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Effect of isothermal annealing on the hydrolytic degradation rate of poly(lactide-*co*-glycolide) (PLGA)

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Abstract

Isothermal crystallization through annealing at 115 °C was conducted to increase the degree of crystallinity of poly (lactide-*co*-glycolide) (PLGA). The maximum increase in the degree of crystallinity (~21%) was achieved after 60 min of annealing. The crystal size/perfection was observed to increase with annealing time. The annealed PLGA films were then hydrolytically degraded in phosphate buffered saline solution of pH 7.4 at 37 °C for up to 150 days. Minimal mass loss was observed throughout the time investigated, suggesting that the samples were still in the first phase of degradation. The increase in the degree of crystallinity of the PLGA samples annealed at 15 and 30 min was found to retard their overall rate of hydrolytic degradation, when compared to those samples with higher initial crystallinity (annealed for 45 and 60 min) that had faster degradation rates. The increased degradation rate at higher crystallinity was associated with the loss of amorphous material and the formation of voids during annealing, which decreases the glass transition temperature and increases the average water uptake in the samples annealed for longer times. Therefore, the increase in degree of crystallinity is found to retard hydrolytic degradation but only to a certain extent, beyond which the formation of voids through annealing increases the rate of hydrolytic degradation.
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Keywords: Poly(lactide-*co*-glycolide); PLGA; Biodegradable; Hydrolytic degradation; Crystallinity; Isothermal annealing

1. Introduction

The use of biodegradable polymers in the biomedical field has become increasingly important in the recent years due to their special ability to degrade. Poly(lactide-*co*-glycolide) (PLGA) is an example of such a biodegradable polymer, and has been extensively used in various biomedical applications [1]. It has been an excellent candidate for such applications because of its good biocompatibility, low toxicity, satisfactory mechanical strengths and excellent biodegradability [2].

PLGA is hydrolytically unstable, and its degradation mechanism in vivo has been credited to the random hydrolytic chain scission of ester bonds in its backbone, with possible enzyme catalytic effects [2]. This continual ester hydrolysis reaction forms carboxylic acid end groups that further catalyze the hydrolysis reaction, and leads to the eventual formation of lactic and glycolic acids [2–4]. In vivo, lactic and glycolic acids enter the tricarboxylic acid cycle and are metabolized and subsequently eliminated from the body as carbon dioxide and water [2,4]. It is generally accepted [5–7] that the hydrolysis of most polyesters, such as PLGA, proceeds according to the reaction shown in Fig. 1.

The kinetics of this reaction are given by

$$\mathrm{d[COOH]}/\mathrm{d}t = k'[\mathrm{ester}][\mathrm{H_2O}][\mathrm{COOH}] = k[\mathrm{COOH}] \quad (1)$$

where [COOH], [ester] and [H_2O] are the concentrations of carboxyl end groups, ester and water in the polymer matrix respectively, and it is assumed that in the early stages of the reaction the concentration of water and ester are constant. By assuming that $[\mathrm{COOH}] = 1/M_n$,

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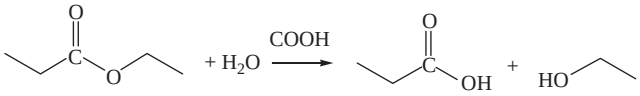

Fig. 1. Hydrolysis of ester bonds in biodegradable polyesters.

it can thus be shown that,

$$\ln M_n = \ln M_{n,0} - kt, \quad (2)$$

where $M_{n,0}$ is the initial number-average molecular weight of the polymer. The rate of degradation of PLGA is dependent on several factors [2,8], one of which includes its degree of crystallinity.

The percentage or degree of crystallinity reflects the extent of formation of crystalline regions within a polymer matrix. The degree of crystallinity has been shown to affect mechanical strength and swelling behaviour of the polymer, properties that influence the polymers suitability for end applications [9]. The formation of crystals in lactic/glycolic acid polymers is mainly dominated by the monomer used. Apart from the intrinsic susceptibility of a given polymer to form crystalline regions, the processing history of a polymer, such as its heating and cooling rate, also has significant effects on its crystallinity [10].

