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Radiation sterilisation of doxorubicin bound to poly(butyl cyanoacrylate) nanoparticles

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Abstract

Doxorubicin-loaded poly(butyl cyanoacrylate) (PBCA) nanoparticles were prepared by anionic polymerisation under non-aseptic conditions. The feasibility of sterilisation of this formulation using either γ-irradiation or electron beam irradiation was investigated. The irradiation doses ranged from 10 to 35 kGy. *Bacillus pumilus* was used as the official test microorganism. The bioburden of the untreated formulation was found to be 100 CFU/g. Microbiological monitoring revealed that at this level of the bioburden the irradiation dose of 15 kGy was sufficient for sterilisation of the nanoparticles. The formulation showed excellent stability with both types of irradiation in the investigated dose range. The irradiation did not influence the physicochemical parameters of the drug-loaded and empty nanoparticles, such as the mean particle size, polydispersity, and aggregation stability. The molecular weights of the PBCA polymer as well as the polydispersity indices (M_w/M_n) remained nearly unchanged. The drug substance was stable to radiolysis. Additionally, the presence of irradiation-induced radicals was evaluated by ESR spectroscopy after storage of the particles at ambient temperature. The paramagnetic species found in the formulation were mainly produced by irradiation of mannitol and dextran used as excipients.

Keywords: Irradiation; Sterilisation; Nanoparticles; Poly(butyl cyanoacrylate); Doxorubicin

1. Introduction

Parenteral drug delivery systems based on polymeric nanoparticles have been intensively investigated during the last decades covering a wide variety of drug substances. Application of the nanoparticles as drug carriers offers various advantages compared to conventional dosage forms, such as predictable optimisation of the drug biodistribution and enhanced efficacy (for recent reviews see Couvreur and Vauthier, 2006; Moghimi, 2006). In particular, poly(alkyl cyanoacrylate) nanoparticles appear to be a promising and versatile drug delivery system which could allow a considerable extension of the drug activity spectrum, as it was recently demonstrated for doxorubicin. Indeed, the clinical applications of doxorubicin, a potent anticancer antibiotic, were somewhat limited since this drug is a P-gp substrate and cannot be delivered efficiently to P-gp-expressing multidrug-resistant tumours or to tumours protected by the P-gp-expressing blood–tissue barriers such as the blood–brain barrier. At the same time, doxorubicin-loaded poly(isohexyl cyanoacrylate) nanoparticles enable an effective chemotherapy of a chemoresistant tumour, hepatocellular carcinoma (Barraud et al., 2005), whereas polysorbate 80-coated poly(butyl cyanoacrylate) (PBCA) nanoparticles facilitate the delivery of doxorubicin to the brain and considerably increase its efficacy against an intracranial glioblastoma in rats producing a long-term remission in >20% animals (Steiniger et al., 2004). Moreover, the latter formulation displayed a significantly reduced cardio- and testicular toxicity

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compared to the conventional dosage form (Pereverzeva et al., 2007).

These excellent results suggest that the formulations based on poly(alkyl cyanoacrylate) nanoparticles have a high potential for clinical use; yet an important aspect of this technology, i.e. the method of sterilisation, remains to be established. Indeed, the development of a suitable method for the sterilisation of nanoparticulate drugs, and poly(alkyl cyanoacrylate)-based formulations in particular, is challenging since the applicability of the commonly used techniques is limited by the physicochemical parameters of these nanoparticles. The PBCA nanoparticles are sensitive to heat: Sterilisation of these nanoparticles in suspension by autoclaving (121 °C, 10 min) produced an irreversible loss of their stability due to aggregation and sedimentation (Sommerfeld et al., 1998). Similar results were obtained after sterilisation of the freeze-dried PBCA particles in a formaldehyde stream at 60 °C (Sommerfeld et al., 1998). Sterile filtration obviously also is not applicable since the mean particle size (usually 150–300 nm) is similar to the pore size of the filters (220 nm). Therefore, apart from manufacturing of the particles under aseptic conditions which is a cost-consuming procedure, sterilisation by irradiation appears to be the only feasible alternative also offering the advantages of low chemical reactivity, low measurable residues, and fewer variables to control.

