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Gamma irradiation effects on stability of poly(lactide-co-glycolide) microspheres containing clonazepam

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Abstract

This work was aimed at evaluating the effects of γ irradiation on the stability of microspheres made of a poly(lactide-co-glycolide) copolymer (PLGA) and loaded with 15% w/w of clonazepam (CLO). The influence of CLO on PLGA radiolysis mechanisms and the identification of possible irradiation markers were also investigated. Microspheres were prepared by means of a spray-drying method. γ Irradiation was carried out either under vacuum or in air, at a dose of 25 kGy, by using a ^{60}Co source. The stability of CLO loaded microspheres was evaluated over a 6-month period on the basis of drug content and dissolution profile. Radiolysis mechanisms were investigated by using electronic paramagnetic resonance (EPR) analysis. The microspheres irradiated under vacuum were stable over the considered period of time. After irradiation in air, CLO release rate increased by ~10%, and did not change further in the following period of storage. The EPR analysis showed some radicals arising from both the polymeric matrix and the active ingredient. Polymer/CLO spin transfer reactions suggest that CLO had a radio-stabilising effect on the polymeric matrix. In the loaded microspheres, the intensity in time of the CLO radical signal is sufficient for its possible use as irradiation marker.

Keywords: Gamma irradiation; Clonazepam; Microspheres; Poly(lactide-co-glycolide) copolymer (PLGA); Electronic paramagnetic resonance (EPR)

1. Introduction

Aliphatic polyesters based on lactic and glycolic acids are the most widely used polymers for the preparation of biodegradable microspheres intended for parenteral use. As polylactide (PLA) and poly-(lactide-co-glycolide) (PLGA) are moisture and heat-sensitive polymers, they are good candidates for γ sterilisation. Nevertheless, ionising radiation induces dose-dependent cross-linking and/or chain scission and concomitant molecular weight loss of these

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polymers [1–3]. The radiolytic degradation of PLA or PLGA increases in the presence of oxygen. During the γ irradiation process, the oxidation of the polymer can decrease cross-linking, and increase degradation or lead to chain scission [4].

The effects of γ irradiation on the microparticulate systems made of PLA or PLGA are also influenced by drug loading, and are not easily predicted because of the different chemico-physical characteristics of the active ingredient and its interactions with the polymeric matrix. Volland et al. showed a decreased captopril release from γ irradiated microspheres [5]. In contrast, Yoshioka et al. described an increased progesterone release from irradiated microspheres with increasing irradiation dose [6]. Mohr et al. showed accelerated kinetics of estradiol release with increasing irradiation doses, due to dose dependent polymer degradation [7]. The same work also showed estradiol grafting to PLGA as a consequence of degradation.

The influence of drug loading on polymer degradation is also discussed. PLA degradation was independent of methadone loading [8], but it was higher when prometazine loading increased [9]. Again, Bittner et al. showed that PLGA degradation rate slowed down following the incorporation of tetracycline in the microspheres [10]. Although the effects of ionising radiation on PLA and PLGA molecular weight and drug release have been discussed in a number of papers, the stability of the irradiated biodegradable microparticulate systems has been scantly investigated.

In this work, the effects of γ irradiation either under vacuum or in air at a dose of 25 kGy on the stability of microspheres made of PLGA and loaded with clonazepam (CLO) was evaluated. A minimum absorbed dose of 25 kGy was regarded as adequate for the purpose of sterilising pharmaceutical products without providing any biological validation [11]. CLO was selected as model drug because it is a benzodiazepine compound with marked antiepileptic properties used in the chronic therapy of myoclonic seizures in children. Thus, a controlled release drug delivery system intended for parenteral administration appears of interest.

The CLO microparticulate system made of a poly(lactide-co-glycolide) 50:50 copolymer was prepared by the spray-drying method [12]. The influence of CLO on PLGA radiolysis and the identification of possible irradiation markers were investigated by using electronic paramagnetic resonance (EPR) analysis. The identification of possible irradiation markers could be very useful to prove performed irradiation on the final product.

The stability of CLO loaded microspheres irradiated either under vacuum or in air was evaluated over a 6-month period on the basis of their drug content and dissolution profile.

2. Materials and methods

Poly(lactide-co-glycolide) 50:50, Resomer® RG 503 (PLGA), inherent viscosity 0.39 dl/g, 34 000 MW, was from Boehringer Ingelheim (Ingelheim am Rheim, Germany). Clonazepam was supplied by Roche Spa (Milan, Italy). L-alanine and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were from Fluka (Milan, Italy). Paraffin wax was from Aldrich (Milan, Italy) and sodium lauryl sulphate was from Carlo Erba (Milan, Italy).

Unless specified, all other compounds were of analytical grade.

2.1. Preparation of microspheres

Microsphere preparation was performed by using the spray-dryer Lab-Plant model SD04 (Lab-Plant, West Yorkshire, UK) as described in a previous work [12]. Placebo microspheres were obtained by spraying 2% w/w solution of PLGA in methylene chloride through a standard nozzle (inner diameter: 1 mm). The process parameters were set as follows: inlet temperature: 50°C; outlet temperature: 35–36°C; flow rate: 10 ml/min.

CLO loaded microspheres were prepared by spraying — under the same conditions as above — 2% w/w solution of PLGA in methylene chloride in which CLO had been previously solubilised in the following ratio: PLGA:CLO 85:15 w/w. After preparation, the microspheres were lyophilised by using a Modulyo 4K Freeze Dryer (Edwards, UK), and stored in an airtight container at 4°C.

2.2. γ Irradiation of placebo and CLO loaded microspheres

Placebo and CLO loaded microspheres were ir-

radiated by using ^{60}Co as irradiation source (Applied Nuclear Energy Laboratory (L.E.N.A.), University of Pavia). Irradiation was performed at room temperature either under vacuum or in air; a 25-kGy dose at 1.3-kGy/h dose rate was applied.

2.3. Morphology

Microparticle size and morphology before and after irradiation were evaluated. The microsphere shape and surface were analysed using SEM (JSM-T 800, Jeol Italia, Pieve Emanuele, Italy). The samples were sputtered with an Au/Pd coating in an argon atmosphere. The microsphere size was determined by light blockage method and an HIAC/ROYCO apparatus, model 3000, equipped with an HC60 sensor. Samples of microspheres were suspended in filtered and bidistilled water, and analysed while gently stirring. The results are the average of five determinations.

