

Some observations on radical yield in a γ -irradiated microbial copolyester

Malcolm B. Huglin and Paul S. Wormald

Department of Chemistry and Applied Chemistry, University of Salford, Salford M5 4WT, UK

(Received 5 December 1996; revised 1 March 1997)

Poly(3-hydroxy butyrate-co-3-hydroxy valerate) containing 11 mol% 3-hydroxy valerate (HV) units has been subjected to γ -irradiation in vacuo at 293 K to various doses up to 30 kGy. Radical yields estimated by ESR at 293 K increased linearly with dose up to 5 kGy. This dependence, in conjunction with the corresponding linear one measured for a γ -irradiated standard (glycine), yielded a value of $G(\text{radical}) = 1.7 \pm 0.5 (\text{h eV})^{-1}$ for the polyester, which is discussed in relation to values reported for microbial polyesters of different contents of HV. The radical yield remained constant over a measurement period of 200 min, whereas, after the ESR tube was opened to the atmosphere, the radical yield fell by 80% over the same time, indicating quenching by oxygen. The production of peroxidised polymer on continuous γ -irradiation in air was confirmed qualitatively. © 1998 Elsevier Science Ltd. All rights reserved.

(Keywords: γ -irradiation; G (radical) value; ESR)

Introduction

Poly(hydroxy alkanooates) are biodegradable, thermo-plastic materials, which can be produced by a microbial fermentation process. The most common of these polyesters is poly(3-hydroxybutyrate) (PHB) the crystallinity and crystalline melting points of which are lowered by the introduction of 3-hydroxyvalerate units (HV) into the main chain¹. The bacterial mode of synthesis can afford copolymers poly(HB-co-HV) spanning a wide range of compositions. Such materials are chemically stable and contain no markedly labile groups. Hence, although they can form miscible blends with some other polymers², chemical modification to supplement their properties is difficult.

γ -ray induced graft copolymerisation is a general method of effecting chemical modification. It can employ several specific techniques all of which rely on the existence of a high G-radical value for the substrate polymer. Even for such a radiation stable³ polymer as poly(ethyleneterephthalate), careful optimisation of conditions has enabled grafting by the mutual irradiation technique to be effected⁴. Fundamental radiation chemistry of simple esters and non-biodegradable aliphatic polyesters has been studied^{5–7} with particular reference to elucidation of the nature of the species produced via ESR. The work by Miller and Williams⁸ on PHB and poly(HB-co-HV) focused primarily on changes in mechanical properties induced by γ -irradiation.

Reported work on γ -ray induced grafting to these microbial polyesters is extremely scant⁹. However, the nature of radicals and measured G (radical) values in PHB and P(HB-co-HV) have been presented by O'Donnell and co-workers¹⁰. The composition of the copolymers was different from that of the copolymer under consideration here. Moreover, a rather contentious issue arises in the discussion of the results of O'Donnell *et al.* (see later).

For these reasons a re-examination is made here of the radical yield in a γ -irradiated microbial polyester. The findings should have relevance to the feasibility of radiation grafting to this polymer and possibly point also to the most suitable grafting technique.

Experimental

The sample of P(HB-co-HV), obtained from Zeneca Bioproducts Division, was refluxed in chloroform for 24 hr, after which the solution was filtered through Whatman GF/C filter paper to yield a clear product. The polymer was isolated by precipitation in a six fold excess of methanol followed by filtration and drying to constant weight in vacuo. For convenience of packing into ESR tubes the polymer was ground to a particle size not exceeding 1 mm by sieving through a mesh. Copolymer composition was determined by NMR on a solution in D-chloroform. Glass transition temperatures (T_g) were measured by DSC using a Mettler TA 300 instrument at a heating rate of 10 K min⁻¹.

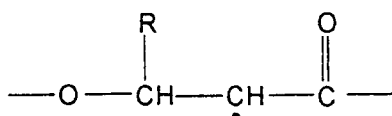
For the ESR studies the polymer was packed into a quartz, constant bore Wilmad 700-PQ clear fused ESR tube, which was evacuated to 1 mPa for 15 hr at ambient temperature and then sealed prior to γ -irradiation. The general procedure for determining the G (radical) value by ESR was similar to that adopted for nylon-6¹¹. However, here a problem was encountered, as the quartz tube exhibited a radical signal after irradiation. This was overcome by tipping the polymer powder to a part of the tube where it could be cooled in liquid nitrogen, while the sample tube itself was flame annealed to remove the signal. The tube was subsequently checked for radical signal to verify that the annealing procedure had removed the radical activity. The polymer was then tipped back into the annealed part of the tube and the spectrum then collected. The samples were irradiated up to 30 kGy using a ⁶⁰Co γ -irradiation source at a dose rate of 0.7 kGy hr⁻¹. The irradiations were carried out at 293 K and the incident dose rate was measured by Fricke dosimetry.