As demonstrated previously, γ-irradiation was successfully applied for sterilisation of drug-loaded PLGA (Bozdag et al., 2005; Mohr et al., 1999) as well as poly(isobutyl cyanoacrylate) nanoparticles (Paramonov et al., 1996). In particular, γ-irradiation of ampicillin bound to poly(isobutyl cyanoacrylate) nanoparticles with the doses of 24.2 or 27.4 kGy did not affect its antibacterial activity; there also was no significant influence of the irradiation on the release kinetics of the drug from the particles. However, the efficacy of sterilisation as well as the optimal irradiation dose and the impact of irradiation on the ingredients were not evaluated in this study. At the same time, these parameters are critical for the final assessment of suitability of the radiation sterilisation for poly(alkyl cyanoacrylate) nanoparticles. Indeed, application of this method for polymeric drug delivery systems also could be associated with certain problems, such as drug radiolysis or cross-linking or backbone scission of polymers induced by irradiation, which may influence their chemical and physical properties (Fernández-Carballido et al., 2006; Hausberger et al., 1995).

The objective of the present study was the development of a method for radiation sterilisation of the above described promising nanoparticulate formulation of doxorubicin based on PBCA nanoparticles.

2. Materials and methods

2.1. Reagents and chemicals

n-Butyl-2-cyanoacrylate was provided by Sichel-Werke, Hannover, Germany. Doxorubicin and doxorubicinol were a gift from Sicor, Rho, Italy. All other reagents were obtained as follows: dextran 70,000 and mannitol, from Merck, Darmstadt, Germany; polysorbate 80 from ICI Chemicals, Essen, Germany. Other chemicals were supplied by Sigma (St. Louis, USA).

2.2. Preparation of doxorubicin-loaded nanoparticles (Dox-NP)

Doxorubicin-loaded poly(butyl cyanoacrylate) nanoparticles were prepared by anionic polymerisation in the presence of the drug as previously described by Gulyaev et al. (1999). 1% of *n*-butyl-2-cyanoacrylate was added to a 1% dextran solution in 0.001N HCl under constant stirring. 30 min after the start of the polymerisation, doxorubicin was added to a final concentration of 0.25%. After a total of 4 h, the mixture was neutralised with NaOH, filtered through a G 1 glass filter (Schott, Mainz, Germany), filled into vials (2 ml/vial), and freeze-dried after addition of 3% (w/v) mannitol as a cryoprotector. The mean particle size was measured by photon correlation spectroscopy (Coulter N4 MD, Coulter Electronics, U.K.). The amount of free doxorubicin in the preparation was assessed indirectly by a spectrophotometric assay of the free drug after separation of the particles by centrifugation (150,000 × *g*, 45 min). The empty (placebo) nanoparticles were prepared using a similar technique.

2.3. Synthesis of poly(butyl cyanoacrylate)

For the preparation of the sample of poly(butyl cyanoacrylate), *n*-butyl-2-cyanoacrylate (0.2 ml) was added dropwise into 0.002N solution of HCl. Then the pH of the reaction mixture was adjusted to 8.0 with a 1N solution of NaOH. After 3 h, the precipitated polymer was separated by filtration, washed with distilled water, and air-dried.

2.4. Irradiation

The lyophilised samples of doxorubicin substance empty nanoparticles (NP), and doxorubicin-loaded nanoparticles (Dox-NP) ($n = 4$) filled into glass vials (20 mm × 55 mm) were subjected to radiation sterilisation. γ-Irradiation was performed using a ^{60}Co-radiation source (MRH-γ-100, Issledovatel, Moscow, Russia) at a dose rate of 0.9–1.0 kGy/s. Irradiation by electron beams was performed using a linear electron accelerator (LUE 8-5M Moscow, Russia) with an electron energy of 8–10 MeV. The radiation doses were 10, 15, 25, and 35 kGy. Irradiation was performed at ambient temperature. The dosimetric control was performed using a radiochromic film dosimeter (SODP F 5/50, FTRI, Rosstandart, Russia) which was placed inside the vials. Non-irradiated samples served as control.

2.5. Analysis of doxorubicin by HPLC

The analysis of doxorubicin was performed by isocratic HPLC (SP 8000, Spectra Physics, U.S.A.) using a Separon C8 column (250 mm × 3 mm, 7 μm). The chromatograms were registered at 254 nm using a spectrophotometric detector (SP 8300, Spectra Physics, U.S.A.). A mixture, acetonitrile–0.05% aqueous solution of trifluoroacetic acid (35:65), was used as the mobile phase; the flow rate was 0.8 ml/min.

