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Effect of gamma-irradiation on cladribine and cladribine-containing biodegradable copolymers

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Abstract

The aims of this study were to assess the effects of sterilization with gamma-irradiation on (i) bulk cladribine and (ii) cladribine-containing biodegradable copolymers. The stability of cladribine upon irradiation was confirmed by TLC, HPLC, UV, IR, DSC, rentgenography and electron microscopy. The stability of copolymers containing cladribine upon irradiation was assessed by IR, DSC and EPR. In vitro kinetics of nucleoside release from the copolymers before and after irradiation were compared, and only slight changes were found. Results of our study indicate that gamma-irradiation can be safely applied for the sterilization of cladribine or cladribine-containing copolymers for medical purposes.

Keywords: Biodegradable copolymers; Cladribine; Gamma-irradiation

1. Introduction

Malignant brain tumors are currently incurable [1]. In particular, chemotherapy is not effective because of the life-threatening peripheral (usually bone marrow) toxicity which appears at drug doses far lower than those required for evoking tumor eradication or growth suppression [2]. Intracerebral implantation of biodegradable copolymers containing cytotoxic drugs is a strategy which may circumvent this problem by establishing a high and sustained concentration of a cytotoxic compound at the tumor site, while peripheral toxicity is avoided [3].

Cladribine, a cytotoxic nucleoside analog, is very active towards some hematological malignancies. Its dose-limiting toxicities are bone marrow suppression and opportunistic infections related to immunosuppression [4,5]. The drug also displays marked

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activity against cells of primary brain tumors (gliomas) in vitro [6], but maximal tolerated doses are not effective in glioma patients [7].

We have developed a series of biodegradable copolymers loaded with cladribine which, in model conditions approximating the intracerebral environment, release micromolar quantities of the nucleoside over several weeks [8]. The prerequisite for their application in a clinical setting is that they can be sterilized without decomposition or a drastic change in properties. Exposure to gamma-rays is a frequently used and convenient method of sterilization of copolymers loaded with drugs, although it may change their properties [9]. However, the stability of cladribine upon gamma-irradiation may raise concern, since UV light causes rapid decomposition of this compound [10]. The aim of the present study was to assess the effects of sterilization with gamma-irradiation on cladribine itself and on the kinetics of release of this drug from cladribine-containing copolymers.

2. Materials and methods

2.1. Cladribine and cladribine-containing copolymers

Pharmaceutical grade (>99% by HPLC, water content <0.1%) bulk cladribine was obtained free of charge from the Foundation of the Development of Diagnostics and Therapy (Warsaw, Poland).

Two cladribine-containing copolymers (Table 1) were prepared according to the procedures described in previous work [8]. In brief, the synthesis of the copolymer of glycolide and lactide (≠1317) was performed in THF solution at 20 °C, using butyl lithium as initiator of the polymerization reaction. The synthesis of the lactide and caprolactone block copolymer (≠1284) was conducted in the presence of zirconium(IV) acetylacetonate at 100–150 °C. The copolymers were precipitated with methanol and dried at 50 °C under vacuum. The copolymers were then dissolved in methylene chloride. Cladribine was dissolved in dimethyl sulfoxide, added to the copolymer solutions and stirred. The mixture containing nucleoside was cast on a glass plate and the solvent was evaporated at ambient temperature. The resultant films, 0.1–0.3 mm thick, were then dried under reduced pressure and stored in a desiccator at room temperature. Their composition was established from ^{1}H and ^{13}C NMR spectroscopic data collected with a Varian Unity Inova spectrometer, and average molecular weights were determined by liquid chromatography and gel chromatography with the use of a Waters ALC/GPC 3 M and a RI Spectra Physics SP 8800 chromatographic system.

2.2. Exposure to gamma-irradiation

Portions (100 mg) of cladribine, or 10×10 mm pieces of cladribine-containing copolymers, were placed in 3 ml glass vials closed with a plastic stoppers. An Issledovatel RChM-gamma 20 cobalt-60 radiation source was used to irradiate the samples. Cladribine samples were irradiated with doses of 15, 20, 25, 100 or 200 kGy, and samples of copolymers were exposed to irradiation of 15, 20 or 25 kGy.

