

Degradation of poly(lactide-*co*-glycolide) (PLGA) and poly(L-lactide) (PLLA) by electron beam radiation

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Abstract

This paper seeks to examine the effects of electron beam (e-beam) radiation on biodegradable polymers (PLGA and PLLA), and to understand their radiation-induced degradation mechanisms. PLGA (80:20) and PLLA polymer films were e-beam irradiated at doses from 2.5 to 50 Mrad and the degradation of these films were studied by measuring the changes in their molecular weights, FTIR spectra, thermal and morphological properties. The dominant effect of e-beam irradiation on both PLGA and PLLA is chain scission. Chain scission occurs first through scission of the polymer main chain, followed by hydrogen abstraction. Chain scission, though responsible for the reduction in the average molecular weight, T_c , T_g and T_m of both polymers, encourages crystallization in PLGA. PLLA also undergoes chain scission upon irradiation but to a lesser degree compared to PLGA. The higher crystallinity of PLLA is the key factor in its greater stability to e-beam radiation compared to PLGA. A linear relationship is also established between the decrease in molecular weight with respect to radiation dose.

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1. Introduction

Poly(lactide-*co*-glycolide) (PLGA) and poly(L-lactide) (PLLA) have been extensively used as a controlled release carrier for drug delivery due to their good biocompatibility, biodegradability and mechanical strengths [1,2]. PLGA and PLLA are both hydrolytically unstable, and although insoluble in water, they degrade by hydrolytic attack of their ester bonds [3], resulting in the formation of lactic and glycolic acids [3–9]. Conventionally, the rate of hydrolytic degradation for these biopolymers is controlled by altering their physical properties; such as their molecular weights, degree of crystallinity and glass transition temperature (T_g) [6–8].

Radiation has been known to alter the physical properties of polymers through main-chain scission and cross-linking [10,11]. According to the principles of radiation chemistry, free radicals are formed when

macromolecules of polymers are excited under ionizing radiation [12], where they are then free to react with one another or initiate further reactions among the polymeric chains, thus giving rise to changes in material properties. The combination of two radicals leads to cross-linking or recombination in the amorphous and crystalline regions, respectively [13], whereas chain transfer and the subsequent splitting results in chain scission [14]. Usually both these processes take place simultaneously for many polymers [15].

Semi-crystalline polymers, such as PLLA, are non-homogenous with a two-phase system consisting of amorphous and crystalline regions. During irradiation, energy is deposited uniformly and radicals are formed throughout the polymer in both the amorphous and crystalline regions [16]. However, crystalline regions consist of chains that were more oriented and closely packed compared to the more open amorphous regions. As a result, oxygen, stabilizers and specific active radical species are excluded from the crystalline phase, and the irradiation chemical reaction paths in the amorphous and crystalline phases will therefore be different. The close proximity of the polymer chains in the crystalline

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structure also encourage trapped free radicals to recombine, thus reducing the number of effective chain scissions, through an effect known as the “cage effect” [17].

Although some work has been reported on the effects of gamma radiation on poly(lactic acid) [18], little is known of the effects of electron beam (e-beam) radiation on the morphological, thermal and degradation behaviour of PLGA and PLLA. This paper reports on the investigation into the effects of e-beam radiation on the morphological, thermal and degradation behaviour of both PLGA (amorphous) and PLLA (semi-crystalline) films, establishes a relationship between the changes in molecular weight and radiation dose of these biopolymers, and suggests the possible radiation degradation mechanisms. The motivation behind this work is to investigate into the use of e-beam radiation as a possible means of controlling the rate of hydrolytic degradation of PLGA and PLLA.

2. Materials and methods

PLGA (80:20) and PLLA were purchased from Purac Far East, Singapore (manufactured by Purac, Holland). Films of PLGA and PLLA were prepared by a solvent casting method, in which the biopolymers are first dissolved in dichloromethane (DCM), purchased from E. Merck, Germany, using a weight ratio of 1:12 (polymer: DCM). The solution was then spread over a glass plate to give a film of approximately 0.7 mm in wet thickness, and the solvent slowly evaporated in air at room temperature for 48 h to prevent the formation of air bubbles. The remaining solvent was then removed in an oven at 40°C for a week.

Thermogravimetric analysis (TGA) conducted on the films show no significant amount of DCM present in the films after drying. The dry thickness of the film was then measured to be about 0.055 mm, which was below the maximum film thickness (0.065 mm) allowed for a 100% full depth penetration of the electron beam.

Each of the PLGA and PLLA film samples was irradiated using the Energy Sciences Inc. (ESI) Electron Beam Accelerator at room temperature (25°C), humidity and in the presence of air. The maximum accelerating voltage used was 175 kV, with the radiation dose ranging from 2.5 to 50 Mrad.