2.5.1. Sample preparation

The freeze-dried nanoparticulate formulations were solubilised in acetonitrile, as follows: 3 ml of acetonitrile were added into each vial; then the vials were sonicated for 15 min, and 6 ml of water were added for polymer precipitation. The suspension was quantitatively transferred into a 50 ml volumetric vessel and diluted to this volume with the mobile phase. 2 ml of this mixture were centrifuged for 15 min at 3000 rpm. The supernatant was filtered through a nylon filter (0.2 μm) (sample solution).

The retention time was ∼12 min for doxorubicin and ∼10 min for doxorubicinol. The amount of by-products was calculated as the ratio of the area of the additional peaks to the total peak area on the chromatogram.

2.6. Assay of doxorubicin content

The content of doxorubicin in the nanoparticulate formulation was measured spectrophotometrically after its dissolution. 3 ml of the mixture water–DMF (2:7) was added into a vial containing the freeze-dried formulation (∼80 mg), and the mixture was kept for 2 h at ambient temperature. The resulting clear solution was quantitatively transferred into the 50 ml volumetric vessel and diluted to this volume with the same mixture. The concentration of Dox in this solution was measured spectrophotometrically (Thermo Spectronic Heλios, Unicam, Great Britain) at 480 nm.

2.7. Molecular mass distribution of PBCA

The molecular weights of PBCA were determined using gel-permeation chromatography (GPC). The system consisted of a pump (HPLC pump 64, Knauer, Berlin, Germany), refractive index detector (RefractoMonitor IY, Milton Roy, U.S.A.), and a spectrophotometric detector (3100 Milton Roy, U.S.A.), PL®-Gel 5 μm columns 10^2 Å and 10^3 Å (Phenomenex, U.S.A.). The polystyrene standards of narrow polydispersity with molecular masses in a range from M_w 510 to 95,000 Da (Waters, U.S.A.) were used for column calibration. A mixture of chloroform and methanol (97:3, v/v) was used as a mobile phase at the flow rate 1 ml/min. The detection wavelength was 264 nm.

2.7.1. Sample preparation

Chloroform was added to the nanoparticle preparations. The samples were stored overnight at ambient temperature for polymer dissolution, and then the excipients that were insoluble in chloroform (dextran and mannitol) were separated by filtration. The filtrates were diluted with the mobile phase. The polymer concentration in the samples was ∼1 mg/ml. All measurements were performed in duplicate.

2.8. Electron spin resonance (ESR) spectrometry of the samples after irradiation

The ESR spectra were measured using an X-band (9.4 GHz) spectrometer (Spin, St.-Petersburg, Russia) at a microwave power of 1 mW with a high frequency modulation of 100 kHz. A single crystal of $CuCl_2 \times 2H_2O$ was used as an absolute standard of spin number for quantitative determination. The number of radicals in the experimental samples was estimated by comparison of second integrals of the ESR signals in the studied sample and standard. The ESR spectra were recorded for the samples irradiated at a dose of 15 kGy after one year of storage. The amount of the radicals in the samples also was measured in the samples subjected to γ-irradiation to the dose of 15 kGy using a K-120000 ^{60}Co source and incubated for 1 and 20 min post-irradiation at ambient temperature or 10 min at 40°. In order to clarify the role of specific components in radical formation, the ESR spectra of irradiated PBCA, dextran, and mannitol were also measured. All measurements were performed in duplicate.

