Potentiometric Method for Determination of Microclimate pH in Poly(lactic-*co*-glycolic acid) Films

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ABSTRACT: The acidic microclimate pH (μpH) in poly(lactide-*co*-glycolide) (PLGA) devices has been identified as a major source of instability of encapsulated proteins and acid labile small molecules. A fast and reliable potentiometric technique for μpH measurement in thin PLGA films was developed and validated. Standard glass pH electrodes were coated with PLGA films, and the zero-current potential was measured with respect to a calomel reference electrode. The measurement was independent of external media pH but responsive to addition to the polymer films buffering salts from lyophilized solutions of various pH values. The contribution of additional diffusion and interfacial potentials in the electrochemical cell due to the presence polymer coating was determined to be roughly ≤0.2 pH units by two independent measurements and slightly pH dependent. A highly acidic μpH (μpH <3) developed in PLGA 50/50 films (∼30−100 μm thick) after 1 day of incubation in a physiological buffer at 37 °C and remained acidic for 4 weeks. The μpH varied significantly with the thickness of the coating during extended incubation. The μpH of a 7 μm thick coating rose to a neutral value (pH 6.5) after 1 week of incubation, but it remained acidic (pH 2−3.5) for 250 and 30 μm thick coatings. Hence, the microclimate pH measurement described here may be useful for mechanistic evaluation and μpH control of biodegradable polymer systems encapsulating labile molecules.

Introduction

Poly(lactic-*co*-glycolic acid) (PLGA) devices are the vehicles of choice for the controlled release of therapeutic proteins, peptides, vaccines, antibiotics, and anticancer drugs and are increasingly being examined for delivery of naked DNA.[1] This polymer is biodegradable, and upon penetration of water the hydrolysis of ester bonds (i.e., biodegradation) leads to a decrease in polymer molecular weight, liberation of monomers and oligomers, and subsequent device mass loss (i.e., bioerosion). PLGA hydrolysis is characterized by two reactions: a random chain and end-group scission. The end-group scission has been reported to occur roughly 10 times faster than the random chain scission.[2,3] Depending on the device geometry, size, and porosity, the acidic polymer degradation products can either be released or accumulated in the microclimate, which can lower the so-called microclimate pH (μpH), the pH in aqueous pores of the polymer. Depending on the pH−rate profile specific for the encapsulated molecule of interest, an acidic microclimate can be either stabilizing or destabilizing inside PLGA devices. For example, the acidic microclimate in PLGA 50/50 microspheres is the major factor that explains the exceptional stability of encapsulated, base-labile camptothecins.[4] By contrast, this acidic microclimate also leads to the acid-induced aggregation of encapsulated bovine serum albumin (BSA)[5,6] and the acid-induced deformylation of the anticancer drug, vincristine.[7] Therefore, understanding and control of the PLGA μpH are critical for the stabilization of encapsulated substances.

It is well established that an acidic microclimate (pH < 3) develops inside large (∼1−2 mm dimensions) poly(DL-lactide) (PLA) specimens,[8] leading to acceleration of acid-catalyzed degradation of the polymer core.[9−11] It is reasonable to assume that below some length scale of PLGA (e.g., <100 μm in diameter) the diffusion path length is too short for the degradation products to accumulate in the microclimate, and hence, an acidic μpH may not develop in small PLGA devices such as microspheres. Indeed, accelerated degradation of the polymer core was not observed for PLA microspheres of 125−250 μm particle size.[12] However, the μpH of PLGA 50/50 microspheres was reported to be in the neighborhood of 2 by confocal microscopy with the use of pH-sensitive fluorescent probes[13,14] and ∼3.4 and <4.7 by electron paramagnetic resonance (EPR) with the use of the pH-sensitive spin probes.[15,16] Unfortunately, both confocal microscopy imaging and EPR are laborious techniques, and the pH probes are sensitive only in a narrow pH range; thus, the microclimate pH was evaluated for only a few specific conditions. The microclimate pH of PLGA microspheres is expected to be quite different, depending on the selected polymer, encapsulated substance, and device size and porosity. Each of these properties all of which can influence polymer hydrolysis and acid transport. In addition, during microsphere incubation at 37 °C for extended periods of time (1−3 months) PLGA degradation and acid transport are likely time-dependent, causing significant changes in μpH.

In this study a potentiometric method for PLGA μpH measurement was developed and validated. This technique involves the coating of a standard glass pH electrode with a thin PLGA film and measuring the zero-current potential with respect to a reference electrode (Figure 1A). A calibration was performed to deduce the μpH of a PLGA film from the measured cell potential. The electrodes were coated under a variety of conditions, and μpH was measured during polymer

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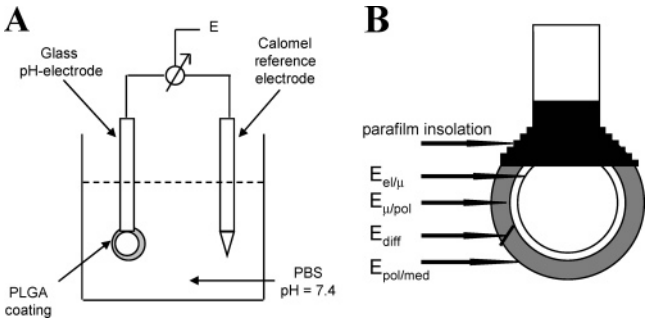

