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Plasticizing effect of water on poly(lactide-co-glycolide)

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Abstract

The purpose of this research was to evaluate the effect and nature of hydration on the glass transition temperature (T_g) of poly(D,L-lactide-co-glycolide) and investigate the physical state of water within the polymer during hygrothermal aging. The polymer was incubated in water at 23, 30, 37 and 55 °C, while the vapor sorption studies were carried out at 37 °C using saturated salt solutions. The water content and the thermal behavior of PLGA-water system were assessed by Karl Fischer titration and modulated differential scanning calorimetry, respectively, the hygrothermal aging was monitored by gel permeation chromatography. Water depressed reversibly the T_g by about 15 °C regardless of the incubation conditions. The T_g then remained constant at ~30 °C for five days, except when degradation occurred. A broad ice melting peak was detected around 0 °C. In the sorption studies, a linear correlation (r^2 0.9837) between the T_g and the moisture content was observed in the range of 0.3–2.6% w/w, but there was no discernible endothermic event associated with the melting of ice. Data were found to fit reasonably well to the Gordon–Taylor/Kelley–Bueche equation. There were no differences between bulk and vapor water aging. It is proposed that the water responsible for plasticizing the polymer was non-freezable (bound) water and the small fraction of such water which was absorbed at high relative humidity caused polymer degradation in the same manner as bulk water.

1. Introduction

Amorphous polymers are characterized by glass transition temperature (T_g), which is the transition point between a highly viscous brittle structure called glass and a less viscous, more mobile, rubbery state. The rubbery state (above the T_g), represents a liquid-like structure with high molecular mobility and is thus more prone to physical and chemical changes than the glassy state. The T_g value of a glassy polymer can be modified by blending with a small amount of a low molecular weight substance. Plasticization occurs

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when a small molecule, called a plasticizer, blended with a glassy polymer results in a decrease of the T_g of the polymer and its elastic modulus [1]. Such plasticization normally increases polymer flexibility or mobility. The reverse phenomenon, i.e. an increase in T_g, such as occurs with the addition of an agent, like a drug substance, has been defined as antiplasticization [2]. Therefore, the performance of a polymer can be modified in the presence of a plasticizer, or another substance, depending on the nature of the association between the various phases.

It has been shown that water molecules absorbed into a polymeric matrix act as an effective plasticizer, causing profound changes in the physicochemical properties of amorphous solids [3,4]. Water, with a reported T_g of about -135 °C [3,4] can act as a very potent plasticizer. In fact, a blend of compatible amorphous materials can exhibit a single T_g that is intermediate to the T_gs of the pure components. Different theoretical equations are also available to predict the T_g of a two amorphous component mixture [3,4]. Moreover, water may form stable bridges through hydrogen bonding resulting in an antiplasticizing effect [5].

Since the thermodynamic properties of the water sorbed into a polymer undergo change with respect to those of bulk water, the nature of the interaction between water and hydrophilic polymers has been investigated using calorimetric and spectroscopic techniques [6,7]. Based on these studies, water has been classified into three "species": (i) non-freezable bound water, (ii) freezable bound water, (iii) freezable free (bulk) water. Non-freezable bound water is defined as the water closely associated with the polymer matrix and does not give rise to observable phase transitions by calorimetric analysis. The freezable bound water is the fraction less closely associated to the matrix that does exhibit a melting/crystallization event remarkably different from that of bulk water. Freezable free (bulk) water shows the melting/crystallization temperature and the relative enthalpy not significantly different from that of bulk water [5,6].

The behavior of water sorbed into polymers can be ascribed to different reasons, such as the effect of capillary condensation, the confinement of water by polymer structure, the formation of clusters, or the strong interactions between the highly dipolar water molecules and the polymer polar groups [8–10]. However, there has been rather limited attention given to the effect of water on hydrophobic polymers, especially polyorthoesters [11–14].

The copolymers of lactic and glycolic acids (PLGA) are among the few polymers approved by the Food and Drug Administration for human clinical applications such as surgical sutures, implantable devices and drug delivery systems [15–17] because of their excellent biocompatibility, biodegradability and mechanical strength. Release of actives from drug delivery systems is controlled by a complex and not completely known mechanism including the diffusion through the polymer matrix and/or the fluid-filled pores and polymer degradation. In this regard, hydration represents the initial and, consequently, the fundamental step in the drug release process because the presence of water, in terms of biologic fluid or in vitro release media, is necessary for drug dissolution and diffusion through the drug delivery system. Additionally, it is essential for initiating and sustaining the hydrolytic degradation/erosion process of the polymeric matrix.

