EFFECT OF GAMMA IRRADIATION ON DRUGS

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Abstract

Several drugs (ceftazidime, vancomycin, glucagon, erythromycin and dobutamine) were studied in order to determine their radiostability. The methods used to measure the degradation of the drug were the potency and the colour change after irradiation. Electron spin resonance (ESR) is currently being used to detect irradiated foodstuffs and may be a promising technique to detect irradiated drugs. Trapped radicals in cefazolin sodium were studied and quantified by ESR for this purpose. It is proposed that the trapped radicals play an important role in the formation of the final radiolytic compounds. The potency of ceftazidime was not significantly modified after an irradiation of 25 kGy, whereas the potency of erythromycin and dobutamine decreased slightly. Glucagon was revealed to be radiosensitive with a significant decrease in its potency after irradiation. The visible spectra of glucagon and dobutamine did not change significantly after irradiation. The absorbance of erythromycin and vancomycin increased after irradiation. According to European Pharmacopoeia standards, the colour change of ceftazidime is unacceptable. The ESR spectra reveal that the trapped radicals in cefazolin sodium are characteristic of an irradiation. The radical concentration is dependent on the irradiation dose and decays over time. Radical concentration in cefazolin sodium was reduced by 99% after 100 days of storage. These radicals are responsible for about 13% of the measured final radiolytic product. Ionic reactions could also lead to final radiolytic products.

1. INTRODUCTION

The growing interest centered on treatment of pharmaceuticals by ionizing radiation arises from the clear advantages this process offers compared to other methods of sterilization. The process can be carried out on the packaged product and may be applied to heat sensitive drugs. Radiation processing is clean and well controlled [1]. One of the major concerns of using ionizing radiation to sterilize pharmaceuticals is the formation of radiolysis products that might affect the quality of the drug. Two studies on the effect of irradiation on drugs were performed.

Studies show varying degrees of damage caused by irradiation on drugs [2–6]. Each drug is analysed individually because of the difficulty in predicting their radiostability. The first study investigates the radio-induced degradation of several drugs (ceftazidime, vancomycin, glucagon, erythromycin and dobutamine). The extent of the drug degradation is estimated by the potency loss and the colour change after irradiation.

In previous works [5, 6], trapped radical identification and final product analysis proved useful to elucidate the radiolysis mechanism. It is postulated that the trapped radicals, formed by the absorption of energy from the ionizing radiation, play an important role in the formation of the final radiolytic products. To optimize and to control the radiosterilization process, the mechanisms leading to the formation of “radio-induced impurities” need to be understood. The second study investigates the radiolysis mechanism in cefazolin sodium.

ESR spectroscopy was used to study the formation and decay of cefazolin sodium radicals. ESR is the most sensitive method for detecting unpaired electrons such as those present in free radicals [7]. These radicals are transient species that can be trapped in the solid for up to several weeks and they provide evidence of irradiation. The radicals of cefazolin sodium are quantified to see if they are responsible for the final radiolytic products.

2. DETERMINATION OF THE DEGRADATION OF DRUGS (PART I)

2.1. Experimental part

2.1.1. Reagents

All products are from Eli Lilly (drug products): Tazidime® (Ceftazidime), Vancocin® (Vancomycin hydrochloride), Glucagon® for injection (Glucagon), Ilotycin® Gluceptate (Erythromycin gluceptate) and Dobutrex® solution (Dobutamine hydrochloride).

2.1.2. Irradiation

Irradiation was performed in dry ice with a Gammacell 220. The dose rate was about 10 Gy s^{-1} and the dose was 25 kGy. Irradiations were performed in triplicate.

2.1.3. Potency

(1) Ceftazidime: Analytical column Merck LiChrophere 100, 5 μm, 4.6 mm×25 cm; Column heater: 30°C; Mobile phase: (water, 25% tetrabutylammonium hydroxyde in methanol, 85% phosphoric acid (900-10-5) - methanol (93 - 7); Flow rate: 1 ml/min; UV detection at 262 nm. Samples were prepared by weighing 16 mg of Tazidime® into a 100 ml volumetric flask and diluting with water.