Figure 1. Schematic representation of the experimental setup for potentiometric μpH measurement. (A) A glass electrode was coated with a PLGA film and a zero-current cell potential (E) was measured with respect to a calomel reference electrode in PBST at 25 °C. (B) Potentials expected to be formed during the measurement include the interfacial potential between aqueous microclimate and electrode ($E_{el/\mu}$), the interfacial potential between microclimate and polymer phase ($E_{\mu/pol}$), the diffusion potential in the polymer (E_{diff}), and the interfacial potential between polymer and the media ($E_{pol/med}$). The measured potential $E = E_{el/\mu} - E_{\mu/pol} + E_{diff} + E_{pol/med} - E_{ref}$.

degradation. This potentiometric method can be used to begin to understand the complex phenomena of μpH variation.

Theory

Measurement of μpH. In the absence of a polymer coating during pH measurement, the zero-current cell potential (E) in an electrolytic cell is equal to the potential difference between the working electrode (E_{el}) and the reference electrode (E_{ref}). The E_{ref} is normally independent of the medium pH since the reference electrode is separated from the medium by a KCl junction. The working electrode senses pH by the change in the glass/medium interfacial potential (E_{el}). The E_{el} according to the Nernst equation is proportional to log a_{H^+} with a proportionality constant of $2.3RT/F = 59.2$ mV/ decade at 25 °C, where a_{H^+} is the H^+ activity in the medium.[17,18] Therefore, the measured E changes with pH according to the glass/medium potential, and all other potentials remain constant under normal conditions (i.e., no alkaline error or elevated ionic strength to overcome the KCl junction potential).

As shown in Figure 1, when a pH electrode is coated with a thin PLGA film and the zero current cell potential (E) is measured with respect to a calomel reference electrode, the presence of a polymer coating introduces several additional potentials contributing to the overall value of E. It is assumed that after the initial hydration of PLGA coating a thin aqueous film forms on the glass surface. Accordingly, the measurement can then be treated as if the pH electrode is immersed in the polymer aqueous microclimate.

Therefore, according to these foregoing assumptions the potentials formed during the potentiometric measurement within the polymer film are as follows: pH-electrode-microclimate potential ($E_{el/\mu}$), microclimate-polymer potential $E_{\mu/pol}$, polymer-medium potential $E_{pol/med}$, and reference electrode potential (E_{ref}) (Figure 1B). Hence, the measured cell potential (E) may be described by

$$E = E_{el} - E_{ref} = E_{el} - E_{\mu/pol} + (E_{\mu/pol} - E_{pol/med}) + E_{pol/med} - E_{ref}$$

where $(E_{\mu/pol} - E_{pol/med}) = E_{diff}$ is a diffusion potential and $E_{\mu/pol}$ and $E_{pol/med}$ are interfacial potentials.[19] The composition of the aqueous layer adjacent to the electrode is assumed to be the same as that of the aqueous pores inside the polymer. Therefore, $E_{el/\mu}$ is proportional to the μpH in the PLGA aqueous pores. To obtain the value of $E_{el/\mu}$, the contribution of $E_{\mu/pol}$, E_{diff}, and $E_{pol/med}$ to the measured overall potential E was estimated experimentally, as described below.

Materials and Methods

Chemicals. Poly(DL-lactide-*co*-glycolide) with a 50:50 monomer ratio and an inherent viscosity of 0.61−0.63 dL/g in hexafluoro-2-propanol was purchased from Birmingham Polymer Inc. The fluorescent dyes, 10-hydroxycamptothecin and fluorescein, were obtained from Dabur India Limited (New Delhi, India) and Molecular Probes Inc. (Eugene, OR), respectively. Standard buffer solutions (pH 2−10) were purchased from Fisher Scientific (Pittsburgh, PA). All other reagents and solvents were of analytical grade and obtained from commercial suppliers.

Coating of Glass Electrodes. Standard glass pH-sensitive Accument electrodes were purchased from Fisher Scientific (Pittsburgh, PA). The electrodes were coated by dipping into a PLGA−acetone solution followed by subsequent quenching in double distilled water at 4 °C for 1 h, similarly as described by Zhou et al.[20] PLGA concentrations of 500, 250, and 125 mg/mL were used in coating to vary the thickness of polymer films. In some instances ground and sieved (<45 μm) powders of buffer salts at 15% (w/w) were suspended in the 500 mg/mL PLGA solution prior to the coating. Coated electrodes were dried for 24 h at a room temperature. To avoid buffer penetration into the microclimate by diffusion around the coating edge, the edge was sealed with paraffin above the ion-sensitive bulb (Figure 1B).