The aim of this work was to provide an understanding of the effect of water, bulk or vapor, on the T_g of poly(lactide-co-glycolide) in the early stage of hydration, prior to the onset of degradation. The physical state of water within the hydrated polymer and the effect of hygrothermal aging were investigated.

2. Materials and methods

2.1. Materials

Poly(D,L-lactide-co-glycolide) (PLGA) RG503H (M_w 30,000 Da) was supplied by Boehringer Ingelheim (Ingelheim, Germany). Polyvinyl alcohol (PVA) (M_w 30,000–70,000 Da) was supplied by Sigma (Aldrich Chemical Company, USA). $MgCl_2$, $Mg(NO_3)_2$, NaCl and KNO_3 were provided by Fisher Scientific (USA). All the solvents were of analytical grade.

2.2. True density determination

The RG503H true density was determined using a helium pycnometer Ultrapycnometer 1000 (Quanta-

chrome Instruments, USA). A 10 cm^3 cell was used for the analysis and the density (g/cm^3) was expressed as the mean of ten successive measures. This value was found to be 1.5804 g/cm^3.

2.3. Hydration studies

2.3.1. Sample conditioning

The effect of water content on RG503H was evaluated in two sets of samples prepared as follows:

About 20 mg of PLGA were suspended in 10 mL of deionized water containing 0.5% PVA and incubated at room temperature (~23 °C) or in a ventilated oven at 30 ± 2, 37 ± 2 and 55 ± 2 °C. At predetermined intervals, the samples were filtered, washed and then hermetically sealed in aluminum pans.

Water vapor sorption studies were performed at 37 ± 2 °C using hermetic chambers containing saturated salt solutions with different relative humidity (RH) (MgCl$_2$: RH=32%, Mg(NO3)$_2$: RH=50%, NaCl: RH=75%, KNO$_3$: RH=90%). Samples of about 5–10 mg were quantitatively placed in open aluminum pans and incubated in the chambers until equilibration. Simultaneously, a quantitative sample of about 1 g was placed in each chamber and the weight monitored. The equilibrium was assumed to be achieved when the 1 g sample weight reached a constant value ($\Delta m \pm 0.0005$ g). Equilibrium for the small samples was assumed to be at the same time of the larger sample. At this time, the pans were hermetically sealed and analyzed.

2.3.2. Water content determination

The moisture in the PLGA samples was measured by a Brinkmann 694 KF Coulombmeter Metrohm (CH). About 50 mg were quantitatively transferred to glass vials and conditioned in hermetic chambers containing saturated salt solutions with different RHs (32%, 50%, 75%, and 90%). The analysis was performed in triplicate. After equilibration, the vials were removed and immediately hermetically sealed with rubber closures. In the case of 90% RH, three samples of 50 mg for each time point (7, 14, 21, 28 and 35 days) were incubated at the aforementioned relative humidity. Another set of 50 mg samples was vacuum dried overnight and analyzed as a control (0.3% RH; 0 time point in Tables 2 and 3). One milliliter of anhydrous methanol was

added to the vials, the vials sealed and then intermittently shaken for 1 h. Samples of 100 μL of methanol were withdrawn from the vials using a syringe and injected into the titration vessel. The water content was measured in replicates of three. The water content (%) was calculated using the following equation:

$$\text{water content}(\%) = \frac{W_s - W_b}{M_0} * 100 \qquad (1)$$

where W_s is the mass of water in the sample, W_b is the mass of water in methanol and M_0 is the initial mass of the polymer.

2.3.3. Thermal analysis

In order to characterize the thermal behavior of the polymer/water system, modulated differential scanning calorimetry (MTDSC) was performed by using a 2920 Modulated$^{\textregistered}$DSC (TA Instruments, USA) equipped with a refrigerated cooling system (RCS). Samples of about 5–10 mg were quantitatively transferred to aluminum pans, hermetically sealed, weighed and subjected to two cooling and heating cycles from -60 to 60 °C at cooling and heating rates of 5 °C/min, a period of 40 s and an amplitude of ± 0.5 °C. Following the procedure, the samples were reweighed to ensure that no water was lost during the two heating cycles. The DSC cell and RCS were purged with dry nitrogen at 60 and 120 mL/min, respectively. The system was calibrated using an indium standard.