2.4. EPR analysis

EPR analysis was performed on CLO, placebo microspheres and CLO loaded microspheres.

Irradiation was performed at the Applied Nuclear Energy Laboratory (L.E.N.A., Pavia University, Italy) by using a ^{60}Co gamma source calibrated against alanine and Fricke dosimeters, at the following conditions: irradiation temperature $T=77$ K (liquid nitrogen), samples sealed under high vacuum in EPR quartz tubes, dose rate 1.3 kGy/h, total dose 25 kGy; irradiation temperature $T=298$ K (source temperature), samples sealed under high vacuum in EPR quartz tubes, dose rate 1.3 kGy/h, total dose 25 kGy; irradiation temperature $T=298$ K, sample containers were open during irradiation, dose rate 1.3 kGy/h, total dose 25 kGy.

After irradiation, part of the sample tubes was flamed by using the sliding technique in order to eliminate the radiation induced quartz paramagnetic centers. During this operation, the sample temperature was not allowed to rise above 77 K for samples irradiated at 77 K.

EPR analysis was performed by using a Varian E-109 spectrophotometer (Palo Alto, CA, US) equipped with a data acquisition system and a temperature control apparatus. The EPR spectra were analysed by computer simulation by using the Hamiltonian:

$$H = -\beta\vec{S}\cdot\tilde{g}\cdot\vec{H} + \Sigma_i\vec{S}_i\cdot\tilde{A}_i\cdot\vec{I}_i - \Sigma_i\vec{I}_i\cdot\vec{H}$$

where H is spin Hamiltonian, β is Bohr magneton, $\tilde{g}$ is g tensor, $\vec{H}$ is external magnetic field, $\vec{S}$ is electron spin operator, $\tilde{A}$ is hyperfine tensor, and $\vec{I}$ is nuclear spin operator.

The spectra of samples irradiated at 77 K, under vacuum, were recorded at 113 K and after annealing at 298 K. Furthermore, the spectra of the samples were recorded also after 18 h at room temperature and after admission of air at 298 K. The annealing procedure was intended to ensure suitable experimental conditions so that the reaction of the primary species trapped during the irradiation at 77 K could take place.

Spectra of samples irradiated at room temperature were recorded immediately after irradiation and at different storage times in order to study radical decay.

The radiolytic radical yields were determined through comparison of the EPR signals areas by using alanine standards with a known number of spins. The alanine standards were prepared by extrusion of alanine powder/wax mixture (~20% wax) in the form of cylinders having a similar geometry to that of the samples. Stable alanine radicals were generated by irradiation; their concentration was determined by comparison with a standard solution of DPPH.

2.5. Water content

The water content of placebo and loaded microspheres was determined by Karl Fischer volumetric titration (Micro KF 2026, Crison, Italy). A sample of 25 mg — precisely weighed — was suspended in methanol, and titrated with a Karl Fischer pyridine-free solution. Each value is obtained from triplicate determinations.

2.6. Differential scanning calorimetry (DSC)

Thermal analysis was performed on samples of CLO, placebo and loaded microspheres by using a DSC 2010 TA (TA Instruments, US).The samples

were heated in closed aluminum pans, at a heating rate of 10°C/min under a constant flow of nitrogen.

2.7. Drug content

A 5-mg amount of CLO-loaded and accurately weighed microspheres was dissolved in 25 ml of acetone. The samples were assayed by HPLC-UV method. The HPLC system was an HP 1100 Chem-Station (Hewlett Packard, Germany). Chromatographic conditions were: column: C_{18} ODS 2 Hypersil 200×4.6 mm (Shandon HPLC, UK); wavelength: 240 nm; mobile phase: acetonitrile/methanol/water (30/30/40, v/v/v); flow rate: 1.5 ml/min; injection volume: 10 μl. The analyses were performed at room temperature. The drug concentrations were determined from the standard curve (5–100 μg/ml) and the method gave 99.8% recovery of theoretical value (C.V. <0.4% on the basis of three determinations).

In the stability study of non-irradiated and irradiated microspheres, the drug content was determined after 0, 30, 60, 180 days in triplicate.

2.8. In vitro release test

In vitro release tests were performed according to the Ph. Eur. III Ed. paddle dissolution method, on CLO loaded microspheres (a) not irradiated, (b) irradiated at room temperature under vacuum, and (c) irradiated at room temperature in air.

A total of 500 ml of phosphate buffered saline solution, pH 7.4 (Ph. Eur. III Ed.) containing 0.15% SDS as wetting agent, was used as release medium. The suspensions were maintained at 37°C while stirring at 100 rpm for 24 h. The amounts of CLO released from the microspheres were spectrophotometrically determined at 240-nm wavelength. The test was performed in triplicate. The test was performed soon after irradiation (time 0), and repeated after 15, 30, 60, 90, 120, 150 and 180 days.

The release rate constant was calculated according to Higuchi's equation as follows: $M_t/M_\infty = kt^{0.5}$ where M_t is the amount of drug released at time t, M_∞ is the drug loaded in the matrix and k is the release rate constant expressed as $h^{-0.5}$.

3. Results and discussion

3.1. Microsphere morphology

Both placebo and drug loaded microspheres had a spherical shape and a wrinkled surface (Fig. 1). The size of over 90% of placebo and CLO loaded microspheres was in the 2–10-μm range (Fig. 2). Irradiation of placebo and loaded microspheres performed either in air or under vacuum caused a change in the particle size distribution (Fig. 2). An increased number of particles in the range 5–10 μm was shown among placebo microspheres (Fig. 2A). CLO loaded microspheres (Fig. 2B) showed the

