

Physical Properties of PLGA Films During Polymer Degradation

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ABSTRACT: The rates of change of polymer properties (glass transition temperature, weight fraction sorbed water, and polymer molecular weight) were determined in poly (DL-lactide-co-glycolide) films under accelerated storage conditions. Films were stored at 70°C and 95%, 75%, 60%, 45%, or 28% relative humidity. Weight fraction sorbed water was determined by thermogravimetric analysis, the glass transition temperature ($T_{g_{mix}}$) of the polymer/water mixture by modulated temperature differential scanning calorimetry, and PLGA number-average molecular weight (M_n) by size exclusion chromatography (SEC).

Rates of moisture increase and $T_{g_{mix}}$ decrease were related to the decrease in PLGA M_n through a modification of the Gordon-Taylor equation. Understanding the relative rates of polymer physical and chemical degradation will allow for improved design of PLGA formulations that control rates of drug delivery and preserve drug stability. © 2009 Wiley Periodicals, Inc. *J Appl Polym Sci* 114: 2848–2854, 2009

Key words: differential scanning calorimetry (DSC); gel permeation chromatography (GPC); glass transition (T_g); Polyesters; thermogravimetric analysis (TGA)

INTRODUCTION

Poly(lactide-co-glycolide) (PLGA) is the most commonly used polymer for pharmaceutical controlled release devices.¹ A polyester, PLGA is biodegradable and biocompatible, hydrolyzing to lactic and glycolic acid monomers. Due to the hydrolysis of PLGA, parameters typically considered invariant descriptions of a solid formulation, including the glass transition temperature (T_g), moisture content and polymer molecular weight, change with time.^{2–8} The change in PLGA properties during polymer biodegradation influences the release and degradation rates of incorporated drug molecules.^{9–14} PLGA physical properties themselves have been shown to depend upon multiple factors, including the initial molecular weight,¹⁵ the ratio of lactide to glycolide,^{6,16} the size of the device,¹⁷ exposure to water (as both a reactant and a plasticizer),^{2,18,19} and storage temperature.²⁰ Although many of these properties are expected to covary,^{2,3,21} there are no published theories that successfully describe the relationships among these parameters throughout

the course of polymer degradation. The Gordon-Taylor equation has been used to relate glass transition temperature and water content in PLGA,^{2,3} but only in systems with limited polymer degradation or over a narrow range of moisture content. Elegant diffusion and reaction-diffusion models have been developed to relate drug release and polymer degradation rates in biodegrading polymers^{17,22,23} but, with the exception of effective diffusion coefficients, the time dependence of polymer physical properties has not been included in these models.

The work reported here addresses PLGA physical properties and their relationships during polymer degradation. PLGA films were subjected to accelerated storage conditions and physical properties were monitored over time. A modified version of the Gordon-Taylor equation was developed and used to relate the glass transition temperature ($T_{g_{mix}}$), water content and polymer molecular weight (M_n) throughout the polymer degradation process. The equation provides good agreement with the measured, time-dependent polymer properties.

MATERIALS AND METHODS

Materials

50/50 Poly (DL-lactide-co-glycolide) (PLGA) (inherent viscosity 0.58 dL/g, 75.4 kDa), polystyrene narrow molecular weight standards (151.7, 66.35, 38.1, 19.88, 10.05, 4.92, 2.35, and 1.26 kDa), and 50/50 PLGA

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broad molecular weight standards (>144.1, 144.1, 75.4 (lot used for film preparation), 38.4, and 10.8 kDa) were purchased from Absorbable Polymers International (Pelham, AL). Tetrahydrofuran (THF) and acetone were purchased from Fisher Scientific (Fair Lawn, NJ).