The driving force for crystallization is an increase in the (negative) interaction energy achieved by efficient molecular packing and by maximization of the number of strongly interacting groups that are in direct contact (e.g. forming hydrogen bonds). Once the molecules discover the most efficient arrangement, they repeat it endlessly in a crystalline lattice, where the smallest repeating entity is the unit cell [11]. The creation of a three-dimensional ordered phase from a disordered state is a two-stage process. The first step in crystallite formation is the creation of a stable nucleus brought about by ordering of chains in parallel array, stimulated by intermolecular forces, followed by the stabilization of long range order by the secondary valence forces which aid the packing of molecules into a three-dimensional ordered structure. The second stage is the growth of the crystalline region, the size of which is governed by the rate of addition of other chains to the nucleus. As this growth is counteracted by thermal redispersion of the chains at the crystal-melt interface, the temperature must be low enough (below T_m) to ensure that this disordering process is minimal. Yet, the temperature should be above T_g to enable sufficient thermal energy for the diffusion of chains into suitable orientations. For polymers in general, measurable rates of crystallization occur between ($T_m - 10$ K) and ($T_g + 30$ K), a range in which thermal motion of the polymer chains is conducive to the formation of stable ordered regions [12].

It is widely accepted that the degree of crystallinity affects the rate of hydrolytic degradation of PLGA but there is a lack of data that relates the degree of crystallinity of PLGA to its hydrolytic degradation rate. The ability to control the degree of crystallinity of PLGA through annealing therefore becomes an attractive study. This paper seeks to study the effects of isothermal annealing on the morphological properties of PLGA films and its subsequent hydrolytic degradation behaviour.

2. Materials and methods

2.1. Preparation and annealing of PLGA films

PLGA(80:20) (IV = 4.8) was purchased from Purac Far East, Singapore (manufactured by Purac Holland). Films of PLGA were prepared using a simple solvent casting method. The polymer was first dissolved in dichloromethane (DCM) at a weight ratio of 1:25. This weight ratio would give a polymer solution with good flow properties, without compromising on solubility. The polymer solution was then poured into a Teflon mould and the solvent was evaporated slowly in air at room temperature for 48 h to prevent the formation of air bubbles. The films were then placed in an oven at 40 °C for a week to evaporate any remaining solvent, leaving behind dried films with thickness about 0.055 mm.

The polymer films were then cut into rectangular strips of dimensions 8 cm × 3 cm and isothermally annealed at 115 °C, which is approximately the crystallization temperature of PLGA [13], for various lengths of time. Different annealing times resulted in different degree of crystallinity in PLGA. The percentage crystallinity (from WAXD) and enthalpy of fusion (from MDSC), for these films were then plotted with respect to annealing time.

2.2. Hydrolytic degradation of annealed PLGA films

PLGA films annealed for 15, 30, 45 and 60 min were used for the in vitro degradation studies, with the non-annealed films (0 min) used as the control sample. Prior to in vitro hydrolytic degradation, the initial mass (m_0) of each PLGA film was recorded. The samples were then placed in 10 ml screw top bottles containing phosphate buffered saline (PBS) solution (pH 7.4) and incubated at 37.0 °C for various lengths of time (up to 150 days). Samples were removed once every 3 days for the first 2 weeks and subsequently once every 10 days. The pH of the solution was monitored over time to ensure a stable pH 7.4 was maintained at all times.

At the designated time intervals, the films were removed from the PBS solution, rinsed with distilled

water and surface dried using water-absorbent paper. The wet mass was measured immediately (m_w). The samples were then dried in an oven at 37.0 °C for 5 days, after which the dry mass was recorded (m_d). The water uptake was calculated by subtracting the dry mass (m_d) from the wet mass (m_w), and taken as a percentage over the dry mass. Mass loss was taken as the difference in the dry mass (m_d) with respect to the initial mass (m_0) of sample. All parameters were normalized by dividing by their initial masses and reported in terms of percentage.