Data were treated with Thermal Solutions software (TA Instruments, UK) and the results expressed as the mean of three determinations.

2.4. Hygrothermal aging

2.4.1. Preparation of the samples for hygrothermal aging studies

A set of samples was incubated in open vials over saturated salt solution, 90% RH, at 37 ± 2 °C. At predetermined intervals, samples were removed, assayed for water content and vacuum dried until constant weight. Another set of samples was incubated in deionized water containing 0.5% PVA and stored at 37 ± 2 °C in an electronically controlled oven. At predetermined intervals, samples were removed, filtered, rinsed with deionized water and

vacuum dried at room temperature until constant weight. Finally, a set of samples was exposed to dry heat in an oven at 37 ± 2 °C.

2.4.2. Molecular weight determination

The molecular weight of polymer during hygro-thermal aging was measured by gel permeation chromatography (GPC). The GPC system consisted of two Ultrastyragel columns connected in series (7.8×300 mm each, one with 10^4 Å pores and one with 10^3 Å pores), a delivery device (Shimadzu LC-6A, Japan), UV detector set at $\lambda = 210$ nm (Shimadzu, Japan) and software to compute molecular weight distribution (Waters, Maxima 820, Medford, USA). Sample solutions in tetrahydrofuran (THF) at a concentration of 5 mg/mL were filtered through a 0.45 μm filter (Millipore, USA) before injection into the GPC system and were eluted with THF at 0.4 mL/min. The weight-average molecular weight (M_w) of each sample was calculated using monodisperse polystyrene standards, M_w 1000–50,000 Da.

3. Results and discussion

3.1. Effect of bulk water on polymer T_g

When PLGA was incubated in bulk water for 1 h, the T_g was depressed from 45.5 to about 30 °C independent of the test temperature (Table 1). Surprisingly, after only 30 min of incubation at room temperature, the polymer was in a hydrated state because the T_g was already depressed to a similar value as obtained after 1 h of incubation (data not shown).

Prolonging the incubation time until five days, no further changes were discernable, except in the case of the sample incubated at 55 °C since a remarkable amount of degradation had occurred (Table 1). In spite of the polymer hydrophobicity, the samples hydrated in a short time. The plasticizing effect exerted by the water absorbed into the polymeric matrix was reversible, since after drying, the T_g of samples returned to near original values. Since GPC showed no substantial lowering of molecular weight of samples incubated at 37 °C or lower over the initial 5 days, the lowering of T_g due to the change in chain length during the plasticization process was felt to be insignificant in comparison to that due to the water absorbed. This result was confirmed also by the observation that after rapid polymer hydration, which leads to T_g depression, no further changes in wet T_g were detected (Table 1).

Water appears to act as a plasticizer within the polymer matrix. This suggests that when the polymeric matrix (i.e. microspheres or implants) absorbs water, be it in the tissue site or incubated in vitro, a rapid hydration occurs. In the present study, the decrease of about 15 °C yielded a T_g of PLGA to a value below the body temperature (T_b). Such transition from a glassy state ($T_b < T_g$) to the rubbery state ($T_b > T_g$) can have a profound influence on the properties of the polymer. Indeed, it is known that in the case of small molecules the diffusion coefficients through the polymer matrix increase several orders of magnitude when in the glass transition region [1,18]. These results suggest that T_g of hydrated PLGA better describes the characteristics of the matrix during utilization and, consequently, it should be taken into

Table 1
Glass transition temperature of hydrated PLGA[a] (Wet T_g) after incubation in deionized water at 23, 30, 37 and 55 °C and recovered T_g (Rec. T_g) after vacuum drying at room temperature until constant weight