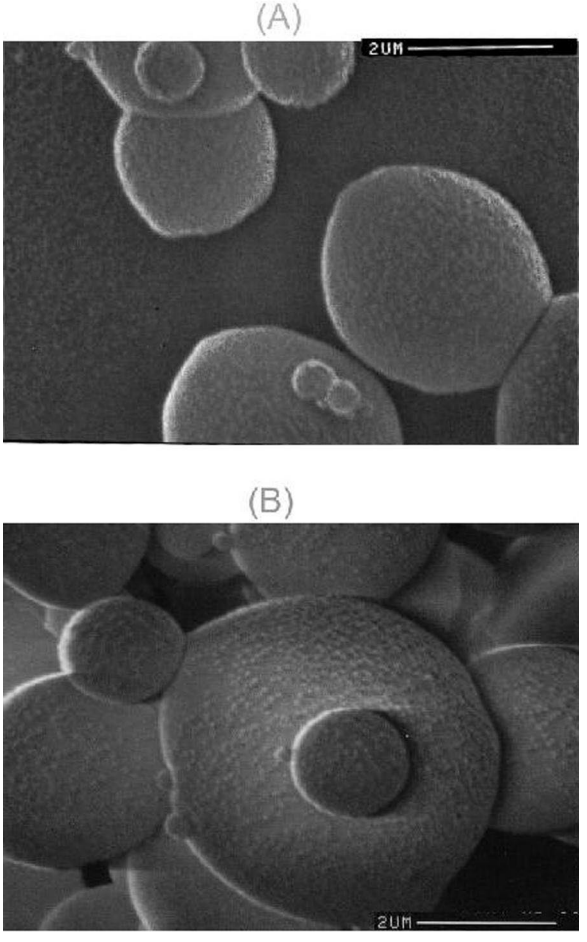

Fig. 1. Photomicrographs of CLO loaded microspheres (A) before irradiation, and (B) after irradiation at 25 kGy in presence of air.

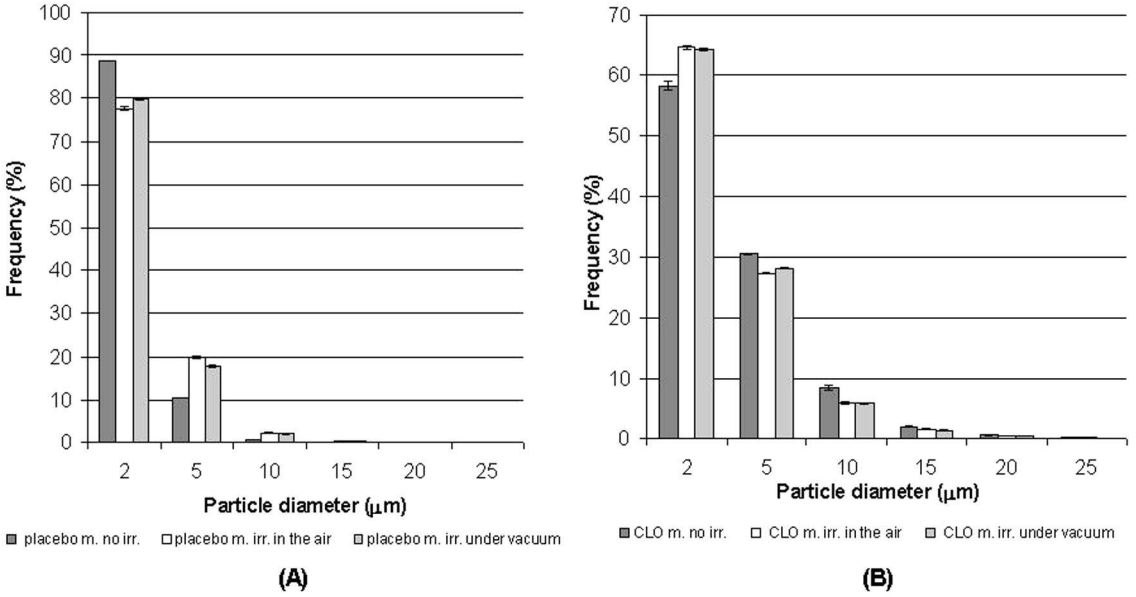

Fig. 2. Particle size distribution of placebo microspheres (A) and CLO loaded microspheres (B) before irradiation, and after irradiation at 25 kGy, under vacuum and in presence of air.

opposite pattern, suggesting that CLO presence affected the surface properties of the irradiated microparticles and, consequently, the aggregation state of the microspheres. The scavenging of the polymer radicals by CLO shown by EPR analysis could have caused a modification of the surface properties of irradiated loaded microspheres compared to the placebo microspheres.

3.2. EPR analysis

3.2.1. Clonazepam

The EPR spectrum recorded at room temperature following γ irradiation at 77 K under vacuum shows a triplet signal (Fig. 3) as a major hyperfine component with g and hyperfine tensor anisotropy consistent with the assignment to a nitroxyl-like radical (Fig. 3A). The presence of other species contributing to the center of the nitroxyl spectrum is inferred. Possible candidates having expected EPR signal compatible with the experimental pattern were shown by computer simulation, i.e. the phenyl radical (Fig. 3B) formed by loss of the Cl atom; the amidyl radical (Fig. 3C) formally generated by the N–H bond rupture; and the carbonyl radical (Fig. 3D) generated after the rupture of the methylene C–H bond in the diazepinone section. Following the primary ionisations, the radiolytic formation tendency of the three species is expected to increase; the dissociative electron capture of the C–Cl bond and the deprotonation of the cation-radicals involving the loss of the amido or methylene hydrogen are favored reaction paths. Furthermore, species D can be formed in hydrogen abstraction processes due to the lability of the activated C–H bond. The EPR spectrum obtained by γ irradiation of CLO in air does not show any significant differences as compared to the EPR spectrum under vacuum probably because the crystal structure of the compound is not permeable to oxygen.

The CLO radical radiolytic yield at 77 K is 0.0030 ± 0.0006 μmol/J. This value is at least one order of magnitude smaller as compared to saturated hydrocarbons; this actually shows the stabilising effect induced by the aromatic component in the drug molecular structure. The radiolysis of aromatic compounds is characterised by fast internal conversion of excited states to low energy levels which are