2.3. Characterization of PLGA films

DSC measurements were performed on a TA Instrument DSC 2920 Modulated DSC (MDSC) apparatus, in which changes in the thermal properties, enthalpy of fusion and crystallization of the annealed and hydrolytically degraded PLGA films were investigated. To avoid oxidative degradation, the sample and reference pans were purged with nitrogen at a constant flow rate of 48 ml min^{-1}. Approximately 5 mg of each sample was heated from −40 °C to 230 °C at a scan rate of 5 °C min^{-1}. The change in ΔH, ($\Delta H = \Delta H_f - \Delta H_c$), gives the polymers' true crystallinity as a direct result of annealing or hydrolytic degradation, since there are no available literature on the enthalpy of fusion for a 100% crystalline PLGA.

Wide-angle X-ray diffraction (WAXD) was performed using the Shimadzu XRD-6000 employing CuKα radiation ($\lambda = 1.5406$ Å). A scan axis of 2θ was used to obtain diffraction patterns of a scan range between 10° and 50°. The θ-fixed angle was kept at 0.5° and a scan rate of 0.2 deg/min was used. The voltage used was 40 kV and the current was 30 mA. The thin-film attachment was set to a rotational mode, which rotates at a speed of 50 rpm. The degree of crystallinity was calculated as the percentage of the scattered intensity of the crystalline phase over the scattered intensity of the crystalline and amorphous phase. The change in full-width at half-maximum (FWHM) is related to the change in the mean dimension of crystallites perpendicular to the *hkl* planes, t, by Scherrer's equation [14]

$$B = \frac{0.9\,\lambda}{t\cos\,\theta}, \tag{3}$$

where B is the broadening of diffraction line on the 2θ scale (radians) measured at its half maximum intensity. The FWHM is strongly affected by crystal defects and distortions, which cause line broadening. In this paper, the total thickness of the crystal, t, in (Eq. (3)) is not calculated. Instead, the variation in the FWHM (B) is only used as a rough indication of the changes in crystallite size as a function of annealing and degradation.

The number-average molecular weight (M_n) of each of the hydrolytically degraded films was determined using the Agilent 1100 series gel permeation chromatography (GPC), which was performed at 30 °C with 80%-tetrahydrofuran and 20%-DCM as solvents [13], using reflective index detector (RID) as the detector. The calibration is done in accordance to polystyrene standards and the flow rate used was 1 ml min^{-1}.

3. Results and discussion

3.1. Isothermal annealing of PLGA

The change in the degree of crystallinity and enthalpy of fusion (ΔH_f) was followed using WAXD and MDSC, respectively as shown in Fig. 2. Similar trends were obtained from both characterization techniques. The results show an increase in the degree of crystallinity with annealing times. The degree of crystallinity increased gradually at first and then becomes more pronounced after 30 min of annealing time. Thermal energy supplied, through annealing, enables the movement and re-orientation of the polymer chains and in the process, assists the chains in discovering the most efficient arrangement. This results in the formation of close-packed structure of crystalline regions from an initially amorphous morphology, thus increasing the degree of crystallinity. Since polymer chain diffusion is time dependent, annealing for a longer period of time allows more chains to diffuse into the suitable orientations for crystalline arrangement.

Fig. 3 shows the wide-angle X-ray diffraction patterns of the non-annealed (0 min) and the 60 min annealed films. The X-ray diffraction pattern of the non-annealed film shows a broad amorphous peak, whereas the X-ray diffraction pattern of the annealed film shows sharp crystalline peaks. The FWHM of the most intense X-ray diffraction peak at 16.35° is plotted against annealing

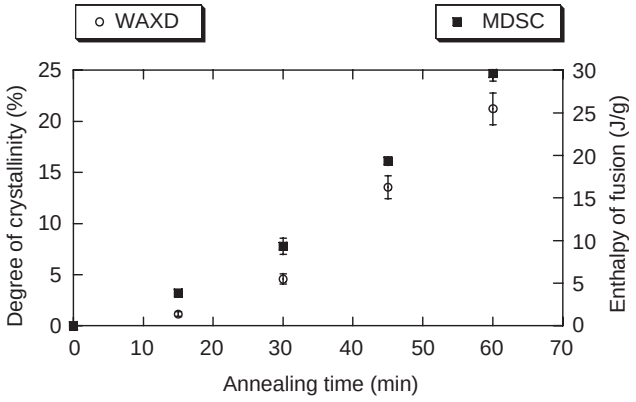

Fig. 2. Increase in degree of crystallinity of PLGA with annealing time.

