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An EPR and ENDOR study of γ- and β-radiation sterilization in poly (lactide-co-glycolide) polymers and microspheres

James A. Bushell [a], Mike Claybourn [b,*], Helen E. Williams [b], Damien M. Murphy [a,*]

[a] School of Chemistry, Cardiff University, Main Building, Park Place, Cardiff CF10 3AT, UK
[b] AstraZeneca, Silk Road Business Park, Charterway, Macclesfield, Cheshire SK10 2NA, UK

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Abstract

EPR/ENDOR spectroscopy was used to characterise the free radicals generated in a series of PLGA raw polymers and microspheres (with lactide : glycolide compositions of (75 : 25), (65 : 35) and (50 : 50)) after exposure to gamma (γ-) and electron beam (β-) irradiation at room temperature. Both sets of irradiated samples produced analogous EPR spectra, indicating that the type and distribution of free radicals generated by γ-irradiation are similar to those generated by β-irradiation. The radicals were identified by EPR simulations as the chain scission species –CHO–($\approx$ 27% abundance),–$C(CH_3)O$– ($\approx$ 23% abundance) and the terminal–$C(CH_3)$–OR fragment ($\approx$ 50% abundance), and these assignments were supported by the ENDOR analysis. The latter two radical species were demonstrated to originate from the lactide component of the PLGA polymer. Overall systematically higher radical concentrations were found as the lactide content of the PLGA raw polymer and microspheres increases (ie., 75 : 25 > 65 : 35 > 50 : 50) for both γ- and β-irradiation. However, while the relative concentrations of free radicals was similar in the raw polymer samples after exposure to γ- or β-irradiation, a substantial difference was found for the microsphere samples; an approximate doubling of the radical content was found in the γ-irradiated PLGA microspheres compared to the identical β-irradiated microspheres.

Keywords: Radiation; EPR; ENDOR; PLGA; Radicals; Sterilisation

1. Introduction

Controlled release drug delivery technology has been expected to become a highly lucrative prospect for the pharmaceutical industry [1], with some estimates on profits being up to double the estimated market value of oral delivery technologies. This high value can be attributed to the potentially high benefits of these systems. Increased appreciation of manipulating pharmacokinetics and pharmacodynamics can result in more efficacious drugs. Perhaps equally important, several of these products have been shown to dramatically improve patient compliance, currently a huge cost to the health care sector [1]. Some of the most effective carriers for drug

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* Corresponding authors.

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delivery systems are prepared from thermoplastic aliphatic polyesters such as poly (lactic acid) (PLA), poly (glycolic acid) (PGA) and their co-polymers (PLGA) [2]. These polymers have several favourable properties including biodegradability, biocompatibility, mechanical strength as well as regulatory approval, making them suitable for a wide range of pharmaceutical applications.

It is essential that drug delivery systems meet the pharmaceutical requirements of sterility. For heat sensitive products, such as PLGA (Tg ~40 °C), ionising radiation is the preferable sterilization method. However, even at the minimal sterilization dose of 25 kGy radiation has been shown to cause degradation as well as morphological changes to the polymeric system and may cause changes to any drug formulation [3]. The majority of research concerning the irradiation of these polymers has focused on the effects of γ-rays emitted from a nuclear source (i.e., ^{60}Co). However, due to favourable practical properties such as dosage rate and cost, electron beam (β-) irradiation is likely to be used more in the future. It has been reported that drug release rates (bupivacaine) are less affected by β- than γ-radiation [4]. Ionisation radiation effects, whether they are caused by a γ or β source, are essentially the same. The main effect on polymers is degradation via main-chain scission and cross-linking [5,6]. For PLGA the dominant effect has generally been seen to be chain scission leading to degradation manifested as a reduction in molecular weight [3,7,8]. Although the main radiation reactions are essentially the same for γ- and β-irradiation, there are differences between the methods of practical operation. The time scales of each reaction are quite different due to the inherent nature of each radiation source. γ-rays are characteristically highly penetrating, with a low dose rate (kGy/hour) whereas β-rays offer lower penetration but at a significantly higher dose rate (kGy/s) [7].

