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Effect of γ-radiation on a polyanhydride implant containing gentamicin sulfate

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Abstract

Septacin™, a polyanhydride implant containing gentamicin sulfate, was sterilized by γ-radiation. Its copolymer molecular weight (M_w by GPC) was increased after this radiation. No cross-linking was shown in the radiated samples as no gel content was found by the filtration method. The chemical structure as detected by ^{1}H NMR for non-radiated and radiated samples was comparable. For samples radiated at higher dose levels (70–100 kGy), the IR spectra showed that the intensity of absorbance attributable to the C–H stretching vibration (at 2852 and 2927 cm^{-1}) was attenuated, indicating free-radical formation or loss of hydrogen atoms from C–H bonds. However, the mass spectra for the γ-radiated and the non-radiated controls after they were completely depolymerized in methylene chloride were virtually identical. Therefore, it could be concluded that the increase in copolymer molecular weight for radiated Septacin was a result of chain extension in the copolymer backbone during radiation. In addition, wide-angle X-ray diffraction and polarizing light microscopy (PLM) revealed a change in the physical structure of the radiated copolymer. There was an increase in crystallinity of the copolymer with increasing radiation doses; the greatest increase in crystallinity occurred at the dose range of 70–80 kGy, which was also shown to result in the greatest molecular-weight increase. The crystalline morphology of the samples as detected by PLM was not altered by γ-radiation, regardless of the dose levels. © 2002 Published by Elsevier Science B.V.

Keywords: γ-Radiation; Polyanhydride; Biodegradable implant; Molecular weight; Gel content; Cross-linking; Chain extension; Crystallinity

1. Introduction

Septacin™ is a polyanhydride (Shieh et al., 1994) (poly(EAD:SA) 1:1 copolymer; Fig. 1) implant containing 20% gentamicin sulfate. It is designed to be used in the treatment of osteomyelitis. The implant releases gentamicin at the implantation site for up to 4 weeks. It is biodegradable (Shieh et al., 1994); hence, a second surgery to remove the implant is not necessary. Septacin is manufactured by an injection-molding method using a mixture consisting of copolymer and gentamicin sulfate. The product is a strand of

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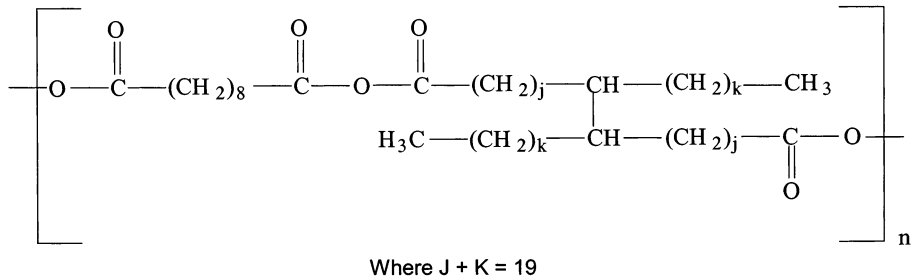

Fig. 1. Molecular structure of the poly(EAD:SA 1:1) copolymer.

five beads; each bead is connected with a linker (Fig. 2). The product is packaged in an aluminum pouch and is sterilized by gamma radiation (γ-radiation). γ-Radiation has been used as one of the sterilization technologies in food and pharmaceutical industries (Briendel, 2000). The current guidance standards for radiation sterilization of healthcare products have also been published (ANSI/AAMI/ISO, 1990). In particular, the validation for establishing a minimum radiation sterilization dose (25 kGy) was documented (AAMI TIR, 1996) and approved in the United States.

Polyanhydride copolymers are semi-crystalline polymers which are composed of two domains: amorphous and crystalline (Tamada et al., 1992). During radiation, radicals may be formed throughout the polymer in both domains (Williams, 1991). The radical reactions may lead to changes in the polymer morphology and the degree of crystallinity (Williams, 1991). After γ-radiation, material properties such as molecular weight, crystallinity, and mechanical properties could be affected by potential molecular changes, including cross-linking, chain scission, the formation of new molecules, and changes in the chemical structure of the polymer (Dawes and Glover, 1996; Burlant et al., 1962; Shultz and Bovery, 1956; Wall and Brown, 1957; Gavrila and Gosse, 1994; Bohm and Tveekrem, 1982; Arakawa et al., 1986; Kanbara et al., 1994; Hooper et al., 1997; Garrett et al., 1991). This study was undertaken to investigate the cause of the molecular-weight increase in the Septacin copolymer during γ-radiation and its impact on the structure of the copolymer.