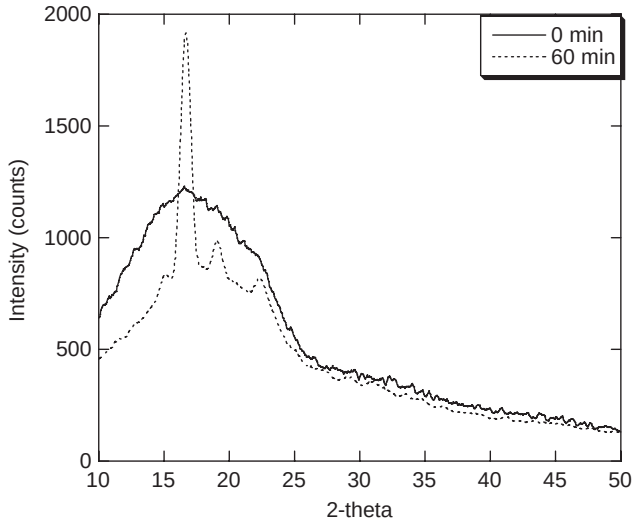

Fig. 3. X-ray diffraction pattern of non-annealed and 60 min annealed PLGA films.

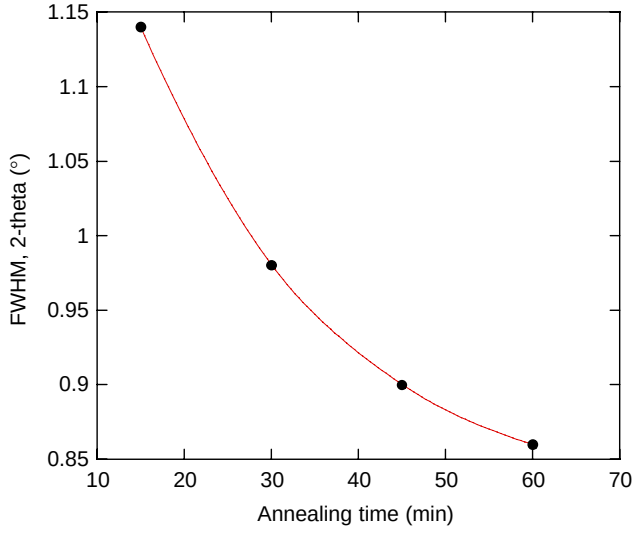

Fig. 4. Plot of FWHM of X-ray diffraction peak 16.35° with annealing time.

time as shown in Fig. 4. The results show a decrease in FWHM values with increasing annealing time. The decrease in FWHM, denotes an increase in the dimension of the crystallites, and/or a decrease in crystal distortion and defects. The formation of crystals in PLGA, through annealing, occurs in two stages; firstly through crystal nucleation, where the creation of a stable nucleus is brought about by ordering of chains in parallel array, followed by the growth of crystals. Crystal growth was therefore responsible for the decrease in the FWHM.

Fig. 5 plots the glass transition temperature (T_g) of PLGA against annealing time. The T_g decreases with increasing isothermal annealing time due to the loss of amorphous material. The decrease in the T_g is also an

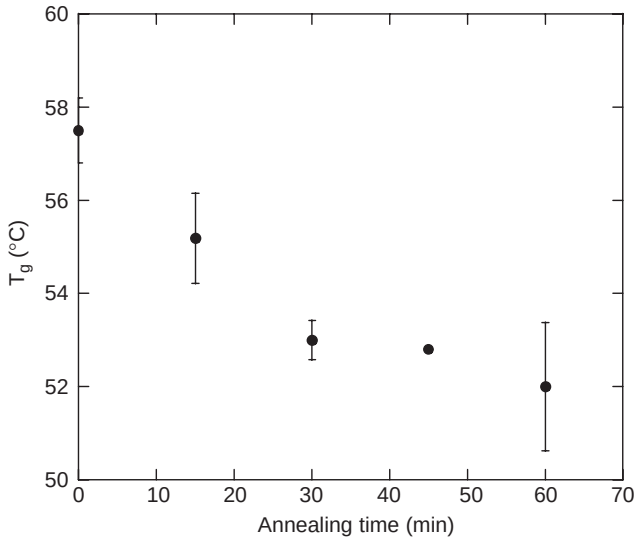

Fig. 5. Plot of T_g of PLGA with annealing time.

indication of voids formation in the annealed PLGA films. This can be explained by the mechanisms of chain movement during crystal formation through annealing. During isothermal annealing, the amorphous chains have sufficient energy to re-orientate and pack themselves in a more efficient manner, resulting in the formation of crystals through nucleation and crystal growth. This results in an even more open amorphous region and the formation of voids at the crystal-amorphous interface due to material loss during crystallization.