EPR techniques have previously been utilized in the study of irradiated polymers [9–11], including the effect of γ-irradiation on poly (lactide-co-glycolide) [12–15]. It has been documented that γ-irradiation of PLGA leads to the formation of radical species from both the lactide and glycolide components of the polymer [16,17]. It is indicated that polymers with free -COOH end groups are more sensitive to γ-irradiation than polymers with esterified -COOH end groups, and that γ-irradiation affects the raw polymer in a different manner to its processed counterpart microspheres [8,15]. However, at the present time there is no information concerning the nature of the radicals produced by electron beam irradiation of PLGA. Therefore, in the current work, we have undertaken a systematic characterisation by Electron Paramagnetic Resonance (EPR) and Electron Nuclear DOuble Resonance (ENDOR) of the free radicals generated in a range of PLGA polymers after exposure to γ- and β-radiation.

2. Methods and materials

2.1. Materials

The 50 : 50 raw polymer was supplied by *Alkermes Inc*., Cincinnati, USA and the 65 : 35 and 75 : 25 raw polymers were supplied by *Birmingham Polymers Inc*., Alabama, USA. Microspheres were manufactured and supplied by *AstraZeneca* in the size range 25–125 mm. The lactide:glycolide compositions used were: (75 : 25), (65 : 35) and (50 : 50) and selected according to their desired physical properties. The samples were analysed in unprocessed and processed microsphere form. The microspheres were prepared from a polymer–dichloromethane gel and hardened via solvent extraction. The polymers were amorphous and were found to have glass transition temperatures (Tg) of around 40 °C as verified by modulated DSC.

2.2. β- and γ-irradiation of unprocessed and microspheric PLGA samples

Samples for irradiation experiments (*ca*. 0.2 g) were evacuated in a quartz tube to a residual base pressure of 10^{-4} Torr using a conventional vacuum manifold. Samples were sealed and irradiated at ambient temperatures (~30 °C). γ-irradiation was performed using a commercial ^{60}Co source (*Isotron, UK*) to a dose of 26.6 kGy. β-irradiation was performed using a 4.5 MeV electron beam accelerator (*Isotron, UK*) to a dose of 32.1 kGy. Irradiated samples were subsequently transferred under a glove box into a 4 mm OD quartz tube for EPR/ENDOR analysis.

2.3. EPR and ENDOR spectroscopy

For EPR or ENDOR analysis, *ca.* 0.1 g of polymer was used in a suprasil EPR tube. The X-band EPR spectra were recorded at various microwave powers (0.1 mW to 100 mW) and at a range of temperatures (120 to 298 K). The EPR spectra were recorded on a *cw*-Bruker EMX spectrometer operating at 100 kHz field modulation and equipped with an ER-4119HS high-sensitivity cavity. EPR spectra were simulated using the *SimEPR32* programme [18]. The X-band ENDOR spectra were recorded on a *cw*-Bruker ESP 300E series spectrometer operating at 12.5 kHz field modulation and equipped with an ESP360 DICE ENDOR unit in an EN-801 ENDOR cavity. The ENDOR spectra were recorded at 130 K, using 8 dB RF power from a ENI A-300 RF amplifier, 251 kHz RF modulation depth and 5 mW microwave power.

In the EPR technique, microwave induced transitions between the two non-degenerate electron spin manifolds ($M_S = \pm ½$) are detected in the presence of an external applied magnetic field, producing an EPR spectrum with magnetic field units. In the ENDOR technique, the changes to the partially saturated EPR transition in the presence of an intense radiofrequency field, which induces transitions between the non-degenerate nuclear spin manifolds (eg., $M_I = \pm ½$ for a proton), are detected producing a spectrum in frequency units.