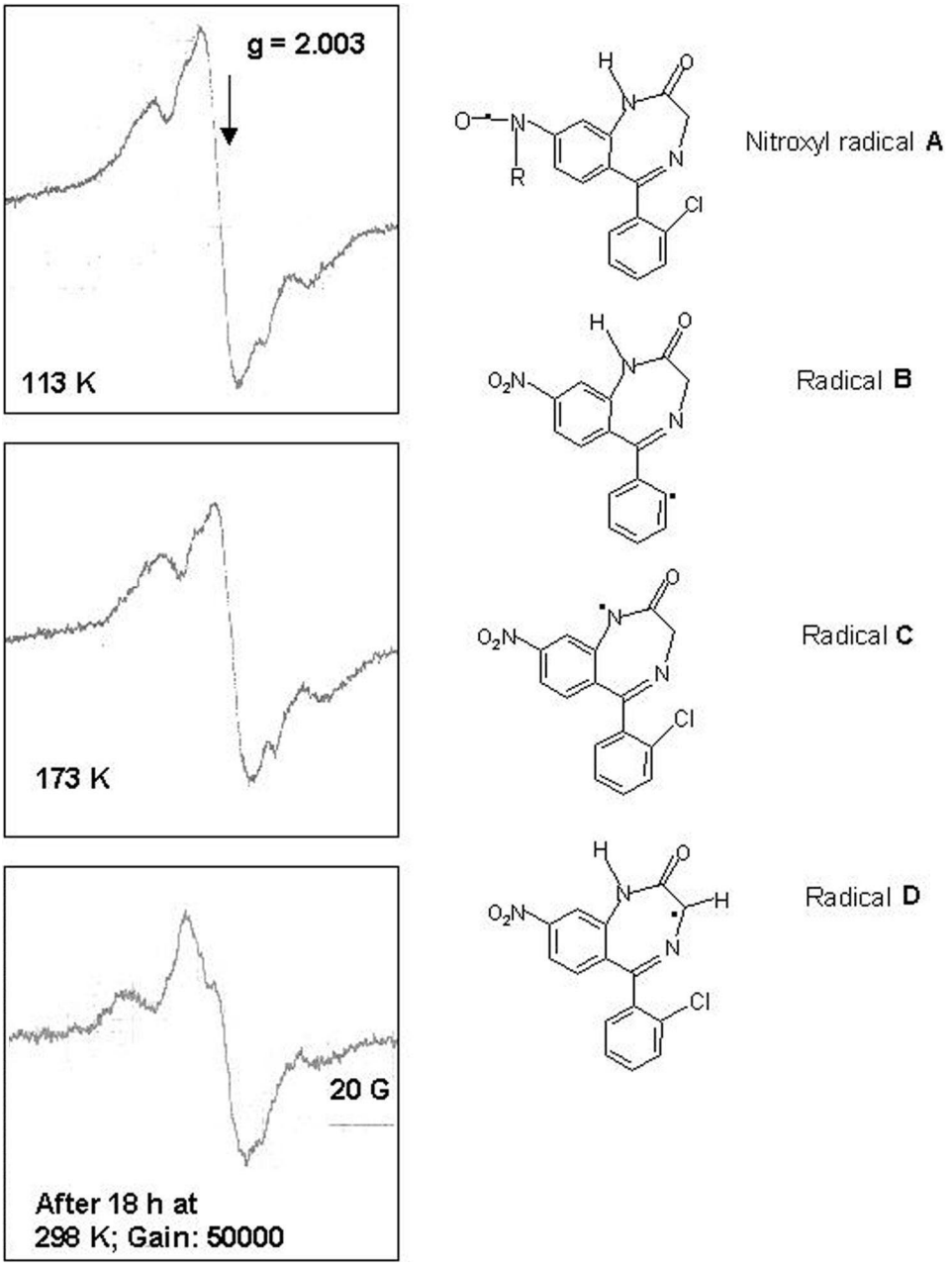

Fig. 3. EPR spectra of the neat crystalline CLO after under vacuum γ irradiation at 77 K: effect of the annealing temperature. Dose rate 1.3 kGy/h; dose, 25 kGy; $G = 25\,000$ except where quoted. The nitroxyl radical A is the stable prominent species contributing to all the spectra. Radicals B, C, D are minor oxygen sensitive components.

often reduced due to emission of radiations or by degradation to thermal energy without leading to molecular decomposition [13].

As expected, nitroxyl radicals show a remarkable stability [14]; the decay rate at room temperature leads to a 33% concentration decrease in 20 days.

3.2.2. Placebo microspheres

When considering the intermediate radicals trapped at 77 K, in the γ radiolysis of the polyester, primary processes are characterised by extensive chain scission with a minor participation of C–H bond ruptures (Fig. 4). When warming above 77 K, thermally activated hydrogen abstractions by the primary radicals at the secondary and tertiary C–H bonds take place. Hydrogen abstraction radicals decay slowly under vacuum at room temperature. On admission of oxygen, polymer radicals are converted into the corresponding peroxyl radicals at a rate