Time (days)	23 °C		30 °C		37 °C		55 °C	
	Wet T_g (°C)	Rec. T_g (°C)	Wet T_g (°C)	Rec. T_g (°C)	Wet T_g (°C)	Rec. T_g (°C)	Wet T_g (°C)	Rec. T_g (°C)
0.041	32.7 ± 0.3	47.7 ± 0.7	29.5 ± 2.0	45.6 ± 1.6	31.7 ± 1.3	49.2 ± 0.8	31.6 ± 0.3	46.5 ± 1.7
0.25	29.8 ± 1.8	45.9 ± 1.5	28.7 ± 1.6	47.8 ± 2.1	30.4 ± 0.1	48.8 ± 0.1	28.8 ± 0.1	46.6 ± 0.3
1	30.8 ± 0.2	47.2 ± 0.1	31.0 ± 0.5	48.3 ± 0.1	30.2 ± 0.6	49.2 ± 1.0	26.7 ± 1.1	41.1 ± 0.9[b]
2	30.3 ± 0.5	47.6 ± 1.0	30.1 ± 0.1	48.2 ± 0.1	29.5 ± 1.2	48.0 ± 0.1	n.d.[c]	n.d.[c]
5	31.2 ± 0.7	48.6 ± 0.1	30.0 ± 0.5	48.5 ± 0.5	27.2 ± 0.1	46.3 ± 0.1	n.d.[c]	n.d.[c]

The results are expressed as mean $\pm$ standard deviation ($n = 2$).
[a] T_g of the raw polymer dried overnight was 45.5 ± 1.3 °C.
[b] GPC analysis suggested that the decrease in T_g value was due to polymer degradation.
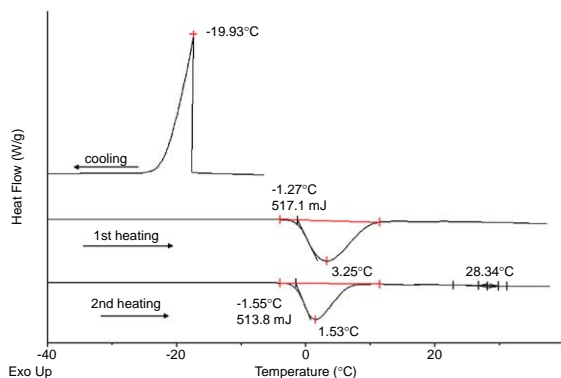[c] n.d.: not determined, because substantial polymer degradation had occurred.

Fig. 1. MTDSC data of PLGA incubated in bulk water for 6 h at 37 °C.

Table 2
Moisture content and glass transition temperature (T_g) of hydrated PLGA[a] after equilibration over saturated salt solution at 0–90% RH at 37 °C

Relative humidity (%)	Moisture content (%, w/w)	T_g (°C)
32	0.82 ± 0.04	42.8 ± 0.6
50	1.26 ± 0.07	37.7 ± 1.1
75	1.70 ± 0.10	35.1 ± 1.0
90	2.61 ± 0.10	28.8 ± 0.5

The results are expressed as mean ± standard deviation ($n = 3$).
[a] After drying the raw polymer overnight, the moisture content was determined to be 0.30 ± 0.04% and the T_g measured was 46.3 ± 1.3 °C.

consideration in the design and characterization of such drug delivery systems.

Fig. 1 shows the typical MTDSC data of the wet polymer after incubation in bulk water at 37 °C for a 6 h period. The broad endothermic peak observed around 0 °C during both heating ramps was attributed to the melting of ice. Since the temperature and the enthalpy of these endothermic transitions were not significantly different from that of bulk water, they were attributed to the freezable free (bulk) water. Direct incubation in water should provide an excess of water, some of which orients in the matrix as clusters or remains adsorbed on the surface, behaving like free or bulk water [8,10]. It has been reported that polar molecules such as water, when sorbed into a matrix, tend to interact with other water molecules to form aggregates or clusters [8].

In the cooling ramp, the exothermic peak was detected at a temperature lower than the one reported for pure water crystallization. This transition was attributed to a supercooling phenomenon, which is characterized also by the asymmetric peak shape [19]. According to Karl Fischer analysis, the amount of water present in this sample was 20.4% w/w (data not shown).