3. Results and discussion

3.1. Polymer analysis

The polymers were analysed for verification of the quality of the materials. They were analysed both before and after irradiation for: water content (Karl Fischer Analysis), molecular weight (Gel Permeation Chromatography) and Tg (Modulated DSC). The results are shown in Table 1. As expected a general decrease was seen in the molecular weight of the samples on irradiation that is more evident in the Mn than Mw results. No considerable change was seen in the Tg or water content of the samples on irradiation. No significant differences were seen between the properties of the γ- or β-irradiated samples. A detailed investigation of the physicochemical characteristics of these polymers, including SEM images, particle size data, acid end group analysis, residual monomer content, residual solvent content, etc., will be reported elsewhere [19].

Table 1

Polymer composition	Mn/Da	Mw/Da	Pd	Water content/ %w/w	Tg Midpoint/ °C
50:50 (RP–NI)	3845	7100	1.85	0.86	33.9
50:50 (RP-γ)	3380	6950	2.06	0.27	37.6
50:50 (RP-β)	3230	6940	2.15	0.27	37.9
50:50 (MS–NI)	3225	6710	2.08	0.48	40.5
50:50 (MS-γ)	3110	6810	2.19	0.33	39.6
50:50 (MS-β)	3130	6660	2.13	0.33	38.6
65:35 (RP–NI)	5475	10,700	1.96	0.46	37
65:35 (RP-γ)	5410	10,250	1.9	0.28	38.2
65:35 (RP-β)	4645	9740	2.1	0.29	40.3
65:35 (MS–NI)	3380	6395	1.89	0.61	41.9
65:35 (MS-γ)	3350	6490	1.94	0.42	42.3
65:35 (MS-β)	3030	6130	2.02	0.3	44.6
75:25 (RP–NI)	4160	7980	1.92	0.47	39
75:25 (RP-γ)	3535	7935	2.25	0.44	39.6
75:25 (RP-β)	3605	7570	2.1	0.27	41
75:25 (MS–NI)	3515	7320	2.09	0.71	42.7
75:25 (MS-γ)	3660	7155	1.96	0.65	38.3
75:25 (MS-β)	3380	7270	2.15	0.3	40.2

RP = Unprocessed *R*aw *P*olymer; *MS* = *M*icro*S*phere Polymer.
NI = *N*on-*I*rradiated; γ = Gamma irradiated; β = Electron Beam Irradiated.

3.2. EPR Analysis of γ- and β-irradiated PLGA samples.

The EPR spectra of PLGA (65 : 35) raw polymer and microspheres after exposure to γ- and -β rays are shown in Fig. 1. Qualitatively, all the spectra display a very similar profile. For example, the spectrum of the γ-irradiated raw polymer (Fig. 1a) is *qualitatively* analogous to the spectrum obtained after exposure to β-rays (Fig. 1b). This indicates that the type and distribution of radicals formed via γ- or β-radiation is the same in both raw polymers and microspheres.

It should be noted, that in a previous paper from our group on γ-irradiated PLGA, the samples employed in the investigation were subsequently found be loaded with a drug and were therefore not pure PLGA polymer as incorrectly reported at that time [13]. Further investigations revealed that the unusual profile of the room temperature spectrum [13] was actually due to a spin transfer phenomena taking place from the irradiated PLGA polymer to the loaded drug, analogous to that reported by Faucitano et al. [12] leading to the formation of the correctly reported alkyl and alkyl peroxy radicals [13]. This explains the different profiles of the pure γ- or β-irradiated PLGA raw polymers and microspheres in this work, compared to the drug-loaded PLGA microspheres in our previous work [13].