Film preparation and storage

Films were prepared by dissolving 100 mg/mL polymer in acetone and sonicating for 30 min to enhance dissolution. A 0.5 mL aliquot of the solution was then pipetted onto 22 mm × 22 mm glass coverslips and allowed to dry at ambient temperature and relative humidity for ~ 18 h. Accelerated degradation studies for all types of analysis were conducted at 70°C and 95%, 75%, 60%, 45%, or 28% relative humidity (%RH) achieved using K₂SO₄, NaCl, KI, NaBr, and MgCl₂ saturated salt solutions, respectively. These conditions reflect film storage at elevated temperature and humidity rather than *in vitro* release studies into buffered solution. Triplicate samples were removed from storage at appropriate times based upon the expected degradation rate of the polymer. Solid samples for thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) analysis were removed from the glass coverslips with a razor blade. Size exclusion chromatography (SEC) samples were dissolved in 5 mL tetrahydrofuran, producing samples with ~ 10 mg/mL PLGA.

Sample analysis

Thermogravimetric analysis

Determination of total sorbed water was accomplished by TGA using a Q 50 TGA (TA Instruments, New Castle, DE). Thermal scans were conducted from ambient (~ 25°C) to 150°C at a scan rate of 1°C/min in an open platinum pan under dry nitrogen purge. Temperature and mass calibrations were conducted using Curie point standards alumel and nickel. Polymer sample mass ranged from 5 to 20 mg. The weight fraction of sorbed water was calculated using eq. (1):

$$w_1 = (\text{mg water}/\text{mg wet solid}) \quad (1)$$

where the weight of water is calculated using the Universal Analysis TA Instruments software to determine mass loss versus temperature. Separate dehydration steps were confirmed by derivative TGA (dTGA),²⁴ allowing for the estimation of total water ($T < 100^\circ\text{C}$) and lactic acid ($T > 100^\circ\text{C}$). Control solutions of various lactic acid concentrations were prepared and analyzed by the same TGA method. Total water content was accurately measured using this method; however, lactic acid was

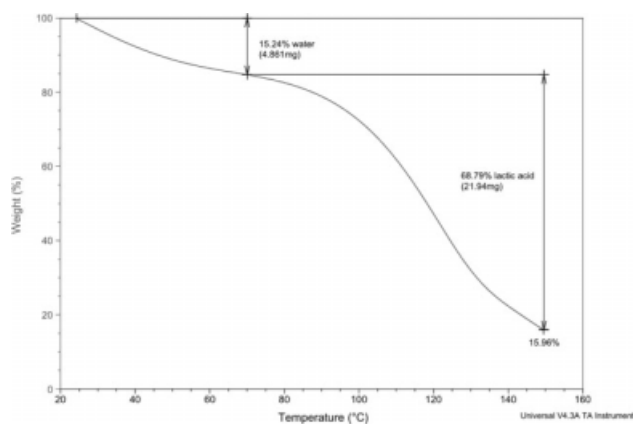


Figure 1 TGA spectrum of an 85% lactic acid solution showing the complete removal of water below 80°C.

not completely removed from the standards in this temperature range (Fig. 1), perhaps due to equilibration with the less volatile dilactide form. The lactic acid removed during TGA analysis at $T > 100^\circ\text{C}$ thus is referred to as “volatile lactic acid monomer.” An example of an 85% lactic acid solution is shown in Figure 1.

Conventional and modulated temperature differential scanning calorimetry (DSC and MTDSC)

Conventional and modulated temperature DSC experiments were conducted using a Q 100 DSC with a refrigerated cooling system (TA Instruments, New Castle, DE). Temperature calibrations were performed using *n*-decane, indium, and tin standards. Indium was also used for enthalpy calibration. Sapphires of known heat capacity (C_p) were used for Tzero calibration. DSC and modulated temperature differential scanning calorimetry (MTDSC) analysis were performed in either standard or hermetically sealed aluminum pans, depending upon the morphology of the sample. Sample mass ranged from 3–12 mg. Conventional DSC experiments were conducted using temperature scan rates of 5°C/min. MTDSC experiments were performed after the first day of storage at scan rates of 1°C/min with modulation amplitudes ranging from ± 0.2 to $\pm 1^\circ\text{C}$ and modulation periods ranging from 30 to 60 sec.

