} - \Delta G^\circ_n) \quad (16)$$

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From eqs 15 and 16, the linear relationship between P_i and t'_R is easily obtained:

$$\log\frac{P_{n+1}}{P_n} = k\log\frac{t'_{R(n+1)}}{t'_{R(n)}} \quad (17)$$

Experimental Section

Materials. Poly(D,L-lactide-*co*-glycolide) 50/50, 85/15 with inherent viscosity (i.v.) at 25 °C of 0.63 (M_w = 85 kDa) and 0.66 dL/g (M_w = 90 kDa) in hexafluoro2-propanol (HFIP), and poly(D,L-lactide) with i.v. of 0.60 dL/g (M_w = 80 kDa) in chloroform were purchased from Birmingham Polymers Inc. Poly(D,L-lactide-*co*-glycolide) 50/50 with i.v. of 0.21 (M_w = 19 kDa) and 0.58 dL/g (M_w = 63 kDa), 85/15, and 100/0 with i.v. of 0.60 dL/g (M_w = 68 kDa) at 25°C, respectively, in HFIP were generously provided by Alkermes Inc. D,L-Lactide and glycolide were generously provided by Purac Biochem. All other chemicals were of analytical grade or HPLC grade and purchased from commercial suppliers.

PLGA Films Coating pH Electrodes. PLGA films coating pH electrodes were prepared similarly as described before.[33] Briefly, standard noncombination-type glass pH-sensitive electrodes were dipped into PLGA acetone solution (500 mg/mL) followed by subsequent quenching in 4 °C water for 1 h. In some cases, glycolic and lactic acids were dissolved in the polymer solution at 75 mg/mL to yield polymer films with 15% w/w theoretical monomer content. As some monomer escapes the polymer into water during encapsulation, monomer loading efficiency, defined as the percentage of monomer encapsulated in the polymer, was determined as described for assay of water-soluble acid content (see below). Coated electrodes were air-dried for 1 h and then vacuum-dried for 36 h at room temperature. Coating thickness of the polymer film (~200−250 μm) was measured by a light microscope with a scale bar. Coated electrodes were incubated under physiological conditions, i.e., in phosphate-buffered saline containing 0.02% w/v Tween 80 pH 7.4 (PBST) at 37 °C.[33] Incubation media were replaced weekly.

μpH Kinetics by Potentiometric Measurement. At predetermined times, the zero-current potential, (E_{cell}) of the incubated electrodes was measured by an Orion pH meter with a calomel reference electrode in PBST (pH 7.4) at room temperature, as previously described.[28] After the removal of the film, electrodes were calibrated by standard buffer solutions at pH 4, 7, 10, and μpH was calculated from the EMF (electromotive force)-pH calibration curve after subtracting the offset pH. The offset pH, due to contributions of additional interfacial and diffusion potentials introduced in the electrochemical cell by the polymer coating, was determined previously from the second-order polynomial function of microclimate pH:[28] pH offset = $-0.0037\ \text{pH}^2 + 0.0195\ \text{pH} + 0.1605$.

Determination of Water Uptake (ϕ_w) and *i*th Water-Soluble Acid Content ($\hat{n}_{HA_i}$). Polymer films were carefully cut, peeled off the electrode bulb, weighed to determine wet mass (M_{film}^{wet}), and freeze-dried on a Freezone 6 freeze-drying system (Labconco Corp., MO) at 133×10^{-3} mbar or less with a condenser temperature of −46 °C for 3 days. After weighing again the dry film (M_{film}^{dry}), water uptake ($\phi_w \equiv [M_{film}^{wet} - M_{film}^{dry}]/M_{film}^{dry}$) was monitored gravimetrically.

Water-soluble acid content was obtained by dissolving dried PLGA coatings in chloroform followed by repeated extraction into water at room temperature.[33] The extraction was repeated 5-fold before combining and freeze-drying the aqueous phases. The dried extracts were reconstituted in ACN, derivatized with bromophenacyl bromide, and analyzed by HPLC (see Supporting Information).[33]

Determination of *i*th Acid Polymer/Water Partition Coefficients (P_i). To reduce equilibration times, very thin polymer films were prepared by dipping glass tubes into a PLGA acetone solution (400 mg/mL for PLGA 50/50 with M_w 85 kDa, PLGA 50/50, 85/15, and 100/0 with M_w 63−68 kDa, and 800 mg/mL for PLGA 50/50 with M_w 19 kDa) followed by subsequent quenching in 4 °C water for 1 h. After drying in air for 1 h and vacuum-drying for 36 h at room temperature, the films were carefully peeled off the surface of the tube and cut into 1×1 cm^2 stripes. Film thickness, determined by a light microscope with a scale bar, was approximately 50 μm (S.D. = 10 μm, n = 10).