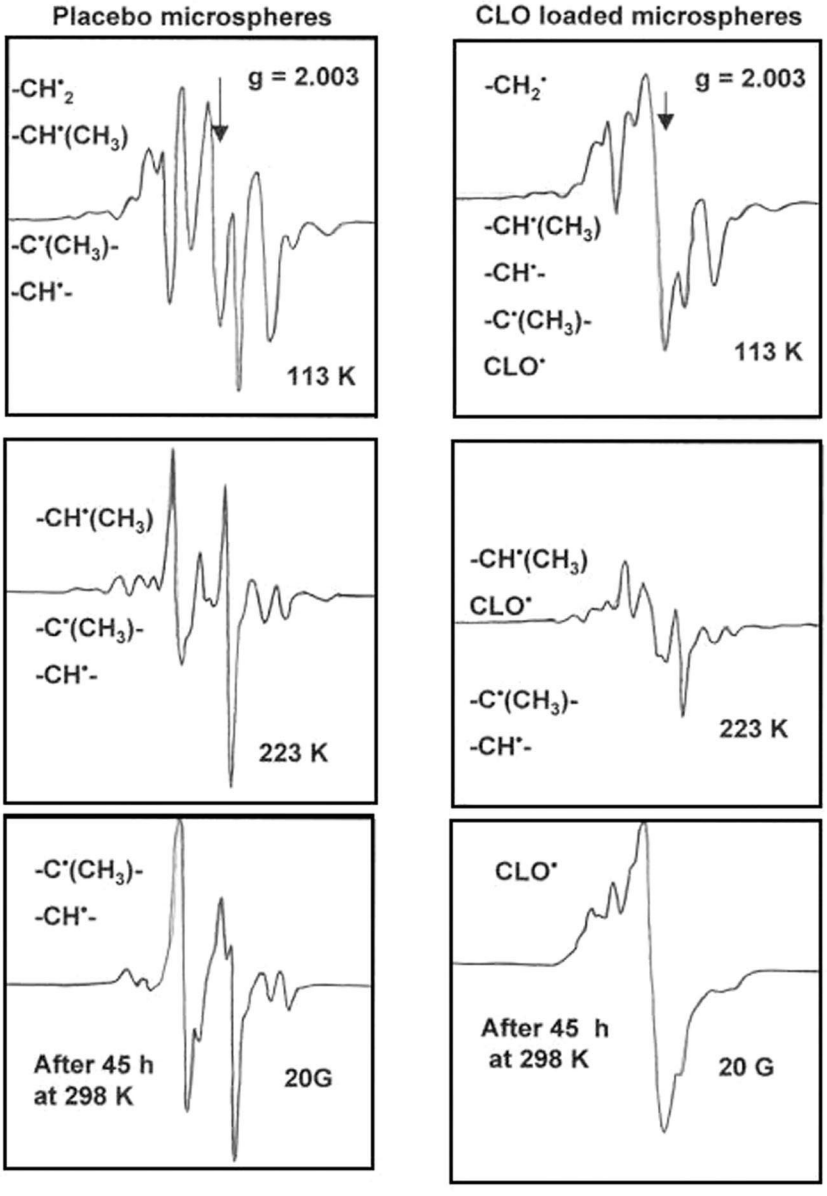

Fig. 4. EPR spectra of placebo microspheres (left column) and CLO loaded microspheres (right column) after under vacuum γ irradiation at 77 K: effect of the annealing temperature. Dose rate 1.3 kGy/h; dose, 25 KGy. CLO• stands for radicals A, B, C, D from clonazepam.

which is almost one order of magnitude faster for the placebo microspheres than that of raw polymer samples.

Peroxyls are expected to initiate a chain hydroperoxidative process. During this post irradiation process, the peroxyl radicals decay half period was ~50 min at room temperature. The presence of the secondary radical $-CH^{\cdot}-$ in relative abundance compared to that of the tertiary radical seems to be at variance with the expected reactivity trend based on the C–H bond strengths, since the tertiary C–H bond breaking energy is lower. This pattern can, however, be explained in terms of stereoelectronic effects favoring hydrogen abstraction from the secondary C–H bond [15]. Prolonged storage at room temperature leads to the appearance of a novel species with a spectrum similar to that of the radical $-C^{\cdot}(CH_3)-$, but characterised by a smaller hyperfine splitting. This decrease is diagnostic of an ether or hydroxyl substituent at the carbon radical center therefore, it is hereby suggested that the signal is assigned to the chain end hydrogen abstraction radical:

$$-O-C(=O)-C^{\cdot}(CH_3)-OH$$

The terminal alcohol units needed for the formation of such species can be generated by the hydrolytic dissociation of glycolide $-CH(CH_3)-O-C(=O)-$ ester bonds.

The radiolysis mechanism, which is described in detail in a previous work [3], is summarised in Fig. 5.

The comparative irradiations of raw polymer and placebo microsphere samples essentially produced the same EPR spectra. This suggests that the polymer morphology does not affect the radiolytic behaviors under vacuum.

3.2.3. CLO loaded microspheres

After irradiation at 77 K under vacuum, radicals generated by both the polymer matrix and the active ingredient are identified in almost equal relative abundant amounts (Fig. 4). When warmed at room temperature, polymer radicals in CLO loaded microspheres decay at a faster rate than in the case of placebo microspheres; consequently, the CLO radical concentration increases steadily attaining 100% after ~45 h. The CLO EPR spectrum finally obtained is very similar to that of neat CLO (Fig. 3), the nitroxyl radical pattern being the dominant component. The presence of species contributing to the central part of the nitroxyl spectrum is also observed. On admission of oxygen, in contrast to the nitroxyl spectrum, these species decay rapidly. This suggests that this component may belong to oxygen sensitive species such as amidyl radicals C or carbon centered radicals B and D. The decay rate of nitroxyl is very slow both in air and under vacuum. As a result, the nitroxyl EPR signal might be used as a radiation marker

$$\text{Polymer/CLO} + \gamma\,(77\ \text{K}) \rightarrow \text{P} + \text{A} + \text{B} + \text{C} +$$

$$\text{D}\,(298\ \text{K}) \rightarrow \text{A} + \text{B} + \text{C} + \text{D}\,(298\ \text{K under air}) \rightarrow \text{A}$$

where P is polymer radicals, and A, B, C and D are CLO radicals as in Fig. 3.

The EPR spectra obtained following irradiations in air at room temperature were the same as those produced by sample irradiation at low temperature

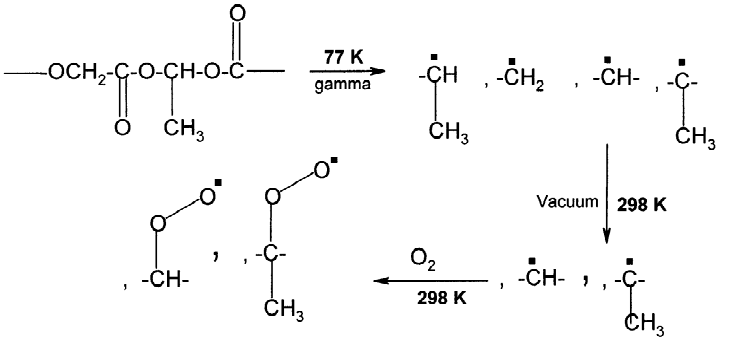

Fig. 5. Proposed mechanism of γ radiolysis of PLGA (placebo microspheres).

followed by prolonged room temperature annealing and admission of air.

3.2.4. Molecular interaction between CLO and the polymeric matrix: evidence of radiostabilisation and spin transfer reactions

CLO in the loaded microspheres has a radiostabilising effect as shown by the 54.1% decrease of overall radical yield with respect to the computed linear contribution of the neat components in the molecular mixture (Table 1). At the same time the *G* (radicals) value for CLO in the loaded microspheres is enhanced by a factor of 40 (Table 1). Such observations point to the existence of significant interactions taking place between the polymer and the active ingredient. The radiostabilisation effect is likely to be related to the aromatic moiety of CLO acting as a channel for degradation of the radiation energy to thermal energy.