Potential Measurement. The zero-current potential was measured by a Corning ion analyzer 250 with an Accumet calomel reference electrode (Fisher Scientific, Pittsburgh, PA). Prior to the coating, the electrodes were calibrated by a three-point calibration in standard buffer solutions at pH 4, 7, and 10 (Fisher Scientific, Pittsburgh, PA). The potential (in mV) was measured in phosphate buffered saline (PBS) containing 0.02% polyoxyethylene sorbitan monooleate or Tween 80 (PBST), pH 7.4, at room temperature. To study the changes in μpH with polymer degradation, coated electrodes (~30 mg coating weight) were incubated in 5 mL of PBST at 37 °C. The buffer was changed periodically, every 1−2 weeks, to ensure perfect sink conditions.

Evaluation of Coating Morphology. The thickness and porosity of PLGA coatings were evaluated before and after 1 week of coating incubation in PBST at 37 °C by scanning electron microscopy (SEM). The coatings were dried and spun-coated with conductive gold palladium prior to analysis. Images were obtained by a Philips XL30 field emission gun SEM set to 10 keV.

To evaluate the homogeneity of the coating, a concentrated stock solution of fluorescent dye 10-hydroxycamptothecin in DMSO was added to the PLGA−acetone solution to yield a theoretical loading of 0.1% (w/w). The coating was hydrated for 1 day in PBST, removed from the electrode, and fixed on the glass slide. The confocal fluorescence image was obtained with a Leica TCS SP2 AOBS confocal microscope equipped with an argon laser. In addition, the thickness and homogeneity of the coatings were routinely evaluated with a light microscope (Axiolab, Zeiss, Thornwood, NY).

Validation of the Potentiometric Method. To evaluate the effect of external media pH on the measured μpH, standard buffer solutions (pH 2−10) were utilized. The effect of possible coating imperfections on the measured μpH was also examined. Following 1 week of electrode incubation in PBST at 37 °C, the coating was removed sequentially by cutting of the polymer coating in ring sections with a blade from the bottom of the electrode bulb to the top. Hence, a larger area of the electrode bulb was exposed after each cut. The exposed

electrode bulb area was roughly estimated with a caliper, and the cell potential of the partially coated electrode was measured in PBST.

The contribution of additional potentials, i.e., interfacial ($E_{\mu/\mathrm{pol}}$ and $E_{\mathrm{pol/med}}$) and diffusion (E_{diff}) potentials, introduced in the electrochemical cell by the polymer coating (Figure 1B) was evaluated. To this end, the coating was carefully removed from the electrode and tested for leaks by addition of a dye solution (0.01 mg/mL fluorescein) on one side of the coating and examination of dye appearance on the other side of the coating. A series of potentials E_1 and E_2 were measured as follows. E_1 was measured when a set of pH and reference electrodes was immersed in the same standard solution (pH 2−8, Fisher Scientific, Pittsburgh, PA). To measure E_2, the removed coating was slowly lowered into standard solution (pH 2−8, Fisher Scientific, Pittsburgh, PA) containing the pH electrode without allowing the solution to overtake the film edge. Then, the inner cavity of the immersed coating was filled with PBST, and the reference electrode was inserted in the PBST. Measurement of the cell potential in this configuration gave E_2. The difference between E_2 and E_1, which corresponded to the contribution of the additional potentials ($-E_{\mu/\mathrm{pol}} + E_{\mathrm{diff}} + E_{\mathrm{pol/med}}$), was plotted as a function of standard solution pH.

The pH offset of measured μpH compared with actual μpH was also examined by equilibrating coated electrodes in acid media. Coated electrodes were incubated in HCl or glycolic acid solutions of pH 1.8 without external solution replacement for a 1 week period until the equilibrium μpH reading was achieved. The difference between the equilibrium μpH reading and the measured pH of equilibrating solutions (i.e., 1.8) was determined.

Microclimate pH Response to Encapsulated Buffer Salts. To correlate the measured coating potential with the polymer μpH, the μpH value was fixed for a short period of time by encapsulation of buffering salts forming microclimate solutions of known pH. Buffer solutions were prepared at 2 M concentration by dissolving several buffering acids and adjusting pH with NaOH and lyophilized for 3 days in a Labconco Freezone 6. Citrate buffer was used for pH 3 and 5, glycolate for pH 4, phosphate for pH 6, and borate for pH 8. The lyophilized salts were ground, sieved (<45 μm), and incorporated into the PLGA coating at 15% (w/w) loading by suspending the salts in the polymer solution before dip-coating. The μpH was determined over the first 24 h of coating hydration in water at 37 °C. The lyophilized salts were dissolved in water. The pH of saturated solutions of salts was determined and correlated with the measured μpH values for coatings containing lyophilized salts.