3.2. Hydrolytic degradation of annealed PLGA films

The water uptake generally increased with degradation time. However, it was very low for all the samples, even after 150 days of degradation. Fig. 6 plots the average water uptake after 150 days of degradation with respect to different annealing times. It can be observed that the non-annealed film (0 min) has the highest average water uptake at about 0.6% of its initial weight. Upon annealing, the average water uptake decreases. However, samples annealed for longer times (45 and 60 min) had higher average water uptake compared with samples annealed for shorter times (15 and 30 min). The average water uptake was highest for the non-annealed film because of its amorphous morphology. Amorphous regions are more open compared with the closely packed crystalline regions, thus allowing more water molecules to penetrate into the amorphous regions compared with the crystalline regions. Hence, the formation of crystalline regions through isothermal annealing effectively inhibits the penetration of water molecules, thus reducing the overall average water uptake. However, for samples annealed for longer times, advance loss of amorphous material and the formation of voids within

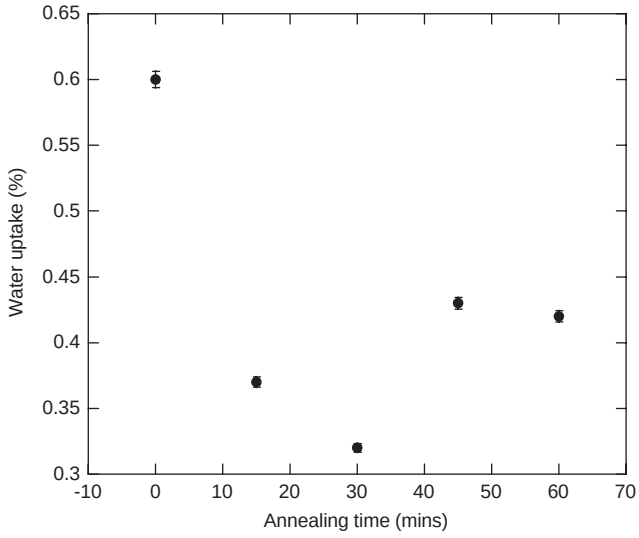

Fig. 6. Average water uptake after 150 days of hydrolytic degradation.

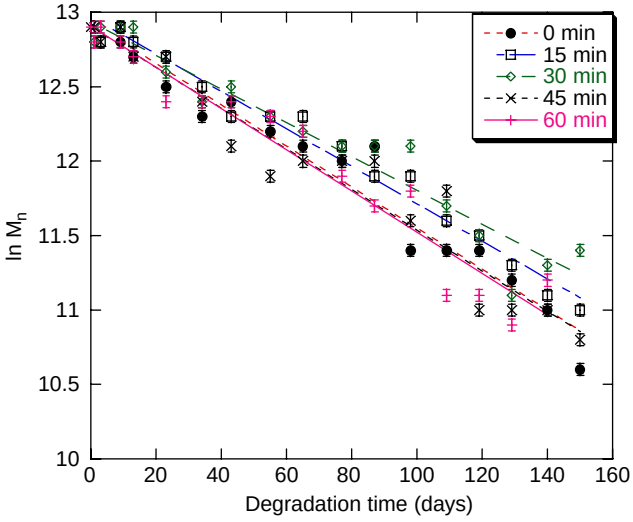

Fig. 7. Plot of ln M_n against hydrolytic degradation time.

the amorphous region and at the crystal interface take place. This would account for the higher average water uptake in the 45 and 60 min annealed films.

