ESR spectroscopy applied to the study of pharmaceuticals radiosterilization: cefoperazone

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Abstract

As an alternative to heat and gas exposure sterilization, ionizing radiation is gaining interest as a sterilization process for medicinal products. Nevertheless, essentially for economic profits, an unauthorized and uncontrolled use of radiation process may be expected. In this context, it is necessary to find methods distinguishing between irradiated and unirradiated pharmaceuticals and, in the absence of suitable detection methods, our attention was focused on ESR spectrometry. In this paper, we examine the potential of ESR as an analytical tool in cefoperazone radiosterilization; this cephalosporin is a potential candidate for radiation treatment due to its thermosensitivity. While the ESR spectra of unirradiated sample present no intensity, a signal, dependent of the irradiation dose, is found exclusively in irradiated samples. The number of free radicals (2×10^{17} radicals per g at 25 kGy) was estimated by comparison of the second integral from radiosterilized samples and DPPH. From this, the G-value could be estimated to 0.3. Limit of detection and limit of quantification are 0.5 kGy and 1 kGy, respectively. Aside from qualitative detection, ESR can be used for dose estimation. The dose–ESR response curves can be simulated by bi-exponential or power functions and the linear function can't be used for simulation even for low doses. Decay of radicals upon storage were simulated using bi-exponential function. The limit of detection of free radicals after irradiation at 25 kGy is 140 days. © 1998 Elsevier Science B.V. All rights reserved.

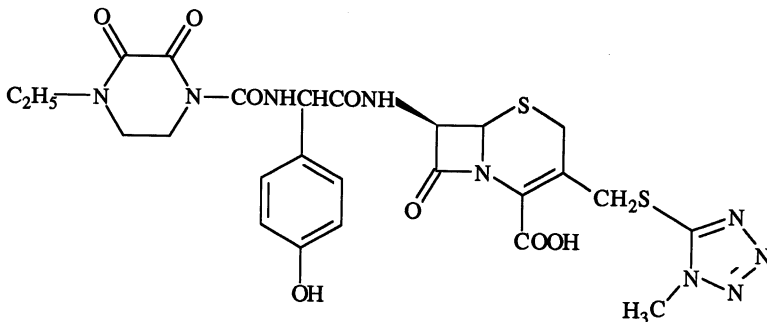*Keywords:* Cefoperazone; ESR; Dosimetry; Decay of radicals

1. Introduction

The sterilization 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 [1–4]. With the publication of EN 552 and ISO 11137 [5,6], there is at least a recognized standard for implementing this technology. Nevertheless, essentially for economic profits, an unauthorized and uncontrolled use of radiation process may be expected. In this context, it is necessary to find methods distinguishing between irradiated and unirradiated pharmaceuticals. In

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the absence of suitable analytical detection methods, our attention [7,8] as well as those of others [9–11] was focused on ESR spectrometry. In this paper, we examine the potentialities of ESR spectrometry as analytical tool in pharmaceutical radiosterilization. A third generation cephalosporin, cefoperazone, was chosen as model; in fact, these products are potential candidates for radiation treatment due to their thermosensitivity [9].

(X-band). For the measurements, 15 mg of substance was weighted with an accuracy of 0.2 mg. 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 spectra) and the signal area (determined by the double integration of the derivative spectral curves; DPPH (1,1-diphenyl-2-picrylhydrazyl radical) was used as reference.

2. Experimental

2.1. Materials

The drug substance was commercial product suitable for clinical use. Cefoperazone was kindly supplied by Pfizer (Amboise, France). This sample was supplied in vials of 1 g sterile powder for injection.

2.2. Irradiation

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 previously calibrated using Fricke dosimetry (ferrosulphate dosimetry). An unirradiated sample was kept as reference. Powder samples were irradiated at room conditions into polycarbonate vials.

2.3. Instrumentation

ESR spectra were recorded at room temperature using a BRUKER ESP 300E spectrometer

2.4. Multivariable regression

Numerical simulations were performed using Levenberg-Marquardt method on a Pentium 75 MHz.

3. Results and discussion

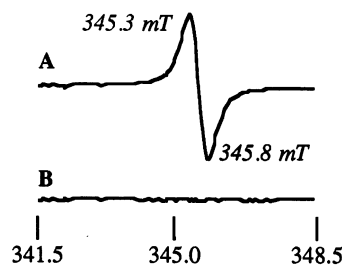While the ESR spectra of unirradiated sample

Fig. 1. ESR spectra of irradiated (A) and unirradiated (B) cefoperazone. 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.
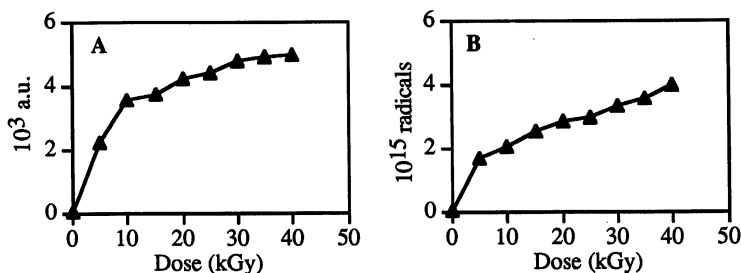
Fig. 2. Dose–ESR response curve. (A) Peak to peak height of the central line of the spectra expressed in a.u. (B) Radical quantitation in the sample (15 mg) after double integration of the spectra.

present no intensity, a signal, dependent of the irradiation dose, is found exclusively in irradiated samples (Fig. 1).