The number and weight average molecular weight (M_n and M_w , respectively) of each of the irradiated sample was determined using the Agilent 1100 series GPC, which was performed at 30°C with 80%-tetrahydrofuran and 20%-DCM as solvents, using Reflective Index Detector (RID) as the detector. The calibration was done in accordance to polystyrene standard with the flow rate at 1 ml min⁻¹.

Ionizing radiation causes the formation and breaking of polymer bonds as a result of intermolecular cross-linking and scission in the polymer. The radiation chemical yields for chain scission (G_s) and cross-linking (G_x), which is defined as the number of such reactions per 100 electron volts of absorbed energy, therefore determines the extent of chain scission or cross-linking during irradiation, and can be calculated from the following equations [19]:

$$1/M_w = 1/M_{w,0} + (G_s/2 - 2G_x)D_x 1.038 \times 10^{-6}, \quad (1)$$

$$1/M_n = 1/M_{n,0} + (G_s - G_x)D_x 1.038 \times 10^{-6}, \quad (2)$$

where $M_{w,0}$ and $M_{n,0}$ are the weight and number average molecular weights of non-irradiated samples. M_w and M_n are the corresponding values following exposure to irradiation dose, D . A ratio of G_s/G_x greater than 4 would indicate that chain scission is more dominant [19].

Changes in the thermal properties and enthalpy of fusion and crystallization were investigated with the use of TA Instrument DSC 2920 Modulated DSC apparatus. The use of the Modulated DSC allows the heat capacity of the sample to be measured in one run with an error of 1% or less. To avoid oxidative degradation, the sample and reference pans were purged with nitrogen at a constant flow rate of 48 ml min⁻¹. The samples were heated from -40°C to 250°C at a scan rate of 5°C min⁻¹, with approximately 5 mg of each sample used in the analysis. The difference between the enthalpy of fusion and the enthalpy of cold crystallization [20] was used as an indirect measurement of the level of sample crystallinity for PLGA with respect to radiation dose. Cold crystallization is defined as the crystallization of the amorphous chains during heating. This enthalpy difference was taken as the true portion that crystallizes upon immediate irradiation. The degree of crystallinity for PLLA was calculated as the percentage of the enthalpy heat of fusion over the enthalpy heat of fusion for a 100% crystalline PLLA ($\Delta H_{f(100\%)} = 95 \text{ J/g}$ [21]).

Wide-angle X-ray diffraction (WAXD) was performed using the Shimadzu XRD-6000 employing Cu K α radiation ($\lambda = 1.5406 \text{ \AA}$), with a thin-film attachment that rotates at a speed of 50 rpm. A scan axis of 2θ was used to obtain diffraction patterns of a scan range between 5° and 40°, with a scan rate of 0.2° deg/min. The θ -fixed angle was kept at 0.5°; and the voltage used was 40 kV and the current was 30 mA. 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 full-width at half maximum (FWHM), B , is related to the mean dimension of crystallites perpendicular to the hkl planes, t , by Scherrer's equation [22],

$$B = 0.9\lambda/t \cos \theta, \quad (3)$$

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. Here, the variation in the FWHM (B) is used as a rough indication of the changes in crystal size as a function of radiation dose.

Small angle X-ray scattering (SAXS) of irradiated and non-irradiated PLGA and PLLA films were obtained using a Bruker AXS Inc. Nanostar system, with Cu $K\alpha$ radiation source ($\lambda = 1.5418 \text{ \AA}$). The samples were analyzed for changes in the SAXS invariant. An X-ray tube voltage of 40 kV and a current of 35 mA were used for all samples. A scan axis of 2θ was used to obtain diffraction patterns of a scan range between 0° and 2.8° , with a scan time of 12 000 s. A vacuum chamber for the incident beam path and scattered beam path was used. A two-dimensional multiwire proportional HI-STAR area detector was used to detect the SAXS signals. One-dimensional diffraction profiles were calculated by averaging the two-dimensional patterns. Corrections were made for sample thickness and sample transmission, and background scattering was subtracted from the SAXS data. The channel numbers of the area detector were calibrated with the use of silver behenate and a rat-tail collagen.

The LabOPhot-2/2a Nikon polarized microscope was used to visually observe changes to the crystals in the polymeric films after irradiation.

The infrared spectra of the films were obtained to verify changes to the structure of PLGA and PLLA before and after irradiation. The Perkin-Elmer system 2000 FTIR was used to characterize the polymeric films. The FTIR spectra were obtained with 16 scans per sample over the range of $4000\text{--}400 \text{ cm}^{-1}$.

3. Results and discussion

PLGA and PLLA films were irradiated with the e-beam source, and the films became brittle to touch with increasing radiation dose. This embrittlement process was presumably due to the degradation of the films as a result of e-beam irradiation.

