# Determination of Water-Soluble Acid Distribution in Poly(lactide-*co*-glycolide)

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**ABSTRACT:** Determination of the kinetics of water-soluble degradation products inside poly(lactide-*co*-glycolide) (PLGA) delivery systems during polymer degradation is important to evaluate the polymer microclimate conditions, particularly microclimate pH changes for optimization of encapsulated drug stability. A pre-derivatization high-performance liquid chromatography (HPLC) method was developed for separation and quantification of water-soluble acid impurities and degradation products in PLGA. Thin PLGA films (~200 μm) were incubated in PBS/0.02% Tween 80, pH 7.4, for 6 weeks. Water-soluble monomers and oligomers were obtained from polymer films after repeated $CHCl_3/H_2O$ extraction and then derivatized into bromophenacyl esters. With the common chromophore, the esters were separated and quantified by HPLC with increased ultraviolet (UV) sensitivity at 254 nm. The total amount of water-soluble acids in the extract was validated by potentiometric titration with tetrabutyl ammonium hydroxide. During the first 3 weeks of incubation of PLGA 50:50 (inherent viscosity = 0.63 dL/g), the principal water-soluble acids in the polymer were glycolic, lactic, and lactoyllactic acids, and an unknown oligomer. After 4 weeks of incubation, a large fraction of higher molecular weight oligomers was observed. Pre-derivatization HPLC can be used to accurately measure water-soluble acid distribution, and may be invaluable to examine the degradation behavior of PLGAs, including the underlying mechanism of polymer microclimate pH development. © 2004 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 93:322–331, 2004

**Keywords:** poly(lactic/glycolic acid) (PLGA); polymer chemical degradation; distribution

## INTRODUCTION

Copolymers of lactic and glycolic acids (PLGAs) are one of only a few biodegradable polymers used in pharmaceutical products or medical devices approved by the United States Food and Drug Administration (US FDA).[1] Initiated in 1960s as resorbable suture/fiber materials,[2] these biodegradable and biocompatible polyesters have been widely investigated as controlled drug delivery systems for the past few decades, resulting in several marketed injectable depots,[3–5] and have an excellent safety record.[6] Recently, PLGAs have been the focus of extensive research for long-term release of therapeutic proteins, and a once-a-month injectable controlled-release system for encapsulating human growth hormone is one of the first products approved by the US FDA among all the new minimally invasive protein delivery system options.[1]

PLGAs are most often synthesized by catalytic ring opening polymerization with lactide and glycolide (i.e., the cyclic dimers of lactic and glycolic acids) as starting materials.[7] The ester backbone undergoes hydrolysis in aqueous environments, such as body fluids, and the polymer eventually degrades to lactic and glycolic acid monomers, which are subsequently eliminated in *vivo* as $CO_2$ and $H_2O$ via the Krebs cycle.[8] In

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addition, varying levels of water-soluble acid impurities are well known to exist in PLGAs, which can influence their solid-state stability, drug encapsulation efficiency, and drug release behavior.[9,10] In general it has been observed that the hydrolytic degradation of the polymer depends on several factors, such as polymer composition, solid-state morphology, polymer molecular weight, crystallinity, and glass transition temperature.[11–14] The process of polymer degradation has been largely investigated by monitoring the time-dependent changes of polymer molecular weight, physicomechanical properties, mass loss, total carboxyl content, and morphology by employing the techniques, such as gravimetry, X-ray diffraction, differential scanning calorimetry, scanning electron microscopy, size-exclusion chromatography, and end group assays.[10,15–18] Such investigations, however, have provided little information about the kinetics of water-soluble acid distribution inside PLGAs during polymer degradation.

It is important to gain detailed knowledge of the existence of the polymer degradation products, especially water-soluble species, inside polymer devices. PLGA-based drug delivery systems are exposed to physiologic conditions for extended periods, and the resulting polymer microclimate can play a critical role for encapsulated drug stability. Recently, it has been demonstrated that protein stability in PLGAs often has been compromised due to the ubiquitous acidic microclimate pH (i.e., μpH) caused by the water-soluble acids in protein regions of the PLGA matrix.[1,19–22] Therefore, exact quantification of the type, amount, and kinetics of water-soluble degradation products to provide valuable information on μpH changes is of interest. Such quantification may also help to elucidate the mechanisms for incomplete release of various drugs, which may be due to the possibility of interactions between certain polymer hydrolytic by-products and those drugs.[23] Moreover, acid-quantification may lead to better understanding of polymer degradation kinetics and mechanisms.[18] Finally, the diffusion of these water-soluble species out of polymer, depending on their type and rate, can likely affect the function of living tissues.[24,25]

