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Influence of radiation treatment on dobutamine

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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 dobutamine hydrochloride. Limit of detection and limit of discrimination are, respectively, 0.5 and 1.5 kGy for dobutamine hydrochloride. Linear regression is applicable for doses lower than 20 kGy. Estimation of the number of free radicals by comparison of the second integral from radiosterilized dobutamine and DPPH standard on the linear part of the curve gives $6 \pm 2\ 10^{15}$ spin/g/kGy. From this result, the G value (number of radicals/100 eV) could be estimated to 0.1 ± 0.04. Decay kinetics for radicals versus storage were considered. Nonhomogeneous kinetics with time-dependent rate appeared valid to reproduce the experimental data. Discrimination between irradiated and unirradiated dobutamine is possible after a storage longer than 2 years. The comparison of the chromatographic profiles of irradiated and unirradiated samples showed minor differences.

Keywords: Dobutamine; ESR; Dosimetry; Decay of radicals; Nonhomogeneous kinetics

1. Introduction

The sterilization of thermolabile medical devices, such as catheters or syringes, with ionizing radiation is successfully practised in many countries. Furthermore, it is possible to sterilize pharmaceutically active substances with ionizing radiation (Jacobs, 1995; Reid, 1995; Boess and Bögl, 1996; Onori et al., 1996). The advantages of sterilization by irradiation include high penetrating power, low chemical reactivity, low measurable residues, small temperature rise and the fact that there are fewer variables to control. Thus, the sterilization can be carried out on finally packaged products.

While the regulations governing the use of radiation processing for pharmaceuticals may vary

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from country to country, all require that the use of the process be documented. With the publication of EN 552 and 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 and recently has proven to be an accurate and reliable technique for dosimetry irradiation of pharmaceuticals (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 dobutamine hydrochloride. We must keep in mind that ESR dosimetry of pharmaceuticals can only be a control a posteriori. Irradiation doses are firstly confirmed by chemical or physical dosimeters in industrial irradiation equipment.

2. Materials and methods

2.1. Irradiation

Samples of dobutamine hydrochloride (a generous gift from Eli Lilly) 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 following the next parameters: sweep field, 336–354 mT; microwave frequency, 9.65 GHz; microwave power, 10 mW; modulation frequency, 100 kHz; modulation amplitude, 0.2 mT; time constant, 164 ms; sweep time, 0.68 min; amplification factor, 2500; peak to peak amplitude determination, 344.1 and 346.1 mT. 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 weighed 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; DPPH was used as reference.

2.3. Multivariable regression

Calculations were performed using WINREG software (Debord, J., Department of Pharmacocinetics, CHRU Dupuytren, Limoges, France, private communication) on a Pentium 75 MHz.

3. Results and discussion

The ESR powder spectrum of dobutamine hydrochloride after gamma irradiation is presented in Fig. 1; the shape of the signal did not depend on dose. No paramagnetic centers were detected in unirradiated samples; the ESR signal recorded is specific for the radiation treatment.

3.1. Dosimetry

Fig. 2 shows a plot of the evolution of the dose–ESR response curves after radiosterilization; the results are the mean of three replicates (R.S.D. $< 1\%$). 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 to be 0.5 ± 0.5 and 1.5 ± 0.5 kGy, respectively. Since 25 kGy was established and accepted by many regulatory authorities (EN 552 and ISO 11137), discrimination from irradiated and unirradiated samples is possible just after irradiation.

Five functions have been tried to fit the data:

Linear regression (Eq. (1); function currently used in food irradiation) for doses between 0 and 20 kGy;

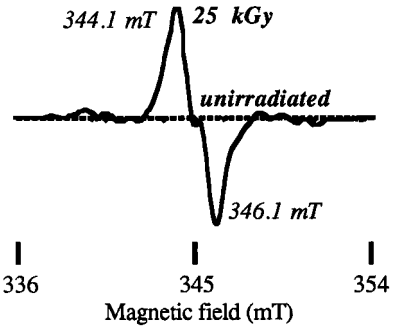

Fig. 1. ESR spectrum.

quadratic fit (Eq. (2); the quadratic term was introduced as a correction to take into account the non-linear shape of the dosimetric curves);

power function (Eq. (3); exponential function (Eq. (4)) and double exponential function (Eq. (5)). The functions used in numerical simulations are given in Table 1.

