

# The Influence of Gamma Irradiation on the Physicochemical Properties of a Novel Triblock Copolymer of $\epsilon$ -Caprolactone and Ethylene Oxide

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## Abstract

A novel triblock copolymer of  $\epsilon$ -caprolactone (CL) and ethylene oxide (E),  $\text{CL}_6\text{E}_{90}\text{CL}_6$ , intended for use in implantable drug-delivery systems, has been subjected to gamma irradiation, in the solid state and in aqueous solution, under different controlled environmental conditions, to assess its stability to a radiation sterilization process.

When copolymer matrices were irradiated with doses of irradiation up to 72 kGy in the presence of oxygen, negligible changes were observed in the molar mass, molecular mobility (assessed by pulsed nuclear magnetic resonance spectroscopy) and thermal properties. However, irradiation of matrices in the absence of oxygen (anoxia) induced the formation of cross-links, as indicated by a reduction in the molecular mobility of the copolymer, but without affecting its molar mass and thermal properties. Gamma irradiation of aqueous solutions of  $\text{CL}_6\text{E}_{90}\text{CL}_6$  in the presence of oxygen induced random polymer chain scission, as evidenced by a reduction in the molar mass, and the formation of a distribution of copolymer chain lengths in solution. Nuclear magnetic resonance relaxation studies showed that irradiation of solutions of  $\text{CL}_6\text{E}_{90}\text{CL}_6$  at concentrations greater than 4% w/v under anoxic conditions with doses of 54 kGy produced polymer gels with a network structure.

These differences in the effects of gamma irradiation on the physicochemical properties of  $\text{CL}_6\text{E}_{90}\text{CL}_6$  might be germane to the method selected for sterilization of the polymer before its use in implantable drug-delivery systems.

Certain minimum performance limits must be imposed upon polymers for use in implantable drug-delivery systems. In an aqueous environment the polymer must not undergo degradation or cross-linking or be liable to leach and adsorb substances unless specifically designed to do so. Polymers with suitable properties have been synthesized directly, or indirectly by the chemical addition of desirable segments or functional groups to existing materials. In this work a copolymer,  $\text{CL}_6\text{E}_{90}\text{CL}_6$ , was synthesized by the block copolymerization of a hydrophilic swellable monomer (ethylene oxide) and a biodegradable hydrophobic component ( $\epsilon$ -caprolactone). The ethylene oxide component ensured swelling of the system upon hydration whereas the  $\epsilon$ -caprolactone imparted degradability and mechanical strength to the system (Kamath & Park 1993).

It is important that implantable polymeric systems are stable to sterilization and that sterilization should not have an undesirable effect upon the functional properties of the polymer (Holland & Tighe 1992). The most expedient method for the terminal sterilization of moisture- and heat-sensitive polymers might be by exposure to ionizing radiation. On this premise it is important to investigate the influence of irradiation on the physicochemical properties of the chosen polymer because radiation-induced changes might affect the solubility, drug-release properties and even the biocompatibility of many polymers. This study is an investigation of the influence of gamma irradiation, under controlled

environmental conditions, on the physicochemical properties of the copolymer  $\text{CL}_6\text{E}_{90}\text{CL}_6$ .

## Materials and Methods

### *Copolymer synthesis*

The block copolymer  $\text{CL}_6\text{E}_{90}\text{CL}_6$  was prepared by using poly(ethylene glycol) 4000 [ $\alpha$ -hydroxy,  $\omega$ -hydroxypolyoxyethylene,  $\text{E}_{90}$ ], to initiate the polymerization of  $\alpha$ -caprolactone at 180°C in the absence of added catalysts; the method used was that of Cerrai et al (1989). Full details of the synthesis, purification and characterization by  $^1\text{H}$  and  $^{13}\text{C}$  NMR and by gel permeation chromatography (GPC) have been published previously (Martini et al 1994). The mole percentage of triblock in the sample, as determined by NMR analysis, was 94%.

### *Irradiation of copolymer samples*

Copolymer samples for irradiation were positioned at fixed distances from a 2000 Ci  $^{60}\text{Co}$  source in Pyrex irradiation vessels. The copolymer matrices and solutions received specified doses of gamma irradiation in the range 1.5–72 kGy at a dose rate of 0.08 kGy  $\text{min}^{-1}$ . The dose rate of the cobalt source was determined by Fricke dosimetry.