To exactly quantify the distribution of the acidic degradation products and monitor their subtle variation during degradation, a simple, yet highly sensitive, specific and reproducible analytical method needs to be developed. High-pressure liquid chromatography (HPLC) has been used to determine polymer degradation products.[26–28] However, these studies either could not achieve simultaneous determination of both glycolic and lactic acids or only identified and quantified monomers, the final degradation products, while neglecting the possible existence of the water-soluble dimers and oligomers. Exact quantification of dimers and oligomers cannot be achieved by simply imposing the same standard curve of monomer on their peak areas, considering potential differences of extinction coefficients for each acid. To date, no efforts have been reported to exactly quantify water-soluble oligomers due to the lack of standards. Furthermore, existing methods directly detect acids at 210 nm, a wavelength very close to the cut-off range of many commonly used mobile phases, which may increase the baseline noise and reduce the sensitivity and detectability.

We sought to overcome the aforementioned drawbacks by simply converting acids to products with the same chromophore, which can be easily and accurately detected by HPLC at a detectable wavelength. In this study, we separated and exactly quantified the water-soluble polymer degradation products inside thin PLGA films during incubation by extracting the water-soluble acids from the polymer, derivatizing them to form stable bromophenacyl esters, and then quantifying them by reversed-phase HPLC.

## EXPERIMENTAL

### Chemicals

Poly(D,L-lactide-*co*-glycolide) 50:50, 85/15 with inherent viscosity (i.v.) of 0.63 and 0.66 dL/g in hexafluoroisopropanol, and poly(D,L-lactide) with inherent viscosity of 0.60 dL/g in chloroform were purchased from Birmingham Polymers, Inc. (Birmingham, AL). D,L-Lactide and glycolide were generously provided by Purac Biochem (Gorinchem, Netherlands). All other chemicals were of analytical grade or HPLC grade and purchased from commercial suppliers.

### PLGA Film Preparation and erosion

The polymer film-coated electrode method, similar to that used previously by our group[29] for measuring microclimate pH inside thin PLGA films, was used to prepare the PLGAs films for analysis. Microclimate pH measurements in the

same specimens and a theoretical model for its prediction will be reported in a future paper. Standard noncombination-type, pH-sensitive, glass electrodes (Fisher Scientific, Pittsburgh, PA) were coated with PLGA by immersing the electrodes into PLGA in acetone (500 mg/mL) followed by subsequent quenching in double-distilled water at 4°C for 1 h. Coated electrodes were dried at room temperature for 1 h before being placed in a vacuum oven for further drying for 36 h at room temperature. A light microscope with a scale bar was used to measure the coating thicknesses of the polymer films, which were ~200–250 μm.

To avoid buffer penetration into the microclimate of the polymer film from the edge of the coating, paraffin was used to seal the surface above the ion-sensitive glass bulb of the electrode before the film coating (~30 mg) was incubated in phosphate buffered saline (pH 7.4) containing 5 mL of 0.02% w/v Tween® 80 (PBST) at 37°C for 6 weeks. Incubation media were replaced weekly to maintain buffer pH, and the incubation tubes were sealed by paraffin to avoid partial evaporation of the fluid.

### Extraction and Preparation of Water-Soluble Acids

At predetermined time points, polymer films were carefully cut, peeled off from the electrode bulb, and freeze-dried before extraction. Each preweighed film was dissolved in 1 mL of chloroform before adding 5 mL of double-distilled water. After mixing by mild vortexing, the biphasic solution was left for 10 min before being separated by centrifugation at 4°C. The upper water layer was quickly removed and frozen with liquid nitrogen. The extraction was repeated five times, and finally the water phases were combined and freeze-dried on a Freezone 6 freeze-drying system (Labconco Corp., Kansas City, MO) at $\leq 133 \times 10^{-3}$ mBar with a condenser temperature of −46°C for 3–5 days. The dried extracts were then dissolved in acetonitrile and further prepared for titration or HPLC analysis as described below.

### Acid Quantification by Potentiometric Titration

The dried water-soluble acid extracts just described were reconstituted in 1 mL of degassed ACN–$H_2O$ mixture (20:80, v/v) and then titrated with 0.1 M tetrabutyl ammonium hydroxide (TBAH) in the same solvent mixture. The electromotive force (EMF) was recorded as a function of the titrant added with a Corning Semi-Micro Combination glass pH-electrode (VWR Scientific, West Chester, PA) attached to an Orion pH meter (Fisher Scientific, Pittsburgh, PA).

### Derivatization of Acids to Bromophenacyl Esters

#### *Acid Derivatization*

Dried water-soluble acid extracts were reconstituted in ACN, and a ≥2-fold mole excess of triethylamine (TEA) and bromophenacyl bromide (*p*BPB) solution in ACN were added. The solution was put in amber glass threaded vials for the protection of the light-sensitive reagent and sealed with rubber-lined closures. The reaction was carried out at 50°C for 3 h, and the resulting solutions were stored at 4°C before HPLC analysis.

