Analysis of the Initial Burst of Drug Release Coupled with Polymer Surface Degradation

Joo-Woon Lee,[1,4] Joseph A. Gardella, Jr.,[1,5] Wesley Hicks, Jr.,[2] Robert Hard,[3] and Frank V. Bright[1]

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Purpose. Local pH effect on the release of a model pH-inert hydrophobic drug coupled with polymer degradation is described at the induction phase of biodegradable polymer erosion for better understanding the nature of initial burst of a drug.

Methods. Using a novel approach with time-of-flight secondary ion mass spectrometry, both surface concentration of Ph_3N and degradation kinetics of PLLA are simultaneously and independently determined from a model Ph_3N/PLLA (20:80 wt%) blend matrix ($t \approx 0.4$ μm on 1.0 cm^2). *In vitro* hydrolysis of the model blend matrix is investigated for short-term periods (<24 h) at physiologic pH and temperature and compared to basic pH.

Results. The rate of PLLA degradation is accelerated by a factor of ~3 when using basic pH *in vitro*, but the rate of Ph_3N accumulation at the surface is accelerated by a factor of ~6.

Conclusions. A new quantitative method has been developed to examine the earliest stages of polymer degradation and drug release. It was applied to a model system that could not be examined by traditional *in vitro* methods. For the model system studied the release of a low molecular weight hydrophobic drug at the induction phase of polymer erosion is related to but not singularly dependent on degradation kinetics.

KEY WORDS: biodegradable polymers; degradation kinetics; drug delivery; secondary ion mass spectrometry; simultaneous surface analysis.

INTRODUCTION

The structure and reactivity of drug delivery formulations based on biodegradable poly(α-hydroxy acid)s, such as poly($_L$-lactic acid) (PLLA) are extremely complex and poorly understood despite the knowledge of hydrolysis and solubility (1,2). Surface hydrolysis may occur at different rates than in the interior because of factors controlling water penetration. It is generally accepted that a homogeneous chain cleavage throughout the matrices is responsible for the erosion of small and thin PLLA matrices (3–6). This bulk degradation follows three major steps in the course of polymer erosion (Fig. 1): 1) Incubation, which reflects the interval required for water penetration. 2) Induction, where the detectable weight loss of the polymer matrix does not start until a critical molecular weight (MW) of the matrix is reached. 3) Onset of polymer erosion (release of degradation products), which results in detectable weight loss and change in degradation rate.

However, the drug release kinetics from drug/biodegradable polymer blend matrices are found to be further complicated because of both polymer erosion and drug diffusion through preformed microporous channels within the matrices (2,7). Factors, such as morphology and crystallinity of a polymer, formulation, drug molecular size, and water solubility, may have significant influence not only on the degradation of drug delivery devices but also on the release profile of a drug. Furthermore, it has been reported in previous studies that it is difficult to predictably control drug release over a desired period (8,9). This is caused by an initial burst (rapid release) of drug combined with the process of relatively faster drug diffusion than polymer degradation of the matrices. Although a number of studies (2,7–9) have been directed toward drug release profiles and correlating these results with polymer degradation kinetics, no attempt has been made to simultaneously determine both at the surface during the induction period of bulk erosion of drug/PLLA blend matrices.

In the present model system of drug delivery, triphenylamine (Ph_3N: *f.w.* 245.33, insoluble in 100g H_2O, pK_b not applicable; 10) has been chosen as a low MW hydrophobic model drug to minimize the diffusion and/or solubility effects of a drug depending on intrinsic basicity in the course of PLLA degradation. The *in vivo* delivery of hydrophobic drugs cannot be approximated using simple release studies into aqueous buffers. Thus, a method to measure surface concentration is a better means to model the availability of a hydrophobic drug *in vivo*.

The *in vitro* hydrolytic PLLA degradation and Ph_3N concentration at the surface of Ph_3N/PLLA blend matrices was investigated as a model drug delivery system. The role of external buffer medium at pH 10.0 also was examined both on the rate of Ph_3N accumulation and on the rate of PLLA degradation at the surface/interface. Although the physiologic pH may govern the macroscopic environment, local pH changes may occur at the interface. Local pH is affected by release of reaction products of the polymer and release of other factors, such as a drug, and by cell or tissue response. These might change the local pH from physiologic to a very different value. Thus, a full examination of pH effect on material degradation and drug release is important for the design of new materials and blends that better control the early stage drug release. The emphasis of the present work is to quantitatively describe how the increase in surface concentration of Ph_3N relates to the surface/interface reactions occurring at the induction phase of bulk erosion of the blend matrices, over which negligible loss of PLLA weight but a decrease in MW in bulk is observed (2,3). This study reports results from the simultaneous surface analysis of both Ph_3N concentration and hydrolytic PLLA degradation within short-term (<24 h) exposures of Ph_3N/PLLA (20:80 wt%) blend matrices to *in vitro* buffered conditions at physiologic temperature (37.0 ± 0.2°C) using time-of-flight secondary ion mass spectrometry (ToF-SIMS).