2. Experimental

2.1. Preparation of copolymer–drug mixture

Copolymer poly(EAD:SA 1:1) with 20% gentamicin sulfate was used to produce Septacin™ beads for this study. The weight-average molecular weight (M_w) and the number-average molecular weight (M_n), both determined by GPC, were 47 000 and 17 100, respectively. This mixture was prepared using a melt-copolymerization method. In this process, a batch-type planetary mixer was used as a reactor as well as a melt-blender. The copolymerization took place under vacuum to remove the residual acetic anhydride and acetic acid produced as reactive by-products.

After the copolymerization was completed, gentamicin sulfate was added to the molten copolymer and mechanical stirring compounded the final mixture. In the final step, the molten bulk drug was poured onto a tray with Teflon®-coated foil, allowed to solidify under ambient conditions, and subsequently cut into small pieces.

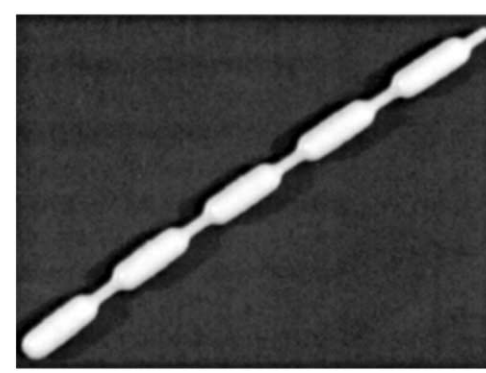

Fig. 2. Bead-with-linker configuration.

2.2. Injection molding of Septacin™ beads

A Gluco P20 injection-molding press was used for molding the Septacin™ beads. It is a plunger-type molder which requires a pre-determined shot size (volume). The mold used features two identical strands with five beads per strand on each side (Fig. 2).

2.3. γ-Radiation process

γ-Radiation was performed at Steris Isomedix, Libertyville, IL using a dosing rate ranging from 10 to 15 kGy/h; the dose levels were from 10 to 100 kGy. To prevent the polymer from degradation during the process, dry ice was used with the samples.

2.4. Analytical methods

2.4.1. Determination of molecular weight

The copolymer molecular weights of Septacin™ samples before and after γ-radiation were measured by a GPC method. Two Waters Styragel HR-5E columns were used in series. Methylene chloride was used as the eluent. Detection was accomplished by using a Shimadzu RID-6A Differential Refractive Index Detector. An Hitachi L-6000 HPLC pump was employed. The injector was a Rheodyne Model 7125 fixed-loop injector with a 10 μl loop. Analytical samples were prepared by dissolving a Septacin bead in 8 ml of methylene chloride and filtering the solution through a glass fiber filter prior to analysis. The analysis was conducted at a 1.0 ml/min flow rate. Data were processed using a PE Nelson Turbo-Chrom Data Acquisition System with TC/SEC software.

2.4.2. Examination of crystalline morphology

For a bead sample, 30 μm cross-sectional slices were produced using a microtome. A polarizing light microscope was used to examine the crystalline morphology of the beads before and after γ-radiation. The sample was placed between two cross-polarizers (90° to each other). If the sample is amorphous, no light will pass into the eyepiece and a dark image is created. However, crystals can reorient light polarization, thus permitting light to pass into the eyepiece and creating a bright image. When the crystals are oriented in certain directions within a sample, the image shows stratification, revealing the crystalline morphology of the crystals. In this manner, the crystalline morphology of the Septacin beads was determined.

2.4.3. Determination of crystallinity

The wide-angle X-ray diffraction (WAXD) method was used to determine the degree of crystallinity of the non-radiated and radiated samples. Each of three sample beads was first cut to half, and six sample halves were positioned side by side in the sample holder and scanned at the same time in one test. The crystallinity was calculated from the area between the scattered (crystalline) and unscattered (amorphous) peaks. The amorphous portion yields only a broad and diffuse peak, and the crystalline portion produces sharp peaks. A formula with which to make a quantitative calculation of the degree of crystallinity is given by (Wunderlich, 1973):

$$\text{Crystallinity}\ (\%) = [I_c/(I_c + kI_a)] \times 100$$

where I_c and I_a are the peak intensity areas for the crystalline portion and the amorphous portion, respectively; k is the calibration constant.