#### *Preparation of Acid Standards*

The standard solutions of bromophenacyl esters of glycolic and lactic acids were prepared by adding TEA and *p*BPB to acid solutions in ACN and allowing the reaction to take place, as already described. For the preparation of the linear dimer of lactic acid (lactoyllactic acid), D,L-lactide was added to water, and hydrolysis was carried out at 80°C for 30 min. The resulting solution was freeze-dried, and the residue consisted of viscous oil, which was then dissolved in ACN and reacted with TEA and *p*BPB. HPLC results indicated that under the hydrolysis conditions used, lactide was hydrolyzed almost completely (~95%), producing ~80% lactoyllactic and 20% lactic acid.

#### *Characterization of Reaction Product by Nuclear Magnetic Resonance (NMR) and Mass Spectroscopic (MS) Analyses*

The HPLC fraction that contained individual bromophenacyl esters of glycolic acid was collected and air-dried to remove organic solvent before freeze-drying. The dried sample was reconstituted in deuterated acetonitrile ($CD_3CN$) or ACN and centrifuged, and the supernatant was removed for NMR and MS analysis, respectively.

Samples dissolved in $CD_3CN$ were immediately transferred to an NMR tube and sealed. The $^1H$ NMR spectrum was acquired on a DRX 500 Bruker spectrometer (Bruker Biospin Inc., Billerica, MA) operating at 500 MHz. The following conditions were used: pulse angle = 90°, pulse width = 11 μs, delay between two successive pulses = 1 s.

Mass spectrometry of supernatant dissolved in ACN was carried out on a Micromass Autospec Ultima Magnetic sector mass spectrometer (Micromass, Wythenshawe, UK). The ions were generated by chemical ionization with ammonia.

### HPLC Analysis

A Waters HPLC system (Waters, Milford, MA) was used for chromatographic separation of bromophenacyl derivatives of water-soluble polymer degradation products. It consisted of a binary HPLC pump, autosampler, and ultraviolet (UV) absorbance detector. Waters Breeze® chromatography software was used to acquire and process data. A 5-μm Symmetry® $C_{18}$ column (25 cm × 4.6 mm i.d.; Waters, Milford, MA) was used with a filtered and deaerated ACN:$H_2O$ (70:30, v/v) mobile phase with the linear gradient to 80:20 (v/v) from 2–4 min. Chromatography was performed at a flow rate of 1.5 mL/min, and the eluent absorbance was detected at 254 nm and room temperature. After 12 min, 100% ACN was used to elute for 8 min.

The analytes in the standard mixture and in the various samples were identified by comparing the retention time of the relevant peaks with those of the corresponding standards injected separately. No distinction could be made between D- and L-lactic acids. Quantification was carried out by integration of the peak areas using the external standard chromatographic method.

### Polymer Film Morphology

The inner side of film coating, which had been adjacent to electrode surface, was freeze-dried and coated with gold-palladium by using Denton Vacuum Desk II Cold Sputter (Enton Acuum Inc., Cherry Hill, NJ). Surface morphology was examined by a Hitachi S-3200N variable-pressure scanning electron microscope (SEM; Hitachi Ltd., Tokyo, Japan).

## RESULTS AND DISCUSSION

### Derivatization of PLGA Water-Soluble Acids

Bromophenacyl bromide (*p*BPB) was utilized here for the preparation of UV-sensitive derivatives of PLGA water-soluble acids. The derivatization reaction proceeds as shown in Figure 1. Anhydrous conditions were required to avoid further hydrolysis of oligomers during the reaction and

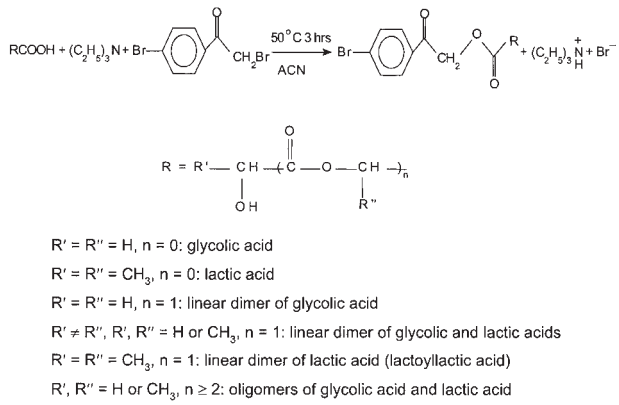


**Figure 1.** Scheme for esterification of water soluble acid extracts with *p*BPB.

to maintain solubility of reaction reagents. The optimization of the reaction parameters (i.e., temperature, time, and reaction reagents concentration) was achieved with the combination of HPLC analysis (data not shown). The reaction product was applied on a $C_{18}$ column and detected at 254 nm with an ACN:$H_2O$ mixture as eluent, and the peak area was used as the parameter for the reaction evaluation. The optimized conditions were selected based on the ease of the procedure, short reaction time, high purity, and high reaction yield.