2.3. Analytical methods

Before and after irradiation, cladribine and cladribine-containing copolymers were weighed and subjected to visual inspection and standard tests for microbiological contamination using the methodology recommended by the Polish Pharmaceutical Society [11] (media, soybean casein digest broth or

Table 1
Copolymers assessed in this study

Copolymer sample	Copolymer composition	Cladribine content (% w/w)	l_{GG}	l_{L}	l_{Cap}	m (kDa)
1317	18% glycolide, 82% DL-lactide	4.5	1.5	6.8	–	30
1284	70% L-lactide, 30% caprolactone	5.5	–	2.7	1.2	40

m, average molecular mass; w/w, weight/weight; l_{GG}, l_{L}, l_{Cap}, average length of glycolidyl, lactydyl or caproil blocks.

thioglycollate resazurine broth; temperature, 25 or 35 °C; incubation for 7 days).

The methods used to assess the stability of cladribine upon irradiation were essentially similar to those described previously for other drugs [12], with some minor modifications. Differential scanning calorimetry measurements of samples of bulk cladribine were performed in the range of temperatures from 20 to 300 °C in an atmosphere of helium at a heating rate of 5 °C/min (Netzsch DSC-204 apparatus and TAA program). Rentgenography and electron microscopy were performed with a HZG 3 diffractometer and a Philips SEM 515 electron microscope, respectively. For IR spectra, a Bruker IFS 113V FT-IR spectrometer (range 400–4000 cm^{-1}) was used. Thin layer chromatography (TLC) was performed with different combinations of stationary phases (cellulose with fluorescent agent, aluminium oxide 60 F_{254}, silica gel 60 F_{254}, silica gel HPTLC 60 F_{254}) and mobile phases [1 M ammonium acetate buffer, acetonitrile–*n*-butanol–0.1 M ammonium acetate–25% NH_4OH (6:1:2:1), chloroform–methanol (4:1), chloroform–ethanol (2:1), methanol–25% NH_4OH (100:1.5), *n*-propanol–water (7:3), *n*-propanol–*n*-butanol–water (2:1:1)] and 0.1% methanol solutions of cladribine were used. The spots were visualized in the light of a quartz lamp of $\lambda = 254$ or 336 nm. For UV spectrophotometry and analytical HPLC, 2 mg/ml solutions of non-irradiated and irradiated cladribine samples in phosphate buffer (pH 4.38) were prepared. The absorbance spectra were measured on a Perkin-Elmer Lambda 20 UV–VIS spectrometer (wavelength range 220–400 nm).

To assess the stability of copolymers upon gamma-irradiation, DSC, IR spectrometry and EPR analyses of cladribine-containing copolymers were performed. Differential scanning calorimetry measurements of samples of copolymers were performed for temperatures from 30 to 300 °C in an atmosphere of helium at a heating rate of 10 °C/min (Perkin-Elmer DSC-7 apparatus). IR spectra were measured with a Perkin-Elmer Spectrum 2000 FT-IR spectrometer (range 400–4000 cm^{-1}), and electron paramagnetic resonance (EPR) analyses were performed on a Bruker ESP 300 X-band spectrometer (100 kHz) with a microwave power of 10 mW and a modulation amplitude of 2 G.

The amount of cladribine in each of the copolymers was determined by dissolving copolymer film discs (previously weighed), approx. 5 mm in diameter, in 2 ml of dimethyl sulfoxide. The content of the nucleoside in the copolymer was then calculated from the result of the HPLC assay. To determine the kinetics of nucleoside release, similar copolymer film discs were placed in separate glass bottles containing 2 ml of artificial cerebrospinal fluid. The bottles were sealed and kept at 37 °C in a water bath. Each day the bottles were opened and the fluid was removed with a pipette and replaced with fresh solution.