Fig. 1 plots the number and weight average molecular weight (M_n and M_w , respectively) of the e-beam irradiated PLGA and PLLA samples against radiation dose. The results show a decrease in average molecular weight with increasing radiation dose. This indicates that chain scission is the dominant process upon e-beam irradiation. The values of G_s and G_x for PLGA and PLLA are then obtained using Eqs. (1) and (2), and tabulated in Table 1. The large G_s/G_x ratios, from Table 1, further illustrate the dominance of chain scission in irradiated PLGA and PLLA films, and this thereby

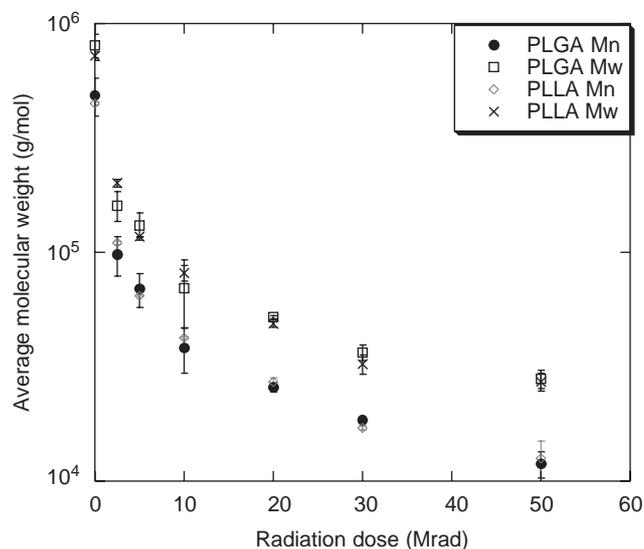


Fig. 1. Number and weight average molecular weight (M_n and M_w respectively) of PLGA and PLLA as a function of radiation dose.

Table 1

Chain scission (G_s) and cross-link (G_x) radiation yields of PLGA and PLLA irradiated in the presence of air

Polymer	G_s	G_x	G_s/G_x
PLGA	0.133	0.006	23.1
PLLA	0.123	0.011	11.0

increases the solubility of these polymers in DCM after irradiation.

It can also be observed, from Fig. 1, that the extent of molecular weight decrease differs for samples irradiated below and above 20 Mrad. The initial radiation doses, up to 20 Mrad, results in a drastic decrease in molecular weight. Subsequently, a more steady decrease in molecular weight was observed for doses greater than 20 Mrad. This difference is due to two different radiation-induced chain scission mechanisms below and above 20 Mrad. The initial drastic decrease in molecular weight is due to backbone main chain scission, where long polymeric backbone chains break into shorter chains, because the energy from the radiation exceeds the attractive forces between the atoms [23]. This happens because the excited states dissipate some of the excess energy by bond scission, resulting in massive formations of alkyl free radicals, within both the amorphous and crystalline regions. However, at higher radiation doses, hydrogen abstraction becomes the key radiation-induced scission mechanism. Due to higher oxygen diffusivity in the amorphous regions, the alkyl free radicals in the amorphous regions react with oxygen to form peroxy free radicals [24,25]. These peroxy free radicals thereby cause chain scission, within the amorphous region and

at the crystal interface, through hydrogen abstraction. Unlike main chain scission, in which a drastic decrease in molecular weight was observed, chain scission through hydrogen abstraction had less pronounced effect on the decrease in molecular weight. Another possibility is that at higher radiation doses, there is a relative increase of cross-linking to chain scission [18]. At such high doses, the number of alkyl free radicals present is larger than the number of peroxy free radicals formed because of limited oxygen diffusion into the polymer. Alkyl free radicals are less effective in causing chain scission compared to peroxy free radicals, and they are more likely to recombine or cross-link in the crystalline and amorphous region respectively.

The ratio of G_s/G_x differs for PLGA and PLLA, as shown in Table 1. The results show that PLGA has a higher G_s/G_x ratio compared to PLLA, indicating that PLGA is more susceptible to e-beam radiation degradation than PLLA. This can be explained in terms of polymer morphology, since PLGA is amorphous and PLLA is semi-crystalline. Due to the close packing of the crystalline structure, the poor diffusion of oxygen into the crystalline region limits the formation of peroxy free radicals and thus, the extent of chain scission. The “cage effect” also encourages the recombination of free radicals in the crystalline region. These factors play an important role in reducing the extent of e-beam degradation in PLLA.

When the reciprocal of the number average molecular weight was plotted against radiation dose, as required in the calculation for G_s and G_x , a strong linear sample correlation coefficient of 0.98 and 0.97 for PLGA and PLLA respectively was obtained. This shows that the decrease in the number average molecular weight can be quantified accurately with respect to radiation dose. Using the least-squares estimate to determine the estimated linear regression equation and the standard error of the estimate [26], the equations obtained for PLGA (Eq. (4)) and PLLA (Eq. (5)) are:

$$1/M_n = 1/M_{n,0} + 1.44 \times 10^{-6}D, \quad (4)$$

$$1/M_n = 1/M_{n,0} + 1.39 \times 10^{-6}D \quad (5)$$

for radiation doses from 2.5 to 50 Mrad, where $M_{n,0}$ is the initial number average molecular weight at 0 Mrad and D is the radiation dose, and the standard error of these estimates are 5.10×10^{-6} mol/g and 6.24×10^{-6} mol/g respectively. These equations will therefore allow for an accurate prediction of the resulting number average molecular weight after irradiation.

