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Radiation-Induced Effects on Cefotaxime: ESR Study

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As an alternative to heat and gas exposure sterilization, ionizing radiation is gaining interest as sterilization process for medicinal products. Detection and dosimetry of pharmaceuticals radiosterilization is a growing concern to numerous government regulatory agencies worldwide. In this context, it is necessary to find methods distinguishing between irradiated and non-irradiated pharmaceuticals. In the absence of suitable detection methods, our attention was focused on electron spin resonance (ESR) spectrometry. A third generation cephalosporin, cefotaxime, was chosen as model; this antibiotic is a potential candidate for radiation treatment due to its thermosensitivity. While the ESR spectra of a nonirradiated sample presents no signal, a signal, dependent of the irradiation dose, is found in irradiated samples. The number of free radicals was estimated by comparing the second integral from radiosterilized samples and a diphenylpicrylhydrazyl reference. Estimation of the number of free radicals gives 1.9×10^{20} radicals mol^{-1} at 20 kGy. From this result, the *G*-value (number of radicals $(100\,eV)^{-1}$) could be estimated to 0.3. Aside from qualitative detection, ESR spectrometry can be used for dose estimation. When quadratic, exponential or bi-exponential functions are applied to the variation of peak to peak amplitude vs. dose, these functions correlate well with the data. However, it is important to notice that linear function correlates well with the data for doses lower than 20 kGy. Since the radiation dose selected must be always based upon the biobur-den of the products and the degree of sterility required (EN 552 and ISO 11137) 25 kGy could no longer be accepted as a "routine dose" for sterilizing a pharmaceutical. Doses from 6 kGy (ISO 11137) could be investigated and linear regression would appear to be the least expensive route to follow. The free radicals concentration appeared to not decrease during the 57 days of storage; the number generated during the irradiation allows the detection of radiosterilized cefotaxime up to two years after irradiation.

Keywords: Cefotaxime, gamma radiation, free radicals, ESR, dosimetry

1. INTRODUCTION

The detection of thermolabile medical devices, such as catheters or syringes with ionizing radiation is successfully practised in many countries. Futhermore, it is possible to sterilize pharmaceutically active substances with ionizing radiation.[1,2] In the 1997 edition of the European Pharmacopeia under the "methods of preparation of sterile products" irradiation is one of

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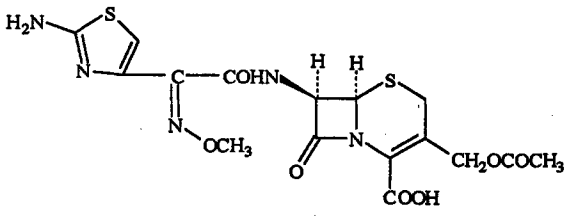
only three processes that can be used as terminal sterilization method. Particularly, gamma sterilization is essentially focused on drugs which cannot be sterilized by conventional methods such as autoclaving, because of their thermosensitivity. With the publication of EN 552[3] and ISO 11137[4] there is at least a recognized standard for implementing this technology. Detection and dosimetry of pharmaceuticals radiosterilization is a growing concern to numerous government regulatory agencies worldwide. In this context, it is necessary to find methods distinguishing between irradiated and nonirradiated pharmaceuticals. In the absence of suitable analytical methods, our attention[5–8] as well as those of others[9–14] was focused on ESR spectrometry. ESR (Electron Spin Resonance) has found, over the last decades, application as a dosimetric tool (alanine dosimetry, individual dose from retrospective dosimetry after radiation accident, ESR dating, identification of irradiated foodstuffs, paramagnetic centers concentration in materials).

The aim of this work was to develop, by mathematical procedures, equations to describe the ESR curves vs. dose and storage time after gamma irradiation; for this investigation, we have chosen a third generation cephalosporin, cefotaxime (Scheme 1). This compound is a potential candidate for radiation treatment due to its thermosensitivity.



SCHEME 1

We must keep in mind that ESR dosimetry of pharmaceuticals could only be a control *a posteriori*; doses are firstly confirmed by chemical or physical dosimeters in irradiation industrial equipment.