[1] Department of Chemistry, University at Buffalo, The State University of New York, Buffalo, New York 14260-3000.
[2] Department of Head and Neck Surgery, Roswell Park Cancer Institute, Buffalo, New York 14263.
[3] Department of Anatomy/Cell Biology, University at Buffalo, The State University of New York, Buffalo, New York 14214-3094
[4] Present address: Department of Chemistry and Biochemistry, University of Texas at Austin, Austin, Texas 78712.
[5] To whom correspondence should be addressed. (e-mail gardella@acsu.buffalo.edu)

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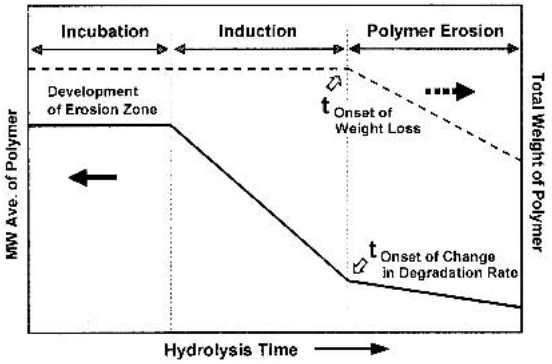

Fig. 1. Descriptive early stage of hydrolytic bulk erosion of biodegradable polymer matrices.

MATERIALS AND METHODS

Materials

PLLA (MW 100,000) was purchased from Polysciences, Inc. (Warrington, PA, USA) and Ph_3N (98%) was purchased from Aldrich (St. Louis, MO, USA). A physiologic electrolyte buffer solution, ISOTON® II (pH 7.4 at 25°C composition: NaCl 7.93 g/L, Na_2HPO_4 1.95 g/L, KCl 0.40 g/L, $Na_2EDTA{\cdot}2H_2O$ 0.38 g/L, NaF 1.30 g/L, NaH_2PO_4 0.19 g/L), was purchased from Coulter Diagnostics (a division of Coulter Electronics, Inc. Hialeah, FL, USA). Sodium carbonate buffer solution (pH 10.0 at 25°C composition Na_2CO_3 0.025 M, $NaHCO_3$ 0.025 M) was prepared with buffer concentrates (DILUT-IT) purchased from J. T. Baker Inc. (Phillipsburg, NJ, USA). High-performance liquid chromatography-grade chloroform ($CHCl_3$) from Aldrich was used for the preparation of ~2% (w/v) Ph_3N/PLLA mixture solutions.

Sample Preparation

Ph_3N/PLLA blend matrices from the ~2% (w/v) mixture $CHCl_3$ solutions were spin-coated onto 10 × 10 mm glass plates at 2000 rpm for 60 s using a Headway Research Inc. Model EC 101 spin-coater. The thickness was measured to ca. 390 nm using a profilometer (Alpha-step® 500, Tencor Instruments). The morphology was clear and flat at 700× magnification of scanning electron microscopy photomicrographs (not shown). To confirm the homogeneous distribution of Ph_3N in the matrices, the back-scattering image was measured using a Hitachi S-4000 scanning electron microscope equipped with a back-scattered electron detector. The image of back-scattered electrons was homogeneous, which supports the interpretation that the drug/polymer blend matrices were homogeneous, that is, no microphase domains of drug were detected in the polymer.

Standard Calibration for Surface Concentration of Ph_3N

Peak intensities representative of the drug and polymer components from the spectrum of untreated Ph_3N/PLLA blend matrices were integrated over two mass ranges: 53 ~59 *Da* for $[C_3H_4O]^{\cdot+}$ = 56 *Da* of PLLA and 241~ 267 *Da* for $[MH]^+$ = 246 *Da* of Ph_3N. In this study, the fragment ion peak, $[C_3H_4O]^{\cdot+}$ = 56.0264 *Da*, from the PLLA repeat unit was used as an internal standard to quantify the surface concentration of Ph_3N (11). The ratio of $[Ph_3NH]^+$ peak intensity divided by the peak intensity of $[C_3H_4O]^{\cdot+}$ was related to the total amount of Ph_3N incorporated in the matrices, and was considered as proportional to the surface concentration of Ph_3N. A standard calibration curve was developed (not shown). Good linearity (R^2 = 0.9984, A = 8.92E-4 in Y – 0.00768 = AX) is obtained from a concentration ratio (X) of 10:90 drug to polymer wt% up to 40:60, followed by the surface saturation of Ph_3N. Hence, the 20:80 wt% ratio of Ph_3N/PLLA blend was chosen for the present study so that significant changes in surface concentration would be detectable.