It is well known that ionising radiation induces dose dependent cross linking and/or chain scission processes in the polymer, with a simultaneous alteration in physical properties of the polymer as observed in Table 1. Chain scission seems the more likely mechanism due to the reduction in molecular weight seen on irradiation of the samples. The radiolytic degradation mechanism of PLGA has been investigated previously, [12,15,17] and it is well known that chain scission processes dominate at low temperatures (eg., 77 K). This results in a wide range of reactive primary and secondary radical species which are visible by EPR. However at elevated temperatures, as in the ambient conditions studied here, H abstraction reactions by the primary radicals occur extensively at the tertiary and secondary C–H bonds on the polymer backbone. This produces a narrower range of relatively stable radicals, such as –CHO– and –C(CH_3)O– which are visible at ambient temperature. It was also recently suggested, [15,17] that C–H bond rupture can also occur at sites α to ether or OH groups producing –C(CH_3)–OH and/or

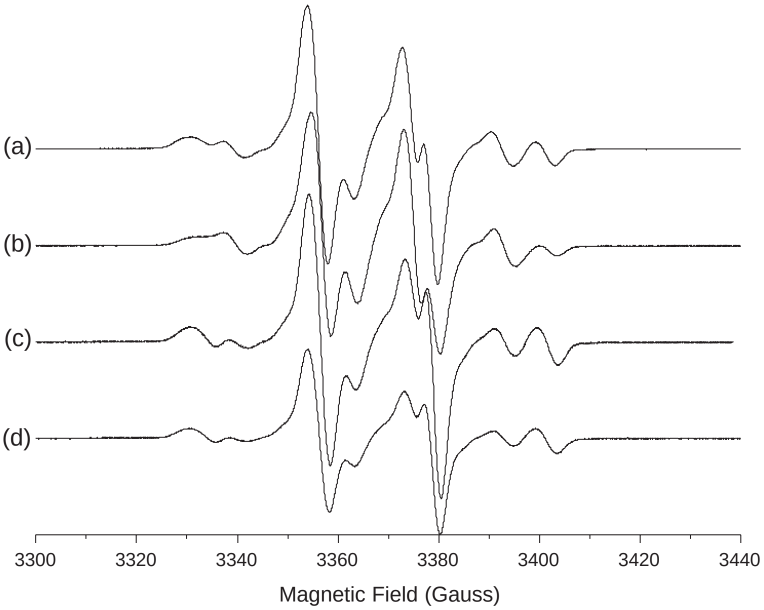


Fig. 1. *cw*-EPR spectra (recorded at 120 K and 0.1 mW) of PLGA (65 : 35) unprocessed raw polymer and microspheres after exposure to γ-(26.6 kGy) and β-beam (32.2 kGy) radiation: (a) γ-irradiated raw polymer, (b) β-irradiated raw polymer, (c) γ-irradiated microspheres, and (d) β-irradiated microspheres.

–$C(CH_3)$–OR which are also visible in the room temperature EPR spectra.

To evaluate the nature of the radicals formed in the irradiated PLGA samples, computer simulations of the spectra were undertaken. The resulting experimental and simulated profile of a representative EPR spectrum (the γ-irradiated PLGA (65 : 35) raw polymer) is shown in Fig. 2.

The EPR spectrum was simulated based on the presence of three major radical species. Radical 1 produces an anisotropic doublet due to the –CHO– fragment ($g_{iso}=2.001$, $a_1=12G$, $a_2=17G$ and $a_3=22G$; Abundance ≈ 27%), radical 2 produces a slightly asymmetric quartet due to the –$C(CH_3)O$– fragment ($g_{iso}=2.0018$, $a_1=24G$, $a_2=24G$ and $a_3=21G$; Abundance ≈ 23%) while radical 3 produces a slightly asymmetric quartet due to the terminal –$C(CH_3)$–OR fragment ($g_{iso}=2.0023$, $a_1=20G$, $a_2=20G$ and $a_3=17G$; Abundance ≈ 50%). The structures of the radicals are illustrated below in Scheme I. For α-protons, as in the –CHO– fragment, a large degree of anisotropy is expected in the hyperfine coupling, based on a predicted hyperfine tensor of ½a, a and $\frac{3}{2}a$ (where a is the isotropic hyperfine coupling term). For β-protons, as in the –$C(CH_3)O$–fragment, the hyperfine anisotropy is substantially less and originates from the difference in angle between the carbon orbital hosting the unpaired electron and the 1 s orbital of the interacting proton. As a result of the amorphous nature of the sample, a broad EPR pattern is produced, displaying all possible orientations (and hence anisotropy) of the α- and β-protons, which have been accommodated in the simulation, producing an excellent fit.