2.9. Bioburden determination

For the determination of the bioburden (initial microbiological contamination) of the non-sterilised samples, a sterile sodium chloride solution in 0.1% polysorbate 80 which improves the detachment of the bacteria from the particles and aids their resuspension was added into the vials with empty and doxorubicin-loaded nanoparticles; the vials were shaken for 10 min, and aliquots (0.5 ml) from each vial were placed in triplicate without further dilution on the preliminarily dried surface of the soya agar in Petri dishes. The dishes were incubated at 32° for 48 h. After incubation, the number of colony-forming units (CFU) was counted. The standard plate count method was used to enumerate the different organisms. Resistance of the microorganisms isolated from the samples to γ-irradiation was evaluated by an indirect method (Pavlov et al., 1991). Briefly, Gram-positive microorganisms isolated from the samples using a standard technique were transferred to agar surfaces in the Petri dishes in the amount of 0.6×10^7 cells/dish. The agar surface was dried, and a sterile paper disc (Whatman 3 MM) was placed on the surface, then 0.01 ml of 0.3% hydrogen peroxide was pipetted in the middle of the paper disc. The dishes were incubated for 1 h at ambient temperature and then for 18 h at 32 ± 1 °C. The sensitivity of the microorganisms was evaluated by the diameter of the growth inhibition zone around the paper disc. The results were interpreted according to following criteria: the absence of the growth inhibition zone is typical for microorganisms with high radioresistance; the average radioresistance is characterised by the growth inhibition zone of 9–11 mm; the growth inhibition zone for the microorganisms sensitive to irradiation is >12 mm. *Bacillus pumilus* E601 (collection of the State Research Center of Biophysics, Moscow) was used as a reference microorganism.

2.10. Sterility testing with contaminated samples

The efficacy of the irradiation sterilisation process was assessed by testing the sterility of the samples contaminated before the irradiation with a spore suspension of *B. pumilus* as well as with a strain exhibiting a radioresistance similar to *B. pumilus* which was isolated from non-irradiated nanoparticle samples. 10^6 spores of either strain were placed into each vial containing empty or doxorubicin-loaded nanoparticles. Before

irradiation, the vials were sealed with rubber stoppers and aluminum caps. After irradiation, the aliquots of the samples were resuspended in a sterile 0.9% sodium chloride solution containing 0.1% polysorbate 80 and transferred to the soya agar medium in Petri dishes. The surviving spores were counted after 48 h incubation at 37 °C. The CFU determinations were performed in triplicate. The sterility assurance level was set at 10^{-6}.

3. Results

3.1. Nanoparticles

The objective of the present study was to determine the maximal possible as well as the optimal irradiation doses for sterilisation of the nanoparticulate formulation of doxorubicin prepared under standard non-aseptic laboratory conditions. The formulation was manufactured by emulsion polymerisation of *n*-butyl-2-cyanoacrylate in the presence of the drug, as described previously (Gulyaev et al., 1999). This method allowed the preparation of the particles with a mean diameter of 174 ± 28 nm; the encapsulation efficiency reached 65% at the drug-to-polymer-ratio of 1:4. Empty (placebo) nanoparticles had a mean diameter of 200 ± 24 nm.

3.2. Microbiological evaluation

The microbiological evaluation showed that the total bioburden of the non-irradiated samples was ∼10^2 CFU/g. The radioresistance of the found microorganisms was evaluated by an indirect method using a previously established method showing the correlation between the sensitivity of microorganisms to irradiation and to hydrogen peroxide (Pavlov et al., 1991). The microflora of the nanoparticles contained only one radioresistant strain of the spore-forming bacteria with the D_{10} of 1.8–2.0 kGy, which is similar to the D_{10} of *B. pumilus* (D_{10} = 2.2–3.0 kGy) used in this study as the official test microorganism for γ-sterilisation (*Eur. Pharmacopeia*, 5th ed., 2005, Chapter 5.1.2.).

For the evaluation of the efficacy of sterilisation, the samples of nanoparticulate doxorubicin and placebo nanoparticles were contaminated before the irradiation with a spore suspension of *B. pumilus* as well as by the radioresistant strain isolated from the samples (10^6 spores of either strain). Then the samples were sterilised by either γ-irradiation or electron beam irradiation. The samples were irradiated to the doses of 10, 15, 25 kGy (standard overkill dose), and 35 kGy. The non-irradiated samples were used as control. As demonstrated by the microbiological assay, sterilisation of the samples was achieved with a dose of ≥15 kGy independently of the type of irradiation.

3.3. Influence on the nanoparticle size and molecular mass distribution of PBCA

The evaluation of the mean particle size by photon correlation spectroscopy demonstrated that the applied doses of both, γ-irradiation and electron beam irradiation, did not influence the mean particle size (Table 1). All samples could easily be reconstituted in water; there was no tendency for particle agglomeration or sedimentation.