3.2. Effect of relative humidity on polymer T_g

After drying under vacuum overnight, the residual moisture content of the raw polymer was 0.30% w/w and T_g was 46.3 °C. When the polymer samples were incubated over saturated salt solutions at a range of 32–90% RH, equilibrium was achieved in four days

(data not shown). PLGA samples exposed to 90% RH absorbed 2.3% w/w water, resulting in a moisture content of 2.6% w/w. The small amount of absorbed water was due to the high polymer hydrophobicity. The T_g was reduced to 28.8 °C, confirming the potent plasticizing effect of water. The polymer incubated at RHs <90% absorbed lower amounts of water leading to lower T_g depression (Table 2, Fig. 2). The depression of T_g was proportional to the water fraction within the polymer/water system, in the range 0.3–2.6% w/w. Indeed, when T_g values were plotted as a function of water fractions, a linear relationship was observed (r^2 0.9837) (Fig. 3). Since the T_g values of the polymer/water systems containing 2.6% or 20% w/w water were comparable and ice fusion could be observed only at 20% of water, and since only water that is in the same phase as the polymer will act as a
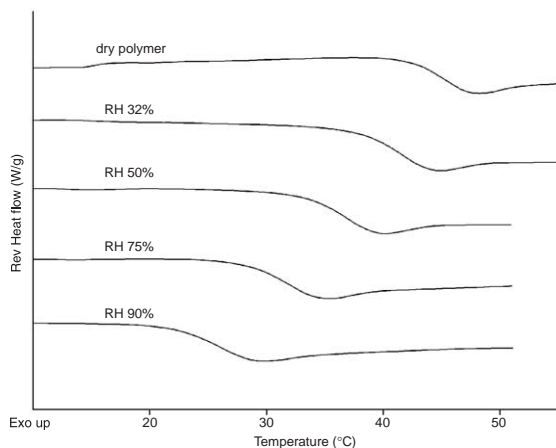

Fig. 2. MTDSC data of PLGA incubated at different relative humidities at 37 °C.
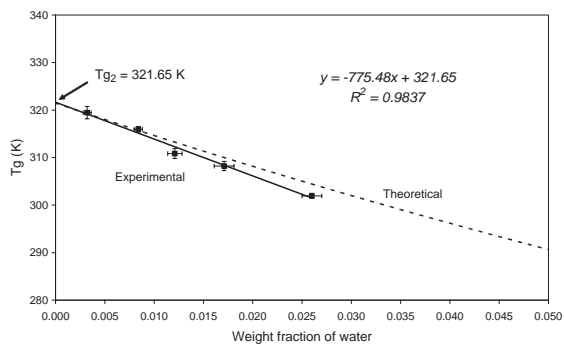
Fig. 3. T_g as function of weight fraction of water. Solid line: experimental data extrapolated to determine T_{g2}; broken line: theoretical values using Gordon–Taylor/Kelley–Bueche simplified equation.

plasticizer, it can be assumed that the amount of water responsible for the maximum plasticizing effect on the analyzed polymer was around 2.6% w/w. The samples at higher water content probably formed a heterogeneous system (binary system) containing also domains of pure water as well as domains of polymer and free water.

In the thermograms of PLGA incubated at different RH (Fig. 4), no melting or evaporation events were detected in the range from −60 to 10 °C. Since freezable water, bound or free, does have a certain degree of crystallinity and shows a melting or crystallization peak, the water absorbed into the matrix, in the range 0.3–2.6% w/w, can be classified as non-freezable (bound) water. Under the proposed experimental conditions, it was possible to detect the presence of non-freezable bound water and freezable free water when the amounts of water were ≤2.6% w/w and about 20% w/w, respectively. However, in previous works [9,10] a hydrophobic polymer, namely poly(vinyl acetate) (PVAc), evidenced the same behavior which was attributed to the formation of water clusters in water-saturated PVAc as well as the migration of a single water molecule during cooling [9,10].

Since the water absorbed into the polymer at different RH was non-freezable, it should be retained in molecular state in the polymeric matrix. The water molecules could directly interact with the polymeric hydrophilic groups such as $C{=}O$ and COO^- by forming hydrogen bonds. While it has been reported that such interactions may cause the presence of non-

freezable water [20], the effect of water confinement in the nanocavity (microenvironment) of the polymer structure should be taken in account [8,9,21].

The same interactions could be responsible for T_g depression. In the case of a hydrophilic polymer, namely poly(vinyl alcohol), using positron annihilation lifetime spectroscopy (PALS) and solid state ^{13}C nuclear magnetic resonance (NMR), an increase in free volume and a lubrication effect were detected in the presence of water [20]. The addition of water molecules into the matrix increased the size of free volume cavities of the polymer. Increments in free volume induced by mixing plasticizers with amorphous polymers, represents one of the mechanisms that can explain the depression of polymer T_g. In fact, the free-volume theory represents one of the three main groups of theories of the glass transition [1].