(2) Vancomycin: Analytical column Merck LiChrosphere 100, 5 μm**,** RP-18, 4.6 mm×25 cm; Column heater: 30°C; Mobile phase: gradient Mobile phase A (triethylamine buffer-acetonitrile-tetrahydrofuran (93-6-1)) - Mobile phase B (triethylamine buffer-acetonitrile-tetrahydrofuran (70-29-1)). 0-12 min: Mobile phase A: 100%; 12–20 min: Mobile phase B: 100%; 23–30 min: Mobile phase A: 100%.; Flow rate: 2 ml/min.; UV detection at 280 nm. Samples were prepared by weighing 40 mg of Vancocin® into a 100 ml volumetric flask and diluting with mobile phase A.

(3) Glucagon: Analytical column Zorbax 300 SB-C8, 5 μm, 4.6 mm×25 cm; Column heater: 35°C; Mobile phase: acetonitrile-0.2 M $NaH_2PO_4.H_20$, 0.04 M cysteine at pH 2.6 (1-3-4); Flow rate: 1 ml/min.; UV detection at 214 nm. Samples were prepared by diluting 1 vial (1 mg of Glucagon) into 1 ml of diluent.

(4) Erythromycin: Analytical column Merck LiChrosphere 100 RP-18, 5 μm, 4.6 mm x25 cm-Column heater: 30°C; Mobile phase: 1 g of sodium pentane sulphonate into 700 ml of water, 300 ml of acetonitrile, 20 ml of triethylamine adjusted to pH 3 with phosphoric acid; Flow rate: 1 ml/min; UV detection at 195 nm. Samples were prepared by weighing 50 mg of Ilotycin® into a 20 ml volumetric flask and diluting with sample solvent (1 g of sodium pentane sulphonate into 700 ml of water, 300 ml of acetonitrile, 20 ml of triethylamine adjusted to pH 7 with phosphoric acid).

(5) Dobutamine: Analytical column Supelco, Supelcosil LC-18, 5 μm, 4.6 mm×25 cm; Column heater: 30°C; Mobile phase: 60:24:16 (v/v/v) mixture of ion pair reagent (15.6 mM 1-octanesulfonic acid solution pH 2.5), acetonitrile, methanol; Flow rate: 1 ml/min.; UV detection at 280 nm. Samples were prepared by diluting 0.5 ml of dobutamine HCl into a 10-ml volumetric flask and diluting with mobile phase.

These HPLC procedures were used only for potency determination and not for the impurity profile evaluation. In the latter case, the main peak is allowed to saturate the signal.

2.1.4. Colour determination

The visible spectra were recorded with a UVIKON 933 spectrophotometer. Water from Millipore-Q was used as a reference.

Samples were prepared by weighing 50 mg of freeze dried product into 1 ml volumetric flask and diluting with water, except for glucagon (1 vial containing 1 mg of glucagon and 49 mg of lactose/1 ml of diluent) and the Dobutrex® solution, which was analysed as received (250 mg of dobutamine/20 ml).

3. RESULTS AND DISCUSSION

3.1. Potency

The potencies of the screened compounds after an irradiation of 25 kGy are summarized in Table I. The potencies are expressed in percentage relative to the potency of the non-irradiated samples: (potency $_{\text{irradiated}}$ / potency $_{\text{non-irradiated}}$)×100.

TABLE I. POTENCIES OF SCREENED COMPOUNDS IRRADIATED AT 25 KGY

Coumpound	Relative potency after irradiation (%)
Ceftazidime	98 ± 2
Erythromycin	97 ± 1
Dobutamine	96 ± 2
Vancomycin	95 ± 3
Glucagon	88 ± 3

Potency was not significantly modified after an irradiation of 25 kGy for ceftazidime; was slightly decreased for erythromycin, dobutamine and vancomycin; was significantly decreased for glucagon. These results are in good agreement with data on the antibiotic activity after irradiation of vancomycin and erythromycin [7]. All the irradiated drugs fell within acceptable limits, except for glucagon.