Analytical HPLC analyses of irradiated cladribine samples and assays of cladribine released from non-irradiated and irradiated copolymers were performed using a Merck Hitachi LaChrom HPLC system with an L-7250 autosampler, an L-7420 programmable detector, an L-7100 pump, a D-7500 integrator and a 250×4.6 mm, 5 μm Supelcosil C_{18} column. The mobile phase was 0.035 M phosphate buffer containing 3% acetonitrile (adjusted to pH 3.0 using

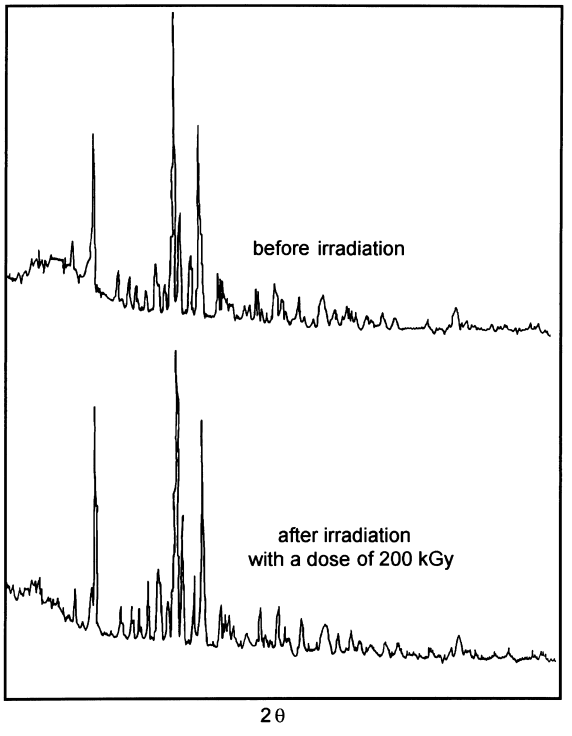

Fig. 1. Powder diffractograms of cladribine.

Table 2
Melting points and enthalpy of gamma-irradiated cladribine measured with DSC

0 kGy	25 kGy	Difference
204.7 °C	186.1 °C	−18.6 °C
200.4 °C	175.8 °C	−24.6 °C
146.6 J/g	131.1 J/g	−15.5 J/g

sodium hydroxide)–methanol (84:16, v/v), with a flow rate of 1 ml/min. The injection volume was 20 μl. UV detection was performed at 264. Calibration curves were generated from the peak areas of freshly prepared standard cladribine solutions.

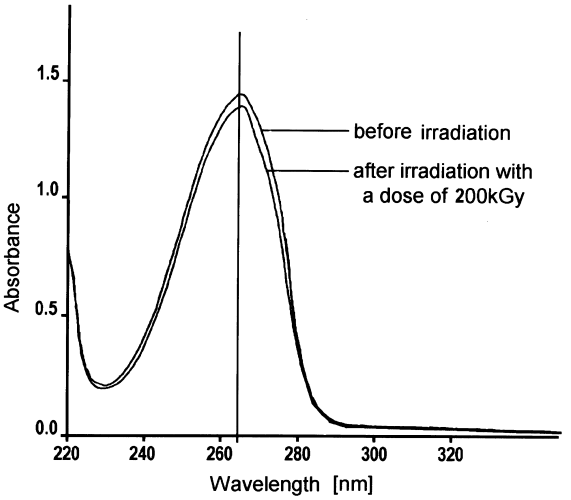

Fig. 3. UV spectra of cladribine.

3. Results

The initial samples of bulk cladribine and cladribine-containing copolymers were found to contain small amounts of bacteria (*Bacillus* and *Clostridium*), but no mould. Each of the tested samples was found to be sterile after irradiation with each of the doses.

Visual inspection revealed discrete changes in the colour of bulk cladribine (slight browning of the