There was no significant mass loss (less than 2.5%) in the non-annealed and annealed films, even after 150 days of degradation. This implies that no or little soluble products and oligomers are formed, even after 150 days of degradation study. This corresponds to the first stage of PLGA biodegradation, which involves random chain scission, where the molecular weight of PLGA decreases significantly, but no appreciable weight loss or soluble monomer products are formed [15].

The natural logarithmic of the number-average molecular weight (ln M_n) of PLGA was plotted against degradation time in Fig. 7. The plots show that the molecular weight (M_n) of PLGA decreases with degradation time, indicating chain scission, due to the hydrolysis of the ester bonds. The degradation rate constants (k) for the films and their respective linear regression coefficients are summarized in Table 1.

From Table 1, it was observed that k differs with films of different annealing times. This implies that the increase in the degree of crystallinity, through isothermal annealing, indeed has an effect on the degradation rate constants. The degradation rate decreases in samples annealed for 30 min and below, but increases in samples annealed above 30 min. The initial decrease in the degradation rate coincides with crystals retarding the rate of degradation, by inhibiting the penetration of water molecules due to the tightly packed crystal structure. This prevents the hydrolysis of the crystalline chains during the initial stages of degradation. However, for films annealed above 30 min, the increase in degree of crystallinity has an adverse effect by increasing the rate of hydrolytic degradation. Annealing for longer times not only results in the formation of crystals but also the loss of amorphous material and the formation of voids, which subsequently allow for higher water uptake (Fig. 6), thus giving rise to a faster rate of hydrolysis (Table 1).

Table 1
Degradation rate constants (k) for different annealed samples

Annealing time	0 min	15 min	30 min	45 min	60 min
Linear regression (R^2)	0.91	0.95	0.94	0.93	0.96
Rate constant (k)	0.0138	0.0126	0.0113	0.0137	0.0152

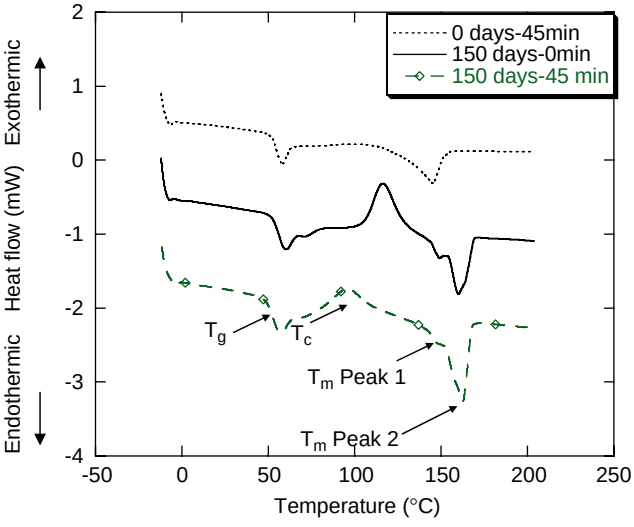

Fig. 8. DSC thermograms of non-degraded (45 min annealed) and degraded (0 and 45 min annealed) PLGA films.

The MDSC thermograms of the hydrolytically degraded films (non-annealed and annealed), as shown in Fig. 8, displayed a cold crystallization peak and two overlapping melting peaks. The formation of the cold

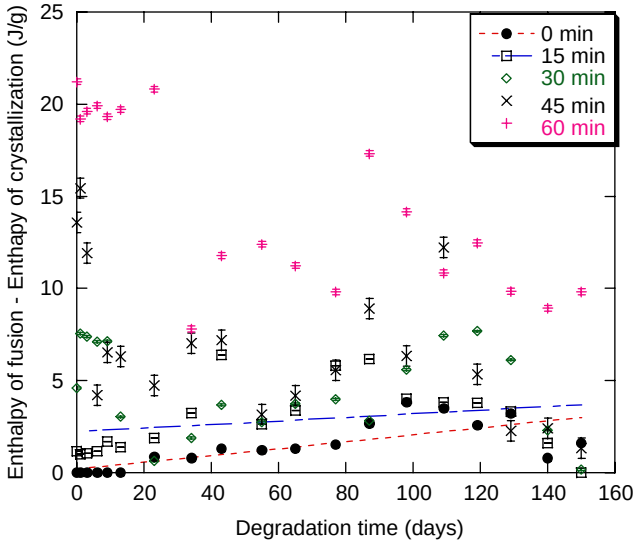

Fig. 9. Difference between enthalpy of fusion and enthalpy of cold crystallization plotted against degradation time.