Each polymer strip was placed in one 4-mL scintillation vial, and the entire film was immersed in 500 μL of aqueous oligomer-containing solutions at known total acid concentration in the range of 6−100 mmol/L. The stock oligomer-containing solutions, mainly composed of glycolic, lactic, and lactoyllactic acids, were prepared by hydrolysis of PLGA 50/50 material at 60 °C, and the resulting individual and total acid concentrations were determined by pre-derivatization HPLC. Each vial containing specific dilutions of the stock solution was capped tightly and incubated at 37 °C, and at predetermined times, film and incubation medium were separated and freeze-dried. The quasi-equilibrium acid content in both the polymer film and the incubation medium were measured similarly by pre-derivatization HPLC.

Data Analysis. Correction for Ionization and Film Water Uptake in Partitioning Measurement. The *i*th acid partition coefficient (P_i) is slightly different from the simple ratio of uncorrected acid concentration in the polymer film (C'_p) to that in the incubation medium (C'_m), since the measured acid contents included acid in polymer and pore phases, and conjugate base in pore and incubation media. Therefore, the corrected acid concentration in the polymer film (C_p) and incubation medium (C_m) were derived according to the equilibria depicted in Figure 1B. The equilibrium P_i was assumed to be equivalent whether the aqueous phase was the incubation media or the aqueous pores in the polymer matrix.

P_i was determined from the uncorrected P_i ($P'_i \equiv C'_p/C'_m$), the media proton concentration ($C_{H^+}^m$), and volume fraction of pore water in the polymer [$\Phi_w = (\phi_w \cdot \rho_p)/(\phi_w \cdot \rho_p + \rho_w)$] as follows (see Supporting Information):

$$P_i = \frac{C_{HA_i}^p}{C_{HA_i}^m} = \frac{C'_p - \dfrac{C'_m}{1-\Phi_w^{-1}}}{C'_m\left(\dfrac{1}{1+\dfrac{K_{a_i}}{C_{H^+}^m}}\right)} = \left(P'_i - \frac{1}{1-\Phi_w^{-1}}\right)\left(1+\frac{K_{a_i}}{C_{H^+}^m}\right) \quad (18)$$

The pK_a of monomers at 1 atm and 25 °C were obtained from the literature (3.82 and 3.84 for glycolic and lactic acids, respectively)[34] and the pK_a of lactoyllactic acid was measured to be 3.1 by potentiometric titration by sodium hydroxide.[35] The increased acidity of the linear lactic acid dimer is not surprising since the δ ketone has an electron-withdrawing ability that is stronger than that of the δ hydroxyl group. The pK_a's of longer-chain oligomers, which may be obtained more precisely by computational chemistry analysis, were simply assumed to be equal to that of lactoyllactic acid. Since the concentration of the oligomers in water pores is small, this assumption is not expected to affect predicted values significantly. PLGA densities were obtained from the manufacturer (ρ_p = 1.37 g/mL for PLGA 50/50, 1.27 g/mL for PLGA 85/15, and 1.25 g/mL for PLA).

Results and Discussion

The role of μpH in PLGA devices has been historically difficult to ascertain because of the absence of simple μpH assays and theoretical models, which delineate physical chemical factors contributing to μpH acidity. The overall goal of

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predicting μpH kinetics required first determination of several variables ($\hat{n}^{T}_{HA}$, X_{HA_i}, P_i, and ϕ_w) defined in the model and development of new analytical methods (pre-derivatization HPLC analysis of oligomer content and composition,[33] and potentiometric determination of μpH[28]). The assembly of those parameters largely independent (P_i) and dependent ($\hat{n}^{T}_{HA}$, X_{HA_i}, ϕ_w) on polymer erosion kinetics is described below, followed by their use to compare predicted and experimental μpH.