The solid samples were found to be radioresistant except for glucagon, because it readily oxidizes into a radical through hydrogen abstraction (R-SH $\rightarrow$ R-S$^{\bullet}$).

3.2. Colour determination

In order to detect a modification in colour after irradiation, the absorption spectra of solutions prepared with non-irradiated and irradiated drugs were compared in the visible light region (380-780 nm). The visible spectra of glucagon and dobutamine were not significantly modified after irradiation. Irradiated erythromycin and vancomycin showed an increase in absorbance. The colour change of ceftazidime was found to be unacceptable according to the European Pharmacopoeia standards.

4. MECHANISMS LEADING TO THE RADIOLYTIC PRODUCTS IN RADIOSTERILIZED CEFAZOLIN SODIUM (PART II)

4.1. Experimental part

4.1.1. Reagent

Cefazolin sodium was supplied from Eli Lilly as the freeze-dried product, Kefzol®.

4.1.2. Irradiation

Irradiation was performed on Kefzol® (freeze dried material) at room temperature, in a Gammacell 220. Glass vials of about 2 g of Kefzol® / vial were irradiated. The dose rate was about 10 Gy s^{-1} and the doses ranging from 2 to 30 kGy. The irradiations were performed in triplicate.

4.1.3. ESR measurements

A Jeol RE series ESR spectrometer connected to a Jeol Esprit 330 ESR system was used. The spectra were recorded at 25°C using the following conditions: Field set: 336.7 mT; Scan range: ± 7.5 mT; Microwave frequency: 9.446 GHz; Microwave power: 0.6 mW; Modulation frequency: 100 kHz; Modulation amplitude: 0.05 mT; Time constant: 0.01 s; Scan time: 2 min. Activated carbon diluted in KCl obtained from Varian (Pitch) was used as external calibration standard. Quartz tubes of about 20 mg of irradiated powder were analysed.

4.1.4. High performance liquid chromatography

The following HPLC system was used: Pump Spectra Physics SP 8800; Auto sampler SP 8880 with injection loop of 20 µl; Variable wavelength detector Spectra 100.

For the potency determination: Analytical column Ultrasphere ODS 5 µm, 4.6 mm×15 cm; Column heater: 30°C; Mobile phase: acetonitrile, triethylamine solution (10 ml/L) adjusted at pH 2.5 with phosphoric acid (9-91); Flow rate: 2 ml/min.; UV detection at 220 nm. Samples were prepared by weighing 25 mg of Kefzol® into a 50-ml volumetric flask and diluting with mobile phase.

For the impurity profile evaluation: Analytical column Merck Licrosphere I 00 RP-18 5µm; 4.6 mm×25 cm; Column heater: 3 0 °C; Mobile phase: gradient acetonitrile-weak solvent (786 mg diethylamine HCl, 2.4 g sodium phosphate monobasic monohydrate into 1 L, pH adjusted to 2.5 with phosphoric acid). Initial composition: weak solvent: 100%. Linearly increase the percentage of acetonitrile at a rate of 1%/min. to a final composition: acetonitrile; weak solvent (30-70). Hold this composition for an additional 10 min.; UV detection at 220 or 450 nm or Beckman Diode Array detector. Samples were prepared by weighing 50 mg of Kefzol® into a 10-ml volumetric flask and diluting with sample solvent (18 g potassium phosphate monobasic dissolved in 1 L water).

4.1.5. Colour determination

The absorbance at 450 nm was measured with a UVIKON 933 spectrophotometer. Water from Millipore-Q was used as a reference. Samples were prepared by weighing 100 mg of Kefzol® into 1 ml-volumetric flask and diluting with water.