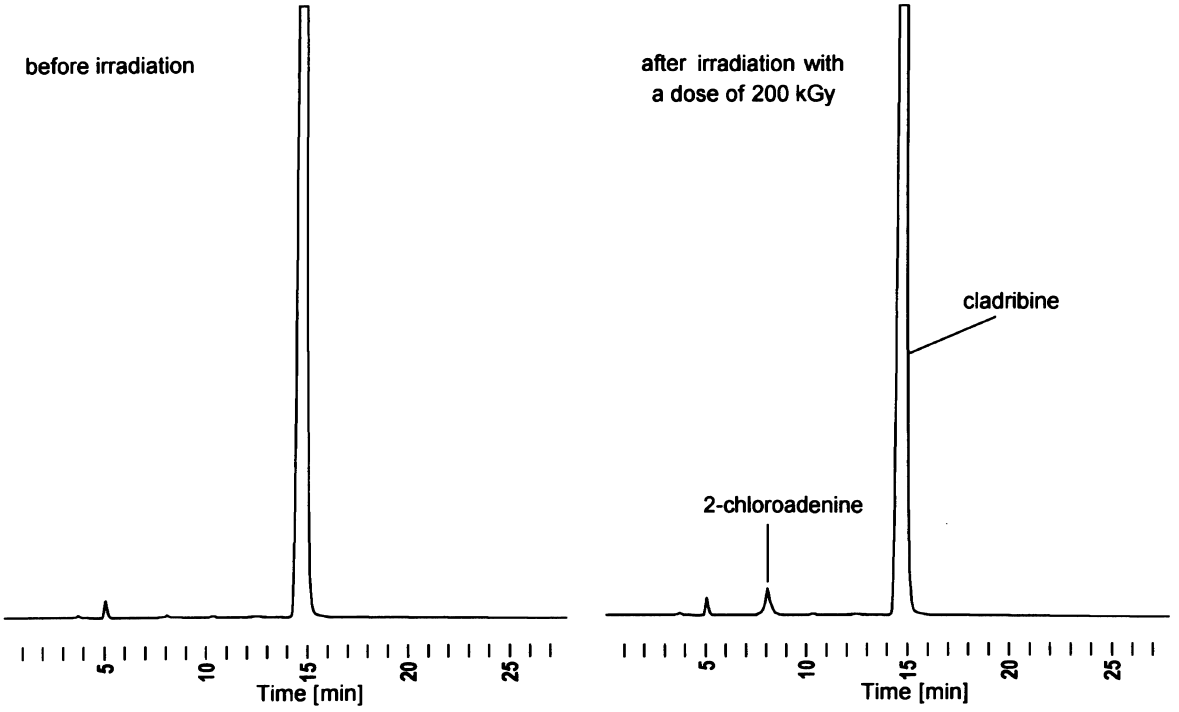

Fig. 2. Evaluation of cladribine with HPLC.

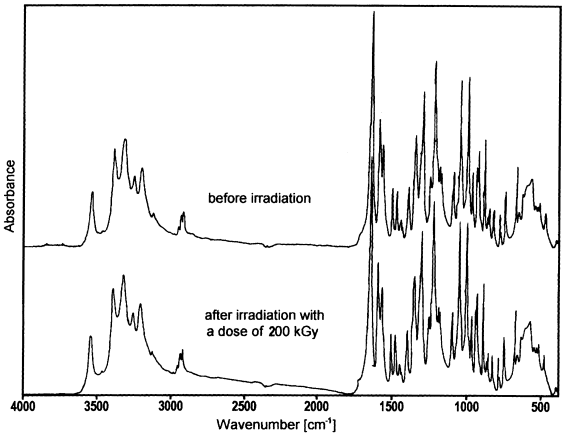

Fig. 4. IR spectra of cladribine.

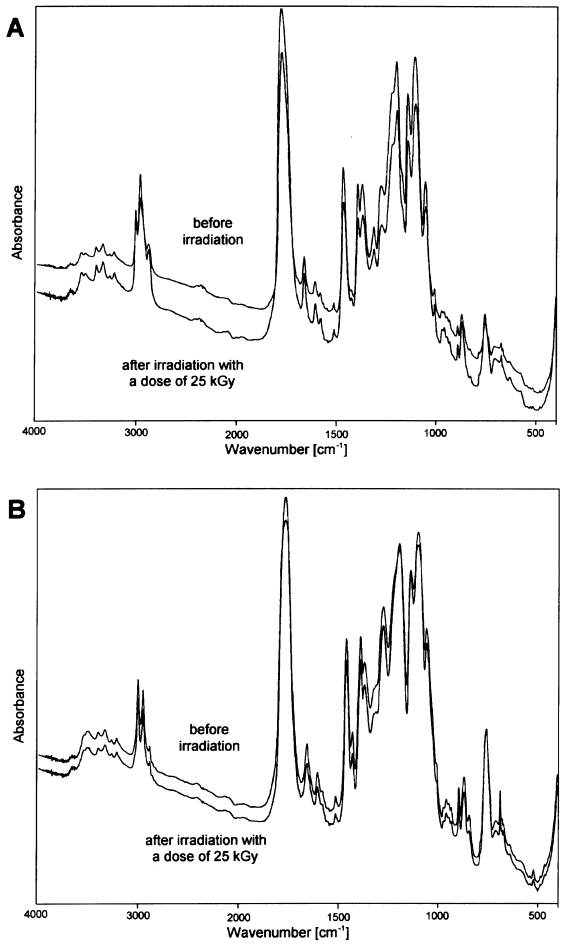

Fig. 5. IR spectra of the copolymers: (A) #1284, (B) #1317.

powder), increasing with the dose of radiation used. However, no change in copolymer appearance was observed.