Table 1
Influence of the type and dose of irradiation on the size of doxorubicin-loaded and empty PBCA nanoparticles

Dose of irradiation (kGy)	Mean particle size[a] (nm)	
	Empty NP	Dox-NP
Before irradiation	301 ± 98	245 ± 83
γ-Irradiation		
10	253 ± 62	215 ± 47
15	235 ± 40	221 ± 58
25	315 ± 90	210 ± 43
35	225 ± 26	214 ± 33
Electron beam irradiation		
15	243 ± 52	219 ± 28
25	239 ± 47	208 ± 26
35	246 ± 55	217 ± 28

[a] Each value represents an average of three measurements.

The molecular mass of PBCA of the irradiated and non-irradiated nanoparticles was assessed by GPC. The measurements performed in duplicate produced close results; the representative values are shown in Table 2. The molecular mass of PBCA in non-irradiated samples of both, empty and doxorubicin-loaded, nanoparticles was ∼2 kDa. Irradiation of the particles with the doses up to 25 kGy did not lead to any considerable changes in the molecular mass distribution of the extractable PBCA. After irradiation with 35 kGy an increase of the PBCA molecular mass (up to 3 kDa) and polydispersity was found in the samples of the drug-loaded nanoparticles.

3.4. Influence on doxorubicin content and stability

The evaluation of the doxorubicin content performed by the spectrophotometric assay of the dissolved formulation demonstrated that this parameter was not influenced by either type of irradiation: all samples contained 4.2 ± 0.2% (w/w) of the antibiotic (∼90% of the theoretical amount).

Table 2
Influence of the type and dose of irradiation on the molecular mass of PBCA in the samples of doxorubicin-loaded and empty nanoparticles

Dose of irradiation (kGy)	Molecular masses[a] (Da)					
	Empty NP			Dox-NP		
	M_n	M_w	M_w/M_n	M_n	M_w	M_w/M_n
Before irradiation	1700	2100	1.2	1350	1900	1.4
γ-Irradiation						
10	1460	2080	1.4	1420	2090	1.5
15	1540	2040	1.3	1700	2100	1.2
25	1820	2400	1.3	1490	1900	1.3
35	1480	1780	1.2	1560	3000	1.9
Electron beam irradiation						
15	1610	2080	1.3	1350	1720	1.3
25	1610	2020	1.3	1320	1800	1.4
35	1620	2030	1.3	1410	1890	1.3

[a] Values shown are representative of two measurements.