5. RESULTS AND DISCUSSION

5.1. Detection of radicals in irradiated cefazolin sodium samples

Only irradiated samples showed ESR spectra (Fig. 1). These spectra, characterized by four lines, are complex and not well resolved and therefore do no allow radical identification. Significant signals were recorded at even low irradiation doses of about 2 kGy; knowing that sterilization requires about 10-25 kGy, it should be no problem to detect irradiated drugs by ESR.

ESR is already being widely used to detect irradiated foodstuffs [8]; a new application would be to distinguish between irradiated and non-irradiated drugs. The use of ESR to detect irradiated drug samples is feasible only if the signal persists during the shelf-life of the drug. As the regulations vary in different countries, a reliable detection method of irradiation is very important.

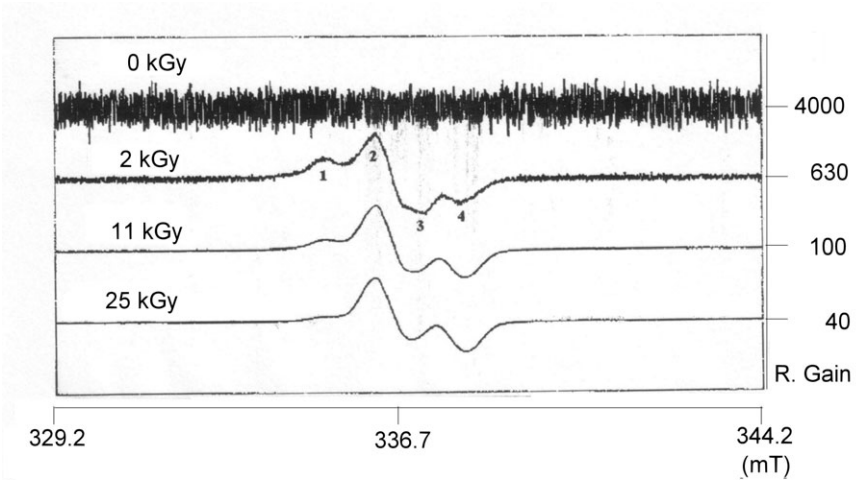

FIG. 1. ESR spectra of Kefzol irradiated at 0; 2; 11 and 25 kGy, recorded 3.05 h after the end irradiation. Different gains were used to record the spectra: from 4000 for the non-irradiated sample to 40 for the sample irradiated at 25 kGy.

5.2. Radical stability

Tests were carried out to investigate whether the storage had an effect on the concentration of free radicals. Storage at ambient temperature in a quartz tube over several weeks (102 days) showed a decrease in the quantity of free radicals. The free radical concentration is estimated by the area of the ESR signal (Fig. 2).

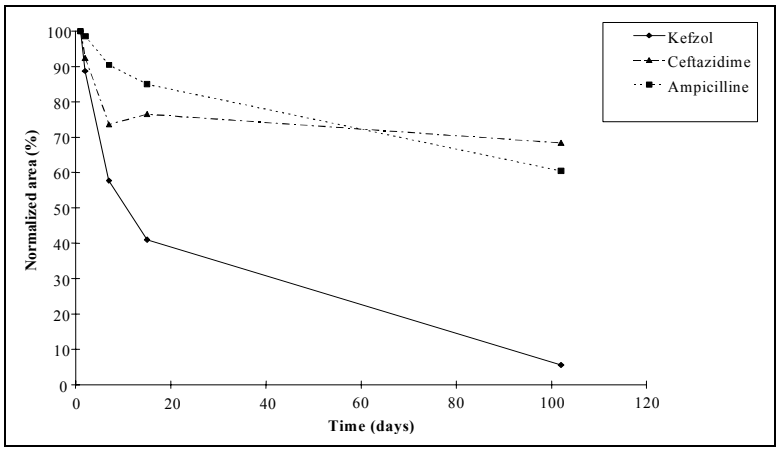

FIG. 2. Radical decay curve in cefazolin, ceftazidime, and ampicillin irradiated at 2 kGy.