Results and Discussion

Morphology of PLGA Coatings. The scanning electron microscopy images of blank PLGA coatings removed from the electrodes after 1 week of incubation are shown in Figure 2. The film thickness was controlled by the concentration of polymer solution used in the electrode coating. The PLGA (i.v. = 0.61 dL/g) solution concentrations of 500 and 250 mg/mL resulted in an approximate coating thickness of 300 and 30 μm, respectively. The thickness of the coating was relatively homogeneous around the electrode as confirmed by the confocal fluorescence microscopy, as seen in Figure 3. An asymmetric coating obtained by this technique consisted of a dense polymer region exposed to the buffer and a more porous surface adjacent to the electrode (Figure 2). Following 1 week of incubation, both polymer surfaces developed an observable microporosity of 5−20 μm pore size. However, the coating cross section remained dense; i.e., a percolating pore network did not appear to develop throughout the coating, ensuring no mixing between the aqueous polymer microclimate sensed by the electrode and incubation medium.

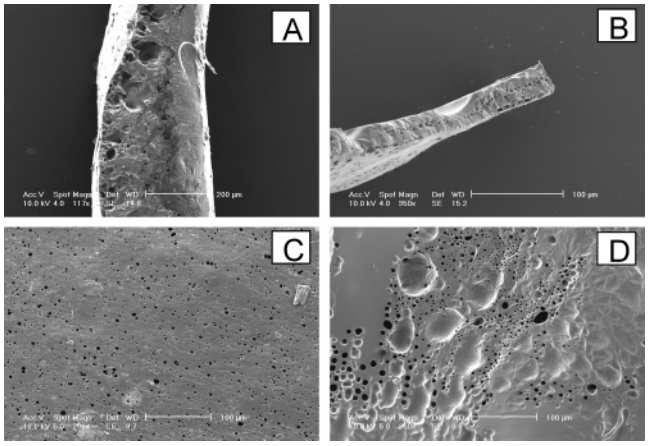

Figure 2. Scanning electron micrograph of PLGA 50/50 coatings removed from the electrode after 1 week of hydration in PBST (pH 7.4) at 37 °C. Coating cross sections (A and B) and surface (C and D) were examined. Coatings were formed by dipping into 500 mg/mL (A) and 250 mg/mL (B) polymer solutions. The buffer side (C) and the electrode side (D) of coating surface are shown for coatings prepared from 250 mg/mL polymer solution.

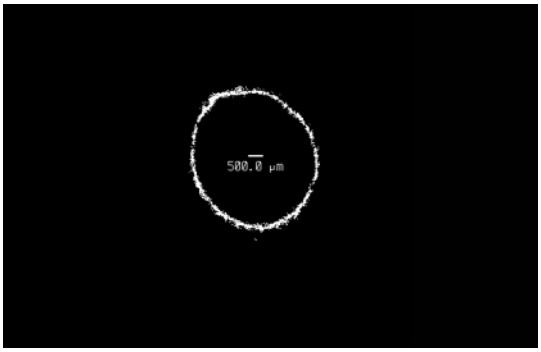

Figure 3. Confocal micrograph of a median cross section of homogeneous PLGA 50/50 (i.v. = 0.61 dL/g) coating. The coating was obtained by electrode dipping into 500 mg/mL polymer solution containing 0.5 mg/mL of fluorescent dye, 10-hydroxycamptothecin. The coating was removed from the electrode after 1 day of hydration in PBST at 37 °C.

Validation of μpH measurement. A drift in the zero-current potential measurement was observed when dried PLGA-coated pH electrodes were first immersed in PBST. After 5−30 min of hydration, a stable cell potential could be recorded. This observation confirms the formation of a thin aqueous film between the surface of a glass electrode and the PLGA coating. The presence of a hydrated layer at the ion-sensitive glass surface is required to obtain a no-drift measurement.[17,18] In addition, after the removal of the polymer coating following PBST incubation, the previously covered electrode surface invariably appeared wet (confirmed by wiping).

After the initial hydration of PLGA 50/50 (i.v. = 0.61 dL/g) coatings the measured cell potential corresponded to an acidic pH of 2.7, according to the electrode calibration prior to the coating. The value of the cell potential was independent of the pH of the medium, in which the electrodes were immersed during the measurement over a pH range from 2 to 10, as seen in Figure 4. This pH independence indicated that the cell potential was responding to the potential from within the PLGA coating.

An appreciable electrical resistance across the polymer film coating was expected due to the low concentra-

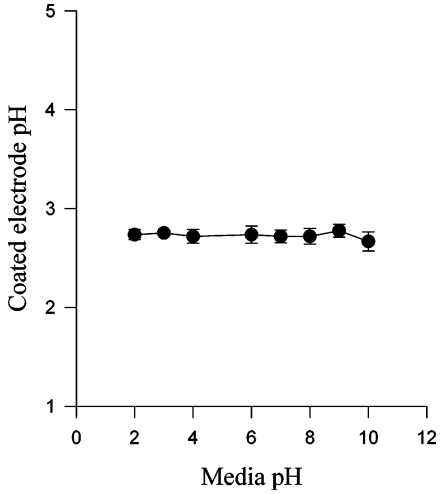

Figure 4. Effect of media pH on the measured μpH of PLGA 50/50 coated electrode. The standard buffer solutions (pH 2−10, Fisher Scientific, PA) were used as an immersion media. The potential was measured after a 1 day incubation of the coatings in PBST (pH 7.4) at 37 °C.