2. EXPERIMENTAL SECTION

2.1. Irradiation

The drug substance was commercial product suitable for clinical use. Cefotaxime was kindly supplied by Roussel Uclaf (Romainville, France) in vials of 1 g sterile powder for injection. The cephalosporin was irradiated with gamma rays (^{60}Co) emitted by an IBL 460 (UFR de Pharmacie, Limoges, France); the dose rate (1347 Gy h^{-1}) was preliminarily calibrated using Fricke dosimetry (ferrosulphate dosimetry). A nonirradiated sample was kept as reference. Powder samples (30 mg) were irradiated at room conditions into polycarbonate vials.

2.2. Instrumentation

ESR spectra were recorded at room temperature using a BRUKER ESP 300E spectrometer (X-Band). Preliminary to the study, care was taken with the use of microwave power and modulation amplitude to avoid saturation. For the measurements, 15 mg of substance was weighted with an accuracy of 0.2 mg. Samples were inserted in standard quartz tube of 4 mm i.d. measured soon after irradiation and stored for radical time-stability study. The evolution of the ESR signal in the ESR signal/dose curves was followed by recording the signal amplitude (peak to peak height of the central line of the spectrum) and the signal area (determined by the double integration of the derivative spectral curves). As a preliminary to the quantitative ESR spectrometry, 1 mg of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma, purity 90%) was ground in a mortar and introduced in a quartz tube. This quartz tube was used as an ESR quantitative reference. Numeric simulations were performed using

Levenberg–Marquardt method with a Pentium 75 MHz.

3. RESULTS AND DISCUSSION

3.1. Detection of Free Radicals by ESR

An ESR powder spectrum of cefotaxime after gamma irradiation is presented in Figure 1. The concentration of free radicals starts to saturate at ca. 20 kGy. As described previously by Ciranni-Signoretti *et al.*[11] the signal is a superposition of a singlet and a triplet (shoulder marked by an arrow in Figure 1), mostly hidden by the singlet. From these authors, the triplet and the singlet lines could be attributed to nitrogen centered (cleavage of bond N–OCH_3) and carbon centered (cleavage of bond CO–CH_3) paramagnetic radicals respectively. Further investigations on the effects of radiation (solid state irradiation and gamma radiolysis,[15] pulse radiolysis[16,17]) show that the 25 kGy irradiation of the solid cefotaxime induced little degradation (five new radiolytic products and less than 0.73% of degradation).[15] One of the degradation products was identified as anticefotaxime corresponding to the isomerization of the oxime ether.

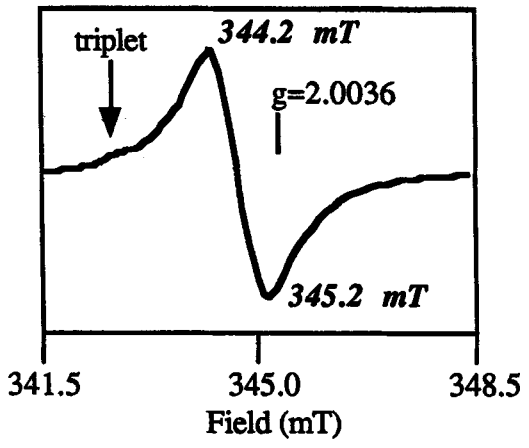


FIGURE 1 ESR spectrum (25 kGy). Conditions: sweep field: 341.5–348.5 mT; microwave frequency: 9.66 GHz; microwave power: 1 mW; modulation frequency: 100 kHz; modulation amplitude: 0.2 mT; time constant: 164 ms; sweep time: 0.68 min; amplification factor: 2500.

Figure 2 shows plot of the evolution of the dose–ESR response curve after radiosterilization. The number of free radicals in the samples is proportional to the area under the ESR absorption curve. For quantitative comparison of different radical species with line width and shape, the second integral of the derivative curve is necessary. The number of free radicals in Figure 2 was estimated by comparison of the second integral from radiosterilized samples and DPPH standard, the spectra of cefotaxime and DPPH were recorded consecutively. To obtain an integrated relative area, all ESR spectra were corrected from baseline drift by a linear function and doubly integrated using the software supplied by BRUKER (ESP 300E data system). Estimation of the number of free radicals gives 1.9×10^{20} radicals mol^{-1} at 20 kGy (6×10^{15} radicals for 15 mg). From this result, the G-value (number of radicals $(100\,eV)^{-1}$) could be estimated to be 0.3.

