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ESR identification of radiosterilized pharmaceuticals: latamoxef and ceftriaxone

J.P. Basly [a,*], I. Longy [a], M. Bernard [b]

[a] *Laboratoire de Chimie Analytique et Bromatologie, UFR de Pharmacie, 2 rue du Docteur Marcland, 87025 Limoges Cedex, France*

[b] *Laboratoire de Biophysique Pharmaceutique, UFR de Pharmacie, 2 rue du Docteur Marcland, 87025 Limoges Cedex, France*

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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. The aim of this work was to develop equations to describe the ESR curves versus dose and storage time after gamma irradiation of latamoxef and ceftriaxone. Limit of detection and limit of discrimination are (0.5 kGy, 1.5 kGy) and (1.5 kGy, 5 kGy) for latamoxef and ceftriaxone respectively. Linear regression is, for latamoxef, applicable for doses lower than 20 kGy. Since the radiation dose selected must always be based upon the bioburden of the products and the degree of sterility required (ANSI/AAMI/ISO 11137), doses in the range 5–20 kGy could be investigated and linear regression would appear to be the least expensive route to follow. Bi-exponential function is of more general applicability to predict irradiation dose in latamoxef. The comportment of ceftriaxone is different. Due to the weak number of free radicals generated during the irradiation, only two models give correct adequacy between experimental and calculated results. Decay kinetics for radicals versus storage were considered. The free radicals decay could be simulated by exponential and bi-exponential functions for latamoxef and ceftriaxone respectively. The limits of detection of free radicals after irradiation at 25 kGy are 140 days for latamoxef and 115 days for ceftriaxone. © 1997 Elsevier Science B.V.

Keywords: Latamoxef; Ceftriaxone; ESR; Dosimetry; Decay of radicals

1. Introduction

Radiation sterilization technology and its applications in the manufacture of pharmaceuticals and cosmetics are being more actively investigated now than at any other time (Jacobs, 1995; Reid, 1995; Tilquin and Rollmann, 1996; Boess and

* Corresponding author. Tel.: + 33 05 55435898; fax: + 33 05 55435801; e-mail: Basly@alpha1.unilim.fr

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Bögl, 1996). The increased use of radiation processing for other industrial purposes (such as the sterilization of medical devices) has led to the development of more efficient and economical irradiation equipment and processes. With the advances made in aseptic processing, we now have products and materials which are much cleaner from a microbiological point of view and thus are likely to require much lower radiation doses to achieve 10^{-6} sterility assurance level (SAL). This change provides an opportunity to terminally sterilize, or at least enhance the SAL, of a much larger number and range of drugs.

While the regulations governing the use of radiation processing for pharmaceuticals may vary from country to country, all require that the use of the process be documented. With the publication of EN 552 and ANSI/AAMI/ISO 11137, there is at least a recognized standard for implementing this technology. From time to time, it may be necessary to determine if a particular drug has been irradiated and to what dose; this is the focus of our research (Basly and Bernard, 1997). Electron spin resonance (ESR) is one of the leading methods for identification of irradiated foodstuffs (Raffi and Kent, 1995) and recently has proven to be an accurate and reliable technique for dosimetry irradiation of pharmaceuticals (Gibella et al., 1993; Ciranni Signoretti et al., 1994; Miyazaki et al., 1994; Onori et al., 1996). ESR yields both qualitative information (i.e. whether or not a sample has been irradiated) and quantitative results (i.e. the dose it received).

The aim of this work was to develop, by mathematical procedures, equations to describe the ESR curves versus dose and storage time after gamma irradiation of two third generation cephalosporins: latamoxef and ceftriaxone. In fact, these products are potential candidates for radiation treatment due to their thermosensitivity.

2. Materials and methods

2.1. Irradiation

The drug substances were commercial products suitable for clinical use. Ceftriaxone and latamoxef were kindly supplied by Roche (Paris, France) and Ely Lilly (Saint Cloud, France) respectively. These samples were supplied in vials of 1 g sterile powder for injection. Cephalosporins were irradiated with gamma rays [^{60}Co] emitted by an IBL 460 (UFR de Pharmacie, Limoges, France); the dose rate was preliminary calibrated using Fricke dosimetry (ferrosulphate dosimetry). An unirradiated sample was kept as reference.