The effect of the concentration of *p*BPB and TEA was examined by varying each concentration independently. Generally, excess *p*BPB was required to obtain a significant yield, indicated by the peak area of the analytes. The maximum yield was obtained when the mole ratio of *p*BPB-to-acid was $\geq$2:1, and there was no significant change in yield with further relative increase in *p*BPB. TEA was used in the reaction to enhance the nucleophilic property of the carboxylate anion. It was observed that, with the mole ratio of TEA-to-acid of >1:1, variation of TEA concentration also did not affect reaction yield. Therefore, a large excess of the reagents apparently is not required or deleterious. The degree of completeness of the derivatization reaction was also evaluated with respect to the effect of reaction time and temperature. The reaction was conducted at 50°C and followed over 24 h. Conversion was not complete after 2 h, but reached a maximum by 3 h. Longer reaction time was not necessary. Examination of the effect of temperature also revealed that, as expected, some increase in temperature was required to reduce derivatization time.

The final standard conditions for the reaction was chosen as follows: reaction temperature, 50°C;

reaction time, 3 h; and acid/pBPB/TEA mole ratio, 1:2:2. The yields were $90 \pm 3$ and $89 \pm 3\%$ (mean $\pm$ SD, $n = 3$) for glycolic acid and lactic acid standards, respectively. The relative purity of the derivative was determined by HPLC, which showed the single dominant peak for monomer standards. The reproducibility of the derivatization was evaluated by conducting the reaction in triplicate and then analyzing each by HPLC (two subsequent injections of each reaction solution), which gave a coefficient of variation (CV) of 6% ($n = 6$).

### Verification of Structure of Synthesized Acid Derivatives

The chemical structure of the derivatization bromophenacyl ester product was confirmed by $^1$H NMR and MS analyses. The peak of the derivatized glycolic acid standard was collected from HPLC and analyzed. The $^1$H NMR ($CD_3CN$) spectrum is shown in Figure 2 with the corresponding peak assignments. Singlets at 2.15 and 4.24 ppm correspond to protons in hydroxyl and methyl groups, respectively, from the glycolic moiety of the derivative. The singlet at 5.40 ppm and quadruple peak at 7.80 ppm were assigned to protons in the acyl group and aromatic ring of the bromophenacyl moiety of the derivative, respectively.

The chemical ionization mass spectrometric analysis with ammonia was used and the spectrum was recorded, as shown in Figure 3. The molecular weight of the derivative was characterized by $[MNH_4]^+$ ions ($m/z = 290$, 292) and some intense fragmentations, such as derived from the *p*-bromophenacyl moiety ($m/z = 183$, 185) and $[CH_2OHCOONH_4]^+$ group ($m/z = 93$). The isotope pattern of the signals was due to the existence of the bromine isotopes.

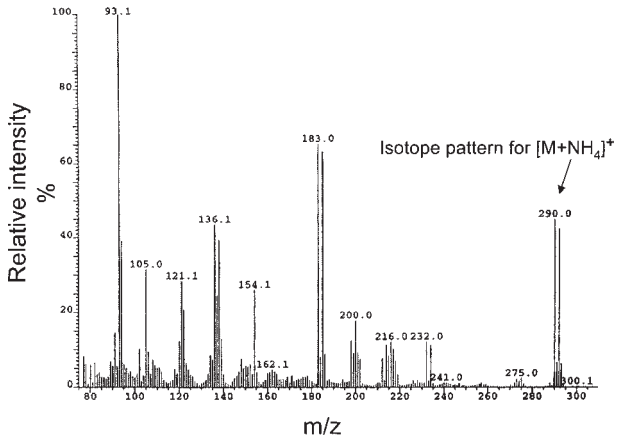


**Figure 3.** Mass spectrum of the reaction product of glycolic acid and *p*BPB in the presence of ammonia.

Based on these results, we confirmed that the derivative is the bromophenacyl ester of glycolic acid. Thus, the different peaks on HPLC chromatograms were safely assigned to the esters of corresponding acid monomers and oligomers.