The sensitivity of the ESR technique was considered in terms of limit of detection (LOD) and limit of quantification (LOQ). The limits were estimated on the basis of the signal-to-noise ratios (S/N = 3 for LOD and S/N = 10 for LOQ) and are 1 ± 0.5 kGy and 2.5 ± 0.5 kGy respectively. Since 25 kGy was established by many regulatory

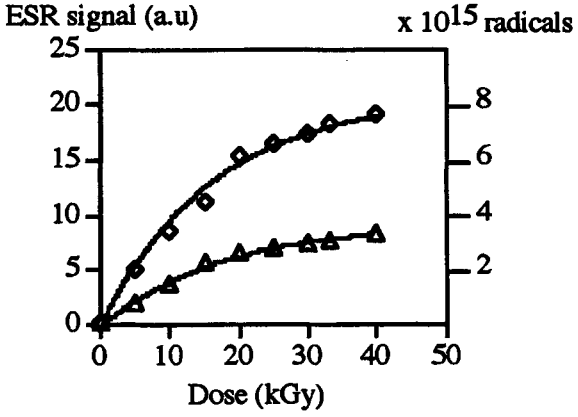


FIGURE 2 Dose/ESR response curves. Triangle: experimental peak to peak height of the central line of the spectra in a.u. divided by 1000; lozenge: radical quantitation in the samples (15 mg) after double integration of the spectra; solid lines are curve fits of exponential function to experimental data.

authorities (EN 552 and ISO 11137) as radiosterilization dose, discrimination from irradiated and unirradiated samples is possible after irradiation.

3.2. Dosimetry

Aside from qualitative detection, ESR spectrometry can be used for dose estimation. For this purpose, four functions have been tried to fit the data plotted in Figure 2:

(1) linear regression,
(2) quadratic fit (the quadratic term was introduced as correction to take into account of the nonlinear shape of the dosimetric curves),
(3) exponential function (first order reaction – this function has been mentioned previously by Desrosiers[18] for estimation of the absorbed dose in radiation-processed food),
(4) bi-exponential function (two irreversible consecutive and unimolecular or pseudo-unimolecular radiolytic reactions, model introduced by Panta[19] for L-alanine ESR dosimetry).

It should be noted that no attempt has been made to force the regression through zero; the numerical results of our fitting are presented in Table I. To be useful, the models described in Table I must be able to predict the irradiation dose. In order to verify the utility of the equations obtained, we have calculated the interpolated (calculated) dose. The procedure was as follows: the interpolated (back-calculated) doses were obtained by entering the measured response (ESR signal) in the models described above. Calculated doses and errors between nominal and calculated doses for each function are given in Table II and Figure 3 shows plot of the errors between nominal and calculated dose vs. nominal dose. Interpolated doses and errors were calculated only on the variation of peak to peak amplitude since previous works showed that this has the advantage of simplicity and has also shown to give better dosimetric results than alternative methods such as double integration.

When Eqs. (2)–(4) (Table I) are applied to the variation of peak to peak amplitude vs. dose, these functions correlate well with the data. However, exponential function, will be sufficient for a dose estimate by retrospective dosimetry. This method presumes a series of additional irradiation doses followed by an ESR measurement at each dose to construct an individual calibration curve for each sample. The advantage of this method is to not require control (non-irradiated) sample. It is important to notice that Eq. (1) (linear function) correlate well (Figure 3 and Table II) with the data for doses lower than 20 kGy. Since the radiation dose selected must be always based upon the bioburden of the products and the degree of sterility required (SAL; Sterility Assurance level) doses from 6 kGy (ISO 11137 – method 2) could be investigated and linear regression would appear to be the least expensive route to follow.