Fig. 2 plots the polydispersity index of PLGA and PLLA against radiation dose. The results show an increase in the polydispersity index for both polymers with increasing radiation dose. This increase is due to poorer chain uniformity resulting from chain branching.

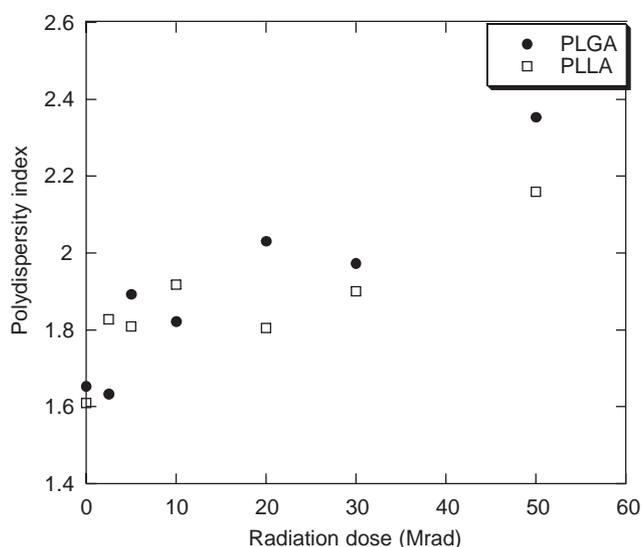


Fig. 2. Change in the polydispersity indices of PLGA and PLLA as a function of radiation dose.

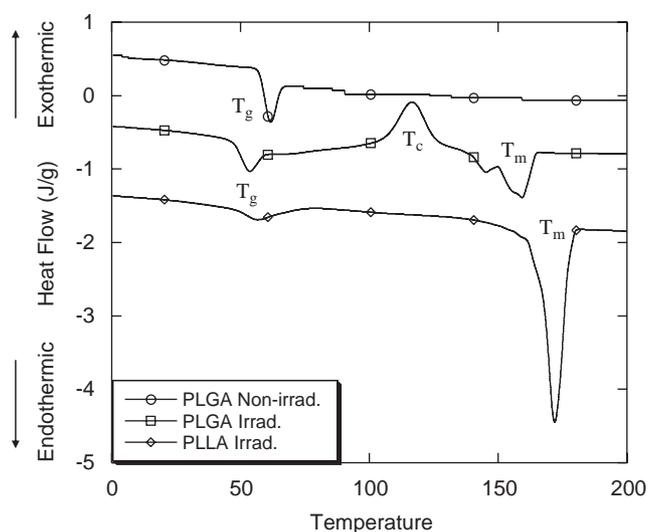


Fig. 3. DSC thermograms of PLGA and PLLA films irradiated at 10 Mrad.

Chain branching arose from the cross-linking or recombination of free radicals. The high polydispersity index at 50 Mrad indicates extensive chain branching at this radiation dose.

Chain scission due to e-beam irradiation results in changes to the physical properties in both PLGA and PLLA. Fig. 3 shows the DSC thermograms for PLGA and PLLA films irradiated at 10 Mrad. The thermogram for the non-irradiated PLGA shows only a glass transition (T_g), whereas the irradiated PLGA thermogram shows additional cold crystallization (T_c) and melting peaks (T_m).

Fig. 3 also shows two differences between the thermograms of the irradiated PLGA and irradiated

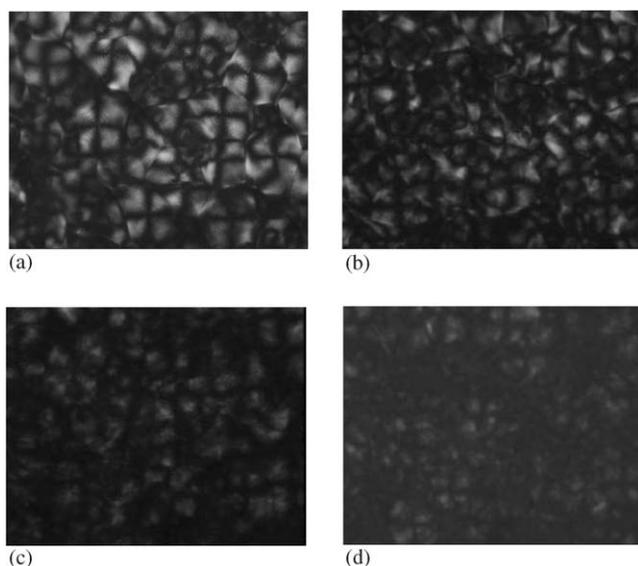


Fig. 4. Polarizing optical microscope pictures ($\times 420$) of irradiated PLLA crystals at (a) 0 Mrad (b) 10 Mrad (c) 20 Mrad and (d) 30 Mrad.