2.4.4. Gel-content determination

This test was performed to detect any cross-linked gel content (Flory, 1953) generated by the radiation process. First, the weights of the test tube and the filtration paper used for the test were measured. Samples were dissolved in methylene chloride in the test tube and then filtered through the filtration paper. The test tube and filtration paper were dried under vacuum to allow the residual methylene chloride to evaporate. The test tube and the filtration paper were then weighed and the gel content was determined as the difference in their weights before and after the test. In addition, the dried test tube and filtration paper were visually examined for the presence of insoluble residues.

Table 1
Molecular weights at different dose levels

Dose levels (kGy)	Weight average (M_w) [a]	Number average (M_n) [a]	Polydispersity (M_w/M_n)
0	55 623	19 321	2.88
10.5–12.6	58 731	19 099	3.08
31.8–35.9	50 453	18 811	2.68
50.0–58.0	63 977	16 358	3.91
72.3–81.9	68 333	15 358	4.45
93.5–103.1	57 205	15 547	3.68

[a] Average of two measurements: duplicate samples and duplicate injections for each sample (four data points) were used for each measurement.

2.4.5. ^1H NMR

The solution was subjected to NMR analysis to detect any structural changes caused by the radiation process. The model of the NMR used for this study was Gemini-200, a 200 MHz ^{1}H NMR. The test was conducted at room temperature. The pulse sequence over 16 repetitions was at a pulse with 90.0° angle to the probe and with a frequency width of 3000.3 Hz. The NMR data were processed using Fourier transform with a file size of 32 768.

2.4.6. Infrared analysis

An FT-IR was used to detect any chemical changes in samples radiated at different dose levels; a non-radiated sample was used as the control. The IR model was a GL-5020 from Mattson Instruments, Inc. The skin and the core of a Septacin bead were first separated and mixed with KBr powder by grinding in an agate mortar at room temperature. A compressed pellet was made under a pressure of 30–40 MPa. All spectra were obtained from the compressed pellets at room temperature using a resolution of 4 cm^{-1}.

2.4.7. Mass spectra

Samples were prepared by dissolving the beads in methylene chloride. Gentamcin sulfate was then extracted by liquid/liquid extraction using 0.1 N H_2SO_4. The polymer/methylene chloride solution was allowed to stand at room temperature for 1 week to effect substantial polymer degradation. The methylene chloride layer was then analyzed by mass spectrometry. Euricic acid dimer (EAD) was dissolved in methylene chloride (at 1.9 mg/ml) and analyzed as a control. Mass spectral analysis was performed using a Finnigan LCQ ion-trap mass spectrometer with an APCI interface. Analysis was conducted by a direct infusion of the sample at 100 μl/min. The spectrometer was scanned from 100 to 2000 amu. Twenty scans were acquired for each sample and the average was reported. The spectra were acquired in the positive ion mode.

3. Results and discussion

The molecular weights of the non-radiated sample and samples radiated at different dose levels are tabulated in Table 1.

The molecular weights of samples radiated at dose levels below 50 kGy did not exhibit significant changes during the radiation process. The most noticeable increases in weight-average molecular weights were observed for samples radiated at the dose level ranging from 70 to 80 kGy. The data also show a decreasing trend for the

Table 2
Degree of crystallinity of Septacin™ beads radiated at different dose levels

Sample number	Dose levels (kGy)	Crystallinity (%)
1	0	32
2	10.5–12.6	33
3	31.8–35.9	34
4	50.0–58.0	36
5	72.3–81.9	41
6	93.5–103.1	42