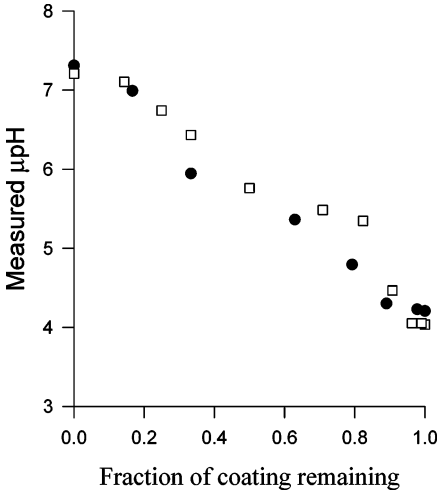

Figure 5. Effect of artificially created imperfections in the PLGA50/50 coating on measured μpH. Coated electrodes were incubated for 1 week in PBST (pH 7.4) at 37 °C. The coating was removed sequentially with larger area or the bulb being exposed each time. The exposed bulb area was roughly measured with a caliper. $N = 2$, (●) and (□).

tion of ions in the PLGA matrix.[21] Therefore, it was possible that after the creation of small holes in the coating the measured pH would represent the pH of the medium rather than the pH of the microclimate due to low solution electrical resistance compared with the polymer. During extended incubation of the electrodes at 37 °C, imperfections in the PLGA coating may develop due to polymer erosion. To ensure the robustness of the μpH measurement, the effect of imperfections in the PLGA coating on the measured pH was investigated. It was observed that with the removal of roughly 10% of the H^+-sensitive bulb area the measured μpH increase was very small, from 4.1 ± 0.1 to only 4.4 ± 0.1. The measured pH increased in proportion with the removed area of coating, from μpH of 4.1 to media pH of 7.4, as shown in Figure 5. Hence, the instant reversal from sensing the μpH to sensing of outside media pH did not occur with the creation of small coating imperfections, indicating that the μpH measurement most likely is not compromised with formation of pores during PLGA erosion.

The pH measured by the glass electrode is determined by the potential along the surface of the H^+-sensitive glass membrane. The different charges on both the polymer-coated and buffer-exposed bulb areas will contribute to the surface potential. Their relative contributions are proportional to the coated and uncoated areas, respectively.[17,18] Hence, a linear increase in measured pH with the increase of coating fraction removed from the ion-sensitive bulb was expected and observed. In addition, the glass pH electrodes used in this measurement possess a high internal resistance (100 MΩ), and therefore, the resistance of the PLGA film should not interfere with the pH measurement.

Contribution of Interfacial and Diffusion Potentials to Measured Cell Potential. To obtain the $E_{el/\mu}$ potential (proportional to μpH), the contribution of extraneous interfacial and diffusion potentials $[E_{\mu/pol} + E_{diff} - E_{pol/med}]$ to the measured cell potential E was required (Figure 1B). This contribution can be measured directly from the difference between the cell potentials measured in the presence and absence of the membrane. To measure the potential across the membrane, the coating was carefully removed from the electrode after 1 day of incubation and examined for the presence of leaks. The PBST solution was placed inside and outside of the PLGA coating. The pH and reference electrodes were placed on the opposite sides of the membrane coating, and the cell potential (E_2) was −15 mV. The cell potential measured without the membrane (E_1) was −12 mV. Hence, the contribution of the extraneous potentials ($E_{\mu/pol} + E_{diff} - E_{pol/med}$) was ($E_2 - E_1$), which was −3 mV or about 0.04 pH units. When the solutions on both sides of the membrane are the same (i.e., PBST), $E_{\mu/pol}$ and $E_{pol/med}$ should compensate for each other.[19] The value of diffusion potential is usually low, and hence, an insignificant contribution of the extraneous potentials was expected and observed.

However, when the cell potential is measured for a coated electrode, the solutions on opposite sides of the membrane have different pH values, i.e., PBST (pH 7.4) on one side and the microclimate solution on the other side. Therefore, the difference between E_2 and E_1 was determined as a function of the solution pH on one side of the membrane (ranged from 2 to 8), while the solution on the other side was PBST (Figure 6). The measured potential difference was very low and in the range of 0.05−0.23 (pH units) depending on buffer pH. Also shown in Figure 6 is a second-order polynomial fit used to obtain an empirical relationship of the pH offset as a function of pH for later use.