The spin transfer effect leading to the enhancement of CLO radicals is proposed to be related to the electron and radical scavenging properties of the nitro group according to the following reaction scheme (Fig. 6). Dissociative electron capture by the C–Cl bond and the hydrogen abstraction at the activated C–H bonds adjacent to the amido group in the diazepinone section are also considered. Following electron scavenging or electron transfer from polymer radicals to the nitro group, nitro anion radicals are formed; these are the precursors of a nitroso derivative. The latter is likely to act as a spin trapping agent leading to the production of the observed nitroxide adducts. The aromatic nitroxyl radicals show generally much lower decay rates as compared to other free radicals and their stability is greatly enhanced when, as in the case of CLO, a substituent is present in the para position. This extra stability coupled with the solid matrix trapping efficiency explains the persistence of such species in irradiated CLO and CLO loaded microspheres.

3.3. Differential scanning calorimetry (DSC)

The water content measured by Karl Fischer titration was less than ~0.4% in both irradiated and non-irradiated microspheres.

Fig. 7 shows the DSC thermograms related to PLGA placebo microspheres and CLO loaded microspheres before and after γ irradiation, performed under vacuum and in presence of air. The following thermal phenomena are observed: (a) an endothermic peak in the region of ~50°C, characteristic of the copolymer but present also in the drug loaded microspheres DSC profiles, which is presumably reckoned with irreversible endothermic reorganisation of the polymer matrix; (b) an exothermic peak in the region of 110–130°C present only in the drug loaded microspheres DSC thermograms; this feature is likely to be related to drug recrystallisation phenomena taking place at temperatures high enough to afford the necessary molecular mobility. The irradiation is seen to cause a shift of the exothermic peak toward lower temperatures whilst the presence of air is essentially without significant effects. This low temperature shift may tentatively be imputed to changes in the polymeric matrices occurring as a consequence of radiolytic events such as chain scissions. Differences in the position of the endothermic peaks in the ~50°C region are also observed as a consequence of the presence of the drug and of the irradiation.

Table 1
Radiolytic radical yields determined by EPR spectroscopy

Sample	Radical yield (μmol/J)
CLO radicals	0.0030±0.0006
Placebo microspheres: polymer radicals	0.26±0.04
Loaded microspheres total radicals (CLO+polymer)	0.12±0.02
Loaded microspheres: CLO radicals	0.065±0.01
Loaded microspheres: polymer radicals	0.055±0.01
Loaded microspheres: calculated total radical yield[a]	0.22±0.04

[a] Calculated from the radiolytic yields of the neat components, polymer and CLO, weighted by the corresponding electron fractions.

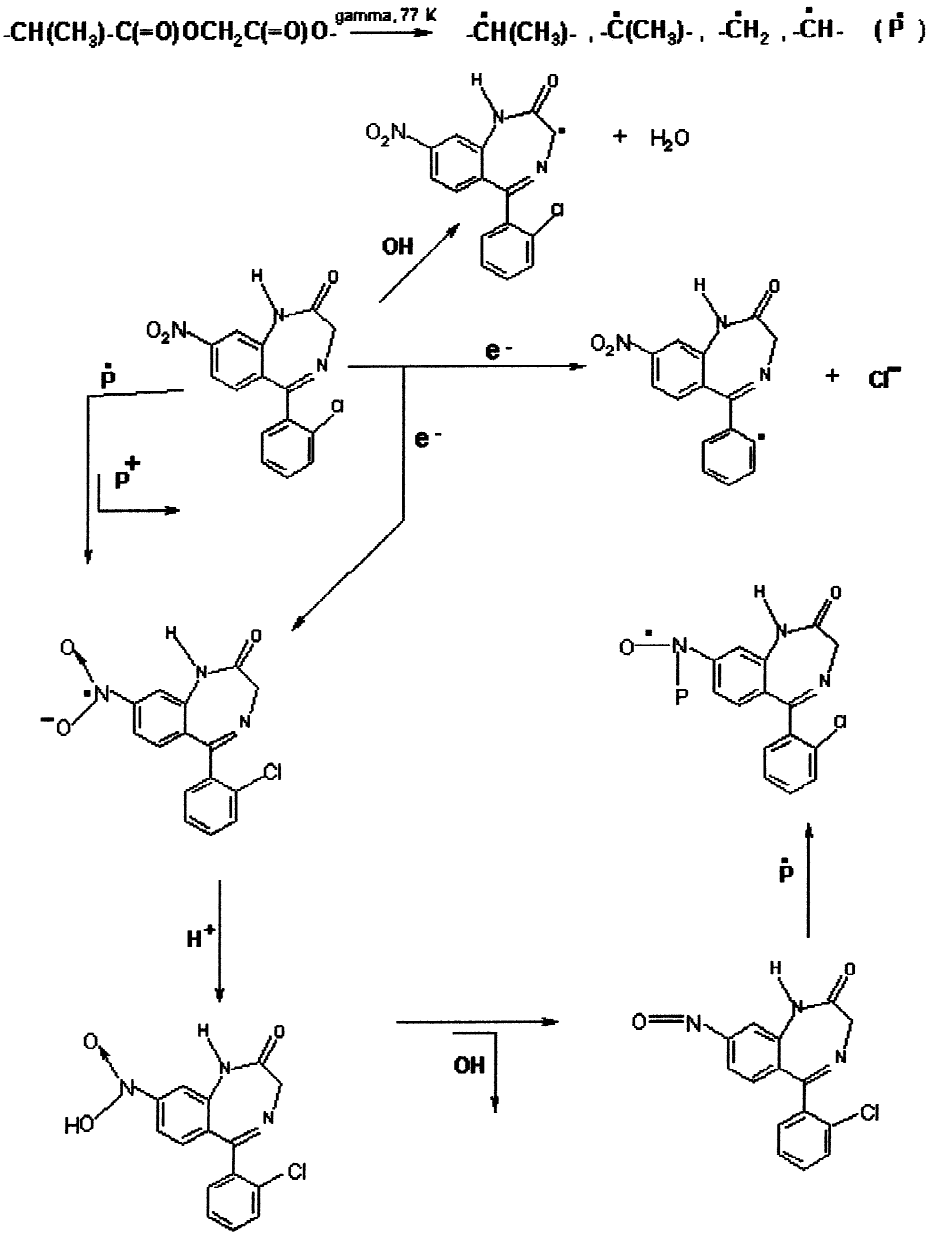

Fig. 6. γ Radiolysis of CLO loaded microspheres: proposed mechanism for spin transfer reaction.