Hydrolysis Treatment

The amount of Ph_3N in Ph_3N/PLLA (20:80 wt%) blend matrices was calculated to *ca.* 0.73 μmol/sample from dissolving the entire film by immersing a sample specimen (film + substrate) in 24 mL of $CHCl_3$ solution at least for 24 h. The *in vitro* hydrolysis of Ph_3N/PLLA (20:80 wt%) blends was conducted at 37.0 ± 0.2°C in two pH saline buffer solutions (pH 7.4 and pH 10.0) to regulate the local autocatalytic effect (12,13) of carboxylic acid end groups generated during the treatment. Each blend matrix was immersed in a separate vial prefilled with 24 mL of buffered solution and reaction vials were placed in an isothermal water bath (Fisher Circulator Model 73) for the predetermined time. All matrices after the allotted times were vacuum-dried at ambient temperature at least for 24 h before being analyzed. The pH values and the extent of Ph_3N diffusion into the buffers were examined after the hydrolysis treatment using a pH meter (Digital Ionalyzer Model 501 of Orion Research Inc.) and a Milton Roy Spectronic 1201 UV spectrophotometer, respectively. Little change in pH value (± 0.1 pH units) was observed and no detectable UV absorption of Ph_3N was measured from the analysis of buffers, from which it can be postulated that the diffusion effect of a drug is minimized in the present model system of drug delivery and the accumulation rate of Ph_3N at the surface of blend matrices represents the amount of a drug available for release of the hydrophobic drug as a function of hydrolysis time.

ToF-SIMS Analysis

Analysis was performed using a Physical Electronics 7200 ToF-SIMS equipped with an 8 KeV Cs^+ ion gun and a channel plate detector. The static mode was used in all acquisitions with primary ion current of 0.3 pA. The pulse width of primary ion current was 1.0 ns. The extractor was operated in the positive ion mode. The total ion dosage in each spectral acquisition was no more than 1×10^{11} ion/cm^2. An electron neutralizer was operated during all spectral acquisitions in pulsed mode at low electron energy with a target current under 1 μA for charge compensation. A time resolution of 1.25 ns per step was used for good S/N ratio at high m/z range. The pressure of main chamber was kept between 10^{-8} and 10^{-10} torr for each analysis. Each of blend matrix was analyzed a minimum of 3 times, typically five times.

RESULTS AND DISCUSSION

As a reference ToF-SIMS spectrum for the untreated Ph_3N/PLLA (20:80 wt%) blend matrices, the surface of the matrices were doped with Na^+ using 1 N NaCl solution, followed by quick dry in vacuum at least for 24 h. This was used

because Na^+-cationization is more predominant in ToF-SIMS ionization process to guarantee that the detected ions from degraded blends were really a result of reaction products and not because of trace amounts of Na impurities. In the low mass range of the spectrum (not shown) fragment ions from PLLA polymer are normally observed, but very little except a noisy background is observed over the range from 600 to 3500 m/z, similar to that of the undoped polymer (13–15). The lack of signals in this high mass portion of the spectrum is likely a result of the strong entanglement of the long chain polymers, which mediates against desorption of polymer-based ions without multiple bond breaking events (13–15). Upon exposure to the hydrolysis, however, low MW PLLA oligomers are gradually generated with increasing hydrolysis time and thus observed at the surface as a series of the hydrolytic degradation as shown in Fig. 2. These degradation-generated oligomers are shorter and less entangled and readily desorb from the surface as secondary ions in the ToF-SIMS experiment (13–15). The distribution of peaks reflects (13–15) the hydrolysis-generated oligomeric PLLA degradation products and the most intense peaks are labeled with an asterisk (*). The spacing between two consecutive peaks is equal to the repeat unit (M_{mon} = 72.02 *Da*) of PLLA, from which the polymer can be identified. * is assigned to a Na^+-cationized PLLA oligomer ion as an intact ion, $[nM_{mon} + H_2O + Na]^+$, where Na^+ comes from the buffer solution (9,10) and participates in the ionization process as an ionization assisting agent (13–15).