PLGA/Water Partition Coefficient of Water-Soluble Acid Degradation Products. To determine directly P_i of water-soluble degradation products, films of PLGA in water were equilibrated in PLGA oligomer solutions produced by accelerated erosion of the polymer. Several complicating factors needed to be resolved to determine P_i, including (1) potential changes in acid levels in the polymer during equilibration caused by hydrolysis of PLGA, (2) potential instability of acids during equilibration, and (3) low levels of larger oligomers prohibiting accurate assay of their content. To overcome these difficulties, several steps were taken, including (1) use of very thin films to minimize the time to equilibrate acids between polymer and water phases; (2) selection of the initial erosion period, in which water-soluble acid content changes in the polymer are low,[33] for equilibration; and (3) use of relatively low-molecular weight acids (glycolic (GA), lactic (LA), and lactoyllactic acids (L_2A)) for direct measurement of P_i. To determine P_i for oligomers of higher molecular weight (which may be unstable during equilibration and whose content in polymer and water phases could not be accurately determined) a linear free energy law was used based on the partitioning of acid derivatives in the biphasic HPLC system and the P_i's measured directly for GA, LA, and L_2A. A typical HPLC chromatogram of derivatized extracted acids after PLGA film was incubated in oligomer-containing solutions for 7 days is shown in Figure S1. As seen in Figure 2A−C, a quasi-equilibrium was reached after 7 days for each low-molecular weight acid in the most rapidly acid-producing PLGA 50/50. At this time point, measured P'_i uncorrected for pH or pore acid content was independent of the initial acid media concentration. The P_i was determined from the slopes of the curves in Figure 2D, which was corrected for ionization and polymer pore volume affects, as described in eq 18, and the corresponding values at infinite dilution[36] are listed in Table 1. The small acids partitioned more favorably in the polymer (P_i range: ~6−20) and increased with the acid hydrophobicity, with 6.3 for glycolic acid to 21 for lactoyllactic acid.

To test the applicability of the adjusted retention time (t'_R) of bromophenacyl esters of the PLGA oligomers during HPLC analysis to predict PLGA/water partition coefficient, the linear correlation between log(octanol/water partition coefficient) of a series of relatively stable hydroxy acids (obtained from ALOGPS 2.1 software[37,38]) and log(t'_R) according to the free energy law eq 15 was examined. As shown in Figure 3A, linearity was observed validating the law for bromophenacyl esters over a wide range of hydrophobicity. Similarly, the free energy law was valid for PLGA/water partition coefficients directly determined for the low-molecular weight acids, as seen in Figure 3B, and provided the slope k in eq 17 necessary to extrapolate P_i's for higher-molecular weight acids. The extrapolated P_i's of four additional acids, identifiable by their retention time in the pre-derivatization HPLC assay (see Figure S2), are also listed in Table 1.

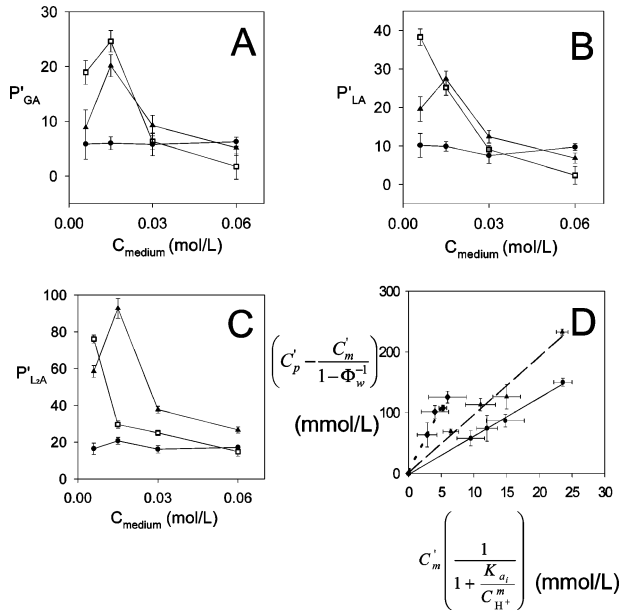

Figure 2. Determination of PLGA/water partition coefficients. Polymer/water equilibrium was reached after 7 days incubation (−□−: 1 day; −▲−: 3 days, −●−: 7 days) and uncorrected partition coefficients (P'_i) were determined for glycolic GA (A), lactic (LA) (B), and lactoyllactic (L_2A) acids (C). (D) Corrected PLGA/water partition coefficients (P_i) for (−●−) GA, (−▲−) LA, and (−♦−) L_2A from linear regression of acid concentration between incubation medium and polymer film after 7 days equilibration according to eq 18 and the equilibrium model (Figure 1B). Slopes corresponded to measured P_i ($r^2 > 0.98$).