2.2. Instrumentation

ESR spectra were recorded at room temperature using a Bruker ESP 300E spectrometer equipped with a variable temperature control apparatus, a data acquisition system and following

Table 1
ESR parameters, limit of detection and limit of quantification

ESR parameters	
Sweep field (mT)	341.5–348.5
Microwave frequency (GHz)	9.66
Microwave power (mW)	10
Modulation frequency (kHz)	100
Modulation amplitude (mT)	0.2
Time constant (ms)	163.84
Sweep time (min)	0.68
Amplification factor	
Latamoxef	500
Ceftriaxone	10 000
Peak to peak amplitude determination (mT)	
Latamoxef	345.4–345.8
Ceftriaxone	344.2–345.1
Limit of detection	
Latamoxef	0.5 ± 0.5 kGy
Ceftriaxone	1.5 ± 0.5 kGy
Limit of quantification	
Latamoxef	1.5 ± 0.5 kGy
Ceftriaxone	5.0 ± 0.5 kGy

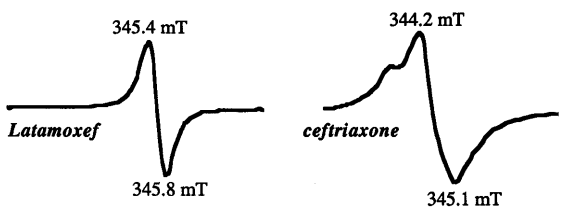

Fig. 1. ESR spectra.

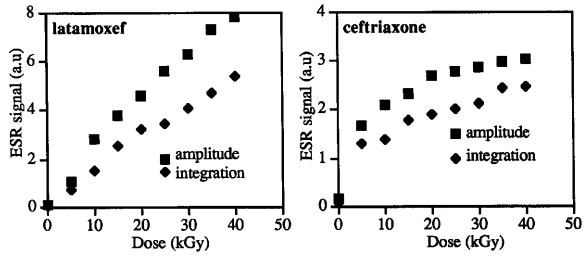

Fig. 2. Dose-ESR response curves.

the parameters described in Table 1. A Bruker strong pitch was used as ESR standard to calibrate the ESP 300E spectrometer before each series of measure.

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 peak to peak amplitude and the second integral of the ESR spectra; the second integral is proportional to the spin concentration (Yordanov and Ivanova, 1994).

2.3. Multivariable regression

Calculations were performed using WINREG software on a Pentium 75 MHz.

3. Results and discussion

ESR powder spectra of latamoxef and ceftriaxone after gamma irradiation are presented in Fig. 1, no paramagnetic centers were detected in unirradiated samples.

3.1. Dosimetry

Fig. 2 shows the 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 limit of detection (LOD), predicted by the $S/N = 3$ criterion and the limit of quantification (LOQ), predicted by the $S/N = 10$ criterion have been determined and are summarized in Table 1. 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.

Five functions have been tried to fit the data:

– Linear regression (equation 1), (function currently 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 described by Poisson statistics (equation 4) and double exponential function (equation 5).

The functions used in numerical simulations are given in Table 2.

It should be noted that background signals (unirradiated sample) were substracted and no

Table 2
Functions used in numerical simulations[a]

Latamoxef
Peak to peak amplitude
ESR signal $= 0.0544 + 0.2329$ D ($r^2 = 0.9805$) 0–20 kGy
ESR signal $= -0.0624 + 0.2736$ D $- 0.0019$ D^2 ($r^2 = 0.9935$)
ESR signal $= 0.3895$ D$^{0.8186}$ ($r^2 = 0.9909$)
ESR signal $= 15.0750$ [1-exp $(-0.0183$ D)] ($r^2 = 0.9932$)
ESR signal $= -5.0422$ exp $(-0.0728$ D$) + 4.3123$ exp $(0.0161$ D$)$ ($r^2 = 0.9911$)
Integration
ESR signal $= -0.062 + 0.1635$ D ($r^2 = 0.9963$) 0–20 kGy
ESR signal $= -0.0260 + 0.1680$ D $- 0.0009$ D^2 ($r^2 = 0.9928$)
ESR signal $= 0.2192$ D$^{0.8661}$ ($r^2 = 0.9920$)
ESR signal $= 13.1747$ [1-exp $(-0.0128$ D)] ($r^2 = 0.9929$)
ESR signal $= -3.4226$ exp $(-0.0415$ D$) + 3.3502$ exp $(0.0144$ D) ($r^2 = 0.9933$)
Ceftriaxone
Peak to peak amplitude
ESR signal $= 0.9317$ D$^{0.3100}$ ($r^2 = 0.9948$)
ESR signal $= -2.0532$ exp $(-0.1983$ D$) + 2.0678$ exp $(0.0084$ D$)$ ($r^2 = 0.9945$)
Integration
ESR signal $= 0.5994$ D$^{0.3696}$ ($r^2 = 0.9864$)
ESR signal $= -1.2485$ exp $(-0.3592$ D$) + 1.2526$ exp $(0.0167$ D$)$ ($r^2 = 0.9869$)

[a] Test of selection of the functions: $r^2 > 0.98$.