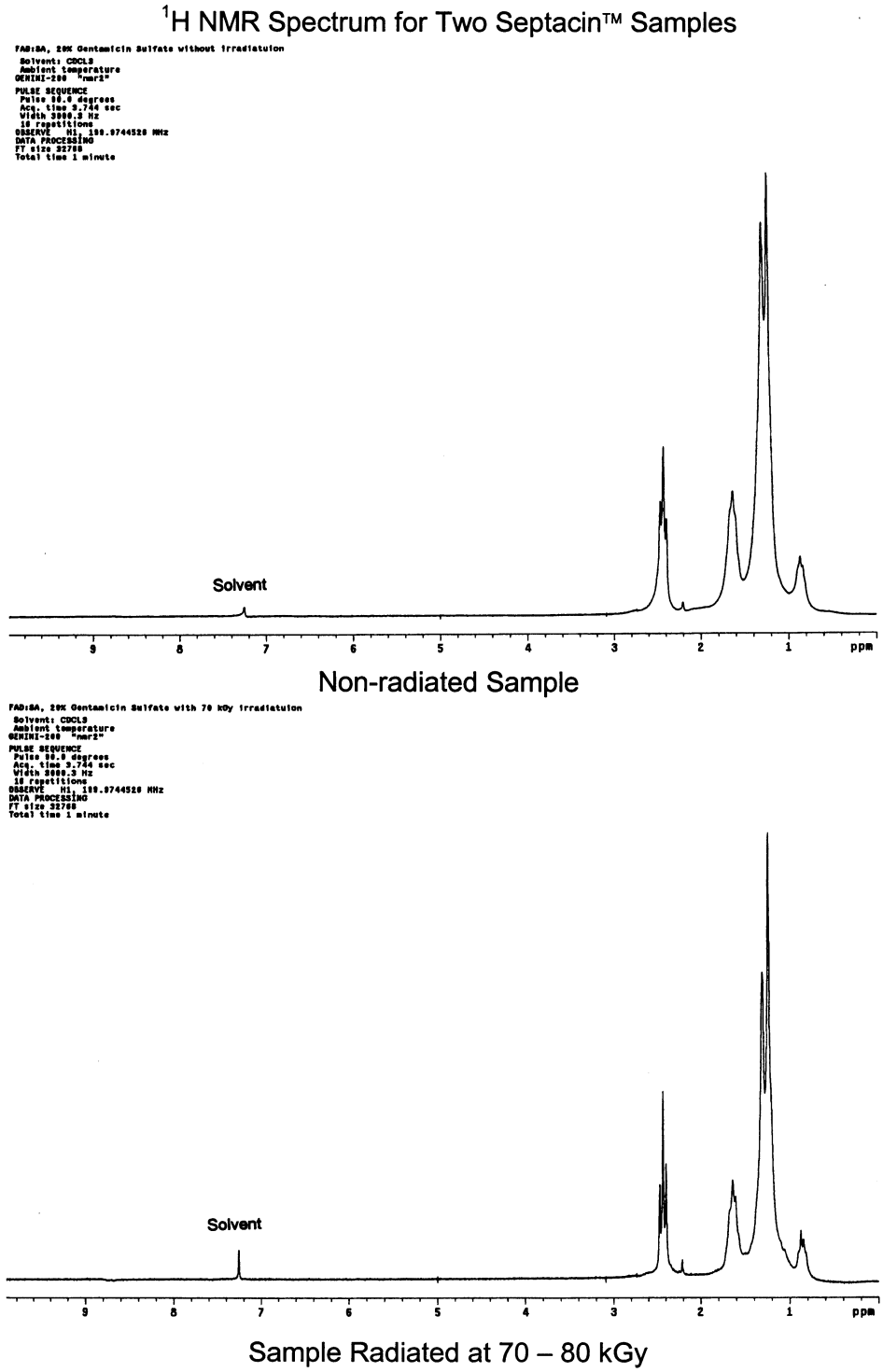

Fig. 3. ^{1}H NMR spectrum for two Septacin™ samples.