Approximately 100 days after the irradiation of cefazolin, the radical concentration decreased by 99%, but the ESR signal was still easily detected. J.P. Basly, et al. suggests that the decay of free radicals can be divided in two phases: the first, corresponding to a “fast” exponential decay and the second, corresponding to a “slow” linear decay [9–12]. The slow decay results from the reorganization of micro-volumes in the solid matrix [1]. This process is very slow and would account for the limited diffusion of radicals and molecular species in the solid. Radicals can then disappear by recombination, but the phenomenon is slow and explains why radicals can be trapped during several months, and sometimes years.

The conservation of the radicals in irradiated cefazolin samples is poor compared to other antibiotics (ceftazidime and ampicillin for instance). Generally, in those antibiotics, 40 to 60% of radicals are still present several months after irradiation [5, 7–9]. One exception was reported for cefuroxime sodium samples [7]. The conservation of radicals must be related to the physical properties of the solid. If the solid allows rapid diffusion, the radical concentration will decrease rapidly, and inversely, if the diffusion is slow, the radical concentration will decrease slowly.

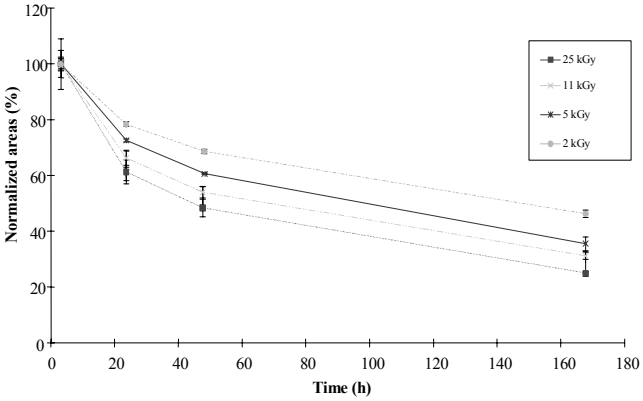

FIG. 3. Radical decay in irradiated Kefzol normalized at 3.13 h versus time. Normalized area (%) = (area at time t / area of the same samples at time t = 3.13 h) ×100 (t is the delay (h) between end of irradiation and ESR measurements) (n = 3).

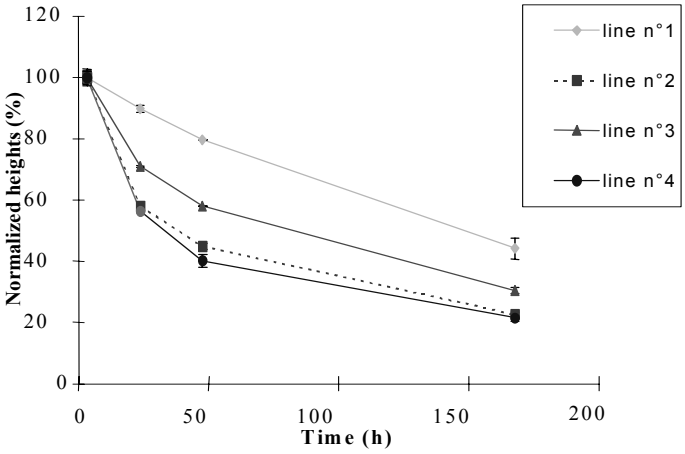

FIG. 4. Lines' height normalized at 3.13 h of Kefzol irradiated at 30 kGy versus time (n = 3). The height was defined by the distance from the positive or negative top of the peak to the baseline.

The rate of decay of radicals in irradiated cefazolin samples was found to be dependent also on the irradiation dose as shown in Fig. 3. The first phase of radical decay is faster when the irradiation dose is higher. Moreover, the shape of the ESR spectra depends on the storage time (Fig. 4). Lines 2 and 4 decrease with the same rate and are probably due to the same radical. The decrease of lines 1 and 3 is slower. This difference in the variation of the relative intensities of the lines indicates the presence of different radicals. A mixture of different radicals in irradiated pharmaceuticals has been previously reported [5, 13].