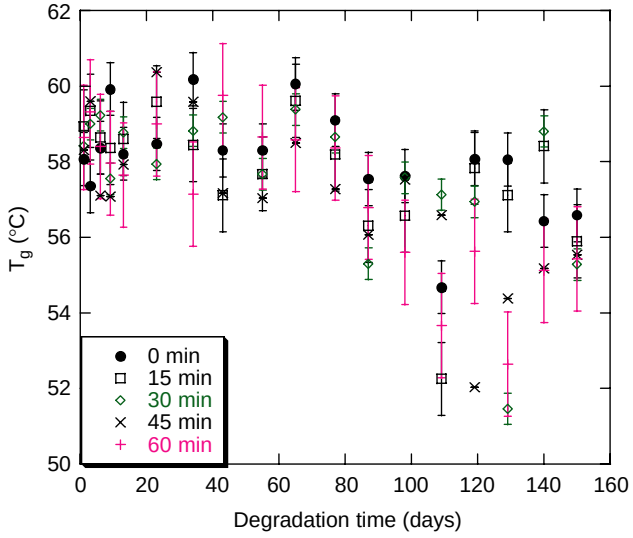

Fig. 10. Plot of T_g against degradation time.

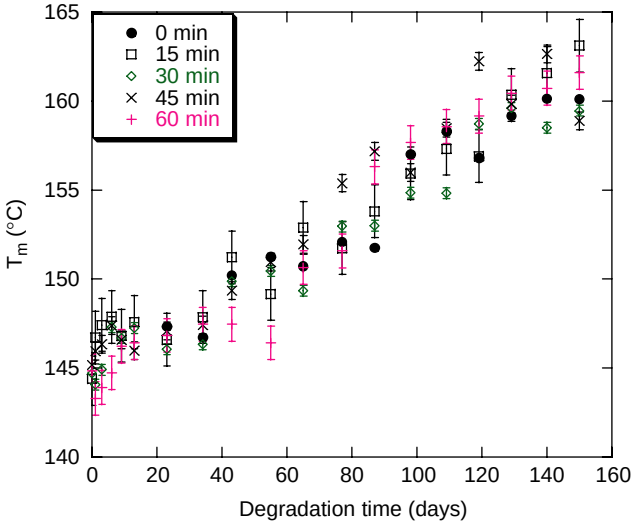

Fig. 11. Plot of T_m (Peak 2) of hydrolytically degraded PLGA films against degradation time.

crystallization peak was due to the re-orientation of the shorter amorphous chains, formed through chain scission, during degradation. These shorter chains are better able to re-orientate and form crystals when thermal energy is supplied during the heating scan. One of the melting temperature peaks (Peak 1) is due to the crystals present after hydrolytic degradation (through annealing and the product of chain scission) and the other melting peak (Peak 2) is due to the crystals formed through cold crystallization, from the cold crystallization peak.

The difference (ΔH) between the enthalpy of fusion (ΔH_f) and the enthalpy of cold crystallization (ΔH_c), of the films immediately after hydrolytic degradation was plotted against degradation time, as shown in Fig. 9. There is a slight increase in ΔH with degradation time for the non-annealed and 15 min annealed films. The increase in the ΔH for the amorphous and lowly crystalline films was probably due to the re-orientation of the amorphous chains, through the effect of chain scission, during hydrolytic degradation. These shorter chains have better mobility and are thus able to re-orientate to form crystals. Fig. 10 plots the T_g of the hydrolytically degraded PLGA film against degradation time. The decrease in T_g supports the increase in chain mobility and flexibility of these shorter amorphous chains, due to continuous hydrolysis. These shorter amorphous chains have better packing capabilities, thus encourages the formation of crystals. This in turn increases the T_m, as seen from Fig. 11, which is the plot of T_m (Peak 2) against degradation time.