3.1. Dosimetry

Fig. 2 shows plot of the evolution of the dose-ESR response curve after radiosterilization; the results are the mean of single determination on three samples (RSD < 2%). 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 0.5 ± 0.5 kGy and 1 ± 0.5 kGy, respectively. Since 25 kGy was established and accepted by many regulatory authorities (EN 552 and ANSI/AAMI/ISO 11137) discrimination from irradiated and unirradiated samples is possible just after irradiation.

In the absence of saturation, the number of free radicals in the sample 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 free radicals number (2×10^{17} radicals per g at 25 kGy) was estimated by comparison of the second integral from radiosterilized cefoperazone and DPPH standard. From this, the G-value (number of radicals per 100 eV) could be estimated to 0.3 ± 0.1.Five functions have been tried to fit the data:
   Linear regression (Equation 1) (function used in food irradiation);

Quadratic fit (Equation 2); the quadratic term was introduced as correction to take into account of the non-linear shape of the dosimetric curves;
Power function (Equation 3), exponential function (Equation 4) and double exponential function (Equation 5).

It should be noted that ESR signal were divided (by 10^3 for signal amplitude and by 10^{15} for double integration) and no attempt has been made to force the regression through zero. Using a test ($r^2 > 0.980$), only two functions were selected (Equation 3 and 5) and the numerical results of our fitting are given below:

Peak to peak amplitude	Double integration
ESR signal $= 1.509D^{0.329}$ ($r^2 = 0.985$)	ESR signal $= 0.763D^{0.437}$ ($r^2\ 0.994$)
ESR signal $= -3.749\exp(-0.1673D)$ $+3.805\exp(0.0067D)$ ($r^2 = 0.993$)	ESR signal $= -1.875\exp(-0.2702D)$ $+1.903\exp(0.0183D)$ ($r^2\ 0.997$)

To be useful, the models described must be capable of predicting the irradiation dose. In order to verify the utility of the equations obtained, we have calculated the interpolated doses. Briefly, the interpolated (back-calculated) doses were obtained by entering the measured response [ESR ratio] in the models described above. Fig. 3 shows the ratio (calculated dose/nominal dose) versus nominal dose.
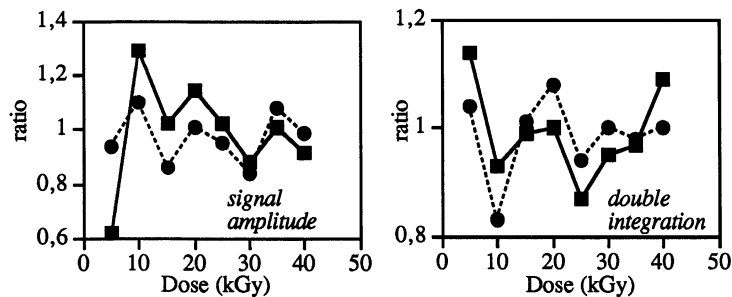
The following statements can be established:

Fig. 3. Ratio (Calculated/nominal dose) versus nominal dose. (■) Equation 3 (power function); (●) equation 5 (bi-exponential function).

The dose-response curves can be simulated by bi-exponential or power functions; however, for doses lower than 10 kGy, we could remarked on Fig. 3 the low accuracy between calculated and nominal doses using power function.

The linear function can't be used for simulation even for low doses. This remark agree with the results obtained previously by Miyazaki et al [11] for ceftazidime.

3.2. Decay of radicals upon storage

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. Fig. 4 plots the evolution of the percentage of free radicals versus storage (peak to peak amplitude). The results indicate that the decay cannot be explained by homogenous first-order or second-order kinetics, so numerical simulation were performed using a bi-exponential model [12]. The results are given below:
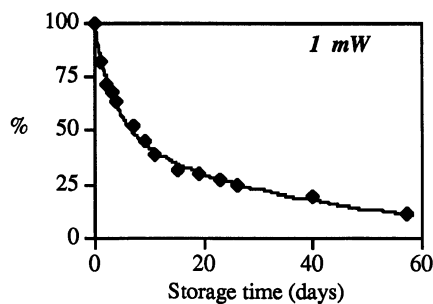

Fig. 4. Decay of radicals upon storage. Continuous line: simulated curves.

$$Bi - exponential function$$
$$\text{Free radicals (\%)} = 48.40 \exp(-0.2552t)$$
$$+ 48.56 \exp(-0.0251t)$$
$$\times (r^2 = 0.993)$$

where t was the time of storage in days.

The decay of free radicals could be divided in two phases:

Phase 1 corresponding to a fast recombination (coefficients 48.40 and 0.2552);

Phase 2 corresponding to a slower recombination (coefficients 48.56 and 0.0251).

Two interpretations could be considered:

The phase 1 could agree with a surface recombination and the phase 2 to a solid diffusion mechanism [7];

Phase 1 and phase 2 could correspond to different radicals with different decay kinetics [9].

After 26 days and 57 days of storage, the losses of free radicals were, respectively, 77 and 89%. The weak stability of the radicals allows the detection of radiosterilized (25 kGy) cefoperazone (S/N > 3) during 140 days.

4. Conclusion

The detection method based on ESR dosimetry seems promising. ESR could provide the proof of radiosterilization of cefoperazone; consequently ESR could allow verification of the commercial exchange (struggle against unauthorized and uncontrolled use of radiation process). ESR dosimetry requires only small samples (less than 50 mg), minimal time and effort for sample preparation; the measurement by ESR is non-destructive. Esti-

mation of the irradiation dose for cefoperazone is more problematic; it requires a knowledge of the irradiation date, a parameter not always accessible, especially in unauthorized and uncontrolled use of radiation process.

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