Water-Soluble Acids in PLGA 50/50 Films during Polymer Degradation. As shown in our previous paper,[33] water-soluble acid content in PLGA 50/50 was shown to be relatively constant (~0.03−0.04 μmol/mg) during the first 2 weeks of the 6-week incubation in PBST at 37 °C. By contrast, the acid content increased dramatically after 3 weeks near the induction time to polymer mass loss, the lag time before medium to high-MW PLGAs release significant matter to the incubation medium (low-MW PLGAs exhibit no lag time).[35] Separation of these acids revealed that glycolic, lactic, lactoyllactic acids, and the hypothesized lactic acid tetramer are the major components in this initial period with particularly high levels of lactoyllactic acid from residual lactide, the cyclic lactic acid dimer used for ring-opening polymerization.[33,39] The relatively low composition of glycolic acid was observed, which is not surprising since glycolic acid has been observed to be released out of the polymer ~3−4 times faster than lactic acid.[39,40] There was also no sign of a linear glycolic acid dimer in the polymer, owing to the aqueous instability of the ester without the steric methyl group.[33] After 3−4 weeks of incubation, the composition of low-molecular weight acids decreased with a steady increase in PLGA oligomers, possibly due to mobilization of polymer chains, which facilitates random scission relative to end scission of the polymer.[29]

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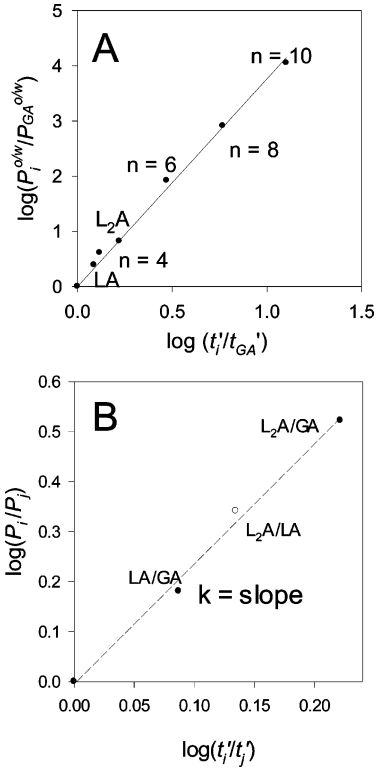

Figure 3. (A) Linear free energy relationship between predicted octanol/water partition coefficient ($P_i^{o/w}$) and adjusted HPLC retention time (t_i') of bromophenacyl esters of hydroxy acids: lactic acid (LA), lactoyllactic acid (L_2A), and ($HOOC(CH_2)_nOH$) with varying chain length (n). Range and ordinate were correlated with $r^2 = 0.99$. (B) Linear free energy relationship between PLGA/water partition coefficient (P_i) and adjusted retention time (t_i') for bromophenacyl esters of small hydroxy acids. The value of k was determined from the linear relationship between $\log(P_i/P_{GA})$ and $\log(t_i'/t_{GA}')$ where i is LA and L_2A ($r^2 = 0.99$) and GA is glycolic acid. The dependent value (○) was not included in the regression.

Table 1. Experimental and Extrapolated P_i of Water-Soluble Acids for PLGA 50/50

acid	t_R' (min)[a]	PLGA–water partition coefficient P_i
glycolic acid	0.63	6.3[b]
lactic acid	0.83	9.5[b]
lactoyllactic acid	1.13	21[b]
oligomer 1[c]	1.33	30[d]
oligomer 2[c]	1.53	42[d]
oligomer 3[c]	1.93	73[d]
oligomer 4[c]	2.13	93[d]

[a] HPLC retention time of acids derivatized with bromophenacyl bromide adjusted for void volume. [b] Measured from the slopes of curves in Figure 2D. [c] Oligomers distinguished by t_R' values (see Figure S2). [d] Extrapolated using k from Figure 3B and eq 17.