As for samples annealed for 30 min and above, a definite trend could not be obtained from the ΔH due to the large scattering of data. In samples annealed for 30 min and above, the initial crystallization of the polymer through annealing along with recrystallization of shorter chains due to random hydrolytic chain scission during degradation plus the cold recrystallization during the thermal scan gave rise to the multiple endotherms observed (Fig. 8). This in turn, made it difficult to calculate the enthalpy of fusion due to the presence of overlapping melting peaks. Hence for the large data scatter.

Fig. 12 plots the change in the FWHM of the most intense diffraction peak at 16.35° with degradation time for samples annealed at 30, 45 and 60 min. The FWHM increases in the first 20 days of degradation for the samples, suggesting a decrease in crystal perfection, due to chain relaxation in the crystalline structure as water is absorbed into the polymer. This coincides with the initial increase in the T_m (Peak 1) after 20 days, as

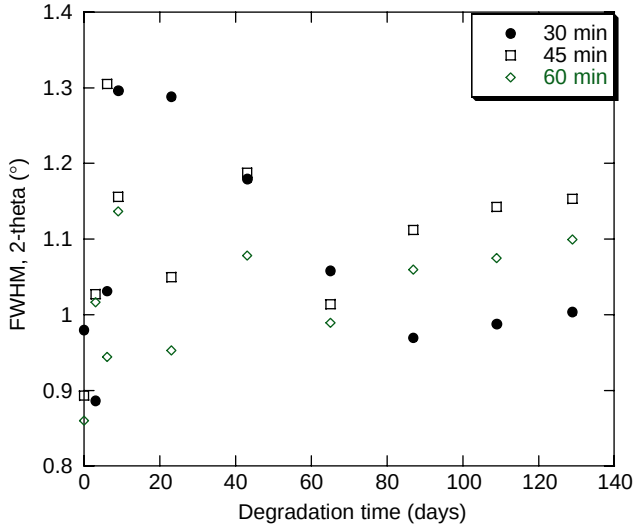

Fig. 12. Plot of change in FWHM of hydrolytically degraded PLGA films.

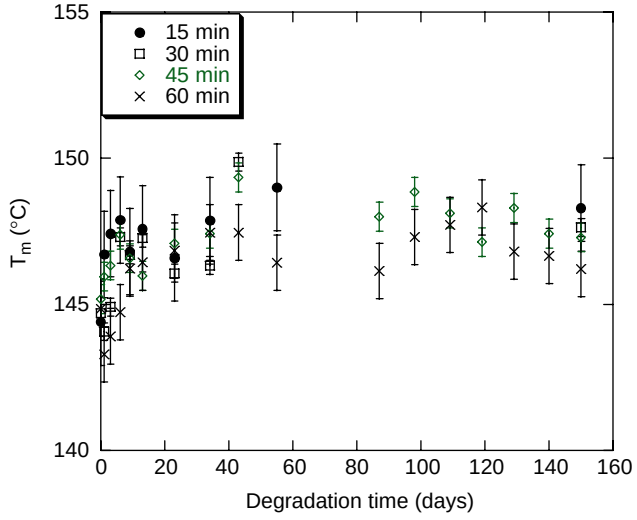

Fig. 13. Plot of T_m (Peak 1) of hydrolytically degraded PLGA films against degradation time.

illustrated in Fig. 13. The increase in the T_m is due to the increase in lamellae thickness, which may have resulted in the decrease in crystal perfection of the crystal unit cell. Subsequently, the FWHM remains relatively unchanged.

4. Conclusions

Annealing of an initially amorphous PLGA at 115 °C causes the formation of crystals and increases its degree of crystallinity with increasing annealing time. The formation of crystals results in the formation of voids and a more open amorphous region, which causes a decrease in its T_g with annealing time. Voids formation from annealing results in a higher than expected average water uptake for samples annealed for longer times (45 and 60 min) compared with the samples annealed for shorter times (15 and 30 min). This increase in the average water uptake therefore increases the hydrolytic rate constants for these films. For the lowly crystalline 15 and 30 min annealed films the formation of voids is insignificant, and the presence of closely packed crystalline structure inhibits the penetration of water molecules into the polymer, resulting in a lower degradation rate constant. Therefore, crystals are found to retard hydrolytic degradation but only to a certain extent. The maximum retardation in hydrolytic degradation rate is 18% for PLGA films annealed at 115 °C for 30 min.

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