PLLA. Firstly, the thermogram for PLLA, unlike PLGA's, did not show a T_c peak and secondly, PLGA displayed two distinct T_m peaks, whereas only one T_m peak was observed for PLLA. The absence of the T_c peak in PLLA is due to the significant decrease of its semi-crystalline morphology. Fig. 4(a) shows a polarizing optical microscope picture of the characteristic Maltese cross pattern for PLLA spherulites. The presence of these existing crystals allows chain folding and continuum crystal growth, thereby eliminating the need for crystal nucleation and thus the absence of a cold crystallization peak. The formation of two T_m peaks in PLGA is due to the structure of the chains after irradiation. One possible reason is that the recombination of free radicals can result in a linear-chained or a branch-chained polymer. The linear PLGA chains allows for closer packing, which explains for the higher melting temperature, and the lower melting peak for the more branch-structured PLGA chains. Another possible reason is that the irradiation of PLGA at room temperature in the presence of oxygen produces two different peroxy free radicals as determined by Montanari et al, as shown in Fig. 5 [25]. Each of these free radicals can recombine with other PLGA chains to give chains of differing molecular structures, and different packing capabilities.

Fig. 6 plots the difference in the enthalpy of fusion and enthalpy of cold crystallization of PLGA against radiation dose. The results, used as an indirect measurement of the degree of crystallinity (DOC), show an increase in the DOC of PLGA with increasing radiation dose. Chain scission during e-beam irradiation gives rise to shorter polymer chains, which have better mobility and are able to align themselves and pack more

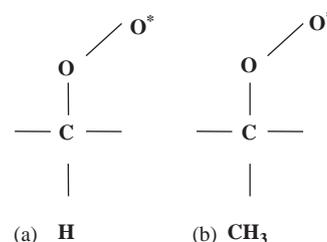


Fig. 5. Proposed free radicals formed during irradiation of PLGA (a) peroxy free radical with hydrogen side group (b) peroxy free radical with methyl side group [25].

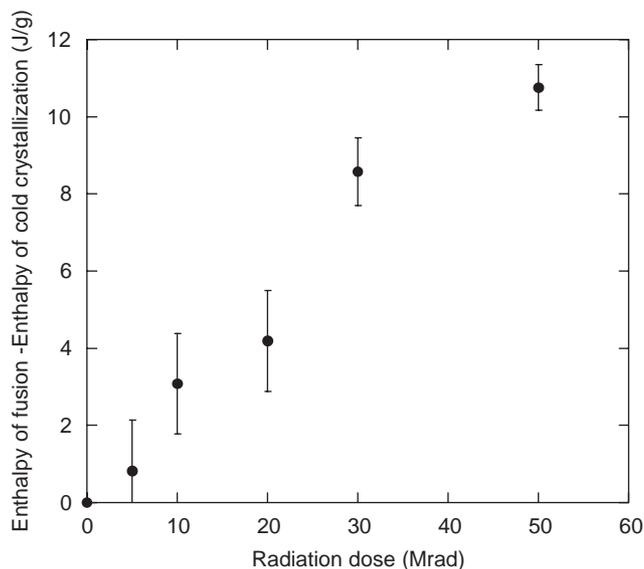


Fig. 6. Change in the difference between enthalpy of fusion and enthalpy of cold crystallization of PLGA films as a function of radiation dose.

easily to form a crystalline phase, and thus also resulting in the formation of a T_c peak (Fig. 3). With increasing radiation dose, more chains are scission, promoting crystallization and increasing the degree of crystallinity in PLGA.

The change in the degree of crystallinity (DOC) of PLLA with increasing radiation dose is shown in Fig. 7. The results obtained from the MDSC and WAXD show similar trends, where an initial increase of DOC (up to 5 Mrad) and a subsequent decrease in DOC was observed. The initial increase in DOC is due to the re-orientation of the shorter chains of PLLA, and the subsequent decrease in the DOC above 5 Mrad is due to the recombination of the free radicals within the crystalline regions. Due to the close pack structure of crystals in PLLA, the free radicals within the crystalline regions are encouraged to undergo recombination, resulting in more branched and non-uniformed chains of PLLA, as observed from the increase in the polydispersity index (Fig. 2), which have poorer packing capabilities. The decrease in DOC is also attributed to crystallinity damage [27] as observed from the polarizing