The glass transition temperature of the wet polymer mixture ($T_{g_{\text{mix}}}$) was determined for all storage conditions as an estimate of the mobility that would be experienced by an incorporated drug molecule.²⁵ The water sorbed by the PLGA films during degradation acts as a plasticizer, increasing the mobility of the polymer matrix as a function of M_n .^{26,27} $T_{g_{\text{mix}}}$ was calculated from the midpoint of the transition using the Universal Analysis TA Instruments

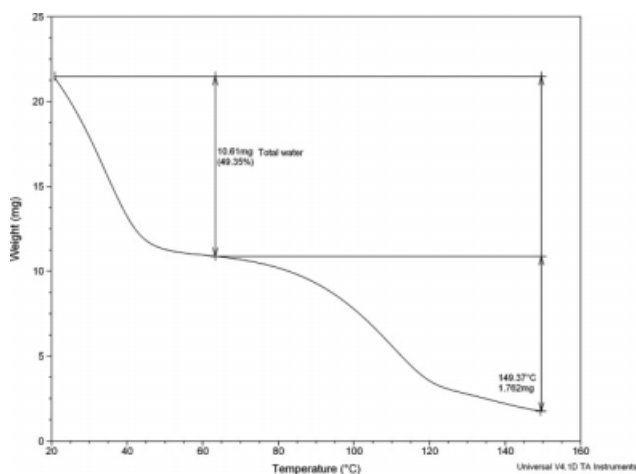


Figure 2 An example TGA spectrum showing the analysis of water in PLGA films (data from PLGA storage at 75% RH and 70°C for 120 h).

software. Studies at 95% and 75% RH were analyzed in triplicate; additional studies used single samples due to the instrument time required per sample and minimal sample variability (± 0.2 to 8°C).

To determine the T_g of PLGA in the absence of water, DSC analysis was performed for samples stored at 95% RH.²⁵ These samples were subjected to a heat-cool-heat cycle. The first heating cycle used conventional heating, with a scan rate of 10°C/min to 150°C to facilitate water and volatile monomer removal. Samples were then cooled to a temperature between -70 and 0°C depending upon the degree of PLGA degradation. The second heating cycle used MTDSC, with parameters similar to those above. T_g of the dry polymer was measured during the second heating cycle, calculated from the midpoint of the transition.

Size exclusion chromatography (SEC)

SEC analysis was performed using a Shimadzu HPLC system (Shimadzu, Kyoto, Japan) consisting of a Shimadzu SCL-6B system controller, Shimadzu LC-6A pump, Shimadzu SIL-10A autoinjector and cooler, and a Shimadzu SPD-6A UV detector with UV detection at 215 nm. Separation was performed using tetrahydrofuran with a flow rate of 0.8 mL/min and a Phenomenex Phenogel 5 μ 10³ Å column (Phenomenex, Torrance, CA) with 1–75 kDa separation capacity. Polystyrene narrow molecular weight standards ranging from 1.26 to 151.7 kDa were used for calibration. Peak position analysis was performed and a plot of log M_p (known peak molecular weight) versus retention time constructed.²⁸ Correlation of polystyrene and PLGA MW separation was confirmed using broad PLGA molecular weight standards ranging from 144.1 to 10.8 kDa (data not shown).