Copolymer matrices were prepared by dry compression using a modified compression apparatus (Engineering Laboratory Equipment) designed to deliver low compressional forces, typically 750 N. These discs were irradiated in Pyrex vessels (3 cm diameter  $\times$  12 cm height) under both atmospheric and anoxic conditions, at ambient temperatures. Anoxic conditions were obtained by evacuation of the samples to a pressure of  $5 \times 10^{-4}$  torr using a high-vacuum line.

Aqueous solutions containing known concentrations of copolymer were irradiated under both atmospheric and anoxic conditions at ambient temperatures, using the same irradiation dose regimen as above. Before irradiation samples of copolymer solution (10 mL) were de-aerated by repeated exposure to  $7.5 \times 10^{-2}$  torr then agitation of the solution on a flask shaker at 60 shakes  $\text{min}^{-1}$  for 15 min. Irradiated copolymer was recovered by freeze drying and its physicochemical properties investigated by use of pulsed NMR, GPC and differential scanning calorimetry (DSC) to detect morphological and structural changes induced by the irradiation.

#### Pulsed NMR

Relaxation studies were performed on a 4% w/w solution of the irradiated copolymer in deuterated chloroform using a Jeol EX270 NMR instrument. Deuterated chloroform, rather than deuterated water, was used as solvent to prevent changes in mobility arising from the formation of micelles from  $\text{CL}_6\text{E}_{90}\text{CL}_6$  in aqueous solution (Martini et al 1994). Spin-spin ( $T_2$ ) relaxation times were calculated from the single exponential decay of signal amplitudes with time,  $t$ :

$$A_t/A_0 = e^{(-t/T_2)} \quad (1)$$

where  $A_t$  and  $A_0$  are the signal amplitudes at time  $t$  and time  $t=0$ , respectively, after the pulse of frequencies. Analysis of data was by the Carr–Purcell–Meibloom–Gill method using the software supplied by the manufacturers.

#### GPC measurements

The GPC system consisted of 4  $\mu$ -styragel columns (Waters Associates) with tetrahydrofuran (THF, analytical grade; Fisons) as the eluent; the flow rate was 1 mL  $\text{min}^{-1}$  at room temperature. Samples were dissolved in THF (a non-aggregating solvent) at a concentration of 0.2% w/v and the solution was injected into the column. The internal marker was dodecane (BDH). A differential refractometer (Waters Associates, model 410) was used as detector. Calibration was performed with a series of polyoxyethylene standards of known molar mass. The molar-mass distributions were obtained via an on-line microcomputer and analysed using GPC 6000 software (Jones Chromatography).

#### DSC measurements

A Seiko 220C DSC instrument was used in this study. Samples of copolymer or irradiated copolymer (7–8 mg) were weighed into aluminium pans (50  $\mu\text{L}$ ) and hermetically sealed by means of a Seiko crimper. The previous thermal history of the copolymer was removed by heating samples to temperatures above the melting point of the copolymer and then cooling rapidly at 300°  $\text{min}^{-1}$  to  $-100^\circ\text{C}$ . The samples were scanned at 10°  $\text{min}^{-1}$  from  $-100$  to  $+80^\circ\text{C}$  to determine thermal transitions.

#### Swelling measurements

The gels formed after exposure of copolymer solutions to known doses of radiation were dried to a constant weight under vacuum at 30°C for 24 h. The xerogels so formed were placed in sealed pre-weighed vials containing 5 g of singly distilled water. Although equilibrium water content was achieved after 24 h, samples were usually left in contact with water for up to 72 h. After this time the gel samples were removed and dried

thoroughly between filter paper before weighing. The cross-linking density,  $N$ , and molar mass per network chain,  $M_C$ , of the gels were calculated from equations 2 and 3, respectively.

$$\ln \phi_1 + \phi_2 + \chi \phi_2^2 = -(N/N_A)V_1^0 \phi_2^{1/3} \quad (2)$$

$$M_C = \rho N_A/N \quad (3)$$

where  $\phi_1$  and  $\phi_2$  are the volume fractions of solvent and polymer, respectively,  $\chi$  is the Flory–Huggins interaction parameter,  $N_A$  is the Avogadro constant,  $V_1^0$  is the molar volume of water, and  $\rho$  is the density of polyoxyethylene. Values of  $\chi$  were taken from the literature (Lakhanpal et al 1968). Approximate values of  $\phi_1$  and  $\phi_2$  in the swollen systems at equilibrium were determined from measurements of the increase of volume of the matrix relative to its original dry volume.