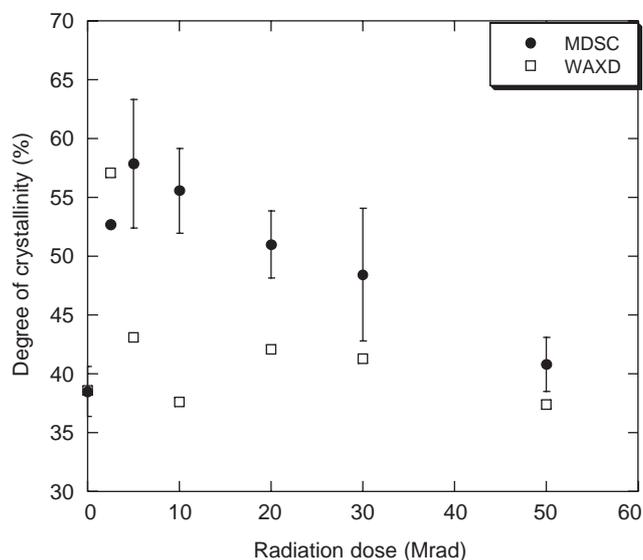


Fig. 7. Change in degree of crystallinity of PLLA films as a function of radiation dose.

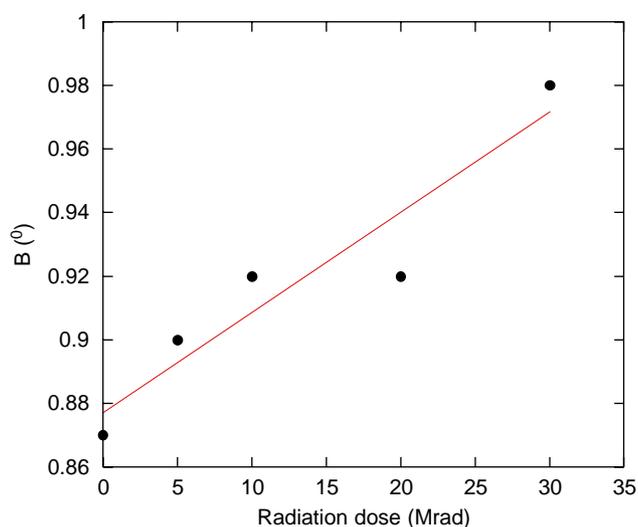


Fig. 8. Change in FWHM (B) of PLLA as a function of radiation dose.

optical microscope pictures in Fig. 4. Crystallinity damage occurs due to peroxy free radicals attacking and destroying the crystalline integrity of the crystals beginning from the crystal interface and moving towards the core of the crystal. Fig. 8 plots the FWHM (B) of PLLA against radiation dose. The increase in B therefore confirms that radiation-induced crystallinity damage results in a decrease in crystal size.

The thermal properties of PLGA and PLLA are affected by chain scission. Fig. 9 plots the cold crystallization temperatures (T_c) of PLGA against radiation dose. The results show that the T_c decrease with increasing radiation dose. Fig. 10 plots the glass transition temperatures (T_g) of PLGA and PLLA against radiation dose. The results show a decrease in

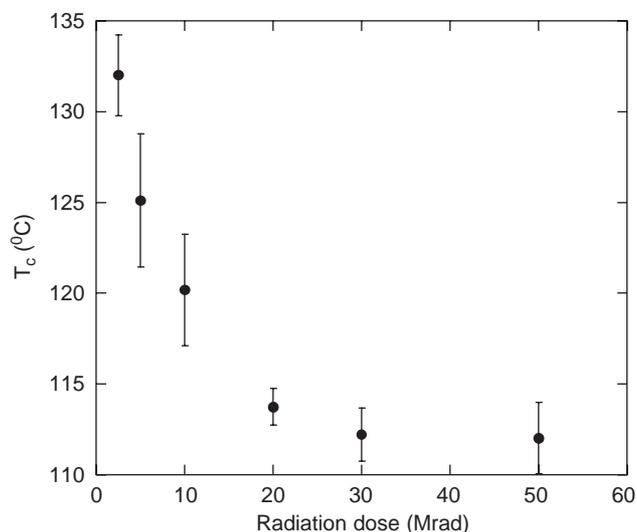


Fig. 9. Cold crystallization temperature (T_c) of irradiated PLGA films as a function of radiation dose. A heating rate of $5^\circ\text{C}/\text{min}$ was used.

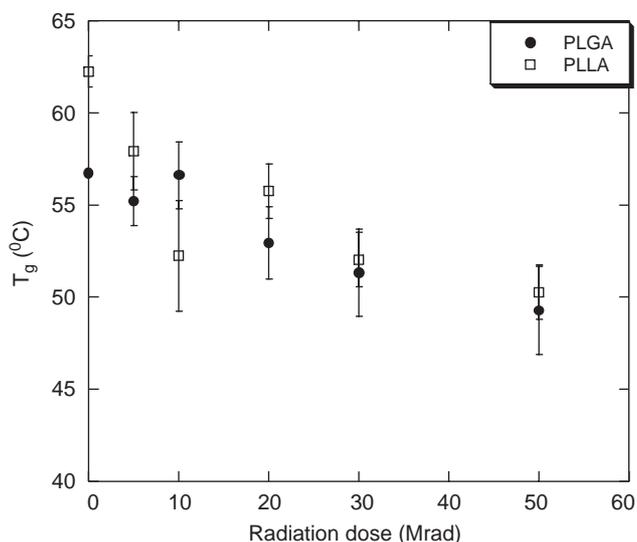


Fig. 10. Glass transition temperature (T_g) of PLGA and PLLA as a function of radiation dose. A heating rate of $5^\circ\text{C}/\text{min}$ was used.