The detailed mechanistic considerations by which these radicals are generated by ionising radiation in PLGA have been discussed elsewhere, [15,17] and does not need to be described further here. However, it should be noted that the –$C(CH_3)O$– and –$C(CH_3)$–OR species are associated with the lactide component of the co-block polymer, while the stable –CHO–radical is associated with the glycolide component of the polymer (see the scheme on the next page). Therefore, the individual EPR peaks associated with these radicals should vary slightly depending on the lactide:glycolide ratios. To investigate this influence of lactide : glycolide ratio on radical distribution, three different ratios of raw PLGA (75 : 35, 65 : 35 and 50 : 50) were γ-irradiated to an identical dose. The resulting EPR spectra are shown in Fig. 3a–c, respectively. The relative intensities of the visible peaks due to radicals 2

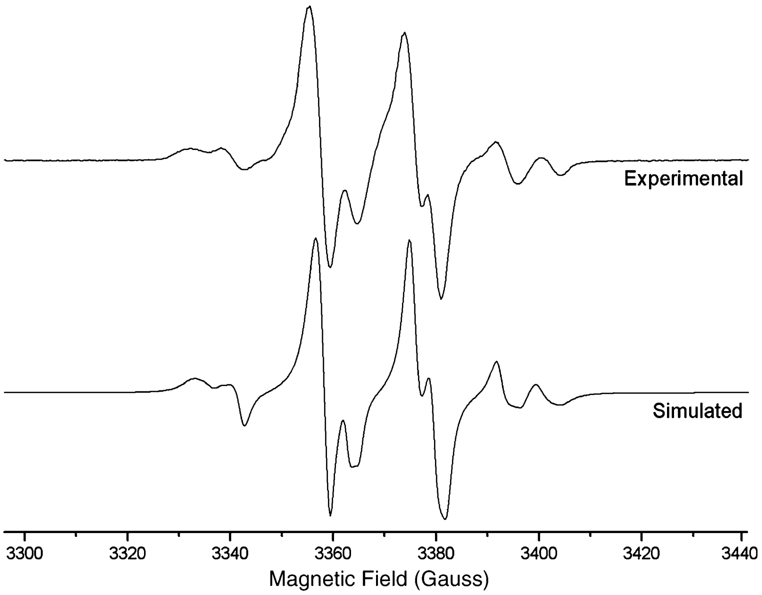
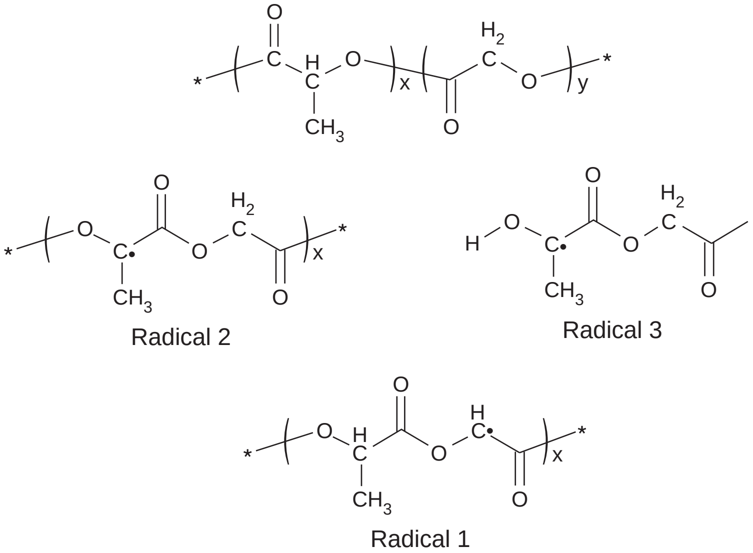


Fig. 2. Experimental and simulated EPR spectra of γ-irradiated PLGA (65 : 25) raw polymer.