## Results and Discussion

#### Irradiation of $\text{CL}_6\text{E}_{90}\text{CL}_6$ discs in the presence and absence of oxygen

Fig. 1 shows a plot of the number-average molar mass ( $M_n$ ) determined by GPC for  $\text{CL}_6\text{E}_{90}\text{CL}_6$  after irradiation in the form of dried matrices with known doses of radiation in the presence of oxygen. It can be seen that there was no change of molar mass with increasing irradiation dose and the presence of oxygen did not alter the effect of the radiation. Other workers (Gilding & Reed 1979) have reported that poly-(glycolic acid) polymers underwent a 70% reduction in number-average molar mass after irradiation with doses of 25 kGy. Similarly, Reed & Gilding (1981) reported that poly(ethylene oxide)-poly(ethylene terephthalate) copolymers underwent a 50% reduction in molar mass after irradiation with 25 kGy doses. However, in neither of these reports was the influence of environmental conditions during the irradiation procedure specified.

The enthalpies of melting determined from DSC are presented in Table 1. The similarity of the enthalpy values over the range of irradiation doses suggests that the crystalline domains of the copolymer were unaffected by gamma irradiation.

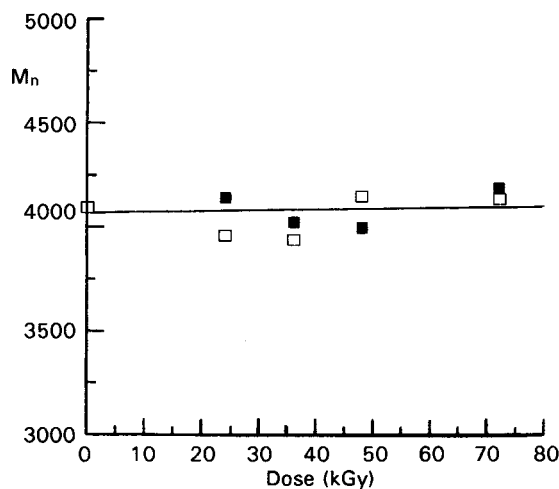


FIG. 1. Plot of the number-average molar mass of  $\text{CL}_6\text{E}_{90}\text{CL}_6$  as a function of irradiation dose for copolymer matrices irradiated in oxygen (■) and under anoxic conditions (□).

diation. No discernible glass-transition temperature could be detected by DSC for samples analysed before or after irradiation.

The sharpness of polymer melting point is influenced by factors such as polymer purity, crystallinity and polydispersity. The melting points were well defined with no apparent shift in the maximum melting point temperature with increases of irradiation dose; a mean melting point of  $50 \pm 0.5^\circ\text{C}$  was obtained for all the irradiated polymers. The sharpness of the melting points of this copolymer suggest that any cross-linking or main-chain scission resulting from irradiation was not sufficient to be detected by DSC. These findings are in contrast with those of other workers who have examined the influence of gamma irradiation on polymer melting point. Gupta & Deshmukh (1983) have reported a reduction in the melting point of poly(lactic acid) samples from  $140$  to  $127^\circ\text{C}$  when irradiated in air with  $5.0\text{-kGy}$  doses. They suggested that shortened polymer chains were produced by main-chain scission and, therefore, that the chain mobility of the polymer was higher. However, when Gupta & Srivastava (1982) irradiated phenolic polyesters in air with  $20\text{ kGy}$  doses, random chain scission of weak links in the polymer backbone caused 'chain stiffening' and an increase in the polymer melting point from  $237.5$  to  $241.5^\circ\text{C}$ .