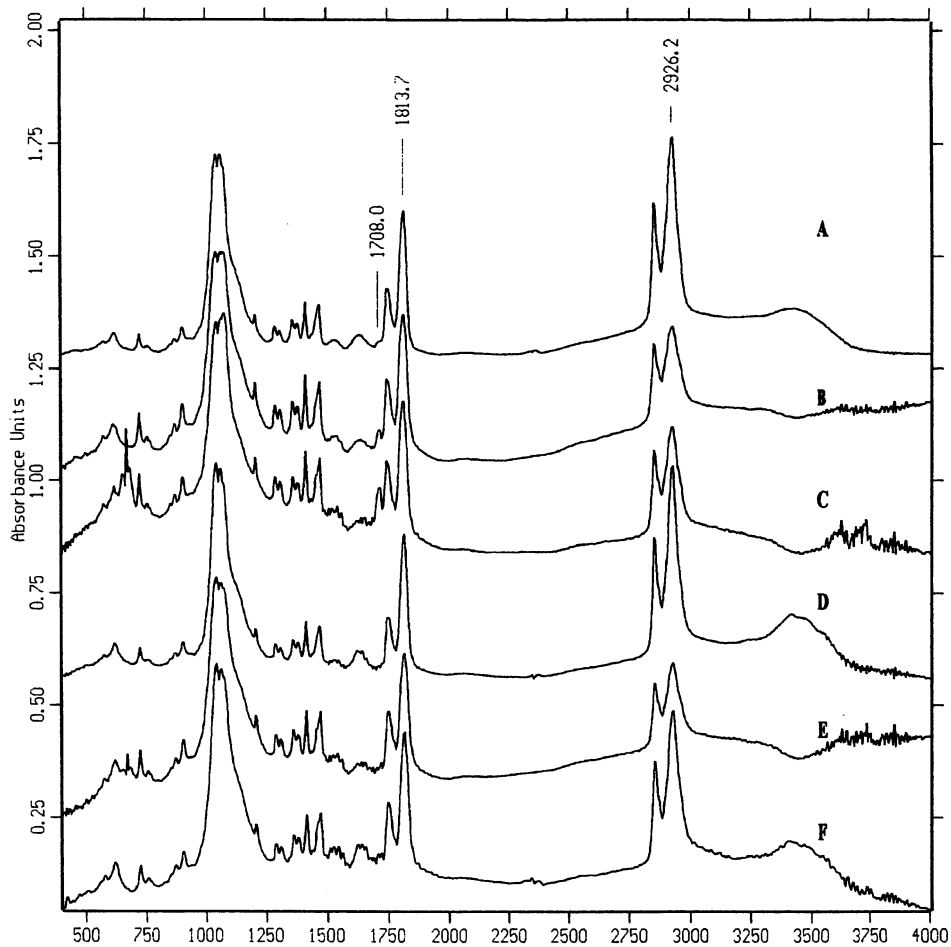

Fig. 4. FT-IR spectra at the skin and core of Septacin™ beads non-radiated and radiated at 70–80 and 90–100 kGy (scaled at 1813 cm^{-1}). (A) Skin of the sample, non-radiated. (B) Skin of the sample, radiated at 70 kGy. (C) Skin of the sample, radiated at 90 kGy. (D) Core of the sample, non-radiated. (E) Core of the sample, radiated at 70 kGy. (F) Core of the sample, radiated at 90 kGy.

copolymer number-average molecular weight with increasing doses. At the highest dose used in this study (94–103 kGy), the copolymer weight-average molecular weight and number-average molecular weight were shown to decrease, which could be caused by chain scission of the copolymer during radiation at this high dose.

Polydispersity is an index of the molecular-weight distribution, i.e. the distribution of polymer chains with different sizes. The greater the value, the broader the molecular size distribution in the polymer. During the radiation of Septacin™, there could be two parallel reactions occurring at the same time: chain scission and chain extension and/or branching. Polydispersity reached a peak value at the dose-level range of 70–80 kGy, indicating that there was a greater number of longer chains being produced by chain extension/branching than the shorter chains generated by chain scission. This phenomenon is further evidenced by the greatest increase in the copolymer weight-average molecular weight and the lowest number-average molecular weight at this dose range. Cross-linking is another potential

reaction that can occur during the radiation process; it can also lead to molecular-weight increase in the copolymer. Additional experiments were conducted to determine whether or not cross-linking did occur in Septacin.

No gel content was detected by the filtration test for radiated Septacin samples (at dose levels ranging from 10 to 100 kGy). Visual examination did not reveal any insoluble residue in the dried test tube or on the filter paper. The absence of gel in radiated Septacin samples indicates that there was no significant cross-linked network formation (Flory, 1953) in the copolymer during radiation.

The NMR spectra of non-radiated Septacin samples and samples radiated at the dose level ranging from 70 to 80 kGy are shown in Fig. 3. Although the greatest increase in copolymer molecular weight was observed at this dose range (Tables 1 and 2), there was no significant change in chemical structure of the radiated copolymer as detected by ^{1}H NMR.

There is no difference between the IR spectra for the non-radiated Septacin sample and those for samples radiated at dose levels below 50 kGy. However, for samples radiated at higher dose levels (70–80 and 90–100 kGy), it was shown that the intensity of absorbance attributable to the symmetric and axisymmetric C–H stretching vibration (at 2852 and 2927 cm^{-1}) was weaker than that for the non-radiated sample (skin and core portions) (Fig. 4).