Data analysis

Nonlinear regression was performed using Origin 7.0 with instrumental error weighting (Microcal Software, MA). ANOVA and F-test comparisons of linear regressions were performed to evaluate the statistical similarity of slopes.²⁹

RESULTS

TGA

TGA of the PLGA films produced sigmoidal curves with two distinct weight loss steps after 24 h of storage at 70°C and at all relative humidity conditions (Fig. 2). Following the first weight loss step, sample mass typically plateaued at ~60°C. Based on comparison with the lactic acid solution control referenced in section “Thermogravimetric analysis” (Fig. 1), weight loss in this temperature range is assigned to the removal of the total water of hydration. As this transition is complete below the vaporization temperature of free water, this is most likely an indication of polymer bound water. Removal of bound water from polymer matrices at temperatures <100°C has been demonstrated previously for PLGA² and PVA.³⁰ The second weight loss step began at ~80°C and sample mass reached a plateau at ~150°C. Weight loss in this temperature range is assigned to removal of volatile lactic acid monomer. Plots of total water sorbed versus storage time were sigmoidal for most RH conditions except 28 and 75% RH (Fig. 3), consistent with a lag time for water sorption by the PLGA films. The uptake of moisture at 28% RH did not reach a plateau within the time period of the study. The maximum weight fraction of total water in the samples ranged from 0.62 to

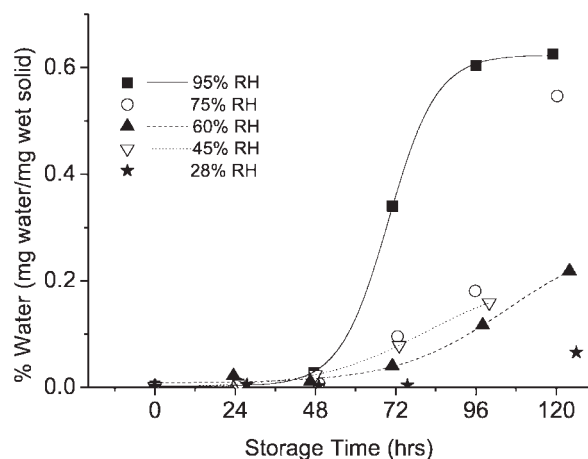


Figure 3 Weight fraction water sorbed by PLGA versus storage time at 70°C and 95% (■), 75% (○), 60% (▲), 45% (▽), or 28% (★) RH. Data shown as weight fraction (mg water/mg wet solid).

0.16 for the high (95 and 75%) and intermediate (60 and 45%) RH conditions (Fig. 3).

DSC

MTDSC analysis of hydrated polymer samples in closed pans gives a measure of the glass transition temperature of the water-polymer mixture ($T_{g_{mix}}$). $T_{g_{mix}}$ decreased upon storage at 70°C for all RH conditions [Fig. 4(a)]. The decline in $T_{g_{mix}}$ with storage time is commonly associated with PLGA hydrolysis, due to both increased water sorption² and decreased PLGA M_n .¹⁵ Attempts to correlate changes in $T_{g_{mix}}$ to %RH of storage were complicated by the low number of data points at the 95 and 75% RH conditions. Slopes could not be accurately compared using ANOVA due to the minimal degrees of freedom for these data sets compared with those at the intermediate and low RH conditions. Visual comparison suggests that the high and intermediate RH conditions produce a similar decline in $T_{g_{mix}}$ with