An indication of the influence of gamma irradiation on polymer molecular mobility was provided by pulsed NMR, a technique which has previously been applied in the study of the effects of gamma irradiation on polymeric systems (Charlesby & Folland 1980; Charlesby 1982). Figs 2 and 3 show relaxation curves obtained for  $\text{CL}_6\text{E}_{90}\text{CL}_6$  matrices irradiated in the presence and absence of oxygen, respectively. Exponential decay of  $A_t/A_0$  was observed with relatively little change in the  $T_2$  values for samples receiving irradiation doses of up to  $72\text{ kGy}$  in the presence of oxygen (see Table 2). In contrast, a decrease in  $T_2$  values with increase of irradiation dose was noted for polymers irradiated under anoxic conditions at doses exceeding  $24\text{ kGy}$  (Table 2).

$T_2$  measurements provide a sensitive probe for investigating factors which modify long-range motion, such as molar mass, chain entanglement, network formation and radiation-induced cross-linking (Charlesby 1982). The decrease of  $T_2$  observed in Table 2 for samples irradiated under anoxic conditions is indicative of hindered chain mobility resulting from the formation of a network structure, the network protons being forced to relax at a faster rate back to their equilibrium condition by the constraints of the network. It is concluded, however, that the extent of cross-linking was limited because it was insufficient to have any measurable effect on the molar mass and thermal properties. The lack of variation of  $T_2$  values

Table 1. Enthalpies of melting of  $\text{CL}_6\text{E}_{90}\text{CL}_6$ , irradiated in matrices in oxygen and under anoxic conditions.

Dose (kGy)	Enthalpy of melting	
	In oxygen ( $\text{J g}^{-1}$ )	Under anoxic conditions ( $\text{J g}^{-1}$ )
0	$103.5 \pm 2.6$	$103.5 \pm 2.6$
24	$106.3 \pm 3.2$	$103.3 \pm 2.0$
54	$102.6 \pm 2.8$	$107.9 \pm 4.0$
72	$104.1 \pm 3.6$	$102.8 \pm 2.3$

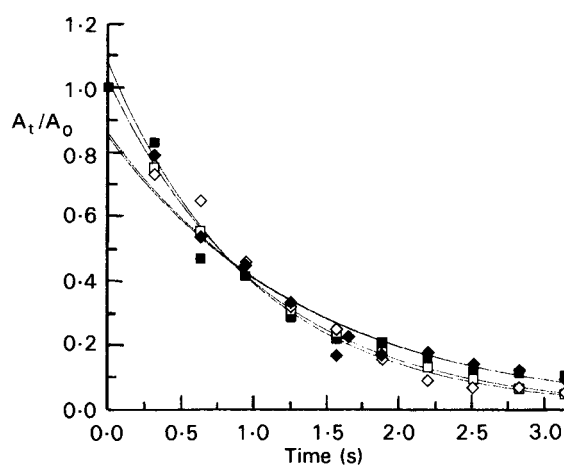


FIG. 2. Relaxation spectra for matrices of  $\text{CL}_6\text{E}_{90}\text{CL}_6$  irradiated in oxygen with doses of  $0\text{ kGy}$  ( $\square$ ),  $24\text{ kGy}$  ( $\blacksquare$ ),  $54\text{ kGy}$  ( $\diamond$ ) and  $72\text{ kGy}$  ( $\blacklozenge$ ).

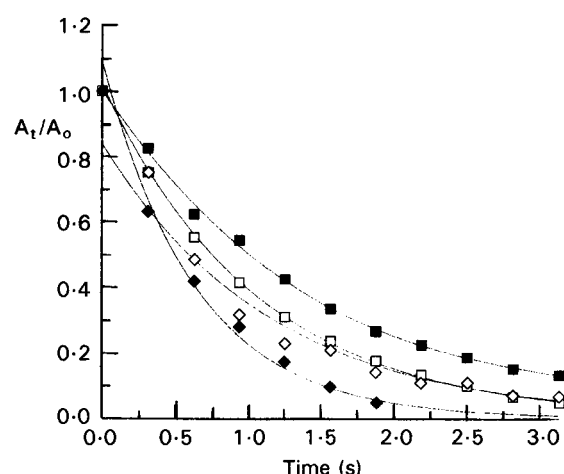


FIG. 3. Relaxation spectra for matrices of  $\text{CL}_6\text{E}_{90}\text{CL}_6$  irradiated under anoxic conditions with doses of  $0\text{ kGy}$  ( $\square$ ),  $24\text{ kGy}$  ( $\blacksquare$ ),  $54\text{ kGy}$  ( $\diamond$ ) and  $72\text{ kGy}$  ( $\blacklozenge$ ).