5.3. The dose effect on the radical concentration

Fig. 5 shows the relative radical concentration in cefazolin samples irradiated at different doses.

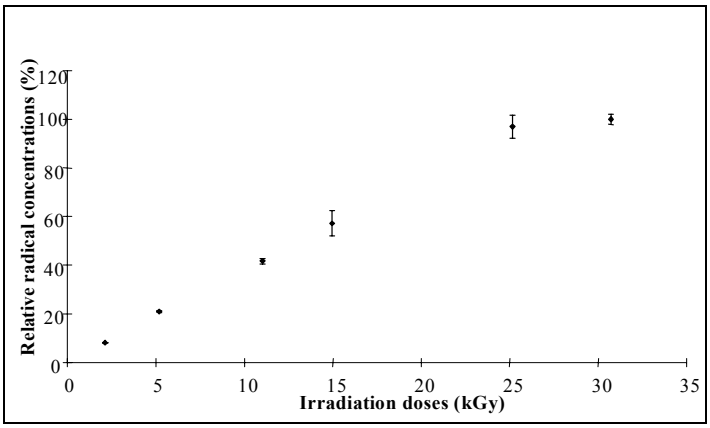

FIG. 5. Relative radical concentrations in Kefzol versus irradiation doses. The measurements were performed 3.13 h after the end irradiation (n = 3).

The concentration of radicals increases linearly with the irradiation dose up to 15-30 kGy. At higher irradiation doses, the growth of radicals is slower and reaches a plateau. The deviation of the radical yield from linearity is common and similar findings are found in other studies [10–12, 14–15]. Rapid processes remove radicals during the irradiation other than those occurring after the radiolysis. The radiolytic compounds accumulating in the solid may scavenge part of the energy used to produce radicals, and this would explain the dose effect.

5.4. Estimation of the radiosensitivity

The final radiolytic products may originate from the radicals formed during the radiolysis. An investigation to see if the radical yield (G) reflects the radiosensitivity of cefazolin sodium was performed. The radical yield (G) is defined as the number of moles of radicals formed per Joule of energy absorbed.

The radical yield (G) was calculated to be 1×10^{-7} or 7×10^{-7} mol J^{-1}, depending on the quantitative reference used for ESR, activated carbon diluted in KCl from Varian (Pitch) or DPPH (2,2-di(4tert-octylphenyl)-l-picrylhydrazyl) respectively. These radical yields are estimates because the ESR response depends on the nature of the radicals. Ideally, the calibration curve must be made using similar radicals. Radicals from irradiated cefazolin were not identified because reference standards were not available.

The average G-value of cefazolin radicals (4×10^{-7} mol J^{-1}) is of the same magnitude as that of other antibiotics such as ampicillin (4×10^{-7} mol J^{-1}) and ceftazidime (0.6×10^{-7} mol J^{-14}), as well as other drugs like dopamine, norepinephrine and cefotetan [11, 12].

Using the average G-value of 4×10^{-7} mol J^{-1} and supposing that all the radicals lead to final products, the potency loss of cefazolin was estimated to be 0.5% after an irradiation of 25 kGy. An irradiation of 25 kGy (25,000 J Kg^{-1} . 4×10^{-7} mol J^{-1}) produces 0.01 moles of radicals per kilo. If each radical induces the degradation of one molecule of active compound, one kilogram of cefazolin sodium looses 0.01 moles or 5×10^{-3} kg of cefazolin sodium (MW 476.5) after 25 kGy. This corresponds to a 0.5% loss.

The potency of cefazolin samples irradiated at 25 kGy, measured by HPLC, was found to be 3.85 ± 0.9% lower than the potency of the non-irradiated samples. The radiation damage is then under-estimated by 13% (0.5% instead of 3.85%). The following hypotheses are proposed:

— The delay between the end of irradiation and the G-value determination was minimized as much as possible (a few hours), but since the radical decay is fast in the first phase after irradiation, a part of the radicals were not taken into account for the G-value calculation.
— Some final radiolytic products are formed during the radiolysis. Only some of the radicals are trapped and may be observed by ESR. A large proportion of the final products are formed by immediate recombination.
— Ionic reactions like ion-molecule reactions (resulting from electrons trapped before diffusing back to the cations), give final radiolytic products. Ions are formed and may react before geminate neutralization. This hypothesis is supported by the thermoluminescence generally observed in irradiated food or drugs [16]. This results from the late neutralization when the electron is de-trapped by thermal effect.