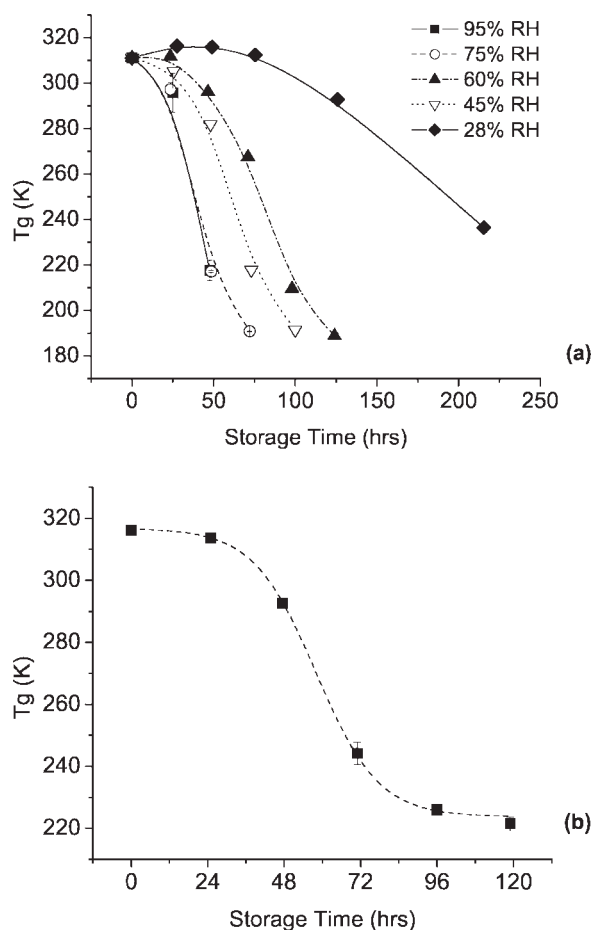


Figure 4 Glass transition temperature ($T_{g_{mix}}$) of wet PLGA films versus storage time at 70°C and various relative humidity conditions (a) and glass transition temperature (T_g) of PLGA versus storage time at 70°C and 95% RH (b). The T_g of dry polymer was determined using open-pan DSC analysis.

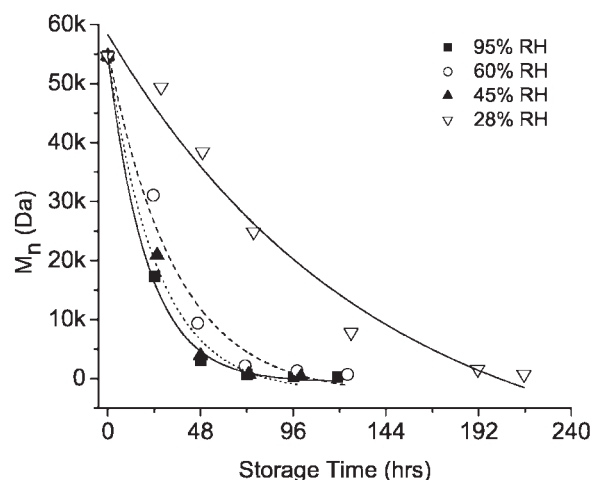


Figure 5 Plot of the number-average molecular weight of PLGA versus storage time at 70°C and various relative humidity conditions.

time, whereas storage at 28% RH results in a slower rate of $T_{g_{mix}}$ change. F-test comparisons of the 60% and 28% RH conditions, which have the same degrees of freedom, show that these rates of change of $T_{g_{mix}}$ are statistically different at the 0.05 confidence level.

Open-pan analysis of PLGA also showed a decrease in polymer T_g with storage time at 70°C and 95% RH [Fig. 4(b)]. This relationship is sigmoidal, which may reflect an initial decrease due to reversible plasticization of water^{2,3} followed by more rapid decrease due to polymer hydrolysis.

SEC

SEC separations of PLGA films dissolved in tetrahydrofuran were multimodal before storage. The initial number-average molecular weight (M_n) was 59 kDa, with minor peaks at 750–700, 400–350, 150, and 80–60 Da. Regardless of the %RH of storage, the M_n of PLGA decreased with storage time (Fig. 5). A minimal decrease in M_n was observed upon film formation; the M_n of films before storage is 55 kDa. During storage, M_n decreased exponentially with time, experiencing little to no lag time upon exposure to any of the relative humidity conditions at 70°C. Data were fit using a first order exponential decay. ANOVA and F-test comparisons indicate that the rates of change of M_n are not statistically different; however, visual comparison suggests that storage at 28% RH results in a slower rate of PLGA M_n change.