Table 2.  $T_2$  values for matrices of  $\text{CL}_6\text{E}_{90}\text{CL}_6$  irradiated in oxygen and under anoxic conditions.

Dose (kGy)	$T_2$ value	
	In oxygen (s)	Under anoxic conditions (s)
0	$1.07 \pm 0.050$	$1.07 \pm 0.050$
24	$1.09 \pm 0.012$	$1.17 \pm 0.010$
54	$1.11 \pm 0.037$	$0.93 \pm 0.023$
72	$1.12 \pm 0.037$	$0.71 \pm 0.046$

with irradiation dose observed for samples irradiated in oxygen reinforce the findings of GPC and DSC showing no appreciable irradiation-induced effects on the physicochemical properties of the copolymer. Cross-linking of poly(lactic acid) irradiated with doses up to  $25\text{ kGy}$  under nitrogen, i.e., anoxic conditions, was reported by Gupta & Deshmukh (1983). Negligible cross-linking was observed after irradiation with similar doses in air.

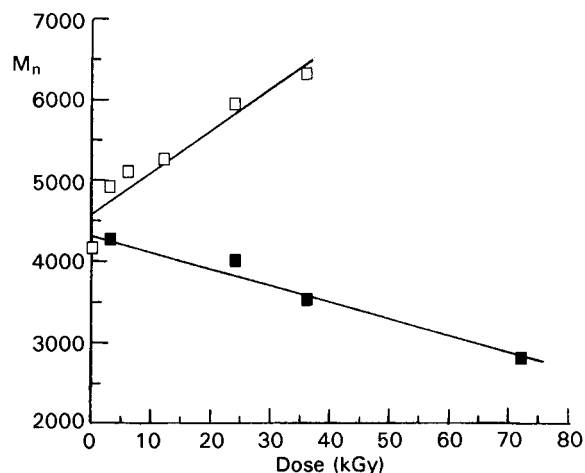


FIG. 4. Plot of the number-average molar mass of  $\text{CL}_6\text{E}_{90}\text{CL}_6$  as a function of irradiation dose for copolymer irradiated in 4% w/w aqueous solution in oxygen (■) and under anoxic conditions (□).

*Irradiation of  $\text{CL}_6\text{E}_{90}\text{CL}_6$  solutions in the presence and absence of oxygen*

Fig. 4 shows the number-average molar mass measured by GPC after irradiation of a 4% w/w aqueous solution. It was only possible to determine the molar mass of  $\text{CL}_6\text{E}_{90}\text{CL}_6$  in solutions irradiated with doses of less than 40 kGy under anoxic conditions, because the highly cross-linked copolymers formed at higher doses were insoluble in THF and so could not be assessed by GPC. Polymers irradiated under anoxic conditions showed a progressive increase in  $M_n$  with increasing irradiation dose, suggesting the formation of larger aggregates owing to cross-linking of copolymer chains. In contrast, when the polymer was irradiated in solution in the presence of oxygen the  $M_n$  values decreased significantly as the irradiation dose was increased over the range 0 to 72 kGy, suggesting polymer-chain scission.