### HPLC Separation and Determination of Water-Soluble Acids

The HPLC baseline separation of a standard mixture of derivatized glycolic, lactic, and lactoyllactic acids is shown in Figure 4. The peaks were assigned by matching each peak with the separate HPLC chromatograms of the individual acid standards. A typical HPLC chromatogram of water-soluble degradation products of PLGA 50:50 film following three weeks of incubation in PBST buffer is shown in Figure 5. The well-resolved lactic and lactoyllactic acid peaks in the

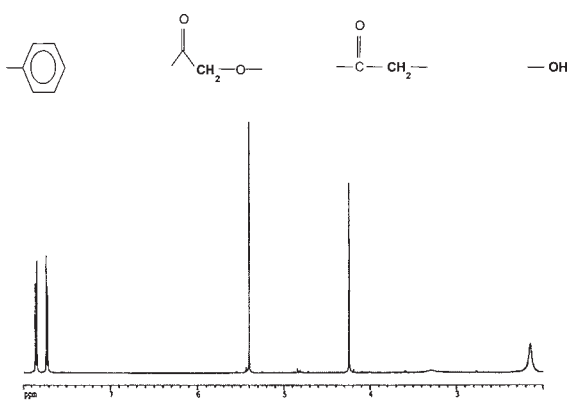


**Figure 2.** $^1$H-NMR ($CD_3CN$) spectra of the ester product of glycolic acid and *p*BPB collected from HPLC.

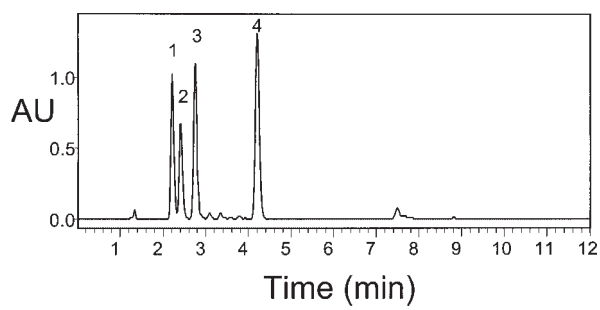


**Figure 4.** HPLC chromatogram of the mixture of reaction product of *p*BPB and three standards, (1) glycolic acid, (2) lactic acid, and (3) lactoyllactic acid, and (4) excess reagent *p*BPB.

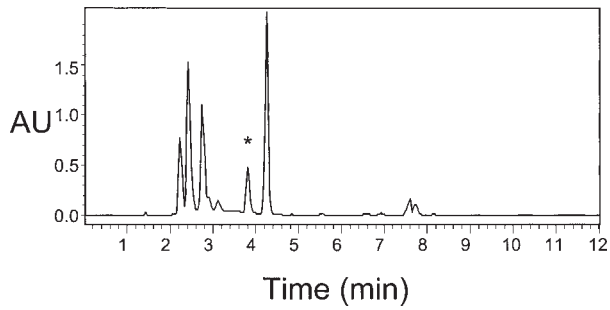


**Figure 5.** Typical HPLC chromatogram of water-soluble PLGA 50:50 degradation products after conversion to bromophenol esters after polymer film incubation for 3 weeks in PBST at 37°C. Key; (*) peak of unknown oligomer believed to be a tetramer of lactic acid.

sample chromatogram displayed the exact same result as in the separation of standard mixture, which indicates the absence of a linear dimer peak of glycolic acid that is expected between lactic and lactoyllactic acid peaks. The possibility of an overlap of lactic and glycolic acid dimer was ruled out because the linear dimer of glycolic acid was observed to be highly unstable in aqueous solution.[28] It was observed that during the first 3 weeks of incubation, the main components of water-soluble acids were glycolic, lactic, and lactoyllactic acids, and one unknown oligomer.

Identification of the unknown oligomer was problematic, because we did not have the corresponding standard to identify this oligomer and the derivatized monomers and oligomers are not easily ionized by liquid chromatography–mass spectrometry (LC–MS) systems. Therefore, we sought to speculate about the unknown oligomer identity from the adjusted retention time of its bromophenacyl ester. For a homologous series, the adjusted retention time ($t'_R$) has the following linear relationship with the number of the repeating unit in their structures:[30]

$$\log t'_{R(m)} - \log t'_{R(n)} = b(m - n) \qquad (1)$$

where $m$ and $n$ are the number of the repeating unit of any two members in the homologous series, and $b$ is the slope. At the constant ratio of ACN/$H_2O$ at 70:30 as mobile phase, $t'_R$ values for lactic and lactoyllactic acids were measured as 0.98 and 1.35 min, respectively, which makes the theoretical $t'_R$ for a tetramer of lactic acid ~2.56 min. Because this theoretical value was very close to the $t'_R$ value of the unknown oligomer (2.50 min) and this oligomer peak was present in PLA films not containing glycolic acid, it is reasonable to speculate that this oligomer is the tetramer of lactic acid.

In addition to the four dominant peaks just described, there were several tiny peaks appearing in the chromatogram after 3 and 7 min. The latter can be attributed to the esters of oligomers of higher polymerization degree, but their relative amount was small. However, after 4 weeks, more oligomer peaks appeared and became more pronounced with time. Some of these peaks could not be baseline separated. A further improvement of the resolution may be difficult under the current HPLC column conditions. For those incompletely separated oligomers, mean retention time was used in the later polymer degradation behavior analysis. Pure ACN was used to elute the column after 12 min to flush out the possible longer chain oligomers, but no additional peaks were found.