Scheme 1. Schematic representation of the PLGA structure and radicals.

and 3 (from the lactide component) decrease more rapidly compared to the peaks associated with radical 1 (from the glycolide component), as the lactide content decreases. The peaks responsible for these three radicals are labelled in Fig. 3 as peaks 1, 2 and 3 corresponding to radicals 1– 3, respectively.

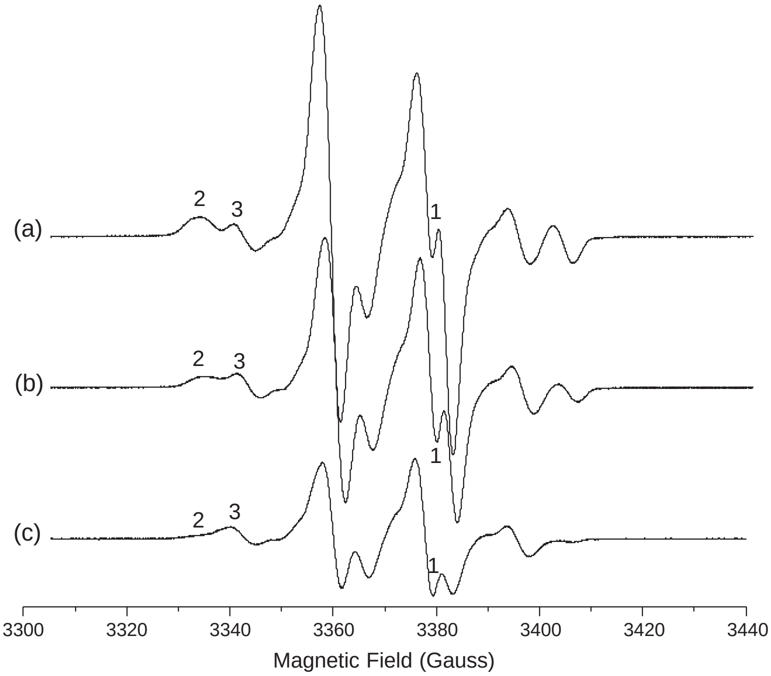


Fig. 3. *cw*-EPR spectra (recorded at 120 K and 0.1 mW) of unprocessed PLGA raw polymers, (a) (75 : 25), (b) (65 : 35) and (c) (50 : 50), after exposure to 26.6 kGy γ-rays.

3.3. ENDOR analysis of γ- and β-irradiated PLGA samples

In many instances, very small hyperfine couplings to remote nuclei cannot be detected in the EPR spectrum, owing to the intrinsically large linewidth of the signals in the amorphous polymer (*ca*. 7 MHz). Such weak couplings can however be resolved in the corresponding ENDOR spectrum, where the resolution enhancement is provided through the considerably narrower line widths (*ca*. 0.5 MHz) and the field dependencies of the ENDOR spectra. Therefore, while the EPR spectrum is composed of a multiplicity of overlapping lines from different signals, the ENDOR spectrum can reveal the hyperfine couplings from a specific radical species if the correct magnetic field position is selected. According to the EPR simulation shown in Fig. 2, the peak component at 3430.5G (position I) should be dominated by couplings due to the –C(CH_3)–OR radicals, while the peak at 3410.5G (position II) should contain an overlapping mixture of signals from –CHO–, –C(CH_3)O– and –C(CH_3)–OR.