DISCUSSION

Characterizing PLGA matrices is difficult because the physical and chemical properties are in constant

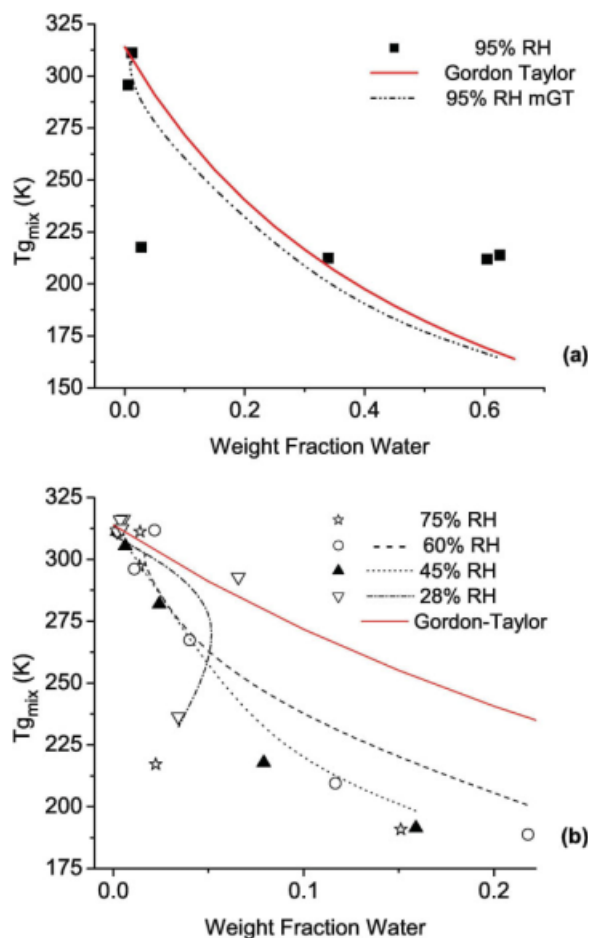


Figure 6 $T_{g,mix}$ versus total water content of PLGA films stored at 70°C and 95% RH (a) and other relative humidity conditions (b). Solid red line represents the Gordon-Taylor approximation of this relationship. Additional lines represent the modified equation that includes PLGA Tg as a function of PLGA M_n [see eqs. (4) and (5)] for each %RH condition (Fig. 4). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

flux as the polymer degrades. The results reported here show the change in moisture content, Tg, and PLGA molecular weight as a function of time for PLGA films stored at different conditions. These physical properties are expected to covary to a significant degree. The change in each PLGA physical property with storage time should be linked to the change in polymer molecular weight, since this is the most basic measurement of the hydrolysis of the polymer. The Gordon-Taylor equation relates the Tg of a noninteracting binary system (e.g., polymer, water) to the Tg's of the individual components and their weight fractions. This equation is often used to describe the plasticizing effect of water in a polymer matrix^{2,3,31,32} and is given by the following:

$$T_{g,mix} = [(w_1 T_{g1}) + (Kw_2 T_{g2})] / [w_1 + Kw_2] \quad (2)$$

where $T_{g,mix}$ represents the glass transition temperature of the wet polymer matrix; w_1 and w_2 are the

weight fractions of the two components, water and polymer, respectively; and T_{g1} and T_{g2} are their glass transition temperatures. K is a constant whose value can be calculated based upon free volume theory, using the Simha-Boyer rule³:

$$K = \rho_1 T_{g1} / \rho_2 T_{g2} \quad (3)$$

where ρ_1 and ρ_2 represent the densities of the two components.^{2,3,33} For this analysis, the Tg of water was estimated to be 135 K,³ the $\rho_{water} = 1 \text{ g/cm}^3$,³⁴ the $\rho_{PLGA} = 1.2 \text{ g/cm}^3$ (Absorbable Polymers International, Pelham, AL), $T_{g2} = 314 \text{ K}$ (DSC measurement of intact polymer, data not shown), and w_1 and w_2 were taken from TGA data (Fig. 3) and allowed to vary with time.