3.3. Decay of Radicals Upon Storage

A suitable technique for the detection of irradiated pharmaceuticals should meet two

TABLE I Coefficients of numerical simulations[a]

Eq. no.	Function	Peak to peak height			Radicals quantitation		
		ESR signal	r^2	F	ESR signal	r^2	F
(1)	Linear	$0.1584 + 0.3367D$	0.990	294	$0.2118 + 0.2841D$	0.991	345
(2)	Polynomial	$0.0435 + 0.4207D - 0.0055D^2$	0.991	387	$0.1288 + 0.3455D - 0.0040D^2$	0.993	494
(3)	Exponential	$9.2696[1 - \exp(-0.0553D)]$	0.991	807	$7.9341[1 - \exp(-0.0619D)]$	0.985	445
(4)	Bioexponential	$-8.6914\exp(-0.0594D) + 8.6294\exp(0.0019D)$	0.994	384	$-7.3907\exp(-0.0553D) + 7.4429\exp(0.0034D)$	0.994	272

[a] Peak to peak height values were divided by 1000 and radicals number values were divided by 10^{15}.

TABLE II Calculated doses and errors[a]

Function	Linear		Polynomial		Exponential		Bi-exponential	
Dose (kGy)	Cal. dose (kGy)	Error (%)	Cal. dose (kGy)	Error (%)	Cal. dose (kGy)	Error (%)	Cal. dose (kGy)	Error (%)
5	5.5	9.4	5.0	0.0	4.7	−6.0	4.4	−12.0
10	10.4	4.0	9.8	−2.0	9.3	−7.0	9.0	−10.0
15	16.5	9.7	17.2	14.7	17.1	14.0	16.8	12.0
20	19.3	−3.3	21.6	8.0	23.0	15.0	22.2	11.0
25	20.4	−18.4	23.7	−5.2	25.7	2.8	24.7	−1.2
30	21.9	−27.1	26.8	−10.7	30.1	0.3	28.6	−4.7
35	22.5	−35.8	28.5	−18.6	32.4	−7.4	30.5	−12.9
40	24.3	−39.2	37.0	−7.5	41.8	4.5	37.8	−5.5

[a]Calculated doses were determined using equations described in Table I and errors (%) were calculated using the following equation: Error (%) = $|(Dose_{(exp)} - Dose_{(cal)})/Dose_{(exp)}| \times 100$.