The retention time of the high excess derivatization reagent *p*BPB was significantly different from those esters of the primary degradation products. The possibility of a peak overlap from *p*BPB and a possible oligomer peak was discounted because no peak appeared at the *p*BPB retention time when *p*BPB was not used in excess in the reaction solution.

The calibration of peak areas against concentrations generated linear functions ($r^2 = 0.999$) for both glycolic and lactic acid standards within a range 0.25–1.5 μmol/mL. The same slopes were observed as a result of existence of the same chromophore, which verified the accuracy of quantification of oligomers by a sample monomer standard curve. The detection limit was estimated to be ~0.008 μmol/mL, corresponding to $4 \times 10^{-4}$ μmol/mg PLGA for the current analytical conditions. The precision of the method, evaluated by repeated injection of a standard solution with a concentration of 0.5 μmol/mL, was represented by a CV of the retention times of <1.0%, and a CV of peak areas of <2.8% ($n = 6$). The possible interference of the coexisting acid species on individual acid quantification was tested by comparing chromatograms of derivatized pure acid and acid standard mixture, and no significant deviation was observed.

### Water-Soluble Acids Inside PLGA 50:50 Films during Polymer Degradation

Total water-soluble acid content inside PLGA 50:50 films during polymer degradation was obtained by repeated liquid–liquid extraction using chloroform to dissolve the polymer and water to recover the low molecular weight acids.

The completion of the extraction was tested by adding known amount of monomers in the organic phase followed by repeated extraction and acid recovery measurement by potentiometric titration. It was shown that $97 \pm 5$ and $95 \pm 7\%$ (mean $\pm$ SD, $n = 3$) were recovered for glycolic and lactic acids, respectively.

Total water-soluble acid content was quantified separately both by the developed HPLC method and potentiometric titration. TBAH, as a strongly basic quaternary ammonium base, was used as a titrant to avoid the possible alkali error of the glass electrode. A small amount of ACN (20%) was added to maintain the solubility of formed tetrabutyl ammonium salts. Both titrant and samples were prepared in the same solvent system (ACN/$H_2O$ = 1:4), thus hydronium ion activity was measured in the consistent system without the disturbance of the added titrant. Possible interference of carbon dioxide was largely avoided by degassing the solvent before titration. Electromotive force (EMF) was recorded as a function of the quantity of titrant added. The end point corresponds to the inflection point of the potentiometric titration curve and was obtained by taking the first derivative. Typical titration and end-point determination curves for water-soluble acid quantification are shown in Figure 6.

Kinetics of total water-soluble acid content inside PLGA 50:50 films was quantified by titration and is shown in Figure 7A after normalization by dry film weight. The acid content increased dramatically after 3 weeks, which was due to the continuous accumulation of acids from polymer

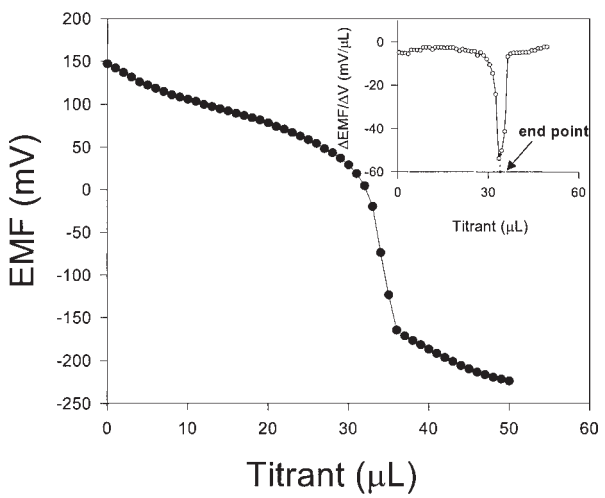


**Figure 6.** Typical potentiometric titration curve (●) and end-point determination (○) of total water-soluble acids extracted from PLGA films.