A plot of this Gordon-Taylor approximation as well as the measured $T_{g,mix}$ versus total weight fraction water content is shown in Figure 6(a,b), and provides a poor fit of the matrix properties for the degrading polymer. Reports by other groups have shown that the Gordon-Taylor equation provides a reasonably good fit of the data at low water content.^{2,3} Here, however, the equation overestimates $T_{g,mix}$ for most conditions and underestimates $T_{g,mix}$ for all systems with high water content ($w_1 > 0.60$). To improve the fit in these two regions, modifications were made to the Gordon-Taylor equation. A limitation of applying the Gordon-Taylor equation to degrading PLGA systems is that the Tg of the polymer changes with time as the polymer undergoes hydrolysis. In PLGA, Tg decreases with a decrease in polymer molecular weight,¹⁵ however, the Gordon-Taylor equation assumes that this change is negligible for molecular weight $>20 \text{ kDa}$.³³ The M_n of PLGA fell below this range for all storage conditions in this study. To address this limitation, the SEC data were used to estimate the changing value of the PLGA Tg.

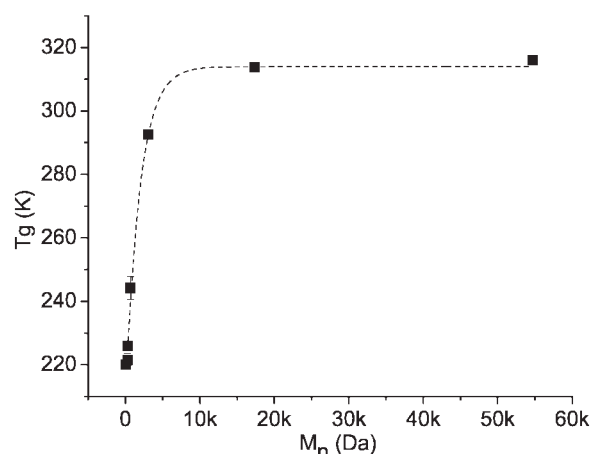


Figure 7 Calibration of PLGA Tg versus PLGA M_n .

TABLE I
Values of Constants Used in eqs. (4)–(5)

Constant	Value ^a	Units
A_1	310 (\pm 0.43)	K
A_2	102 (\pm 2.2)	K
k_1	5.1 (\pm 0.40) $\times 10^{-4}$	Da ⁻¹

^a Value in parentheses represents standard error as determined by nonlinear regression.

A relationship between PLGA M_n and Tg was constructed based upon DSC open-pan analysis of films stored at 95% RH and 70°C, where samples were heated to remove water before Tg determination (Fig. 7). The data were fit with the first order exponential equation:

$$Tg = A_1 - A_2 e^{-k_1 M_n} \quad (4)$$

where A_1 , A_2 , and k_1 are constants, and M_n is determined by SEC (Fig. 5). The values for the constants A_1 , A_2 , and k_1 are shown in Table I. The constants A_1 and A_2 bracket the range of Tg values for the polymer, whereas k_1 gives the dependence on M_n . Due to differences in the polymerization process and polymer end group modification between PLGA manufacturers,^{15,16} these constants may vary depending on the source of PLGA used.

By combining the Gordon-Taylor equation [eq. (2)] and the Simha-Boyer rule [eq. (3)] with eq. (4), changing polymer Tg's can be incorporated into the Gordon-Taylor equation, giving:

$$Tg_{mix} = \frac{w_1 Tg_1 \rho_2 - \rho_1 Tg_1 w_2}{w_1 \rho_2 (A_1 - A_2 e^{-k_1 M_n}) - \rho_1 Tg_1 w_2} \quad (5)$$

where w_1 and w_2 are the weight fractions of water and of PLGA, respectively, at time = t (determined by TGA), Tg_1 is the glass transition temperature of water (135 K), ρ_1 is the density of water (1 g/cm³), ρ_2 is the density of PLGA (1.2 g/cm³), M_n is the number-average molecular weight of PLGA at time = t (determined by SEC), and A_1 , A_2 , and k_1 are constants (Table I). Figure 6 shows the modified equation at various storage conditions. Lines represent the calculated value for Tg_{mix} using the modified Gordon-Taylor approximation [eq. (5)]. The 75% RH condition was not calculated due to inadequate M_n data. The modified equation provides an improved fit, particularly for data obtained at the intermediate and low RH storage conditions up to 0.25 weight fraction total water. However, the fit at 95% RH shows little improvement over the original Gordon-Taylor equation.