To extract a MW average from the distribution of oligomeric degradation products, the spectra were analyzed using a conventional statistical averaging definition for the number average molecular weight (M_n). This was then converted to degree of polymerization (DP). The average DP at time t is defined as the repeating number of M_n of PLLA degradation products:

$$DP = (M_n - 18)/(72) \quad (1)$$

where 18 is the mass of both end-groups and 72 is the mass of PLLA repeat unit. The kinetics expression for the surface degradation by hydrolytic chain scissions of PLLA linkages was derived as a *pseudo* first-order reaction (13,14,16):

$$\ln[(DP - 1)/DP] = -kt + \ln[DP_S - 1)/DP_S] \quad (2)$$

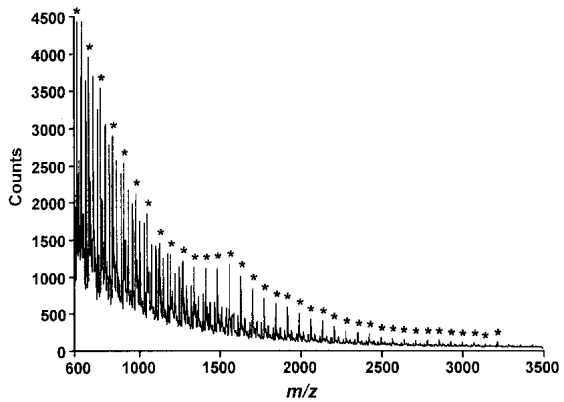

Fig. 2. High mass portion in a positive ToF-SIMS spectrum of Ph_3N/PLLA (20:80 wt%) blend matrices hydrolyzed for 24 h at 37°C in pH 10.0 buffer, where * = $[nM_{mon} + H_2O + Na]^+$ for n = 8~ 44.

where DP and DP_S are the degrees of polymerization of hydrolysis products at time t and being first generated when degradation starts (>0 h), respectively. Therefore, the distribution of PLLA degradation products in high mass range over 600 *m/z* of ToF-SIMS can be converted to the corresponding MWD function in two terms (N_i vs. M_i) of a statistical averaging MW calculation.(13) Using the MWD function, the results of both M_n and resultant semilog terms, ln[(DP – 1)/DP], for kinetics can be calculated. The semilog plots of the kinetic factor are shown in Fig. 3 as a function of hydrolysis time at two pH values. Good linearities are obtained at both pH 7.4 (R^2 = 0.9959) and pH 10.0 (R^2 = 0.9999) buffered conditions. The slope obtained from the linear fitting represents the rate constant of hydrolytic PLLA degradation at the surface of Ph_3N/PLLA (20:80 wt%) blend matrices. The results of linear regression (13,14) of the degradation kinetics represent the characteristic induction phase of the biodegradable poly(α-hydroxy acid) bulk erosion profile. As a result, the rate of PLLA degradation at pH 10.0 (k_{pH10} = −2.0E-4) is 2.67 times faster than that at pH 7.4 ($k_{pH7.4}$ = −7.5E-5) during the 24-h induction period of bulk erosion of Ph_3N/PLLA (20:80 wt%) blend matrices. This result demonstrates the extent of favorable base-catalytic hydrolysis of an ester bond.

To determine the concentration of Ph_3N at the surface, we tested the hypothesis that hydrolysis would change intensities of ions only related to the polymer matrix. A peak at 246 *m/z* in the spectrum of pure PLLA is overlapped with $[Ph_3NH]^+$ = 246 *Da*. The relative intensities, [a peak at 246 *m/z*]/$[C_3H_4O]^{\cdot+}$, of pure PLLA matrices were measured after the hydrolysis under two different pH buffered conditions for 24 h, respectively: 2.62E-3 for pH 7.4 and 2.59E-3 for pH 10.0. They indicate that the relative intensity of the peak at 246 *m/z* ratioed to the intensity of $[C_3H_4O]^{\cdot+}$ in pure PLLA is independent of pH and hydrolysis time. Therefore, it is valid in the present work to use the change in the ratio of intensities, 246 *m/z* divided by 56 *m/z*, as a measure of release profiles