A Joel FEX3 ESR spectrometer operating in the x-band was used to obtain the radical spectrum formed on irradiation. Double integrations of spectrum area (proportional to radical concentration) were made. These areas were compared with those for an irradiated standard, glycine.

Results

Copolymer. The values of T_g for the copolymer and for a sample of PHB were 277 K and 279 K respectively. Comparison of the integrated NMR signals for the protons in the methyl groups in the HB units with those for the protons in the methyl groups in the HV units yielded the copolymer composition as 11 mol% HV.

ESR and radical yield. The ESR spectra were very similar to those obtained by O'Donnell *et al.*, who were able to assign the following radical structure in which R denotes $-\text{CH}_3$ in the HB unit or $-\text{C}_2\text{H}_5$ in the HV unit¹⁰.



To ensure that power saturation did not occur, a microwave power of 1 mW was predetermined as the level where this unwanted effect did not occur. The predetermination involved selecting a power in the linear region of the plot of microwave power against peak intensity.

To ensure that the G value for the polymer was determined in the region where radical yield is directly proportional to dose, several doses were used. Figure 1(a) shows that radical yield (expressed as peak area) is linear in dose only up to 5 kGy; thereafter at higher doses the yield falls off. Figure 1(b) is the corresponding plot for irradiated glycine, which is close to linear in form.

After allowances for packing densities, comparison of the slope of the two linear plots, in conjunction with the value of $4.2 (\text{h eV})^{-1}$ for glycine¹², yields $G (\text{radical}) = 1.7 \pm 0.5 (\text{h eV})^{-1}$ for the polyester.

Figure 2(a) shows that at ambient temperature the radical is stable in vacuo over the time of ESR measurement, 200 min. However, on opening the tube to the atmosphere there is a rapid radical decay, which is presumed to be due to oxygen quenching.

Discussion

The uncertainty limits for the $G (\text{radical})$ value are due to those in the slopes of the lines in Figure 1(a and b) coupled with those in obtaining, cumulative peak areas. In principle, uncertainty in the $G (\text{radical})$ value of the calibrant is normally a dominant factor also. However, although many $G (\text{radical})$ values have been quoted for glycine (see, for

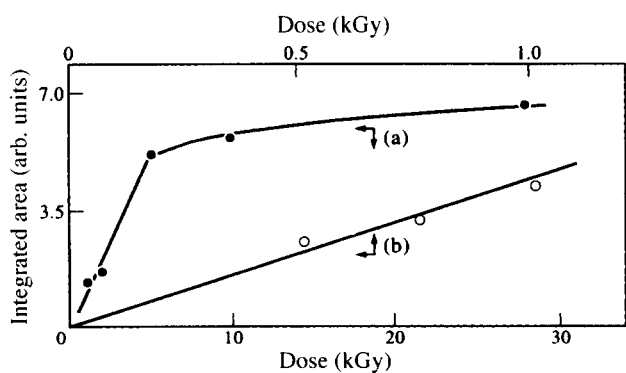


Figure 1 Integrated ESR peak areas as a function of irradiation dose for: (a) poly(HB-co-HV) containing 11 mol% HV; and (b) glycine.

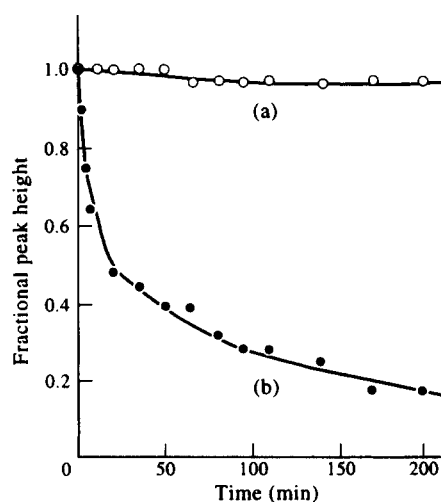


Figure 2 ESR peak height at various times relative to instantaneous peak height after irradiation of poly(HB-co-HV) in vacuo to 13 kGy: (a) sample maintained in vacuo; and (b) sample opened to air.

example Refs. ¹³ and ¹⁴), we believe that full reliance can be placed on the value of $4.2 (\text{h eV})^{-1}$ for glycine in view of the exceptional and meticulous experiments of Baugh and co-workers¹².