Rentgenography (Fig. 1) and electron microscopy (results not shown) did not show any appreciable changes in the structural properties of bulk cladribine after sterilization. The only method which revealed marked changes in the properties of bulk cladribine was differential scanning calorimetry (Table 2).

TLC measurements performed for different combinations of stationary and mobile phases (data not presented) showed no evidence of cladribine decomposition after irradiation, while analytical HPLC revealed 3.5% contamination with 2-chloroadenine in a sample irradiated with 200 kGy (Fig. 2). UV (Fig. 3) and IR (Fig. 4) spectrophotometry showed no significant changes in the spectra following irradiation.

IR spectra (Fig. 5) and DSC calorimetry (Fig. 6) of both copolymers showed no differences between non-irradiated and irradiated samples. Only EPR analyses of copolymer #1284 displayed the presence of the CH_3 radical after gamma-irradiation of a vacuumed sample (10^{-3} mmHg) (Fig. 7).

The kinetics of cladribine release from the copolymers in the in vitro model system changed only slightly by sterilization with gamma-irradiation (Fig. 8).

4. Discussion

Parenteral drug delivery systems have to meet the pharmacopoeal requirements for sterility. Commonly used sterilization techniques, such as sterilization by steam or dry heat, cannot be used for biodegradable aliphatic polyesters of the type glycolide–lactide or lactide–caprolactone since they alter the physical and chemical properties of the copolymer. Moreover, the stability of the drug incorporated in the copolymer matrix has to be taken into account. Therefore, the selection of a suitable sterilization method for such formulations is crucial to ensure their physical and chemical integrity, their performance, and safety in vivo. In this respect, ethylene oxide gas and gamma-irradiation techniques are preferred for the sterilization of biodegradable copolymers.