The *cw*-ENDOR spectra of a representative γ-irradiated PLGA (65:35) raw polymer sample are shown in Fig. 4. The spectra were recorded at the two magnetic field positions marked I and II. The spectrum at position I is composed of a single broad proton coupling of *ca*. 1 MHz, since it is centred on the nuclear Larmor frequency of the proton ($\nu_H = 14.606$ MHz at 3430.5G). The second spectrum obtained at position II contains two distinct protons couplings of *ca*. 1 MHz and a smaller coupling of *ca*. 0.2 MHz, both of which are centred on the nuclear Larmor frequency of the proton ($\nu_H = 14.5211$ MHz at 3410.5G). These two couplings represent two chemically distinct protons environments which interact with the unpaired electron. The larger coupling can be assigned to the weak hyperfine interactions from remote protons of the α-alkoxyl or α-hydroxyl carbon radicals (ie., the protons in the terminal –OH or –OR groups), while the 0.2 MHz coupling most likely originates from distant proton interactions in the surrounding matrix (known as a matrix ENDOR peak). Although poorly resolved, the *cw*-ENDOR spectra support the assignment of the radical structures proposed in Scheme I.

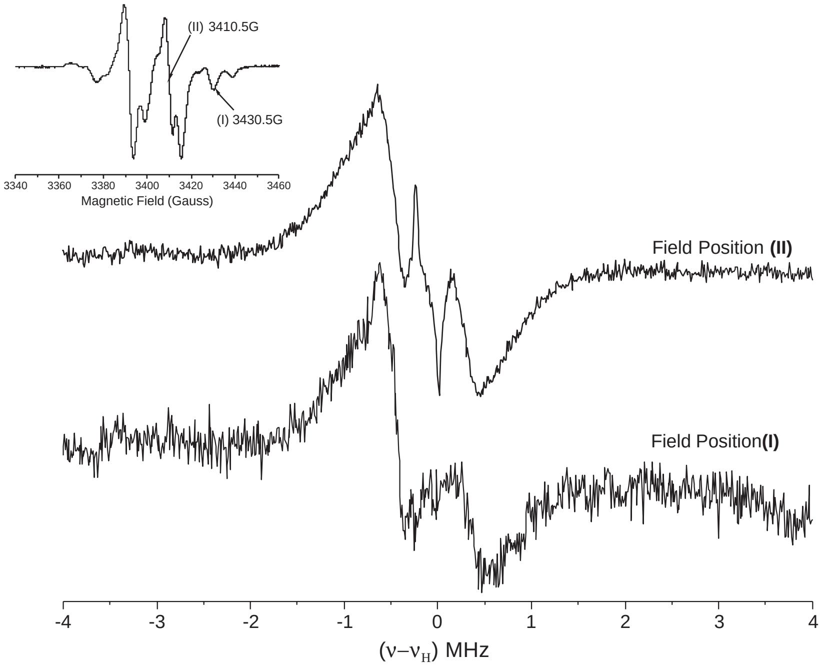


Fig. 4. *cw*-ENDOR spectra of γ-irradiated PLGA (65:35) raw polymer recorded at the magnetic field positions of I (3430.5G) and II (3410.5G).

3.4. Quantitative consideration of radicals

Radical concentration can be measured through double integration of the EPR spectra. The integrated values for the range of γ- and β-irradiated polymers are shown in Fig. 5. The graph clearly shows that radical content is related specifically to the lactide:glycolide ratio. Since a thorough spectroscopic, chromatographic, thermal and particle analysis characterisation of the samples did not reveal any differences between the polymers from the two different suppliers [19], the possibility of impurities or variations in the manufacturing process, can be excluded as being responsible for the differences in radical content. We find systematically higher radical concentrations as lactide content increases (ie., 75 : 25 > 65 : 35 > 50 : 50). This observation is in agreement with the earlier EPR results (Fig. 3), revealing the increase in abundance of Radicals 2 and 3 (which are associated with the lactide component of the polymer) with increasing lactide concentration.