Tracking the pH Offset by Equilibration of Coatings in Acid Media. The very small pH offset of measured μpH compared with actual μpH as observed in the last section was examined further by equilibrating coated electrodes in acid media. Coated electrodes were incubated in HCl or glycolic acid solutions of pH 1.8 for a 1 week period. The kinetics of μpH is shown in Figure 7. After the initial hydration the μpH of 2.5 was detected. The μpH decreased slowly during the incubation in pH 1.8 media with the diffusion of media acidic species into the PLGA coating. After 6 days of incubation the μpH reading equilibrated at roughly pH 2 for both HCl and glycolic acid media, whereas media pH did not change significantly. As in Figure 6, the difference between the measured equilibrium μpH of 2 and

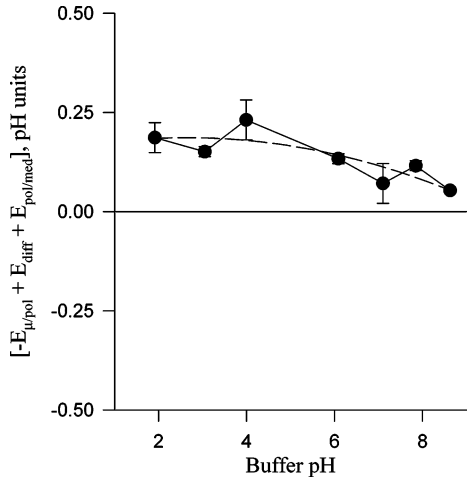

Figure 6. Sum of residual potentials as a function of microclimate pH. Two potentials were measured. The first potential E_1 was measured when reference and pH electrodes were immersed in standard buffer solutions (pH 2−9, Fisher Scientific). The second potential E_2 was measured when pH electrode was immersed in standard buffer solutions pH 2−9 and reference electrode was immersed in PBST (pH 7.4) across the PLGA 50/50 coating. The potential difference in pH units is plotted as a function of buffer solution pH ($E_2 - E_1 = -E_{\mu/\mathrm{pol}} + E_{\mathrm{diff}} + E_{\mathrm{pol/med}}$). The potential difference pH dependence was fit arbitrarily using a second-order polynomial function $y = -0.0037x^2 + 0.0195x + 0.1605$ (- - -). The PLGA membrane used in the measurement was a PLGA50/50 coating hydrated for 3 days, removed from the electrode, and tested for leaks.

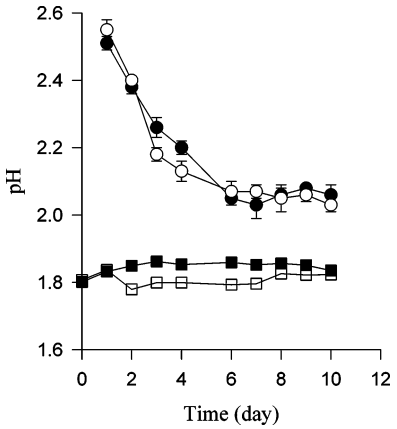

Figure 7. Measured microclimate pH of PLGA 50/50 coated electrode after incubation in HCl (●) and glycolic acid (○) solution of initial pH 1.8 at 37 °C. The measured pH of external media of HCl (■) and glycolic acid solutions (□) remained constant during incubation. (error bars represent $N = 2$, mean ± SD).

the pH of equilibration media of 1.8 suggested a small offset of μpH measurement of roughly 0.2 pH units at this low pH.

Correlation of Cell Potential with Polymer μpH. For further validation of the potentiometric μpH measurement, the coating microclimate was artificially altered and the changes in the measured potential were analyzed. The polymer μpH was fixed for a short period of time by the addition of buffering salts. Concentrated 2 M buffer solutions of pH 3−8 were prepared and lyophilized. The dry ground (<45 μm) buffer salts were then encapsulated in the PLGA50/50 coatings at 15% (w/w) loading. It was hypothesized that water penetration into the polymer would cause the salts to dissolve to a certain extent. Then, the influence of acidic species present in PLGA initially (e.g., acidic impurities) on μpH would be negligible compared to the high buffering capacity of added salts, and the μpH would be fixed therefore at values near to the pH of concentrated solutions of the buffer salts.

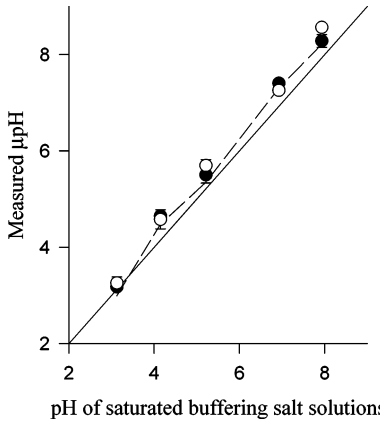

Figure 8. Correlation between measured μpH of coated electrodes containing 15% (w/w) lyophilized buffer salts and pH of saturated solutions of encapsulated buffer salts. The μpH was measured after 1 h (○) and 1 day (●) incubation of coated electrodes in water at 37 °C. The μpH was measured after 1 h and corrected by subtraction of pH-dependent residual potential offset using the polynomial fit from Figure 6 (- - -). The solid line (—) indicates μpH = pH of saturated salt solutions ($N = 3$, mean ± SD).

An excellent correlation between μpH measured after 1 h of coating incubation and the pH of a saturated solutions of the lyophilized salts was observed (Figure 8). The measured μpH was roughly 0.1−0.5 pH units higher than the pH of lyophilized salt solution. The kinetics of μpH during 1 day of incubation of the coating was also examined. The μpH remained almost constant during 24 h incubation, indicating the salt concentration remained at or near saturation during this time. In addition, coatings incubated for 24 h visually appeared to have rough surfaces, indicating the presence of undissolved buffer particles in the polymer. Hence, the correlation between μpH measured after 1 day of coating incubation and the pH of saturated salt solutions was also plotted in Figure 8.