Chemical sterilization with ethylene oxide gas

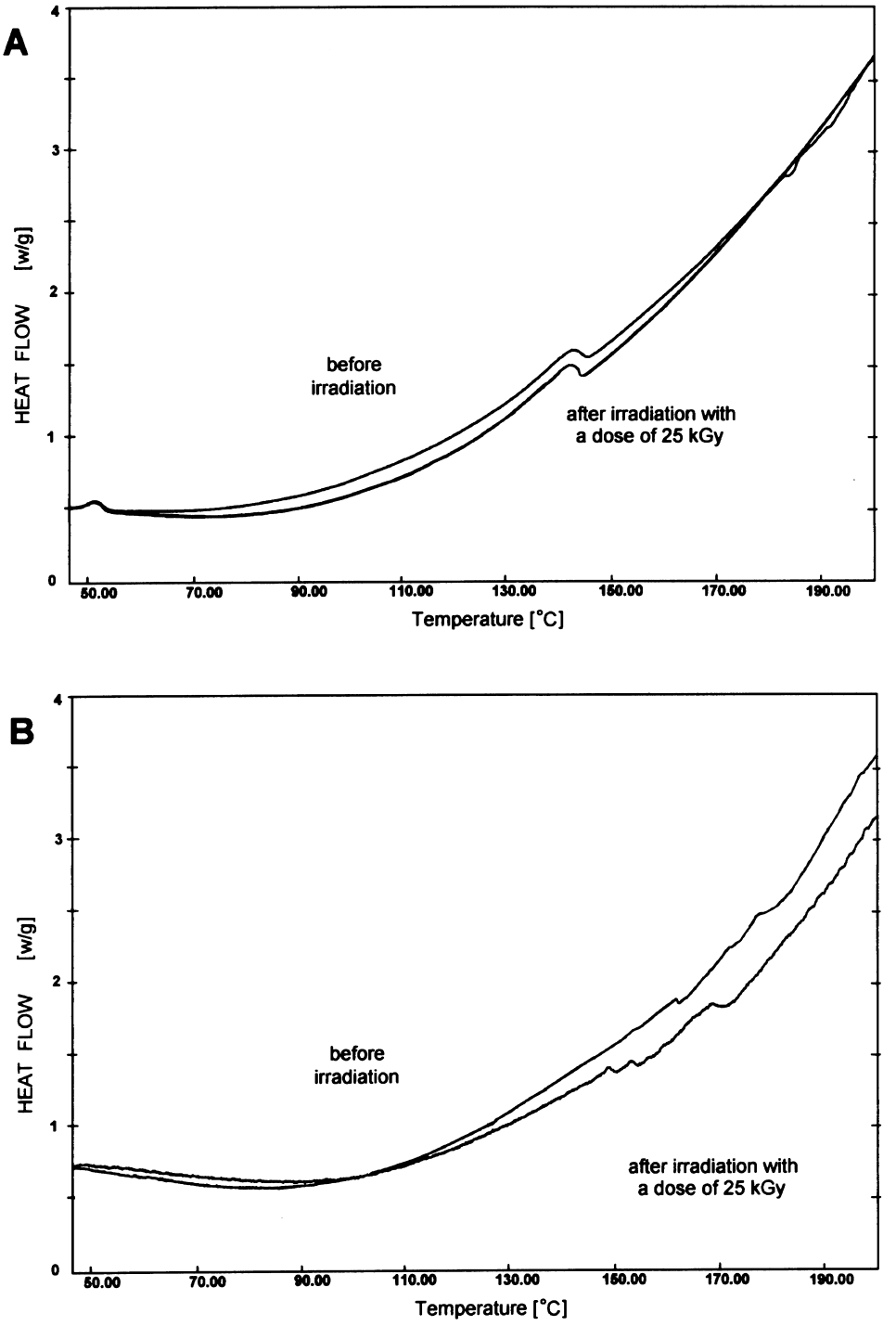

Fig. 6. Differential scanning calorimetry of the copolymers: (A) #1284, (B) #1317.