Polymer Water Uptake and Equilibrium Water Content in the Polymer Phase. Water uptake in PLGA 50/50 films increased slowly from 25% at 1 day to 38% by 4 weeks followed by a sharp decline to 18% by 6 weeks (Figure S3A). The decline can be explained by a change in the physical state of the polymer at this time. Significant mass loss (~50%) was observed over the erosion interval (Figure S3B), and with the drop in polymer molecular weight and glass transition temperature, the physical state of polymer film became highly viscoelastic, which may have caused the closure and loss of water pores.[41]

According to the Flory−Huggins[42] theory of mixing, and interaction parameter, χ (the Gibbs free energy of interaction of a single monomer with water), estimated to be 2.97 from literature,[43] we may estimate the water content in the polymer phase. That is, by setting the chemical potential of water in the pore and polymer phase equal, it is easily shown that

$$\ln a = (1 - \psi_w)\left(1 - \frac{1}{N}\right) + \ln \psi_w + \chi(1 - \psi_w)^2 \quad (19)$$

where a, ψ_w, and N are activity of water (≅1), volume fraction of water in the polymer phase, and ratio of the number average polymer molecular weight (M_n) to molecular weight of monomer units, respectively. From eq 19, ψ_w is ~0.02 v/v, which is consistent with a recently measured value[44] and justifies the negligible water uptake assumption when predicting pH.

Comparison of Experimental and Predicted μpH in PLGA 50/50 Films. Glycolic and lactic acids were encapsulated in PLGA 50/50 electrode-coating films, which was carried out to artificially control the acid content in the microenvironment and test our proposed model. Although both acids had the same theoretical monomer content, due to much lower loading efficiency of glycolic acid (5% for glycolic and 70% for lactic acid, respectively), the μpH of glycolic acid encapsulated samples was much higher. Despite this unexpectedly high μpH, the acid content inside films was also lower, as reflected by the close agreement between predicted and measured μpH during 1 week incubation in ddH_2O, as shown in Figure 4A.

For PLGA 50/50 electrode-coating films incubated for 6 weeks, a highly acidic μpH (~2.6−3.2) was observed during incubation in PBST, an acidic pH range that has been previously implicated to cause unfolding of encapsulated bovine serum albumin to its expanded (E) form, with associated noncovalent protein aggregation and peptide-bond hydrolysis.[20] A decrease in measured μpH by 4 weeks corresponded to the increase of the water-soluble acid content at this time point. As shown in Figure 4B, theoretically predicted μpH for PLGA 50/50 films showed an excellent agreement with the measured values. The concentration of ith acid in water pores, $C_{HA_i(w)}$, calculated by the model (see Figure S4), demonstrated that the primary acids contributing to the acidic μpH were glycolic, lactic, and lactoyllactic acids.

Effect of Polymer Composition. Partition Coefficients of Water-Soluble Acids. See Supporting Information. Variation of PLGA lactic acid content and molecular weight had little effect on P_i of low-molecular weight acids (Figure S5), suggesting the utility of the full range of P_i listed in Table 1 to predict μpH for additional PLGA compositions.

μpH Kinetics and Model Prediction. The effect of copolymer lactic acid content on potentiometrically measured μpH is shown in Figure S3C. Whereas μpH was ubiquitously acidic for the rapidly degrading PLGA 50/50 during the 6-week incubation, μpH in PLA and PLGA 85/15 increased steadily from their initially acidic pH (~3.0) at 1 day incubation, and reached neutral range (5.6−7.4) after incubation for 1 and 3 weeks, respectively. The acidic environment after 1 day incuba-