T_g with increasing radiation dose. These observations further confirm the occurrence of chain scission in the amorphous regions during e-beam irradiation, which result in less chain entanglement and an increase in chain mobility. As a result, lesser energy is required to re-orientate these shorter amorphous chains, and thus also decreasing the T_c (Fig. 9).

Fig. 11 plots the melting temperatures (T_m) of PLGA and PLLA against radiation dose. The results show a decrease in T_m with increasing radiation dose for both PLGA and PLLA. For PLGA, the existence of a T_m is largely due to the melting of the re-crystallized short amorphous chains at its cold crystallization temperature. The decrease in its T_m implies poorer packing capabilities of these crystallized chains, due to chain

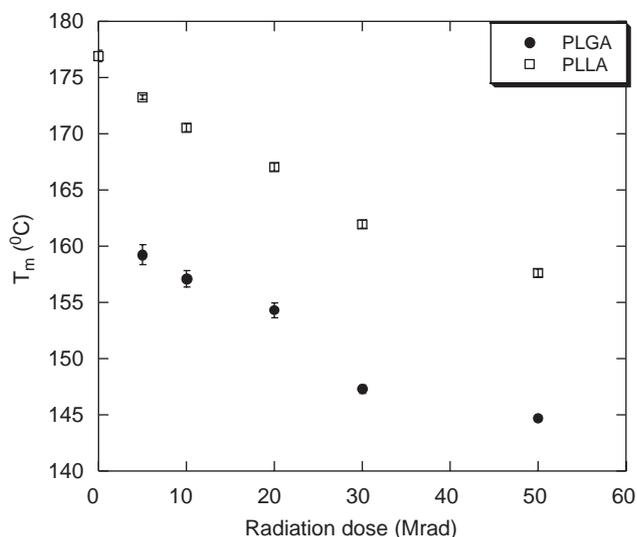


Fig. 11. Melting temperature (T_m) of PLGA and PLLA as a function of radiation dose. A heating rate of $5^{\circ}\text{C}/\text{min}$ was used.

branching and lesser chain uniformity as a result of free radical recombination. The decrease in T_m for PLLA is likewise due to extensive chain branching and the decrease in its crystal size with increasing radiation dose (Fig. 8).

The plot of the SAXS invariant of PLLA with radiation dose is shown in Fig. 12. The results show an initial increase in the invariant. In a 2-phase system with no voids, the invariant is related to the square difference between the electron densities of the amorphous and crystalline phase, as shown [28],

$$I = \omega_a \omega_c (\rho_a - \rho_c)^2, \quad (6)$$

where ω_a and ω_c are the weight fractions of the amorphous and crystalline phase respectively, and ρ_a and ρ_c are their electron densities. From the crystallinity values obtained from Fig. 7, it can be determined that although the individual values of ω_a and ω_c are changing, their product ($\omega_a \omega_c$) remains relatively constant. Therefore, the increase in invariant in irradiated PLLA can be interpreted as due to an increase in the second term of Eq. (6). This increase in electron density difference between the two phases is probably due to the formation of peroxy free radicals by the entering of oxygen into the amorphous region [29], resulting in the difference in bond structures of the amorphous and crystalline chains.

The changes in electron densities between the amorphous and crystalline regions indicate changes to their respective bond structure due to irradiation. Chain scission of PLGA and PLLA results in similar changes in their FTIR spectra; and therefore, only the changes in the FTIR spectra of irradiated PLGA will be discussed. Fig. 13 plots the % transmittance of infrared against the infrared frequency for the non-irradiated and 50 Mrad

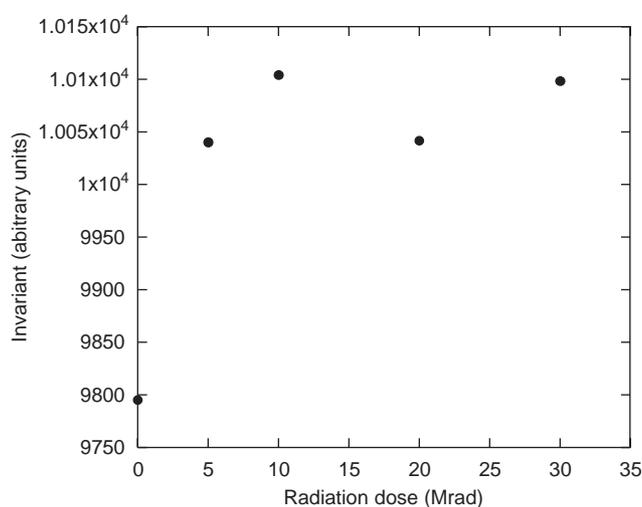


Fig. 12. Change in SAXS invariant of PLLA as a function of radiation dose.