Figs 5 and 6, show the relaxation curves (from NMR) for  $\text{CL}_6\text{E}_{90}\text{CL}_6$  irradiated in aqueous solution in the presence and absence, respectively, of oxygen.  $T_2$  values calculated from a fit of equation 1 to the curves are given in Table 3. In the

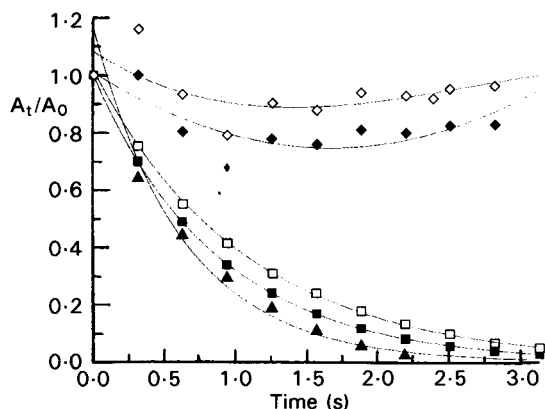


FIG. 5. Relaxation spectra for  $\text{CL}_6\text{E}_{90}\text{CL}_6$  irradiated in 4% w/w aqueous solution in oxygen with doses of 0 kGy (□), 12 kGy (▲), 24 kGy (■), 48 kGy (◇) and 72 kGy (◆).

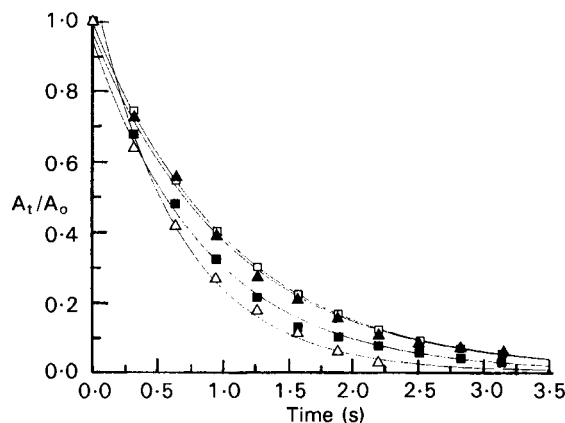


FIG. 6. Relaxation spectra for  $\text{CL}_6\text{E}_{90}\text{CL}_6$  irradiated in 4% w/w aqueous solution under anoxic conditions with doses of 0 kGy (□), 12 kGy (▲), 24 kGy (■), 48 kGy (◇) and 72 kGy (◆).

Table 3.  $T_2$  values for solutions of  $\text{CL}_6\text{E}_{90}\text{CL}_6$  irradiated in oxygen and under anoxic conditions.

Dose (kGy)	$T_2$ value	
	In oxygen (s)	Under anoxic conditions (s)
0	$1.04 \pm 0.050$	$1.04 \pm 0.050$
12	$0.74 \pm 0.060$	$1.01 \pm 0.007$
24	$0.87 \pm 0.050$	$0.82 \pm 0.020$
36	—	$0.71 \pm 0.036$

presence of oxygen the lower  $T_2$  value obtained after a dose of irradiation of 12 kGy was similar to that obtained for a non-irradiated sample, which suggests that an initial phase of cross-linking might have occurred (Fig. 5). The subsequent increase of  $T_2$  after doses of irradiation in excess of 24 kGy indicates the onset of main-chain scission. At higher irradiation doses the extent of this scission was such that exponential relaxation curves were not observed at doses of 48 and 72 kGy. The reason for this non-exponential behaviour was attributed to a range of sizes of copolymer chains in solution and a consequent range of relaxation rates. In contrast, a steady decrease in the  $T_2$  value was observed (Table 3 and Fig. 6) for polymers irradiated with increasing doses in solution under anoxic conditions, indicating that a network structure is being created as a result of irradiation.

Aqueous solutions containing known concentrations of  $\text{CL}_6\text{E}_{90}\text{CL}_6$ , were irradiated with doses of  $\geq 54$  kGy under anoxic conditions. With this dose of irradiation induced gelation of solutions was not observed at polymer concentrations below 4%. The copolymer gels induced by irradiation could be deformed easily under mechanical pressure and had poor handling properties, even when doses of 120 kGy were used. Copolymer solutions irradiated in the presence of oxygen did not produce gels even at doses exceeding 120 kGy. These irradiated solutions were turbid and the extent of the turbidity increased with increasing irradiation dose, probably as a result of the breakdown products of the copolymer. Copolymer solutions were saturated with nitrous oxide both before and during irradiation in an attempt to promote radiation-induced intermolecular cross-linking and the formation of stronger gels