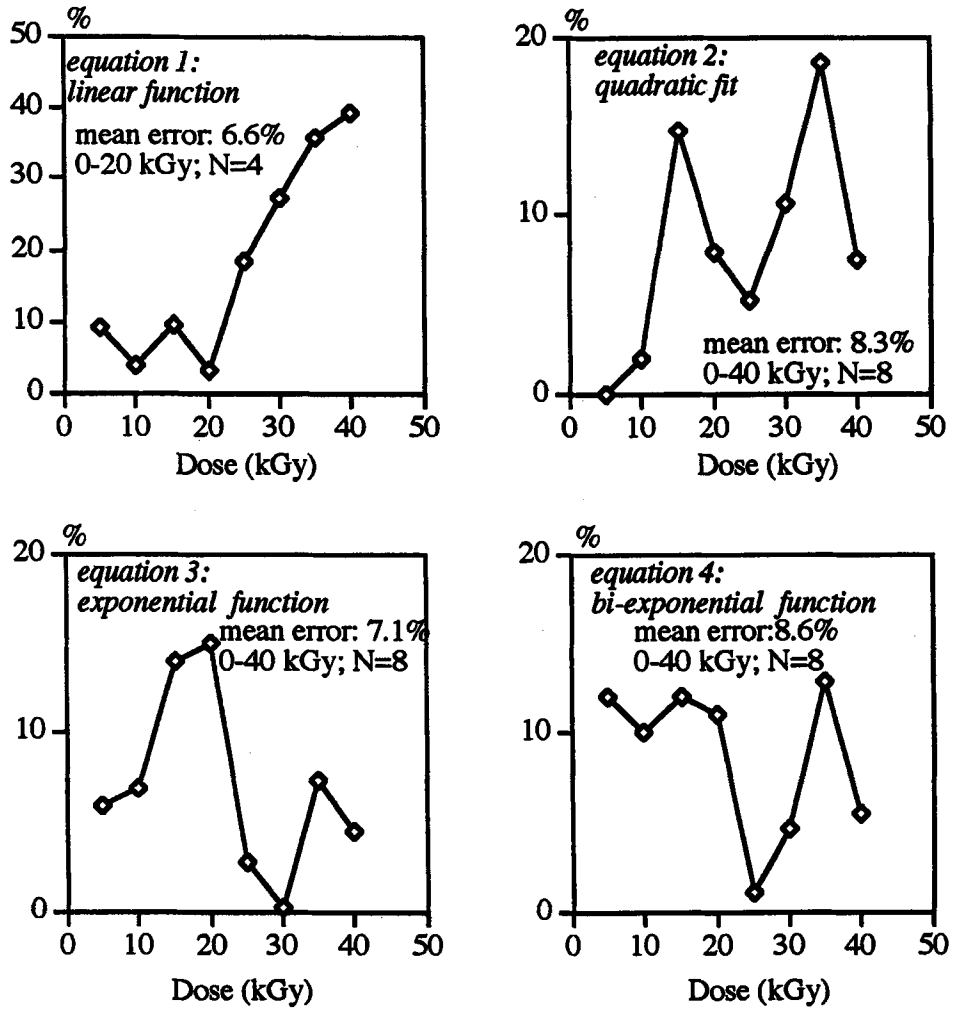


FIGURE 3 Errors (%) vs. dose. Points plotted are errors between experimental and calculated (see Table I) doses using the following equation: Error (%) = $|(Dose_{(exp)} - Dose_{(cal)})/Dose_{(exp)}| \times 100$.

requirements:

- the signal recorded should be specific of the radiation treatment;
- the time stability of the radiation-induced signal should be high enough to allow signal recording over the shelflife of the product.[14]

Tests were carried out to investigate whether storage has an effect on the free radicals concentration. Storage at ambient temperature in a sealed quartz tube over several weeks (57 days) was performed. Figure 4 plots the evolution of the percentage of free radicals vs. storage (peak to peak amplitude). The time limit from the irradiation (25 kGy) for identification of pharmaceuticals radiosterilization by ESR can be evaluated by extrapolation of the amplitude vs. time relationship to the lower limit of detection of the ESR spectrometer. In commercial market of drugs, radicals should be detected up to two years after irradiation.[12] Since the ESR signal appeared to not decrease during the 57 days of storage, it seems possible that the number of free radicals generated during the irradiation allows the detection of radiosterilized (25 kGy) cefotaxime up to two years after irradiation.

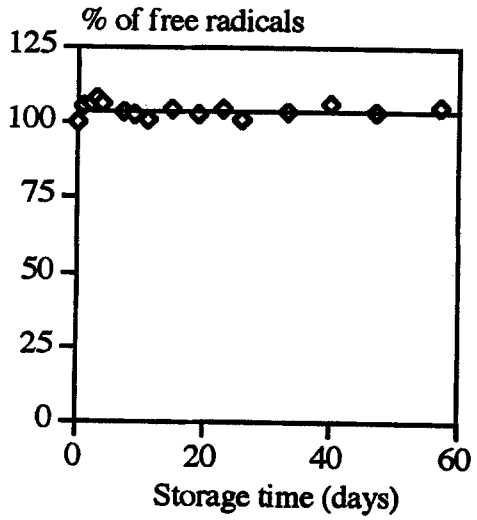


FIGURE 4 Decay of radicals upon storage solid line is curve fit of free radicals decay by a smooth linear function: free radicals (%) = 104.3−0.01*t*.

4. CONCLUSION

The detection based on ESR dosimetry seems promising. ESR could provide proof of radiosterilization; ESR dosimetry requires only small samples (less than 50 mg), minimal time and effort for sample preparation; the measurement is nondestructive. Curie's law implies that the magnitude of ESR resonance is inversely proportional to the sample temperature; hence lower sample temperature could increase the sensitivity of the ESR experiment. A dose estimate from a radiosterilized pharmaceutical can be obtained by reirradiating the pharmaceutical to a number of different doses and measuring the ESR signal intensity at each dose interval, thus generating a dose–ESR response curve for the pharmaceutical being studied. This method, inspired by the protocols described for the detection of irradiated food containing bone, cellulose or crystalline sugars,[20] does not require control (nonirradiated) samples. ESR comparisons involving different laboratories and comparison between ESR and other potential detection methods (such as thermoluminescence) should be interesting.

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