O'Donnell *et al.* used integrated peak areas and converted them to radical yields by comparison with the area of a Varian pitch standard reference. It is interesting to note their derived $G (\text{radical})$ was also *ca.* $1.7 (\text{h eV})^{-1}$ for samples containing 0, 18.4 and 26.3 mol% HV, when they were irradiated at 77 K, i.e. the radiation chemical yield was independent of copolymer composition. G -values were also obtained for some samples irradiated at higher temperatures of which 303 K is the closest to the temperature used in the present work (293 K). For a sample containing 0% mol HV (i.e. PHB) the $G (\text{radical})$ value was practically unchanged, whereas there was a significant fall to a value of $0.27 (\text{h eV})^{-1}$ for both of the two copolymers. It was suggested¹⁰ that the lower G values were due to the T_g values of the copolymers being below the irradiation temperatures, with the inference that enhanced segmental motion facilitated radical decay. Doi has reported that the T_g values decrease from 280 to 257 K as the HV content increases from 0 mol% HV to 95 mol% HV¹⁵. T_g values here lie within this reported range, as should also the (unspecified) ones for the two copolymers studied by O'Donnell *et al.*¹⁰. The explanation of the latter workers is not wholly convincing, because: (i) the irradiation temperature of 303 K is above the T_g of not only the copolymers but also of PHB; and (ii) if the $G (\text{radical})$ value is not affected by copolymer composition for irradiation at 77 K, there seems no reason why this independence should not be tenable in the vicinity of room temperature also. Indeed the reported value of $1.6 \pm 0.1 (\text{h eV})^{-1}$ for irradiation of PHB at 303 K is in good accord with the present value of $1.7 \pm 0.5 (\text{h eV})^{-1}$ obtained at 293 K for the copolymer.

A rough assessment of radical stability in the present system was made by irradiating a sample in vacuo at 293 K to a dose of 13 kGy. The height of the ESR peak at various times relative to initial peak height had a value of *ca.* unity over a considerable time [Figure 2(a)] and the radical is evidently trapped in the crystalline regions at ambient temperatures. Because the ESR instrument was not equipped for following signals at elevated temperatures, we are unable to speculate on the extent of reduction of

radical concentration on heating to a temperature at which graft copolymerisation might be possible via the trapped radical technique using monomer vapour or liquid monomer. After the initial ESR signal was read in a similar experiment, the ESR tube was broken and there was a rapid decay in peak height with time [Figure 2(b)]. It is perhaps not surprising that the decay data do not conform to a simple integral kinetic order of reaction, since the process is heterogeneous and diffusion controlled. A larger scale experiment was conducted in which the copolymer was irradiated to 20 kGy at 293 K with continuous access to air. The production of peroxide and/or hydroperoxide was confirmed by the formation of iodine after the addition of aq. KI to a solution in chloroform of the irradiated polymer. No iodine was produced in a blank experiment on unirradiated polymer. The potential of peroxidised polymer as an initiator for graft copolymerisation to poly(HB-co-HV) is to be investigated. Since the G (radical) value obtained by ourselves is adequately large, the mutual irradiation technique at ambient temperature will also be examined.

Acknowledgements

Thanks are due to Biopol for providing the sample, Bonded Fibre Fabric for their financial support, Mr D. J.

Barracough for operating the ESR spectrometer and Dr P. J. Baugh for his helpful advice regarding ESR spectroscopy.

References

1. Doi, Y., *Microbial Polyesters*, VCH Publishers Inc., New York, 1990, p. 53.
2. Verhough, H., Ramsey, B. A. and Favis, B. D., *Polymer*, 1994, **35**, 5155.
3. Campbell, D., Akaki, K. and Turner, D. T., *J. Polym. Sci.*, 1966, **4**, 2597.
4. Huglin, M. B. and Zlatev, V. B., *Eur. Polym. J.*, 1973, **9**, 761.
5. Ayscough, P. B. and Oversby, J. P., *J. Chem. Soc. Faraday Trans. I.*, 1972, **68**, 1153.
6. Sevilla, M. D., Morehouse, K. M. and Swarts, S., *J. Phys. Chem.*, 1981, **85**, 918.
7. Nozawa, Y., *Bull. Chem. Soc. Jpn.*, 1970, **43**, 657.
8. Miller, N. D. and Williams, D. F., *Biomater.*, 1987, **8**, 129.
9. Mitomo, H., Enjoji, T., Whatanabe, Y., Yoshii, F., Makuuchi, K. and Saito, T., *J. Macromol Sci.-Pure Appl. Chem.*, 1995, **A32**, 429.
10. Carswell-Pomerantz, T., Hill, D. J. J., O'Donnell, J. H. and Pomery, P., *J. Radiat. Phys. Chem.*, 1995, **45**, 737.
11. Huglin, M. B. and Smith, J., *Makromol. Chem.*, 1979, **80**, 2775; *Idem. Ibid.* 1980, **181**, 529.
12. Baugh, P. J., Kershaw, K. and Phillips, G. O., *J. Chem. Soc.*, (B), 1970, 1482.
13. Henriksen, T., *Rad. Res.*, 1962, **17**, 158.
14. Rotblat, J. and Simmons, J. A., *Phys. Med. Biol.*, 1963, **7**, 489.
15. Doi, Y., *Microbial Polyesters*, VCH Publishers Inc., New York, 1990, p. 123.