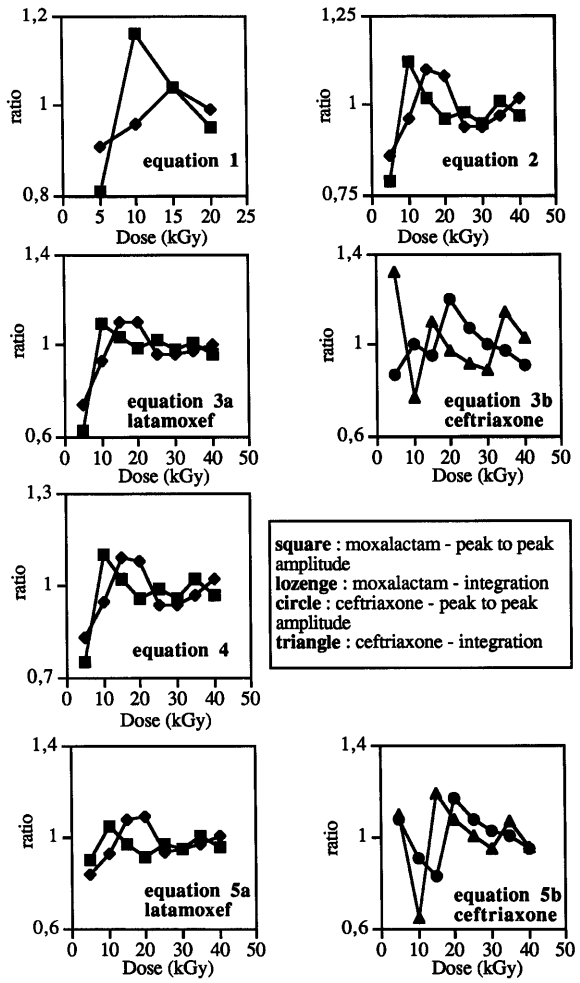

Fig. 3. Ratios vs. irradiation dose.

attempt has been made to force the regression through zero.

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 and regression statistics were applied. Fig. 3 shows the ratio (calculated dose/nominal dose) versus nominal dose. The following statements can be established:

(a) for latamoxef, equation 1 (linear regression) is applicable for doses lower than 20 kGy. Since the radiation dose selected must always be based upon the bioburden of the products and the degree of sterility required (EN 552 and ANSI/AAMI/ISO 11137), 25 kGy could no longer be accepted as a 'routine' dose for sterilizing a pharmaceutical. Doses in the range 5–20 kGy could be investigated and linear regression would appear to be the least expensive route to follow notwithstanding the low accuracy of measurements for low doses. Equation 2 (quadratic fit), equation 3 (power function), equation 4 (exponential function) and equation 5 (bi-exponential function) are of more general applicability to predict irradiation dose for latamoxef. However, the best results, especially for low doses, were obtained with the bi-exponential model (equation 5).

(b) the comportment of ceftriaxone is different. Due to the weak number of free radicals generated during the irradiation, only two models (equation 3 and equation 5) give correct adequacy ($r^2 > 0.98$) between experimental and calculated results.

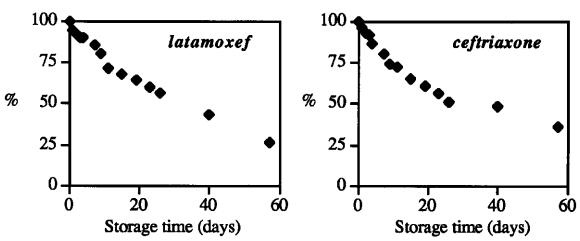

Fig. 4. Decay of radicals upon storage.

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 (68 days) was performed. Fig. 4 plots the evolution of the percentage of free radicals versus storage.

The free radicals decay could be simulated by exponential and bi-exponential functions for latamoxef and ceftriaxone, respectively.

latamoxef free radicals (%) $= 96.76 \exp(-0.0215\ t)$ $r^2 = 0.9902$

ceftriaxone free radicals $(\%) = 32.31 \exp(-0.1014\ t) + 67.88 \exp(-0.0104\ t)$ $r^2 = 0.9938$

where t was the storage time in days.

After 26 and 57 days of storage, the losses of free radicals were respectively (43.3%, 73.3%) for latamoxef and (48.8%, 64%) for ceftriaxone. In the commercial market of drugs, radicals should be detected up to two years after irradiation (Miyazaki et al., 1994); the limits of detection of free radicals ($3 \times$ unirradiated sample signal) after irradiation at 25 kGy and storage at ambient temperature are 140 days for latamoxef and 115 days for ceftriaxone.

4. Conclusion

It is worth noting that, at present, ESR is the only technique which proved to be suitable for identification and quantification purposes in irradiated pharmaceuticals. Moreover, other features such as sensitivity, precision, ease and non-destructive readout make ESR superior to other proposed analytical techniques.

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