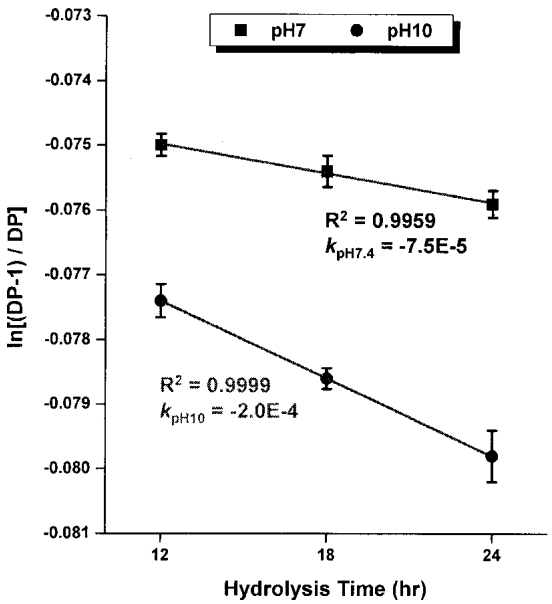

Fig. 3. Semilog plots of (DP-1)/DP for hydrolytic PLLA degradation products generated at the surface of Ph_3N/PLLA (20:80 wt%) blend matrices in two pH-buffered conditions, respectively.

that represent a change in surface concentration of Ph_3N. The surface concentration of Ph_3N from the 20:80 wt% blend matrices has been measured as a function of hydrolysis time at two buffered pHs and compared with the corresponding concentration from a series of Ph_3N/PLLA blend matrices in Fig. 4 for evaluating the cumulative amount of Ph_3N. The rate of increase in relative intensity of $[Ph_3NH]^+/[C_3H_4O]^{\cdot+}$ at basic buffered pH (A_{pH10} = 9.36E-5) is 5.92 times faster than that in physiologic buffered condition ($A_{pH7.4}$ = 1.58E-5).

To better understand the role of the environmental pH effect on the drug release behavior coupled with polymer degradation at the surface/interface of PLLA blend matrices, the surface concentration profiles of Ph_3N have been compared with the corresponding hydrolytic degradation kinetics of PLLA in each pH condition. The extent of change in accumulation rate of Ph_3N ($A_{pH10} = 5.92 \times A_{pH7.4}$) is more than two times greater than the corresponding that in hydrolytic degradation rate of PLLA ($k_{pH10} = 2.67 \times k_{pH7.4}$) at the surface of Ph_3N/PLLA (20:80 wt%) blend matrices.

CONCLUSION

A new quantitative method has been developed to examine the earliest stages of polymer degradation and drug release. It was applied to a model system that could not be examined by the traditional *in vitro* methods. The results from the blend model for drug delivery lead to three important conclusions about this model system for the initial rapid increase in surface concentration of a drug available for release of water-insoluble drugs from a biodegradable polymer matrix. 1) The initial burst of drugs starts during the induction phase. 2) The increased degradation reactivity of polymers in basic pH induces a much faster release of drugs than that in physiologic pH. 3) The change in total weight of the polymer blend matrices is caused by the onset of drug release without a change in polymer weight loss. Extension of these results from this model system is underway.

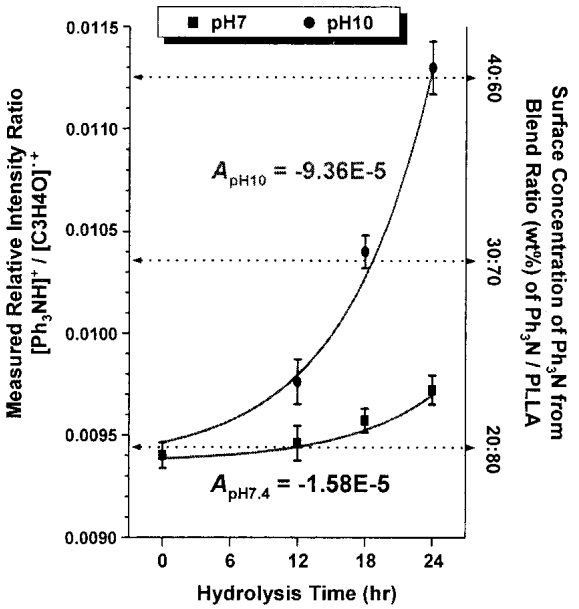

Fig. 4. Left Y axis: concentration of Ph_3N accumulated at the surface of Ph_3N/PLLA (20:80 wt%) blend matrices as a function of hydrolysis time in two pH buffered conditions, respectively. The curves were fit with an empirical exponential expression, ($[Ph_3NH]^+/[C_3H_4O]^{\cdot+}$) = 9.37E-3 + Ae^(t/7.94): A_{pH10} = –9.36E-5 for pH 10.0 and $A_{pH7.4}$ = –1.58E-5 for pH 7.4. Right y axis: surface concentration of Ph_3N obtained from the standard calibration for surface concentration of Ph_3N in experimental section.

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