There are two potential reasons for existence of the 0.1−0.5 pH unit difference. First, the difference can be attributed to the contribution of interfacial and diffusion potentials due to the electrode coating (estimated to be 0.05−0.23 pH units). The dashed line in Figure 8 represents the correlation between μpH measured after 1 h of incubation, corrected for small pH offset (using polynomial function described in Figure 6) and the pH of the saturated salt solutions. This correction places the measurement to within experimental error of measured saturated salt pH values. However, the contributions of interfacial and diffusion potential could be more substantial in this experiment due to the difference of ionic strength on the two sides of the PLGA coating. The microclimate solution is the saturated buffer salt solution while the media solution is PBS. The ionic strength difference on either side of the PLGA coating may increase the contribution of interfacial and diffusion potentials to the μpH measurement. Hence, the difference in this measurement of 0.1−0.5 pH units is slightly higher than the normally observed difference of 0.05−0.23 pH units between measured and actual μpH values.

Second, an additional error in μpH measurement is expected due to the high ionic strength of the microclimate solution resulting from buffer salt encapsula-

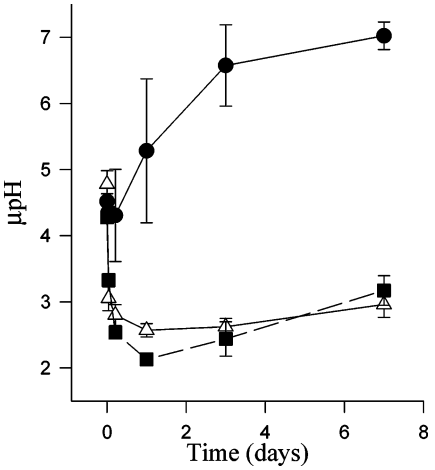

Figure 9. Effect of coating thickness on the μpH of PLGA 50/50 (i.v. = 0.61 dL/g) coatings. The electrodes were coated with 125, 250, and 500 mg/mL polymer−acetone solutions to obtain roughly 7 μm (●), 30 μm (■), and 250 μm-thick coatings (△), respectively. Coated electrodes were incubated in PBST (pH 7.4) at 37 °C. The data were corrected by subtraction of pH-dependent residual potential offset using the polynomial fit from Figure 6 (N = 3, mean ± SD).

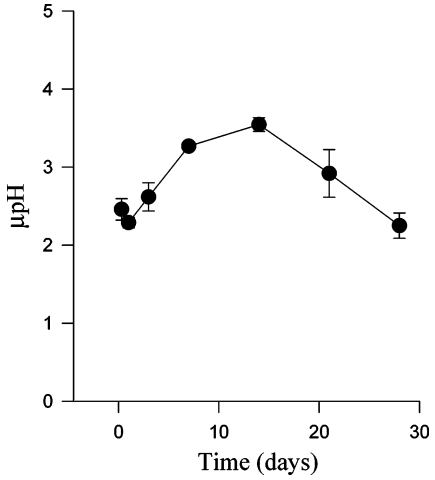

Figure 10. Kinetics of μpH for PLGA 50/50 coating (~30 μm thick) incubated in PBST (pH 7.4). The data were corrected by subtraction of pH-dependent residual potential offset using the polynomial fit from Figure 6 (N = 3, mean ± SD).

tion. High Na^+ concentrations in the microclimate could also interfere with the accuracy of the H^+ activity measurement at the pH electrode.[17,18] The high ionic strength likely disrupted the junction potential during measurement of the saturated salt solutions, causing a small discrepancy in the reference potential between the coated electrode and saturated salt solution experiments.

Effect of Coating Thickness on μpH. Microclimate pH mapping by confocal microscopy with the use of a pH-sensitive fluorescent probe indicated that the μpH in PLGA 50/50 microspheres prepared by solvent evaporation was homogeneous at a value in the neighborhood of 2−3 throughout the particle, with the exception of ~5−10 μm long pH gradient at the microsphere surface.[13] Additional reports indicated that the acidic microclimate did not develop inside PLGA 50:50 microspheres at sizes below 15 μm.[14] Therefore, an acidic μpH was expected for coatings thicker than the length of the pH gradient (~10 μm). To test this hypothesis, the electrodes were coated with 125, 250, and 500 mg/mL PLGA 50:50 solutions to obtain roughly 7, 30, and 250 μm thick coatings (Figure 2A,B). The expected trend was observed (Figure 9). The 30 and 100 μm thick coatings developed an acidic μpH within an hour of hydration (μpH < 3.5), which was sustained during the 1 week incubation period. By contrast, the μpH of 7 μm thick coatings steadily increased form 4.3 to 7.0 during the 1 week of incubation.