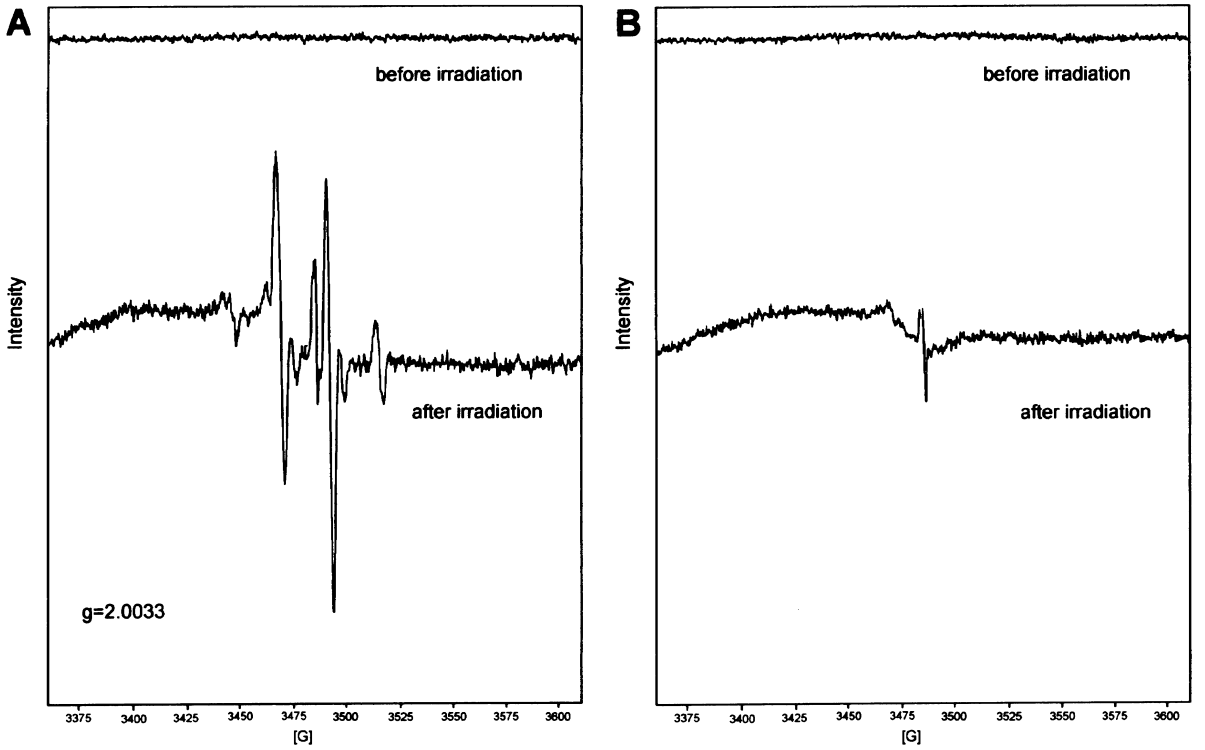

Fig. 7. Electron paramagnetic resonance of the copolymers: (A) #1284, (B) #1317. Samples were sealed under vacuum before irradiation.

offers the advantage of being effective at ambient temperature and for hydrolytically unstable copolymers. Nevertheless, its popularity is decreasing due to the known toxicity and flammability of ethylene oxide. Being a strong alkylating agent, it may also react with functional groups on the copolymer surface, thereby altering its biological properties, and toxicological problems may be encountered due to toxic residues [9,13].

Gamma-irradiation has been shown to affect the properties of drug-loaded polyester microparticles in several ways, such as radiolytic reactions, chain scission and cross-linking, and may also cause gas evolution or free-radical formation. These reactions may have consequences for the nominal drug content or the drug release pattern from copolymers [9,14].

Mohr et al. pointed out that, because of the influence of humidity and oxygen, environmental conditions during irradiation have to be controlled carefully to reduce radiolytic effects on the polymer drug delivery system and to minimize the decrease in mechanical strength of the polymer under irradiation due to polymer chain breakdown [15].

Changes in molecular mass and polymer degradation as a function of gamma-irradiated dose and storage time after gamma-sterilization were also observed [15,16]. Taking into account the relationship between the polymer matrix and drug loaded into the polymer, Bittner et al. reported that there was very little influence of the polymer matrix on the stability of the drug incorporated into a polymer exposed to gamma-irradiation [17]. The same authors observed that the degradation rate of microspheres was slowed after incorporation of tetracycline hydrochloride into the polymeric matrix. However, the influence of microencapsulated drug on polymer decomposition appears to be rather complex. This hypothesis was confirmed by Montarini et al. and Calis et al., with the conclusion that drug incorporated into a polymer may display a radio-stabilizing effect on the polymeric matrix [18,19].