Moreover, water molecules, interacting with the polar chain groups, disrupt polymer chain–chain hydrogen bonding, removing further barriers to bond rotation and chain mobility. One can speculate that only the non-freezable water is responsible for the plasticizing effect on the polymer. In fact, the interactions with the polar chain groups, which contribute to the changes of water thermodynamic characteristics [20,21], could also contribute to the lubrication effect; this represents another mechanism for the depression of polymer T_g in the presence of a plasticizer [20].

Several mathematical models have been proposed in order to predict T_g of compatible blends of amorphous substances [3,4]. In the present study the
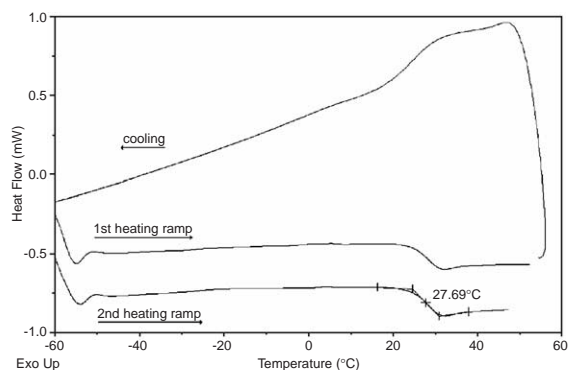

Fig. 4. Typical MTDSC data of PLGA at water content of 0–2.6% w/w, after equilibration at different relative humidities.
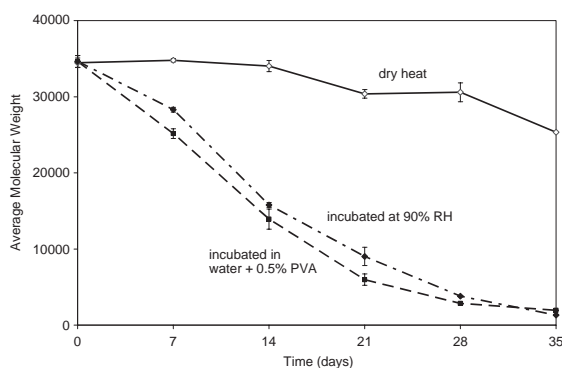
Fig. 5. Variation of weight-average molecular weight (M_w) of PLGA as function of time at 37 °C at various conditions.

Fig. 6. Variation of polydispersity index of PLGA as function of time at 37 °C at various conditions.

Gordon–Taylor/Kelley–Bueche simplified expression was used to predict the T_g of the polymer/water blends ($T_{g_{mix}}$):

$$T_{g_{mix}} = \frac{\left(w_1 T_{g_1} + k w_2 T_{g_2}\right)}{\left(w_1 + k w_2\right)} \quad (2)$$

where w_1 and w_2 are the weight fractions of water and the polymer, respectively; T_{g_1} and T_{g_2} are the corresponding T_g values of each component; the constant k can be estimated using free volume theory with a knowledge of the densities (ρ_1 and ρ_2) of both components, according to the following equation[1]:

$$k = \left(\rho_1 \cdot T_{g_1}\right) / \left(\rho_2 \cdot T_{g_2}\right). \quad (3)$$

The dry polymer T_g value ($T_{g2} = 321.65$ K) was obtained from the linear fitting of the experimental data in Fig. 3 by extrapolation. Fig. 3 also compares the $T_{g_{mix}}$ predicted by Eq. (2) to the experimental data. The experimental T_gs and the theoretical values calculated by Gordon–Taylor/Kelley–Bueche equation correlated well at low water contents over a small range (0–2.6% w/w). The adherence of experimental data to the Gordon–Taylor equation would indicate that the water was fairly evenly distributed in the interior of the polymeric matrix forming a homogeneous system. When the water content was 20% w/w, the experimental T_g did not fit the value predicted by Gordon–Taylor equation (data not shown). This finding confirms that water was miscible with PLGA up to

2.6% w/w and that higher water contents formed a heterogeneous system (binary system).