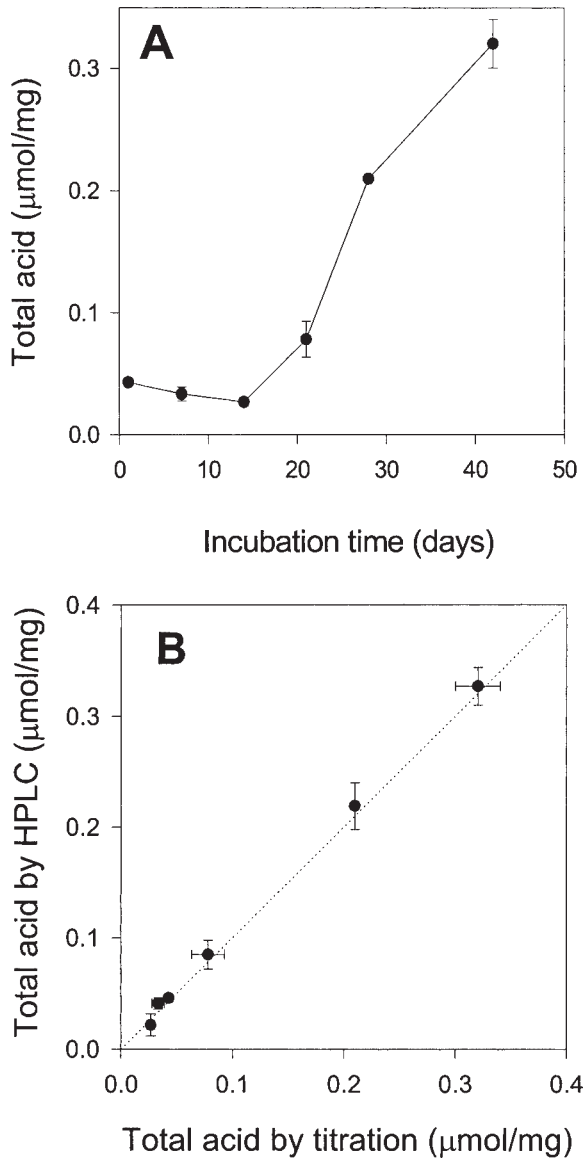


**Figure 7.** Determination of total PLGA 50:50 water-soluble acid content: (A) kinetics of total residual water-soluble acids; (B) comparison of total residual water-soluble acids quantified by potentiometric titration and by HPLC ($n = 3$, mean $\pm$ SE).

degradation and accelerated degradation rate caused by those acids, which auto-catalyze polyester hydrolysis.[18] As seen in Figure 7B, total acid content measurements from potentiometric titration were essentially identical to those data obtained by the prederivatization HPLC method, thus demonstrating the validity of the two analytical procedures.

The relative composition of water-soluble acids is shown in Figure 8. Glycolic, lactic, and lactoyllactic acids, and the hypothesized lactic acid

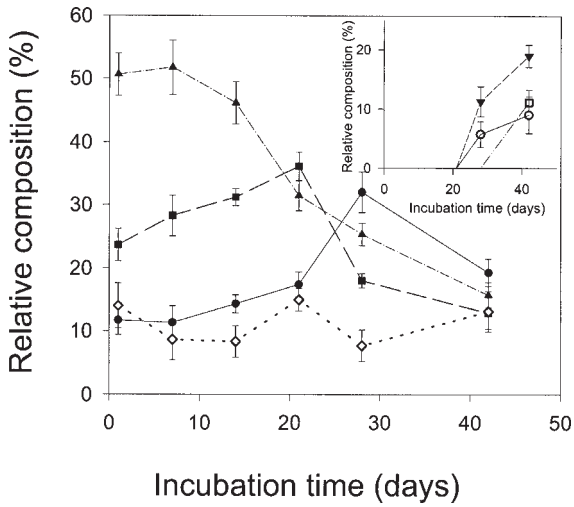


**Figure 8.** Relative molar composition of a variety of water-soluble acids inside the polymer matrix during PLGA 50:50 film degradation. Key: (●) glycolic acid; (■) lactic acid; (▲) lactoyllactic acid; (◇) oligomer 1; (○) oligomer 2; (▼) oligomer 3; (□) oligomer 4 ($n = 3$, mean ± SE).

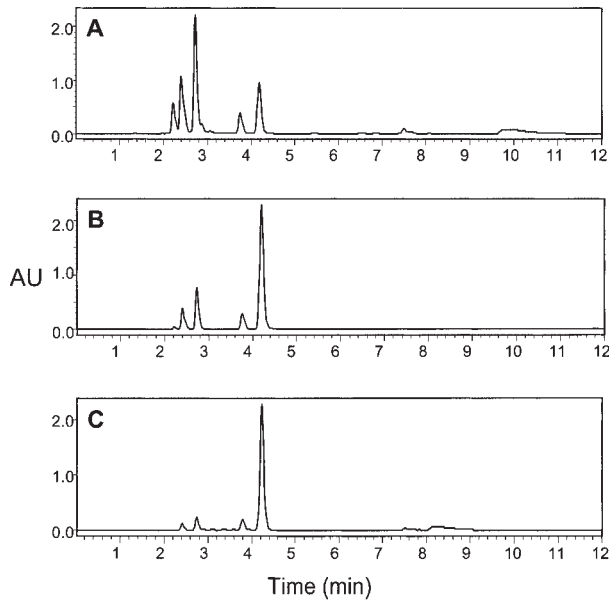


**Figure 9.** HPLC chromatograms of polymer degradation products after conversion to bromophenol esters of (A) PLGA 50:50 (i.v. = 0.63 dL/g), (B) PLGA 85:15 (i.v. = 0.66 dL/g), and (C) PLA (i.v. = 0.60 dL/g) after polymer films were incubated for 1 week in PBST at 37°C.