5.5. Origin of the colour

The main problem encountered with radiosterilized cefazolin sodium is that the reconstituted solution is yellow instead of clear. Even low irradiation doses of about 2 kGy brought about an unacceptable colour change according to European Pharmacopoeia specifications [17]. The extent of the yellow colour change was estimated by the absorbance at 450 nm after water dissolution of the irradiated powder (100 mg/ml) (Fig. 6).

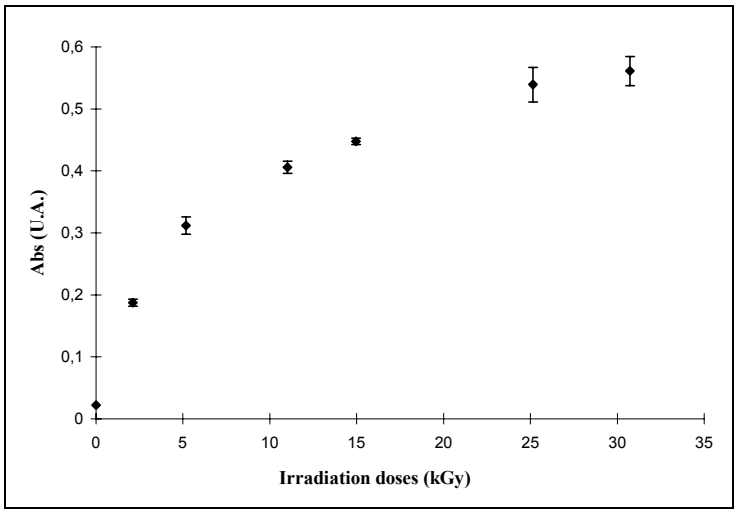

FIG. 6. Absorbance at 450 nm of solutions of Kefzol powders irradiated at different irradiation doses (200 mg of irradiated Kefzol into 2 ml of MilliQ water) (n = 3).

The absorbance is not directly proportional to the dose, it increases exponentially to a maximum. Final radiolytic compounds are responsible for the colour change and not the trapped radicals because these disappear upon dissolution of the solid. The final compounds were not identified because of the complexity of the impurity profile after irradiation [18]. The impurity profile, determined by HPLC, of non-irradiated samples was compared with that of samples irradiated at 25 kGy (Fig. 7).

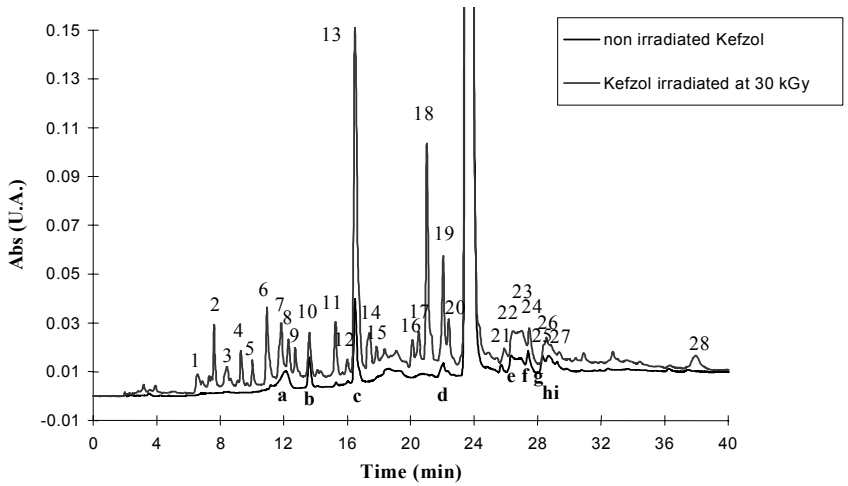

FIG. 7. Superposed chromatograms of solutions (5 mg/n-A) prepared from non-irradiated Kefzol and Kefzol irradiated at 30 kGy. Detection wavelength: 220 nm.