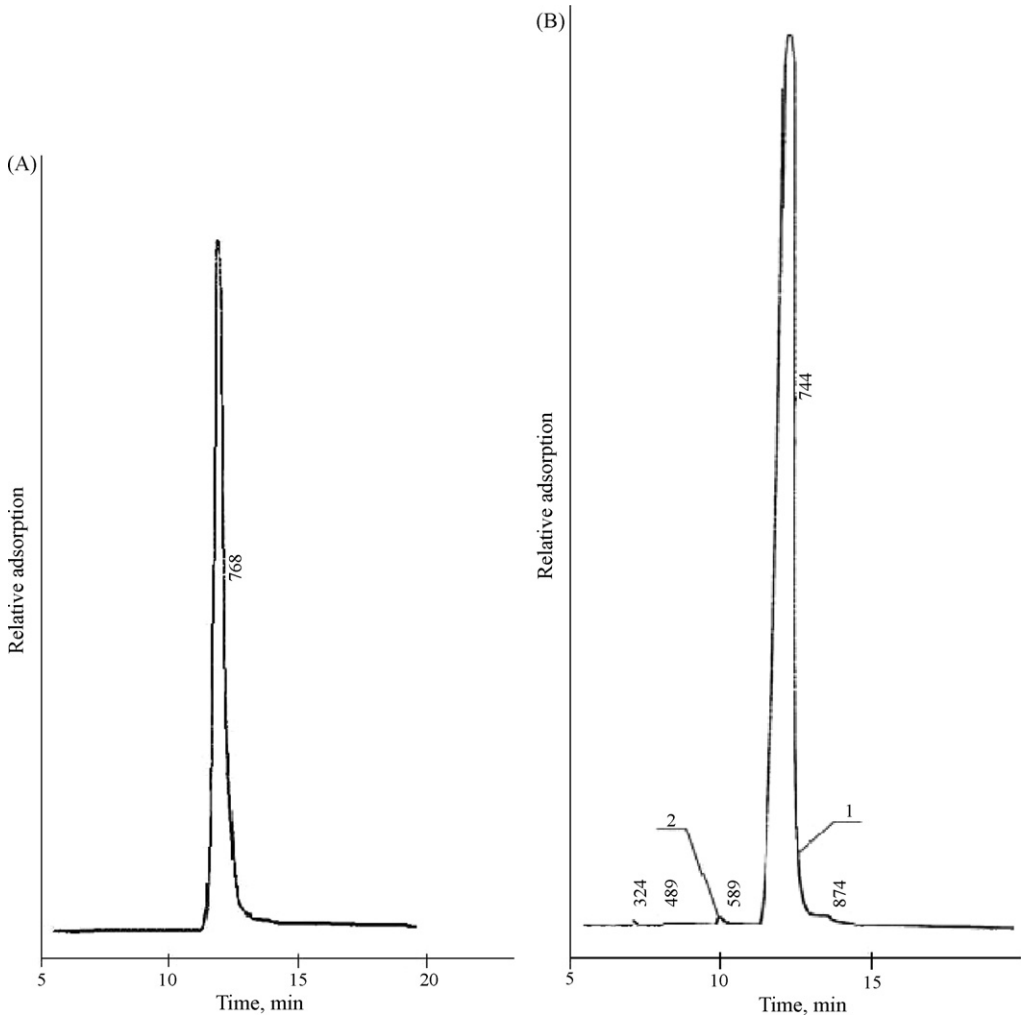

Fig. 1. (A) Chromatogram of doxorubicin substance. (B) Chromatogram of doxorubicin extracted from the nanoparticulate formulation after γ-irradiation to the dose of 35 kGy: 1, doxorubicin; 2, doxorubicinol.

The evaluation of the possible degradation of doxorubicin by either, conditions of the polymerisation process or by irradiation was performed by HPLC (Fig. 1A and B). The analysis of doxorubicin extracted from the non-irradiated sample of the nanoparticulate formulation demonstrated that the sample contained only trace amounts of doxorubicinol and other unidentified impurities; total amount of impurities was below 1%. The chromatogram of doxorubicin extracted from the irradiated samples was similar to that of the non-irradiated sample and did not reveal any evidence of the drug radiolysis at any irradiation dose (Fig. 1B).

3.5. ESR spectroscopy

The presence of the radicals stable at ambient temperature produced by irradiation of the nanoparticle samples was evaluated by ESR spectroscopy. Table 3 shows the results of the measurements for the samples irradiated to the dose of 15 kGy

Table 3
Concentration of radicals in the samples of doxorubicin-loaded and empty PBCA nanoparticles irradiated to the dose of 15 kGy

Irradiation type	Time after irradiation	Temperature of incubation after irradiation (°C)	[R] 10^{-18} (spin/g)[a]	
			Empty NP	Dox-NP
γ-Irradiation	1 min	25	1.7	0.8
	20 min	25	1.5	0.69
	10 min	40	1.2	0.37
	1 year	25	0.5	0.085
Electron beam irradiation	1 year	25	1.0	<0.001

[a] Values shown are representative of two measurements.

Table 4
Concentration of radicals in the samples of dextran, mannitol, and poly(butyl cyanoacrylate) 1 h after γ-irradiation to the dose of 15 kGy

Ingredient	$[R]\cdot 10^{-17}$ (spin/g)[a]
Mannitol	3.1
Dextran	4.2
PBCA	Absent (background level)

[a] Values shown are representative of two measurements.

which was found to be sufficient for sterilisation of the nanoparticles. The evaluation of the samples irradiated with other doses yielded similar results (not shown). The concentration of radicals in the nanoparticles was relatively high, which suggests that the paramagnetic species were stabilised when the irradiation was performed at ambient temperature. The amount of radicals in the drug-loaded nanoparticles was always considerably lower in comparison to the placebo. Although after one year of storage, the concentration of the radicals in the samples decreased 3-fold for the empty and almost 10-fold for doxorubicin-loaded nanoparticles, these samples still contained a considerable amount of radicals. The samples irradiated to the dose of 35 kGy retained considerable amounts of radicals even after four years of storage (not shown). At the same time, heating of the samples at 40 °C for 10 min considerably increased the rate of the radical destruction: their concentration for the empty and doxorubicin-loaded nanoparticles decreased by 30 and 50%, respectively. It is noteworthy that the irradiated nanoparticles reconstituted in water and repeatedly freeze-dried contained practically no radicals; trace amounts of radicals only were found in the sample of the drug-loaded particles after γ-irradiation in the dose of 35 kGy (not shown).