This modified equation still overestimates Tg_{mix} at the 95 %RH condition [Fig. 6(a)] and low water content ($w_1 < 0.25$) [Fig. 6(a)]. Such negative deviations from the Gordon-Taylor equation have been

attributed to decreased entropy in the two component mixture, consistent with induced ordering of the hydrophobic polyester chains and an increase in free volume.³⁵ The modified Gordon-Taylor equation also underestimates the Tg_{mix} at high water content ($w_1 > 0.6$) [Fig. 6(a)] for the 95% RH condition, though to a lesser extent than when original Gordon-Taylor equation is used. The Gordon-Taylor equation requires that the Tg_{mix} should approach the Tg of water (135 K) as the weight fraction of water approaches 1. However, for the 95% RH and the intermediate (60 and 45%) RH storage conditions, the experimental Tg_{mix} values appear to plateau at 220 and 190 K, respectively. Under these conditions, enthalpically favored heterointeractions (PLGA/water) may dominate homointeractions (PLGA-PLGA or water-water) to decrease the free volume in the matrix, resulting in an increased Tg_{mix} .^{35,36} This type of interaction is more likely at later storage time and higher water content, when carboxylic acid and alcohol PLGA degradation products would experience greater hydrogen-bonding with water than the initial hydrophobic polymer.

The poor fit of the 95% RH storage condition using eq. (5) suggests that changes in polymer Tg due to molecular weight changes are not entirely responsible for the lack of agreement of the PLGA data with the Gordon-Taylor equation. Other groups have suggested that a heterogeneous binary system of PLGA-rich and water-rich phases forms in degrading PLGA matrices.² Because the Gordon-Taylor equation assumes ideal mixing, it would not be expected to apply to binary systems, but may provide a better estimate of Tg_{mix} if the concentration of bound water is used rather than the total water content, a suggestion recently made by Blasi et al.² The rapid uptake of water in the 95%RH storage condition may not allow for proper mixing and interaction between the polymer and water phases. Overall, for slower rates of PLGA degradation, the modified Gordon-Taylor equation [eq. (5)] adequately predicts the Tg_{mix} if the weight fraction water and the molecular weight of the degrading polymer matrix are known. Agreement between the data and this equation implies that the PLGA-water system can be treated as a noninteracting mixture throughout the time course of PLGA biodegradation.

These results extend previous findings by other groups. In one instance, the Gordon-Taylor relationship was found to be a good model of the plasticizing effects of water at low concentrations (<3% w/w),² but the relationship was deemed inapplicable at higher total water content due to phase separation. In another report, the Gordon-Taylor equation was a good predictor of Tg in PLGA/water systems at low water concentrations (0–5% w/w)³ and was used to model residual water following formulation. By

including the dependence of Tg on M_n , we have demonstrated that the Gordon-Taylor equation can adequately model degrading PLGA systems with a wider range of moisture content. Additional studies are required to determine the applicability of this approach to PLGA systems at different conditions (e.g., temperature, buffer immersion) and to other biodegradable polymers with varying Tg.

CONCLUSIONS

The moisture, mobility, and molecular weight of PLGA films decreased on exposure to various relative humidity at 70°C. The relative rates of change for these parameters were determined as a function of relative humidity and storage time. Differences in the rates of change for Tg_{mix} and weight fraction water at the 28% RH condition in comparison to the other RH conditions suggests that a threshold of relative humidity exists, leading to a significant decrease in initial PLGA degradation between 45 and 28% RH. A modification of the Gordon-Taylor equation, allowing for changes in polymer Tg as a function of PLGA M_n provided a good description of the relationship among these three parameters.

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