In non-irradiated samples, 9 compounds (named a to i) were detected (A $\geq$ 0.005 at 220 nm; injected solutions: 5 mg/ml). In the irradiated samples 28 compounds (1 to 28) were detected. The majority of compounds are new radio-induced impurities. Peak 13 (or c), already present prior to irradiation, was identified as 5-methyl-1,3,4-thiadiazol-2thiol and presents a significant absorption at 450 nm.

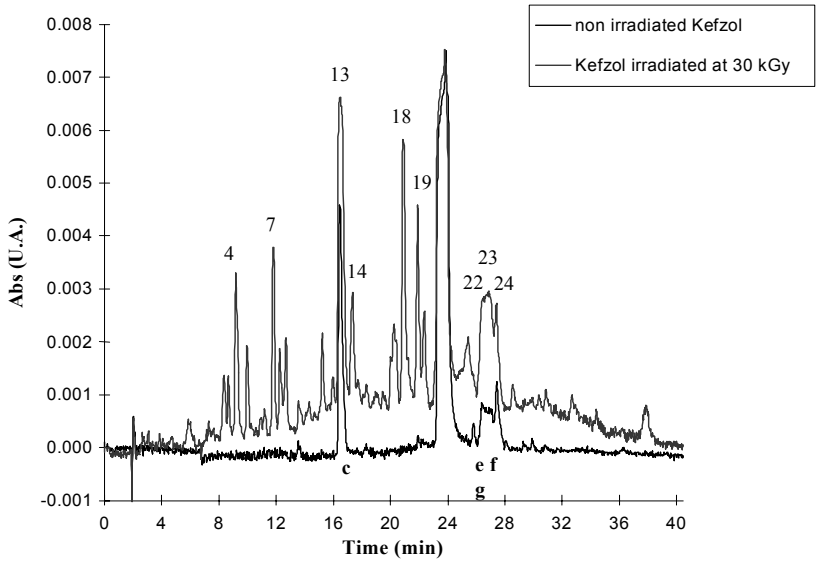

FIG. 8. Superposed chromatograms of solutions (50 mg/ml) prepared from non irradiated and irradiated at 30 kGy Kefzol. Detection wavelength: 450 nm.

As depicted in Fig. 9, the injection of highly concentrated solutions (50 mg/ml) with detection at 450 nm shows that most of the radiolytic compounds slightly absorb at this wavelength. The products of radiolysis are present in traces in the complex chromatogram, rendering their identification impossible [18].

6. CONCLUSION

The potency of the drug after radiosterilization is not the limiting factor, but colour change of the reconstituted solution is. The extent of the radio-induced degradation was limited for erythromycin and dobutamine, according to their potency and visible spectra after an irradiation of 25 kGy. These drugs are good candidates for radiosterilization, unlike the cephalosporins (ceftazidime).

Detection of irradiated drugs by electron spin resonance spectroscopy is promising: ESR is highly sensitive, specific to irradiated drugs, easy to use and does not require much sample. The only drawback is that the radio-induced signals must persist during the shelf-life of the drug. For irradiated cefazolin sodium, the poor radical stability could be a problem. Future testing should be directed towards determining if the ESR signal can still be recorded at the end of the shelf-life.

In a molecular irradiated solid, the radiosensitivity is expressed by the ease of formation of trapped radicals, but for cefazolin sodium samples, trapped radicals cannot explain all of the formation of the final radiolytic compounds. Some of them are coloured compounds and don't come from trapped radicals. Mechanisms leading to final radiolytic compounds in irradiated cefazolin sodium, other than radical reactions, have to be postulated.

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