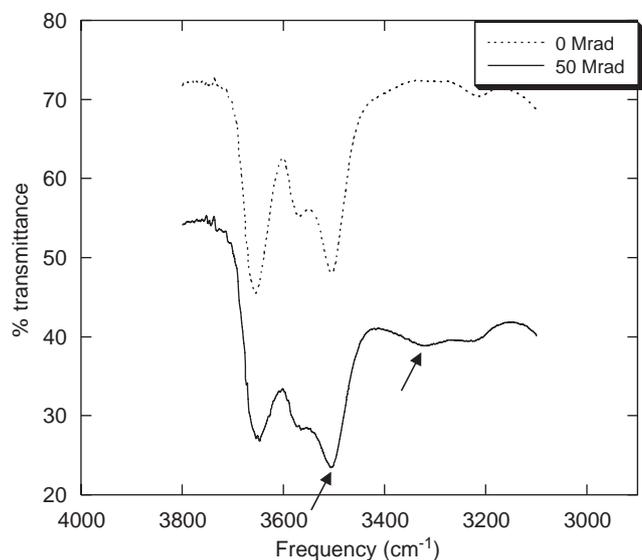


Fig. 13. FTIR spectra of irradiated and non-irradiated PLGA films showing formation of alcohol groups upon irradiation.

irradiated PLGA samples. Irradiation resulted in two differences between the non-irradiated and irradiated samples. One is the formation of a new peak at 3320 cm^{-1} and the other is the increase in peak intensity for peak 3505 cm^{-1} . The formation and increase in peak intensity corresponds to an increase in the alcohol groups formed during irradiation, though a small contribution could also be from water and hydroperoxides produced in the course of oxidation. The formation of these alcohol groups is the result of the peroxy free radicals undergoing degenerate chain branching, giving rise to the formation of alcohol groups as the main termination product.

The degradation of PLGA and PLLA emitted carbon dioxide gas, which was trapped in the irradiated films.

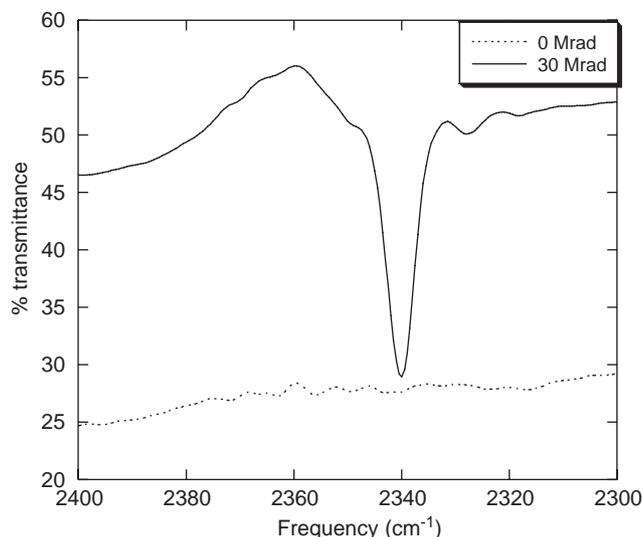


Fig. 14. FTIR spectra of irradiated and non-irradiated PLGA films showing the formation of a carbon dioxide peak upon irradiation.

The plot of % transmittance of infrared against the infrared frequency for the non-irradiated and 30 Mrad irradiated PLGA samples shows the formation of a peak at 2340 cm^{-1} , as shown in Fig. 14. This trapped carbon dioxide gas was only found in all the freshly irradiated samples.

4. Conclusion

PLGA and PLLA degrade through chain scission when exposed to e-beam irradiation in the presence of air, through two main degradation mechanisms, namely backbone main chain scission and chain scission by hydrogen abstraction. This causes a decrease in the average molecular weight (M_n and M_w), and thus a corresponding decrease in the T_c , T_g and T_m of the biopolymers. The decrease in molecular weight was observed to follow a linear relationship with radiation dose. E-beam irradiation causes the formation of a crystalline phase in PLGA but a decrease in crystallinity for PLLA. The semi-crystalline nature of PLLA is the key factor in its greater stability to e-beam radiation as compared to PLGA. Since e-beam radiation decreases the molecular weights and alters the thermal and morphological properties of PLGA and PLLA in a predictable and fairly accurate manner, e-beam radiation can therefore be a potential tool to control the rate of hydrolytic degradation of these biopolymers.

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