The concentration of the radicals also was assessed in the samples of PBCA, dextran, and mannitol after γ-irradiation to the dose of 15 kGy (Table 4). The irradiation of PBCA did not lead to formation of stable radicals, whereas dextran and mannitol exhibited high concentrations of radicals. Accordingly, comparison of the ESR spectra of the nanoparticles with the spectra of mannitol and dextran suggests that the major amount of radicals is produced by irradiation of these components (Fig. 2). The slight difference of the signal line shape in the spectra of nanoparticles (as compared to pure mannitol and dextran) is probably explained by the effect of the polymer matrix environment. The ESR spectra of the drug-loaded and empty nanoparticles were similar.

4. Discussion

At present, two technologies are used for the radiation sterilisation of pharmaceuticals: γ-irradiation that delivers a certain dose relatively slowly over a period of minutes to hours to a large volume of product and electron beam irradiation that can deliver the same dose in a fraction of a second but only to a very small volume of product. Since it could be expected that these two processes may affect the nanoparticles in a different way, both types of irradiation were used in the present study.

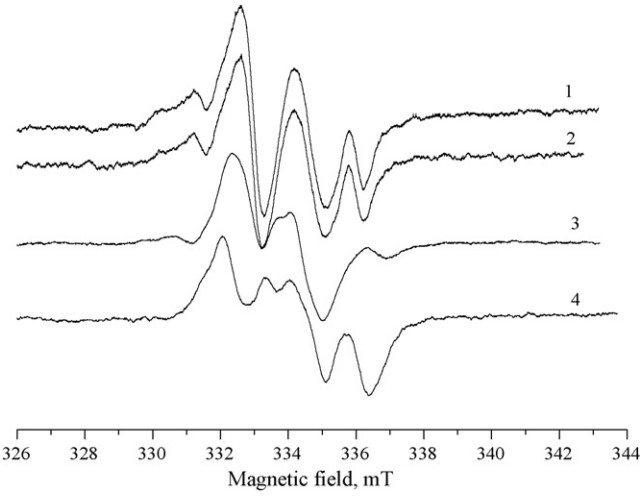

Fig. 2. The ESR spectra of (1) empty and (2) doxorubicin-loaded (2) PBCA nanoparticles, (3) dextran, and (4) mannitol after γ-irradiation to the dose of 15 kGy at ambient temperature.

The irradiation doses were chosen with respect to the initial microbiological contamination (bioburden), the radiosensitivity of the microorganisms, and the required sterility assurance level (SAL). Since the sterility assurance level is normally set at the level of 10^{-6} microorganisms/ml or g of product and since normally an effectivity (F-value) of $n = 8$ is employed for sterilisation, for *B. pumilus* the standard dose of 25 kGy is equivalent to about eight times its D_{10} (2.2–3 kGy). Therefore, it was assumed that at the above level of bioburden the samples could be sterilised with the dose of 25 kGy. The increasing doses of irradiation in the present study were used to determine an optimal sterilisation dose and to evaluate the effect of irradiation on the physicochemical properties of empty and doxorubicin-loaded PBCA nanoparticles. As shown by the microbiological evaluation, an irradiation dose of 15 kGy was sufficient to ensure the required sterility level.

For the evaluation of the influence which the ionising irradiation might have had on the structure of the key ingredients of the formulation – doxorubicin and poly(butyl cyanoacrylate) – the samples were investigated for nanoparticle size and molecular mass distribution of PBCA, as well as for the content of doxorubicin and related degradation products. It was shown that both types of irradiation did not considerably influence the particle size or their resuspendability (Table 1). The molecular mass distribution of PBCA also was not affected with the exception of the sample of the drug-loaded nanoparticles subjected to γ-irradiation at the dose of 35 kGy; the molecular mass of PBCA extracted from this sample increased to 3 kDa (Table 2).