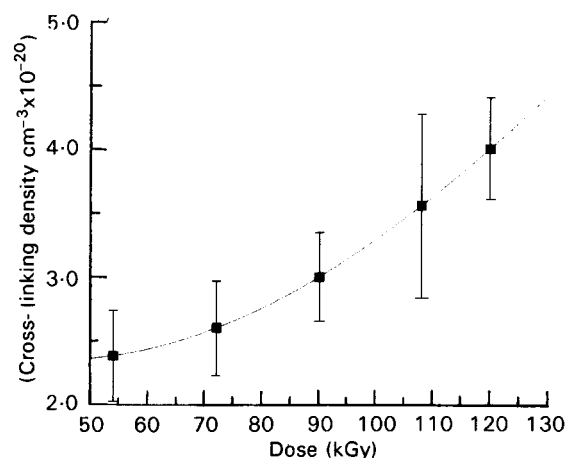


FIG. 7. Cross-linking density, as a function of irradiation dose, of a 4% w/w solution of CL<sub>6</sub>E<sub>90</sub>CL<sub>6</sub> irradiated under anoxic conditions.

(Al-Saden et al 1980). The use of nitrous oxide could increase the number of hydroxyl radicals available for cross-linking. In both experiments, however, nitrous oxide had a negligible effect upon gel properties. Copolymer solutions irradiated under conditions of nitrous oxide saturation had similar  $T_2$  values to those irradiated for comparable times under anoxic conditions.

Swelling studies were performed as a means of assessing the extent of cross-linking in gels formed after irradiation of copolymer solutions with doses greater than 54 kGy under anoxic conditions. Plots of the cross-linking density and molar mass per network chain ( $M_c$ ), as a function of irradiation dose, as calculated by use of equations 2 and 3, are presented Figs 7 and 8. The extent of cross-linking increases appreciably with an irradiation dose of greater than 70 kGy. The reduction in  $M_c$  which accompanies the increase in cross-linking density is expected because the separation between chains would decrease as a greater number of cross-links is formed. Despite the increase in cross-linking density the gels could be deformed easily under mechanical pressure.

In summary, this study has identified differences in the effects of gamma irradiation on the physicochemical properties of CL<sub>6</sub>E<sub>90</sub>CL<sub>6</sub> when irradiated in the solid state and in aqueous solution, and in the presence and absence of oxygen.

Irradiation of copolymer matrices in the presence of oxygen caused no apparent changes in the molar mass, polymer melting point and relaxation spectra ( $T_2$  values). However, after irradiation under anoxic conditions, cross-linking of the copolymer was indicated by the gradual decrease in  $T_2$  values (1.07 to 0.71 s) although the GPC and DSC data revealed no significant changes.

Irradiation of CL<sub>6</sub>E<sub>90</sub>CL<sub>6</sub> in aqueous solution in the presence of oxygen, resulted in a reduction of  $M_n$ , indicative of main-chain scission of the polymer. Irradiation of solutions under anoxic conditions promoted gelation at a concentration of 4% w/w polymer after an irradiation dose of 54 kGy.

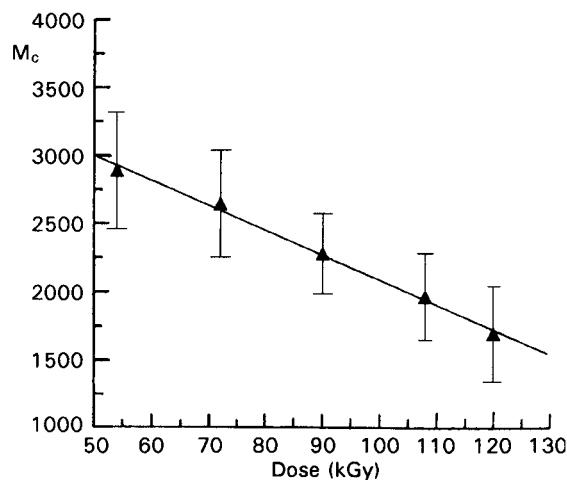


FIG. 8. Molar mass between polymer chains ( $M_c$ ) of a 4% w/w solution of CL<sub>6</sub>E<sub>90</sub>CL<sub>6</sub> irradiated under anoxic conditions as a function of irradiation dose.

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