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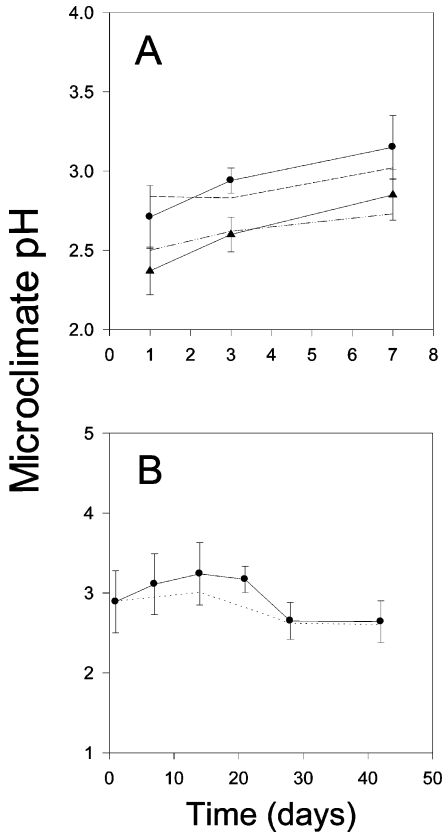

Figure 4. (A) Prediction of microclimate pH in monomer-loaded PLGA 50/50 (M_w 85 kDa) films incubated in ddH_2O for glycolic acid (−●−, - - -) and lactic acid (−▲−, -···-··). Symbols and dashed lines represent data (mean ± SEM, $n = 3$) and predicted curves from eq 11, respectively. (B) Predicted microclimate pH kinetics for PLGA 50/50 films (M_w 85 kDa) incubated in PBS containing 0.02% Tween 80: predicted (···) from eq 11 and experimental (−●−) microclimate pH values.

tion was undoubtedly caused by the acidic impurities in polymer materials after polymerization (e.g., from residual lactide[45]). With longer incubation times, soluble acid residues are likely released from polymer faster than acid production in the slow-degrading PLGA 85/15 and PLA; the resulting low acid content in the polymer matrix was also expected to decrease autocatalytic degradation of the polymer (sequestered acidic degradation products catalyze further PLGA degradation).[15] In Table 2, the predicted μpH is compared with measured values for polymer films during incubation for 1 week after the determination of water-soluble acid content. μpH values were observed to increase for all three polymers during 1 week incubation. In each case, the model predicted measured μpH to within experimental error, and predicted the μpH response to both changes in lactic acid content and time. For example, at 1 week, increasing lactic acid content from 50%, 85%, to 100% yielded steadily increasing experimental (predicted) μpH values of 3.11 ± 0.37 (2.95), 3.31 ± 0.77 (3.20), and 5.60 ± 0.80 (4.80), respectively. Polymer erosion kinetics of these specimens (Figure S3B) were consistent with these data (see Supporting Information).

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Table 2. Predicted and Experimental μpH in PLGA Films as a Function of Composition during Incubation in PBST[a] at 37 °C for 1 Week

polymer (i.v. = 0.60–0.63 dL/g)	experimental μpH ± SEM		predicted μpH [b]	
	1 day	7 days	1 day	7 days
PLGA 50/50	2.89 ± 0.38	3.11 ± 0.37	2.90	2.95
PLGA 85/15	2.83 ± 0.16	3.31 ± 0.77	2.86	3.20
PLA	3.12 ± 0.33	5.60 ± 0.80	3.25	4.80

[a] PBST is phosphate buffered saline plus 0.02% Tween 80. [b] Predicted using eq 14.

Conclusions

The physical chemical basis for μpH development has been elucidated and μpH in thin PLGA films predicted from the water-soluble acid content in aqueous pores. Water-soluble degradation products partition strongly in the polymer phase during biodegradation of PLGAs, which makes most water-soluble acids unavailable for lowering μpH. The methodology described here to determine PLGA/water partition coefficients of water-soluble degradation products may be reasonably applied to other biodegradable polymer systems. Models to predict μpH in the future will need to address more complex systems such as cases where μpH is strongly spatially dependent (e.g., in very small-length scale microspheres/micorparticles), or when encapsulated species (excipients and drugs) affect acid−base equilibria, ionic strength, and water activity. This basic model should provide a basis for future models of higher complexity and improve both μpH control methodologies and design of PLGA systems for delivery of stabilized acid-labile drugs, such as therapeutic proteins.

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Supporting Information Available: Experimental procedures for analysis of acid content by pre-derivatization HPLC, typical HPLC chromatograms of bromophenacyl ester derivatives, derivation of P_i corrected for ionization and polymer pore volume, and the polymer composition effect on P_i and polymer erosion kinetics. This material is available free of charge via the Internet at http://pubs.acs.org.

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