The attenuation in absorbance at 2852 and 2927 cm^{-1} may indicate free-radical formation or loss of hydrogen atoms from C–H bonds. Free-radical formation during the γ-radiation process for polyanhydrides is reported elsewhere (Mader et al., 1996). In Fig. 4, the lowest absorbance intensity at 2852 and 2927 cm^{-1} was shown for sample B, which was radiated at the dose level ranging from 70 to 80 kGy. At this dose, there was also the greatest increase in the copolymer molecular weight. The absorbance at 1708 cm^{-1} by the skin portion of samples B and C is an additional evidence of carbonyl group formation by oxidation reaction during the radiation process. But the core portion did not yield absorbance at this wave number. Since less oxygen diffused into the core portion, the formation of

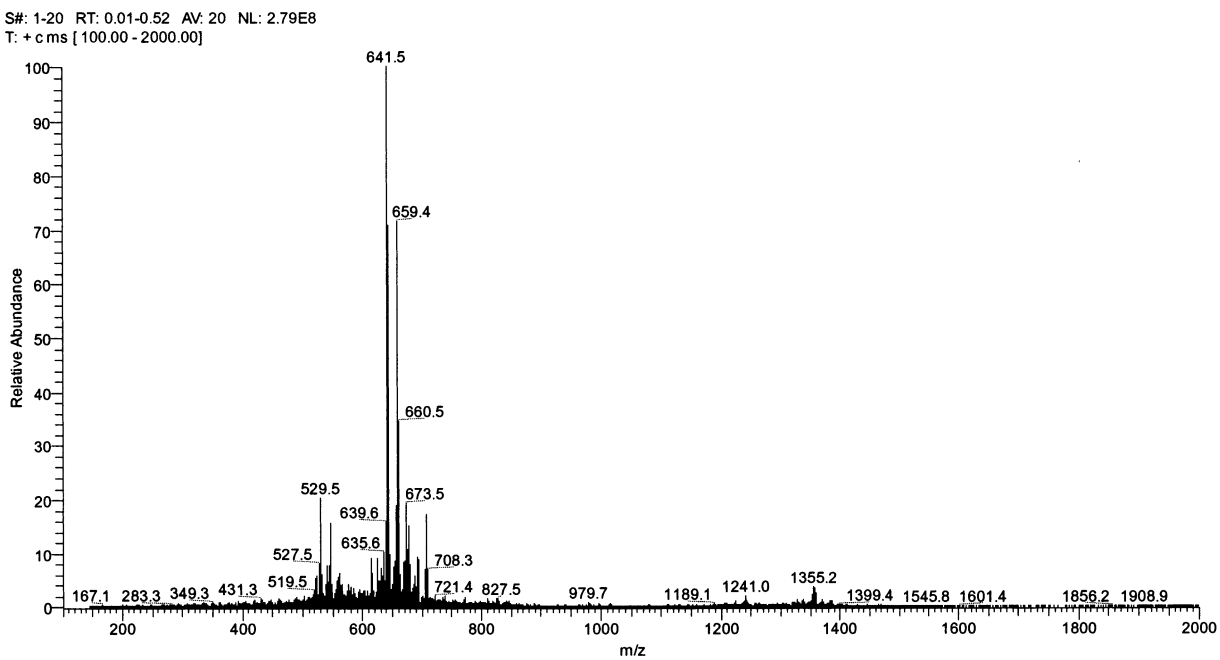

Fig. 5. EAD in methylene chloride.