3.4. Drug content

The drug contents of microspheres did not change after irradiation. Moreover, they did not significantly change during storage over a 6-month period (Table 2).

3.5. In vitro release profiles

As expected, CLO release was controlled by the diffusion of the drug from a monolithic matrix; it followed the square root of time relationship throughout the testing period ($0.9920 < r^2 < 0.9958$)

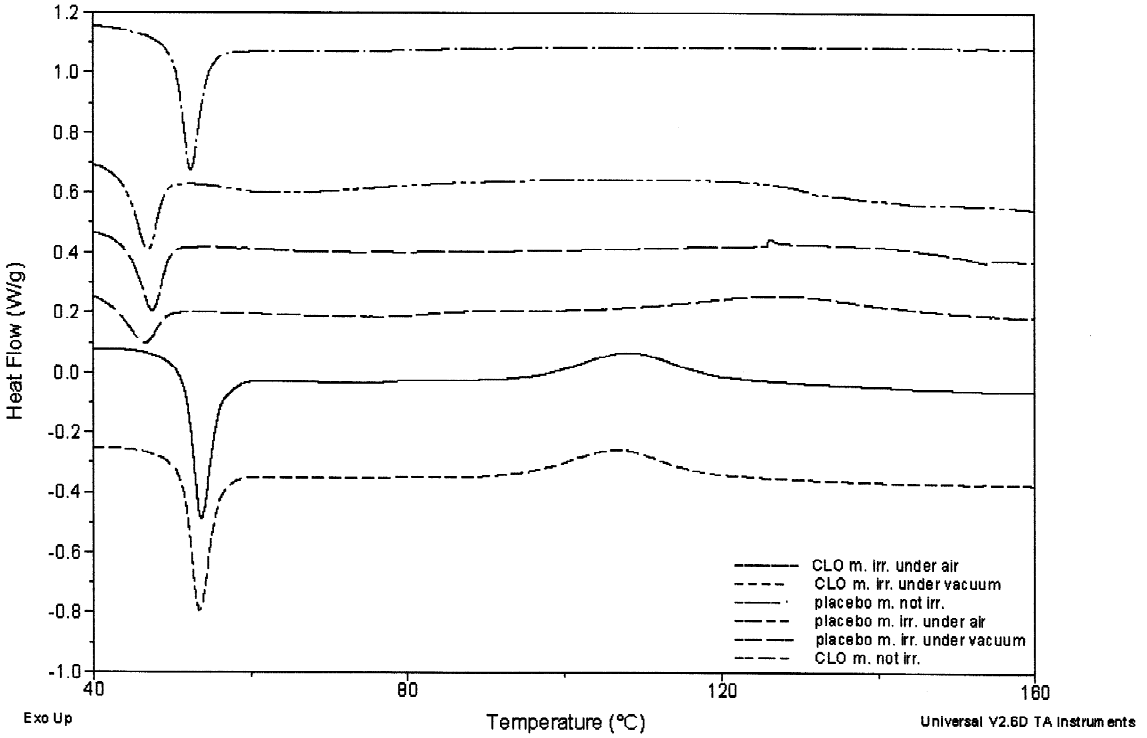

Fig. 7. DSC thermograms of placebo and loaded microspheres irradiated and non-irradiated.

according to the Higuchi model. As an example, the release profiles of CLO from non-irradiated and irradiated microspheres at two different times of storage ($t=0$ and $t=180$ days) are shown in Fig. 8. At the end of the experiments (6 months), the means of the values of CLO amounts released during the stability study were the following:

- 58.3±1.4% w/w non-irradiated microspheres;
- 64.0±2.4% w/w microspheres irradiated in air;
- 61.1±2.0% w/w microspheres irradiated under vacuum.

Release rate constants of CLO are shown in Fig. 9. The values of the constants obtained for non-irradiated and irradiated microspheres under vacuum did not significantly change over the period of 6 months. After irradiation in air, the release rate constants of CLO increased by ~10%, and did not change further for the rest of the storage period. The increased rate constant of CLO release from microparticles irradiated in air can be explained on the basis of the radiolytic degradation of the copolymer [3]. As a matter of fact, the presence of oxygen triggered a hydroperoxidative radiolysis cycle that

Table 2
CLO contents determined in the non-irradiated and irradiated microspheres

Storage time (days)	Non-irradiated microspheres (% w/w)	Irradiated under vacuum microspheres (% w/w)	Irradiated in air microspheres (% w/w)
0	14.6±0.3	14.6±0.4	14.7±0.7
30	14.7±0.4	14.6±0.2	14.6±0.4
60	14.5±0.5	14.3±0.4	14.6±0.6
180	14.4±0.4	14.8±0.8	14.6±0.3