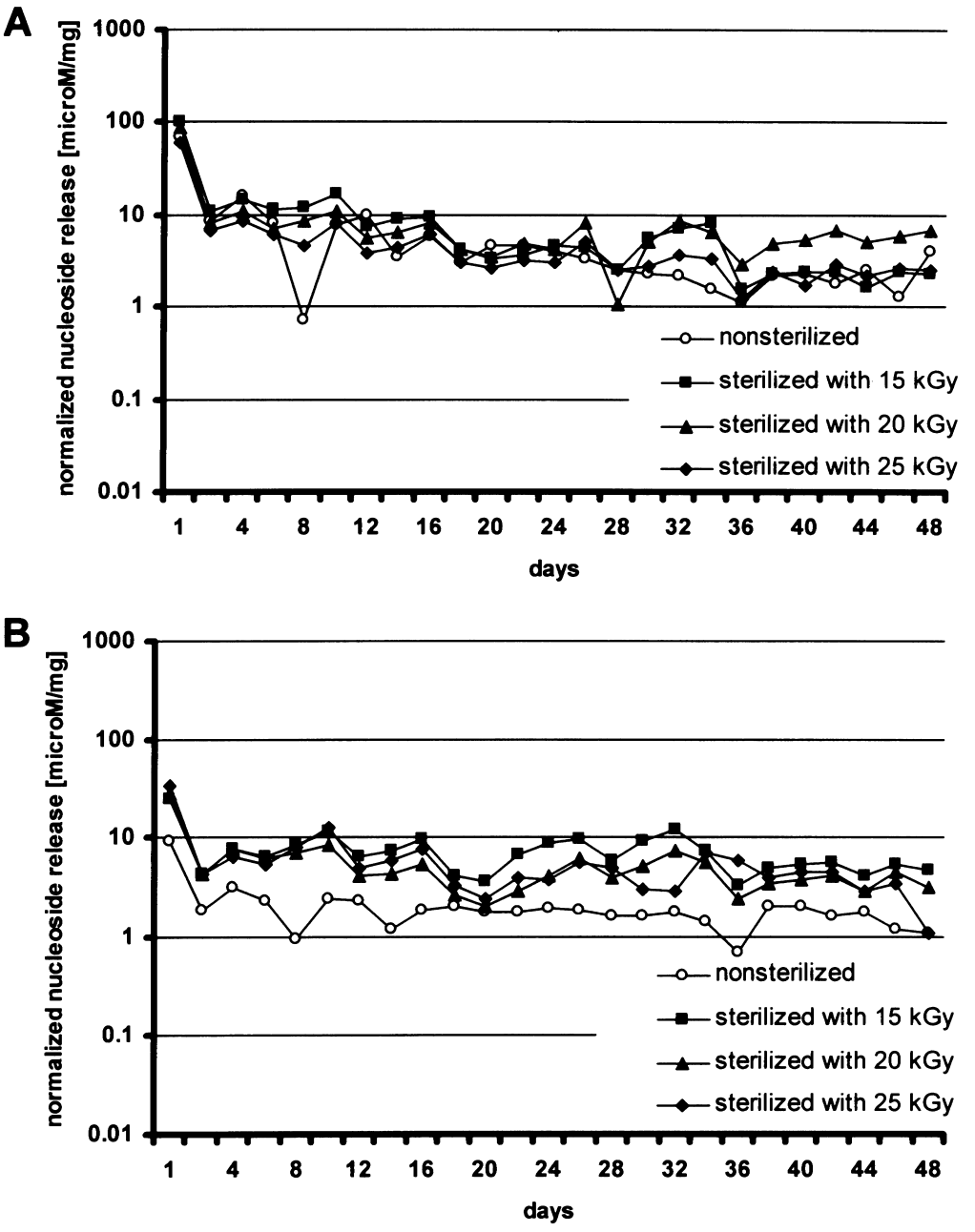

Fig. 8. Kinetics of cladribine release from the copolymers: (A) #1284, (B) #1317 (concentration vs. time).

One should also take into account the composition of the polymeric matrix. Frequently used poly(lactide-co-glycolide) copolymers (PLGA) are commercially available and are very different from the copolymers tested in the present experiments [8]. Different synthesis processes, different methods for introducing the drug into the biodegradable carrier, and different sizes and shapes of the polymer

formulations (microspheres or polymer foil) may be the reasons why the drugs incorporated into PLGA copolymers are released within 2–3 weeks [18,20–22].

In the present study we focused on the chemical and physical properties of cladribine and cladribine-containing copolymers exposed to gamma-irradiation. The absorbed doses of 15, 20 or 25 kGy were regarded as adequate for sterilizing medical devices for clinical use. The stability of bulk cladribine after gamma-radiation (15–25 kGy) was assessed with TLC, HPLC, UV and IR spectrophotometry, DSC, rentgenography and electron microscopy. The only changes due to irradiation were observed with DSC analysis.

Also, the same analytical methods (IR, DSC) applied to cladribine-containing copolymers did not show changes in the stability of the copolymers upon irradiation. The only exception was the EPR analysis of copolymer #1284, in which the free methyl radical was identified in a vacuumed sample after exposure to gamma-irradiation. However, the kinetic properties of the copolymer were not appreciably affected.

Our previous study [8] indicated that continuous release of nucleoside analogs from these specific copolymers may last for over 3 months. An open question remains: whether or not the chemical structure of the drug introduced into the copolymer influences the rate of its release, or the rate of copolymer erosion in the aqueous bioenvironment. Do reactions between the drug and the copolymer matrix play a role in the stability of such a drug delivery system, as suggested by other authors [17]?

We conclude that gamma-irradiation is a suitable sterilization method for parenteral drug delivery systems based on glycolide–lactide or lactide–caprolactone copolymers. Doses of 10–25 kGy are recommended for the sterilization of both cladribine and cladribine-containing copolymers.

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