Kinetics of μpH in PLGA 50/50 Film Coatings. PLGA 50/50 devices are used to deliver drugs for extended periods of time. Therefore, μpH of ~30 μm thick PLGA 50/50 coatings was examined for 1 month during incubation in PBST at 37 °C. As shown in Figure 10, interesting μpH kinetics was observed. A very low μpH of 2.3 after 24 h hydration increased gradually to reach a maximum of 3.5 after 2 weeks incubation and decreased again to 2.4. The increase in μpH can be attributed to the initial release of acidic impurities and less likely the penetration of the buffering ion through the coating during a high permeability phase accompanying water penetration.[22,23] The continuous hydrolysis of PLGA 50/50 is known to cause accumulation of acidic moieties in the polymer. Therefore, this acid accumulation is responsible for subsequent decrease in μpH at later incubation times. Finally, the kinetics, as described in Figure 10, has been found to be reproducible, and a mathematical model that accurately predicts such behavior will be described in the near future.

Conclusions

A novel, fast, and reliable potentiometric method for measurement of PLGA microclimate pH was developed and validated. The potential of a standard glass electrode coated with a thin PLGA film can be used to monitor the microclimate pH. The measured potential was found to be responsive to the changes in μpH but independent of the pH of the outside medium. The contribution of additional interfacial and diffusion potentials to the measured potential of the electrochemical cell was low and could be accounted for by a simple correction.

In addition, it was confirmed that the thickness of the PLGA device was important for μpH development. Thus, the acidic microclimate develops for coatings prepared by the method reported here for thickness above 7−30 μm. It was also observed the μpH of PLGA coating changes with the time of incubation, indicating the influence of various physicochemical processes on the μpH values such as polymer hydration, release of acidic species from the polymer, polymer hydrolysis, and erosion. Hence, the potentiometric method for μpH monitoring can be routinely used to optimize the stability of pH-labile molecules encapsulated in PLGA delivery devices. The potentiometric method for μpH measurement also could be adapted to assess the microclimate of other biodegradable polymer materials.

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References and Notes

(1) Okada, H.; Toguchi, H. *Crit. Rev. Ther. Drug Carrier Syst.* **1995**, *12*, 1−99.
(2) Shih, C. *J. Controlled Release* **1995**, *34*, 9−15.
(3) Shih, C.; Waldron, N.; Zenter, G. M. *J. Controlled Release* **1996**, *38*, 69−73.

(4) Shenderova, A.; Burke, T. G.; Schwendeman, S. P. *Pharm. Res.* **1997**, *14*, 1406−1414.
(5) Zhu, G.; Mallery, S. R.; Schwendeman, S. P. *Nat. Biotechnol.* **2000**, *18*, 52−57.
(6) Zhu, G.; Schwendeman, S. P. *Pharm. Res.* **2000**, *17*, 350−356.
(7) Marinina, J.; Shenderova, A.; Mallery, S. R.; Schwendeman, S. P. *Pharm. Res.* **2000**, *17*, 677−683.
(8) Herrlinger, M. Ph.D. Thesis, University of Heidelberg, Germany, 1994.
(9) Li, S. M.; Garreau, H.; Vert, M. *J. Mater. Sci.: Mater. Med.* **1990**, *1*, 131−139.
(10) Li, S. M.; Garreau, H.; Vert, M. *J. Mater. Sci.: Mater. Med.* **1990**, *1*, 198−206.
(11) Li, S. M.; Garreau, H.; Vert, M. *J. Mater. Sci.: Mater. Med.* **1990**, *1*, 123−130.
(12) Grizzi, I.; Garreau, H.; Li, S.; Vert, M. *Biomaterials* **1995**, *16*, 305−311.
(13) Shenderova, A.; Burke, T. G.; Schwendeman, S. P. *Pharm. Res.* **1999**, *16*, 241−248.
(14) Fu, K.; Pack, D. W.; Klibanov, A. M.; Langer, R. *Pharm. Res.* **2000**, *17*, 100−106.
(15) Mader, K.; Bittner, B.; Li, Y.; Wohlauf, W.; Kissel, T. *Pharm. Res.* **1998**, *15*, 787−793.
(16) Brunner, A.; Mader, K.; Gopferich, A. *Pharm. Res.* **1999**, *16*, 847−853.
(17) Ingold Messtechnik AG, "Practice and Theory of pH Measurement", Ingold, Ordorf, Switzerland, 1989.
(18) Westcott, C. C. *pH Measurement*; Academic Press: New York, 1978.
(19) Morf, W. E. *The Principles of Ion-selective Electrodes and of Membrane Transport*; Elsevier Scientific Publishing Co.: New York, 1981.
(20) Zhou, T.; Lewis, H.; Foster, R. E.; Schwendeman, S. P. *J. Controlled Release* **1998**, *55*, 281−295.
(21) Shenderova, A.; Madou, M. J.; Yao, S.; Schwendeman, S. P. *Proc. Int. Symp. Controlled Release Bioact. Mater.* **1995**, *26*, 5919.
(22) Wang, J.; Wang, B. M.; Schwendeman, S. P. *J. Controlled Release* **2002**, *82*, 289−307.
(23) Kang, J.; Schwendeman, S. P. *Macromolecules* **2003**, *36*, 1324−1330.

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