tetramer were the major components. Lactoyllactic acid content was relatively high during the first 2 weeks, which may be due to the existence of the residual lactide after polymer synthesis and its relatively high stability. Although the linear dimer of glycolic acid was unstable and hydrolyzed to glycolic acid quickly, the lactoyllactic acid can remain intact for a much longer time.[28] A relatively low composition of glycolic acid was observed, which is not surprising because glycolic acid has been observed to be released out ~3–4 times faster than lactic acid.[26,28] After 4 weeks of incubation, all short-chain acids (monomers and dimer) decreased, whereas more oligomers appeared and their relative composition increased. This increase may be caused by mobilization of polymer chains, which facilitates random scission relative to end scission.[31]

### Polymer Composition Effect on Water-Soluble Acid Production and Polymer Degradation

The developed acid derivatization followed by HPLC analysis can be used to analyze the degradation behavior of PLGAs other than PLGA 50:50. The HPLC chromatograms of acids recovered from PLGA 50:50 and 85:15, and PLA films after 1 week of incubation are compared in Figure 9. Water-soluble acids were mainly composed of lactic and lactoyllactic acids and the hypothesized lactic acid tetramer for all of those polymers including PLA. As expected, compared with PLGA 50:50, no substantial peak of glycolic acid ester was observed in PLGA 85:15 films because of the lower glycolic acid content in polymer material. As lactide content in the polymer was increased from 50 to 100%, a reduction in peak areas of corresponding acids was observed, which was due to the slower degradation rate of the lactide-rich copolymer and homopolymer.[27,32]

Significant mass loss was observed after 4 weeks of incubation of PLGA 50:50 films (data not shown), corresponding to the well-known induction time of bulk-degradable PLGA,[33] and PLGA 50:50 films became highly viscoelastic at 6 weeks. By contrast, with the relatively slower degradation rate, PLGA 85:15 and PLA films withstood no significant mass loss or visible changes in physical state during 6 weeks of incubation. The morphology of the inner side of the polymer films (adjacent to the electrode surface) after 4 weeks of incubation is compared in Figure 10. Bulk porosity was the most extensive for PLGA 50:50 films with highest pore volume fractions and pore diameter. Smaller pores were scattered throughout PLGA 85:15 films, whereas PLA films were relatively nonporous.

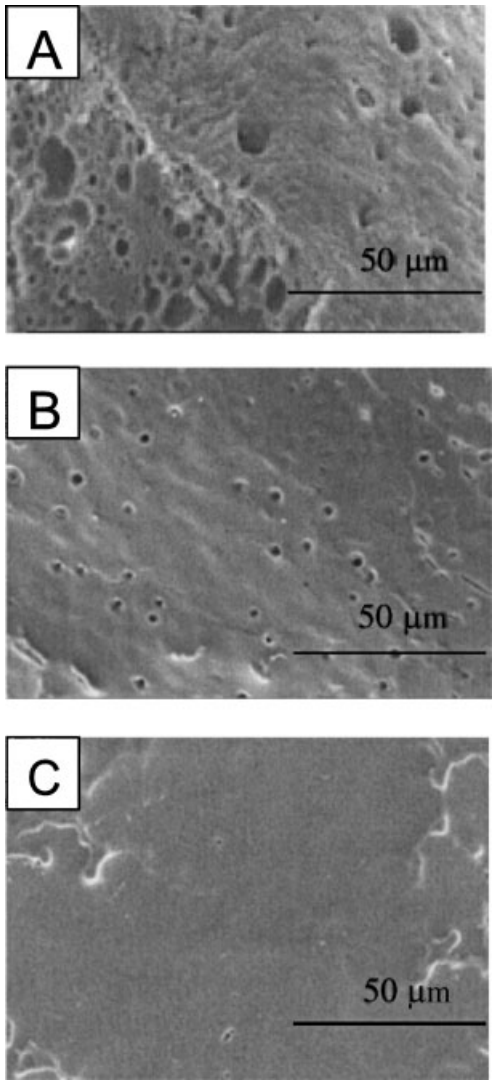


**Figure 10.** Morphology of the inner surface of polymer films after incubation for 4 weeks in PBST: (A) PLGA 50:50 (i.v. = 0.63 dL/g); (B) PLGA 85:15 (i.v. = 0.66 dL/g); and (C) PLA (i.v. = 0.60 dL/g).

In closing, a prederivatization HPLC method was developed to accurately determine the water-soluble acid distribution in degrading PLGAs. This technique may have numerous applications in PLGA characterization, including disclosure of the underlying mechanism of polymer microclimate pH development.

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