It may be noted that the low molecular weights of PBCA observed in this study correlate with earlier findings (El-Egakey et al., 1983; Behan et al., 2001; Bootz et al., 2005). The polymerisation of cyanoacrylates occurring in aqueous media is mainly initiated by OH^-, while H^+ ions simultaneously terminate the reaction. As a result, the molecular weights of polyalkylcyanoacrylates obtained by anionic polymerisation are very low—generally 2–4 kDa. The slight increase in M_w but not M_n that was observed only after γ-irradiation may be indica-

tive of some rearrangements of the polymer chains caused by the radicals which are formed by the radioactive irradiation. This could result in an elongation or branching of the polymer chains, since oligomeric radicals are stabilised within the nanoparticles (Kreuter, 1982). It is, however, unlikely that a major cross-linking of the polymer within the nanoparticles had occurred since it would lead to a loss of polymer solubility, which was not observed.

Interestingly, irradiation considerably affected a lump sample (not nanoparticles) of PBCA (molecular mass ~30 kDa) prepared by anionic polymerisation at pH 7 in the absence of stabilisers. Both types of irradiation, independently of the dose, led to a total loss of the polymer solubility in chloroform. Thus, in contrast to the nanoparticle matrix where the polymeric chains are distributed spatially due to the presence of dextran and mannitol, which increases stability of the system to irradiation, irradiation of the lump sample caused the cross-linking of the polymer.

As shown by the HPLC assay, doxorubicin extracted from the non-irradiated sample of the nanoparticulate formulation contained only trace amounts of doxorubicinol and other unidentified impurities (Fig. 1); the total amount of impurities estimated under the above conditions was below 1%. The assay of the irradiated samples did not reveal any evidence of doxorubicin radiolysis.

The ESR spectroscopy allowed the quantification of the total concentration of the radicals stable at ambient temperature, as well as the qualitative evaluation of the kinetics of their decay. Regarding the role of different components in the formation of stable radicals, it is worth noting that the content of dextran and mannitol in the freeze-dried formulation is considerable and reaches ~80%. The analysis of the data presented in Fig. 2 and Table 4 suggests that the observed radicals result mainly from the irradiation of dextran and mannitol. Absence of the noticeable contribution of radicals from doxorubicin and PBCA suggests that the number of the radicals produced by these ingredients might be low or that the radicals formed are unstable at ambient temperature.

The decay of the radicals at ambient temperature was rather slow: a considerable amount still was present in the freeze-dried samples after one year of storage. However, after reconstitution of the particles in water and subsequent lyophilisation, the radicals practically disappeared; trace amounts were only found in the sample irradiated to 35 kGy (γ-irradiation). It is possible that the radicals are stabilised within the microcrystalline domains of the carbohydrates in the nanoparticle matrix which are destructed upon the contact with water. This result suggests that the presence of the stable radicals in the freeze-dried nanoparticles is not associated with a risk for the patient.

It is noteworthy that the presence of doxorubicin led to a considerable decrease in the concentration of the stable radicals in the nanoparticles irradiated to the dose of 15 kGy. It is possible that doxorubicin serves as a radioprotector providing effective energy transfer due to the aromatic structure. A similar phenomenon was observed also for clonazepam-loaded PLGA microspheres subjected to γ-irradiation (Montanari et al., 2001).

Finally, the results obtained in the present study did not reveal any obvious difference between γ-irradiation and electron beam irradiation in terms of their influence on the investigated physicochemical parameters of the PBCA nanoparticles.

5. Conclusion

The results of the present study demonstrate the feasibility of the radiation sterilisation of doxorubicin formulation based on poly(butyl cyanoacrylate) nanoparticles. At the initial formulation bioburden of 10^2 CFU/g, the dose of 15 kGy delivered by either γ-irradiation or electron beam irradiation appeared to be sufficient for the terminal sterilisation. The formulation also appeared to be stable to radiolysis. The irradiation in the doses up to 35 kGy did not affect stability of the active ingredient and the physicochemical parameters of the formulation such as mean particle size, polydispersity, and aggregation stability; the molecular weights of the PBCA polymer as well as the polydispersity indices (M_w/M_n) were barely influenced. The possible contribution of doxorubicin to the radiolytic stability of the formulation suggests that the parameters of sterilisation for other PBCA-based drugs may need to be adjusted depending on the structure of the active ingredient.

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