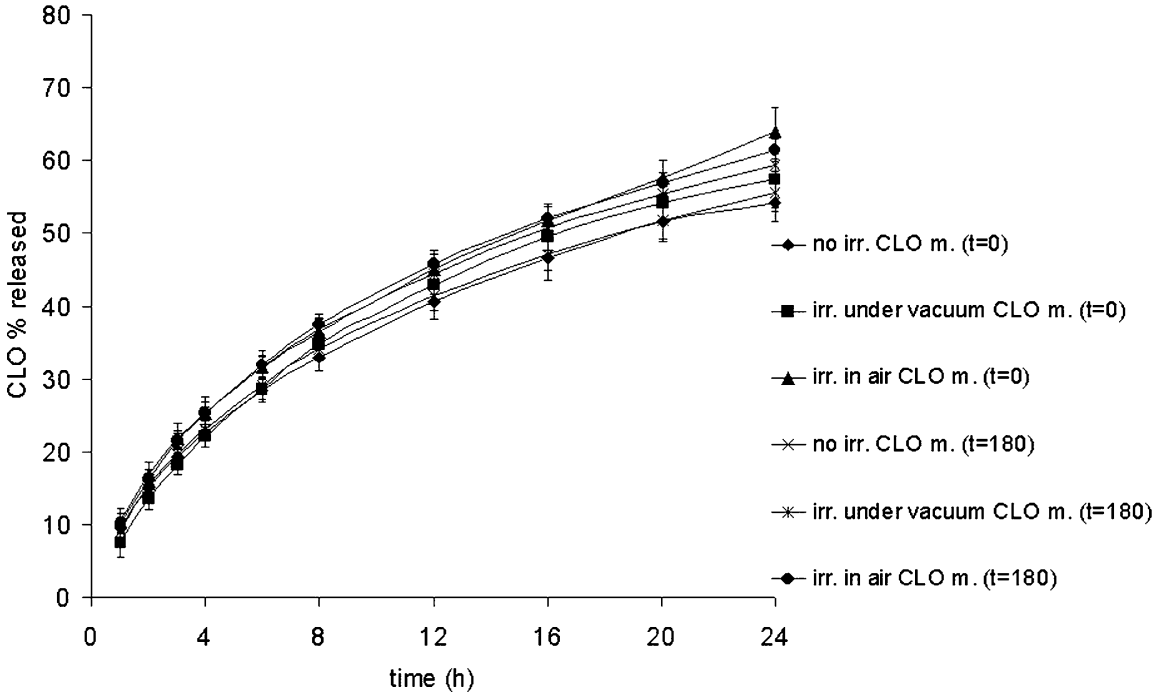

Fig. 8. Release profiles of CLO from non-irradiated and irradiated microspheres at different storage times ($t=0$ and $t=180$ days).

was absent in the microspheres irradiated under vacuum [5].

The increment of CLO release rate shown after irradiation appeared to be in contrast with the shift of PLGA T_g to higher temperatures (Fig. 7), this being an indication of a lower mobility of the polymeric chains and, consequently, of drug diffusion in the matrix. This anomalous pattern, due to radiolytic degradation, could be attributed to a modification of the copolymer wetability and of the degree of hydration.

CLO radiostabilising effect on loaded microspheres as shown by EPR analysis could justify the low modification of the drug release immediately after irradiation and in time.

4. Conclusions

Microspheres containing CLO and irradiated under vacuum were stable over a period of 6 months. Immediately after irradiation in air, CLO release rate constant increased by ~10%, and did not change further in the following storage time. This fact suggests that the PLGA/CLO system was stabilised.

The radicals generated by both the polymeric matrix and the active ingredient were identified and quantified. Evidence of polymer/CLO spin transfer reactions was shown. A radiostabilising effect by CLO on the loaded microspheres was clearly shown by ~54% decrease of the overall radical yield as compared with that of the placebo microspheres.

The observations pertaining to the radiolytic behavior of CLO/PLGA microspheres, with special reference to the spin transfer reactions, the radiostabilisation effects and the detection of intermediate nitroxyl radicals, are suggested to apply also to the other drug delivery systems provided that they contain drugs bearing nitro-aromatic moieties. As a matter of fact, all the above effects are consequences of the electron and free radicals' scavenging properties of the nitro group and of the attitude of the aromatic structures to enhance the energy loss pathways not leading to chemical changes (phosphorescence and fluorescence emission and degradation to thermal energy).

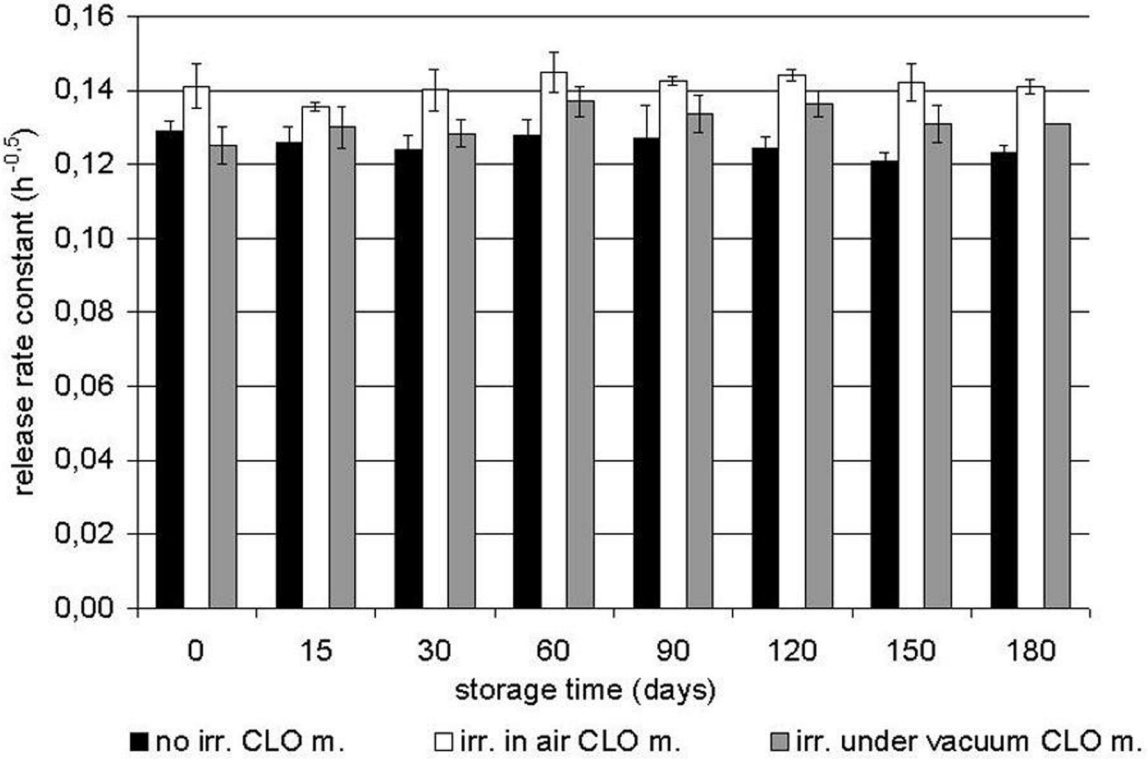

Fig. 9. Release rate constant of CLO from irradiated and non-irradiated microspheres at different storage times.

The persistence of CLO radicals at room temperature in the loaded microspheres was sufficient in order to use them as irradiation markers. On the other hand, this raises the problem of radical toxicity during drug administration. The low concentration of CLO residual radicals ($\sim 10^{-7}$ mol/kg) does not seem to lead to appreciable consequences, especially when the radical fast decay rate on water contact is taken into consideration. The problem of residual radical-induced toxicity in radiation treatment for sterilisation or sanitisation purposes as well as the possibility of using EPR analysis as a specific technique for routine control of irradiated products should be seriously taken into consideration.

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