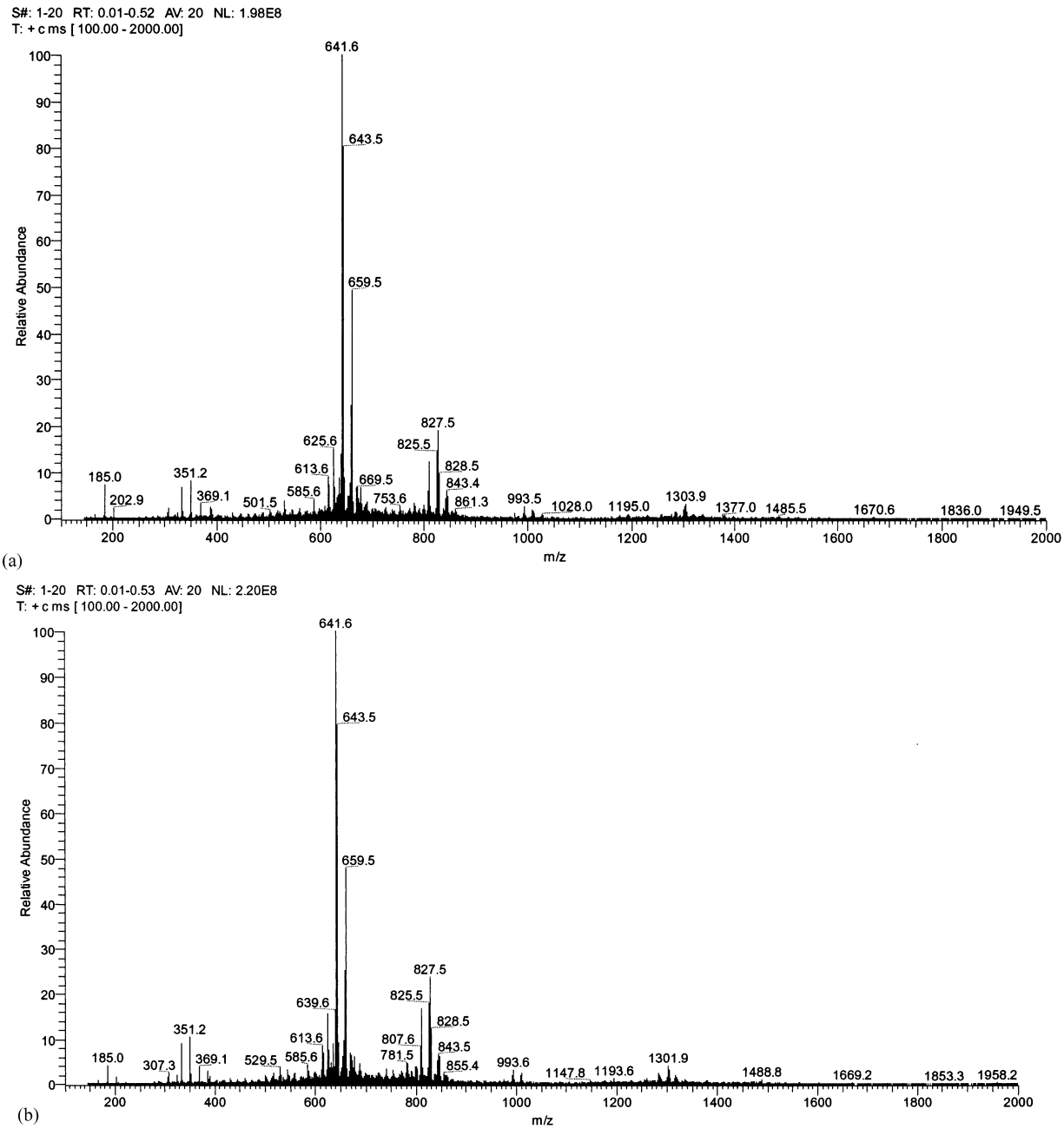

Fig. 6. (a) Non-radiated sample. (b) γ-Radiated sample.

carbonyl groups should decrease from the bead surface towards the core portion. Although no cross-linked structure could be found by means of the gel content test and ^{1}H NMR, the IR results could still indicate the possibility of C–C bond formation resulting from the formation of free radicals on carbon atoms from the breakage of C–H bonds. However, new C–C bond formation

can be further identified by using the mass spectrophotometric method.

Fig. 5 shows the mass spectrum that is obtained with the EAD control. The molecular weight of EAD is 676. The spectrum is abundant with ions 659 and 641, which correspond to $[M - H_2O + H]^+$ and $[M - 2H_2O + H]^+$ for the EAD, respectively. It is quite common for hydroxy-containing compounds (i.e. EAD) to lose water in the gas phase during mass spectral analysis. In addition, there is evidence of the C36 dimer ($M_w = 564$), as evidenced by $[M - H_2O + H]^+$ and $[M - 2H_2O + H]^+$ ions at 547 and 529.

Upon examination of the mass spectra (Fig. 6a and b) for non-radiated and radiated (70–80 kGy) Septacin, one observes the same EAD ions at 659 and 641. In addition, an oligomer consisting of an EAD and sebacic acid monomer, linked via an anhydride bond, is observed, as evidenced by $[M - H_2O + H]^+$ and $[M - 2H_2O + H]^+$ ions at 843 and 825. In addition, there also appears to be an evidence of an oligomer consisting of two sebacic acid monomers plus an EAD, all linked via an anhydride bond $[M - 2H_2O + H]^+$at 1008. The most important finding from this study is that the spectra for the radiated and the non-radiated controls are virtually identical. There is no evidence of any new C–C bond being formed as a result of cross-linking or branching by γ-radiation.

The stratified crystalline morphology was observed in the injection-molded Septacin™ beads (Neilly et al., 1998). Fig. 7 shows the crystalline morphology images of samples listed in Table 1. It is seen that the crystalline morphology was not altered during the γ-radiation process, regardless of the dose levels.