The graph also reveals important differences in the relative abundance of the radicals generated via γ-radiation compared to β-radiation. In particular, the concentration of radicals appears to be systematically less for the samples (both raw polymer and microspheres) exposed to β-radiation compared to γ-radiation. Furthermore, in the case of the microsphere samples, this difference is quite dramatic, with at least a halving of the radical concentrations in the β-irradiated microspheres (columns (d) in Fig. 5) compared to the γ-irradiated microspheres (columns (c) in Fig. 5). It should also be noted that the final γ-ray dosage (26.6 kGy) was even less that the β-ray dosage (32.1 kGy), and despite this, the concentration of radicals formed is higher in the γ irradiated samples. This difference in radical abundance between the γ- and β-irradiated microspheres cannot be rationalised simply in terms of the radiation mechanisms involved, since the mechanism is known to be similar in both cases. While the penetration depth of the β-radiation is far less than γ-radiation, and the radiation exposure time is also substantially less for the equivalent dosage (ie., minutes of exposure time required for β-radiation versus hours for γ-radiation), these radiation effects alone cannot adequately explain the difference in radical abundances encountered in the γ- and β-irradiated microspheres. This is further supported by the fact that the difference in radical concentrations in the γ- and β-irradiated *raw polymers* (ie., columns (a) and (b) in Fig. 5) were not substantially different (ie., the dramatic difference in concentration is only observed in the microspheres).

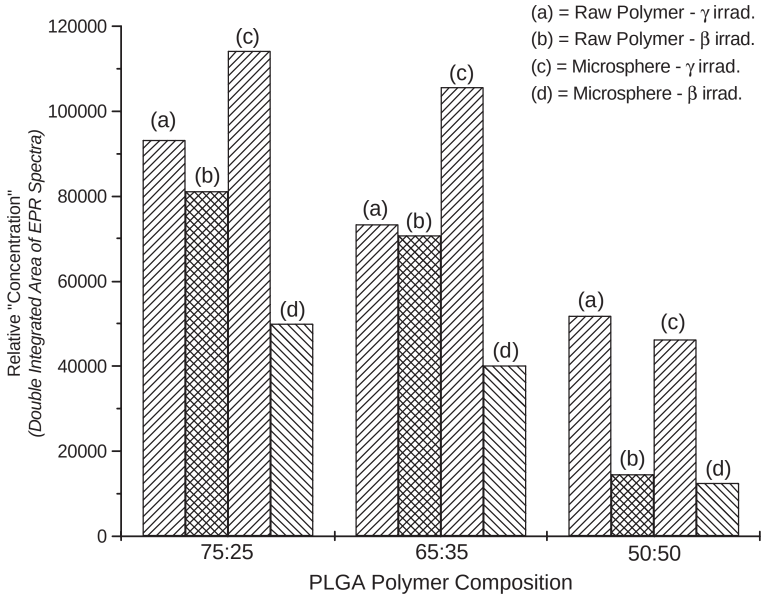


Fig. 5. Double integral EPR signal intensities (relative radical concentrations) of the γ- and β-beam irradiated PLGA polymers (raw polymers and microspheres). All spectra were recorded under identical spectrometer conditions.

4. Conclusion

Exposure of PLGA raw polymers and microspheres to different types of ionising radiation (γ-rays and β-rays) results in the formation of similar types of paramagnetic radical species formed through chain scission and subsequent H abstraction free radical reactions. The polymers were irradiated at ambient temperatures under vacuum conditions. The radicals formed have been identified as –CHO–, –C(CH_3)O– and –C(CH_3)OR, but the relative distribution of the radicals present is found to be soley dependent on the lactide:glycolide ratio and not on the type of radiation used (γ-radiation versus β-radiation). The identity of these radicals was confirmed by computer simulations of the EPR spectra and through complimentary ENDOR analysis. The overall radical content was found to increase with increasing lactide content in the polymer. While the relative abundance of the –CHO–, –C(CH_3)O– and –C(CH_3)OR radicals are similar for both γ- and β-irradiated samples (for a given lactide: glycolide ratio), the overall relative concentration of the radicals was found to be substantially different for the microspheres. Approximately twice as many radical species are generated in γ-irradiated PLGA microspheres compared to β-irradiated PLGA microspheres, regardless of the lactide : glycolide ratios and using approximately similar doses.

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