After incubation in bulk water, the excess water molecules could form clusters [8–10] or form small ice crystals during cooling [10] or remain adsorbed on the polymer surface behaving as bulk water. In fact, as previously shown, quantities of water higher than 2.6% w/w did not cause further reduction of the T_g nor was there evidence of ice melting endothermic peak at about 0 °C. It is hypothesized that only small fractions of non-freezable water may be absorbed into PLGA matrix, due to its hydrophobicity, and they may be responsible of T_g depression.

3.3. Hygrothermal aging

Fig. 5 shows the changes in weight-average molecular weight of PLGA treated at 37 °C with dry heat, incubated in water containing 0.5% PVA, and stored at 90% RH. Initially, the polymer exhibited a rather narrow molecular weight distribution (Fig. 6) and the water content was about 0.3% (Table 3).

Table 3
Moisture content of PLGA after incubation at 37 °C and 90% RH

Time (days)	Moisture content (%, w/w)
0[a]	0.30 ± 0.04
7	2.50 ± 0.10
14	2.87 ± 0.20
21	2.78 ± 0.40
28	6.50 ± 0.40
35	17.30 ± 0.90

The results are expressed as mean ± standard deviation ($n=3$).
[a] After drying overnight.

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[1] $\rho_1 = 1$ g/cm³, $\rho_2 = 1.5804$ g/cm³, $T_{g1} = 138$ K (-135 °C), $k = 0.2714$.

During the incubation in dry heat, the M_w decreased relatively slowly and only at the conclusion of the test period was noticeable degradation detected (Fig. 5). Hydrolytic degradation may commence due to the small amount of water retained in the polymeric matrix. As a consequence of the increased number of carboxylic end groups caused by degradation, an acidic environment could result and subsequently autocatalysis [22,23].

Although PLGA suspended in water or exposed to vapor water showed similar reduction in M_w (Fig. 5), different polydispersity patterns were observed (Fig. 6). During polymer degradation following hydration (Fig. 5), the biphasic profile was characterized by a higher degradation rate during the first 21 days which subsequently slowed during the following 14 days [14]. Since hydrolytic degradation should not become heterogeneous in small low M_w PLGA particles [23], the differences observed in polydispersity were not surprising. In this study the polymer particles were in the 30–70 μm range (data not shown). At such a particle size, soluble oligomers or monomers could readily diffuse from the matrix without causing heterogeneous degradation due to internal autocatalysis. By aging PLGA at 90% RH, the absence of a large amount of bulk water surrounding the polymer binding layer could lead to surface-interior heterogeneous degradation with a greater decrease in M_w.

Table 3 shows that the polymer absorbed <3% w/w moisture in the first 7 days and the water uptake remained almost constant for 21 days. After 7 days of incubation at 90% RH, the polymer formed a transparent film on the bottom of the container as a consequence of drop in T_g, attributed to the presence of water and a decrease in molecular weight. The film formation could affect the water uptake and only after a critical degree of degradation might a more porous structure occur resulting in further uptake/diffusion of water. The critical degree of degradation was apparently achieved at day 21 (Table 3) when the water content began to increase. Moisture uptake into the polymeric matrix of <3% w/w assured the same degradation rate which was measured after incubation in bulk water. To verify this hypothesis, samples were exposed to 90% RH over a 3 day period and subsequently stored at 37 °C in hermetically sealed vials in order to maintain constant moisture content. The var-

iation in M_w over time was superimposable to that of samples that were incubated in bulk water (data not shown). Such results provide evidence that a small non-freezable water fraction can play a fundamental role during the hygrothermal aging of PLGA, due to the direct and strong interaction with the polymeric hydrophilic end groups.

4. Conclusions

PLGA absorbed a small amount of water under high relative humidity and this water acted as a plasticizer lowering the T_g proportional to the amount of water in the matrix. Temperatures of incubation did not influence the plasticizing effect. The larger water content obtained during incubation in bulk water was ascribed to the presence of pure water domains, possibly clusters, which did not affect T_g and behaved as bulk water. It was concluded that water molecules absorbed at high relative humidity were responsible for lowering of the T_g and that such water was distributed evenly through the polymeric matrix as non-freezable water. The small fraction of water absorbed at 90% relative humidity resulted in similar polymer degradation as when the polymer was incubated in bulk water.

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