The degree of crystallinity of Septacin beads radiated at different dose levels was measured by means of WAXD. Data tabulated in Table 2 indicate that there was a slight increase in crystallinity with increasing radiation doses below 50 kGy, but a more noticeable increase was shown at dose of 70 kGy.

Some polymers, when γ-radiated at relatively low doses (< 200 kGy), have been shown to exhibit an increase in crystallinity (Bhateja et al., 1983). This phenomenon is attributed to a chain-

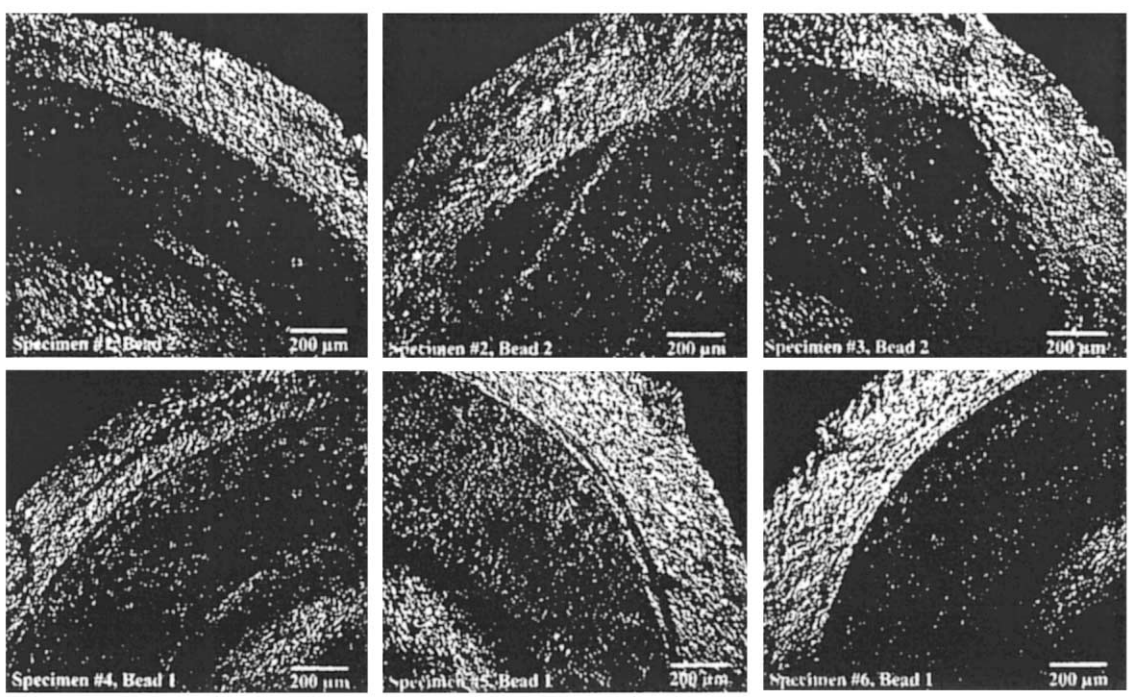

Fig. 7. Crystalline morphology for Septacin™ beads radiated at different dose levels.

scission process that reduces the molecular weight and chain entanglements (Bhateja et al., 1983). The reduction in both properties will increase the mobility of the polymer chains and allow more crystallization during the radiation process (Bhateja et al., 1983). In this study, the reduction in molecular weight for radiated Septacin was not observed. Therefore, the theoretical explanation above may not apply to the Septacin results. In theory, during the radiation process, for every 10 kGy absorbed dose, there is an equivalent heat absorption (McLaughlin, 1989) of 10 kJ/kg. This heat may cause an annealing effect on the copolymer, creating an increase in crystallinity. The effects of annealing on melt-crystallized polymers, including lamellar thickening, increases in the melting point and crystallinity, and changes in mechanical properties, have been well documented (Tadmor and Gogos, 1979).

4. Conclusions

The impact of radiation on a polyanhydride implant, Septacin™, was found to be insignificant with respect to copolymer molecular weight and chemical structure at a dose level below 50 kGy. The greatest increase in crystallinity, molecular weight, and polydispersity was found for Septacin radiated at the dose range of 70–80 kGy. However, no evidence of cross-linking and branching was observed for samples radiated at this dose range by means of the gel content test, ^{1}H NMR, and mass spectroscopy. Although IR spectra have shown evidence of free-radical formation from the cleavage of C–H bonds for these samples, results from this study have led to the conclusion that the molecular-weight increase in radiated Septacin is not the result of cross-linking or branching, but the result of chain extension.

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