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

Table 1
Functions used in numerical simulations

Peak to peak amplitude
ESR signal $= 0.6272 + 0.3672D$ ($r^2 = 0.969$)
ESR signal $= 0.6692 + 0.4148D - 0.0045D^2$ ($r^2 = 0.984$)
ESR signal $= 0.16104D^{0.4902}$ ($r^2 = 0.989$)
ESR signal $= 10.9173\ [1 - \exp(-0.0563D)]$ ($r^2 = 0.996$)
ESR signal $= -7.8152 \exp(-0.0809D) + 7.8381 \exp(0.062D)$ ($r^2 = 0.998$)
Second integration
ESR signal $= 0.5412 + 0.2152D$ ($r^2 = 0.932$)
ESR signal $= 0.4650 + 0.2602D - 0.0029D^2$ ($r^2 = 0.982$)
ESR signal $= 0.9438D^{0.5146}$ ($r^2 = 0.995$)
ESR signal $= 6.6349\ [1 - \exp(-0.0599D)]$ ($r^2 = 0.990$)
ESR signal $= -3.6188 \exp(-0.1257D) + 3.6330 \exp(0.0137D)$ ($r^2 = 0.996$)

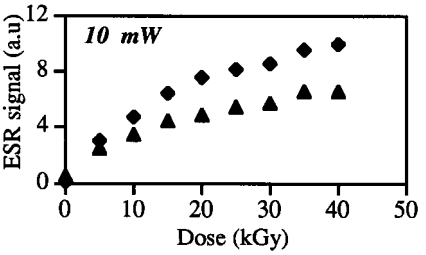

Fig. 2. ESR signal/dose curves.

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 and regression statistics are given below.

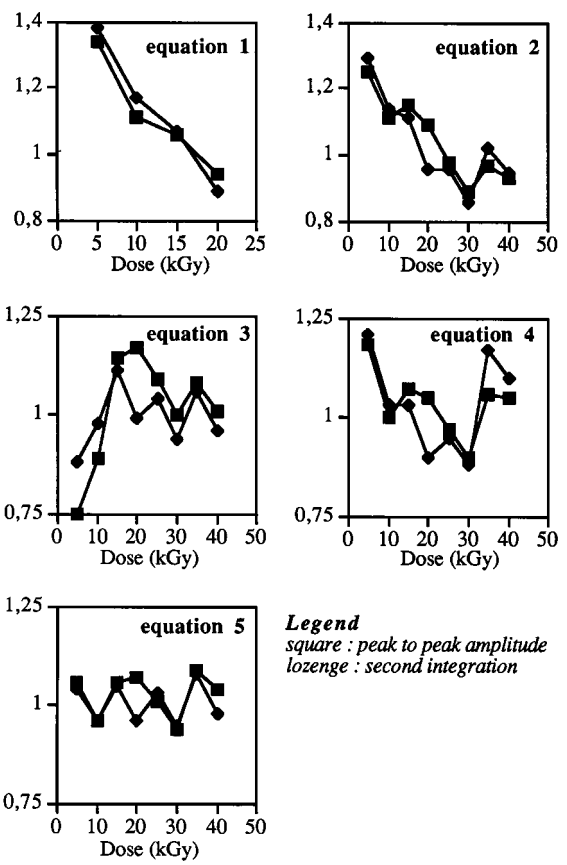

Fig. 3. Ratios (calculated dose/nominal dose) versus nominal dose.

	Peak to peak amplitude	Second integral
Eq. (1)	$D_{cal} = 2.80$	$D_{cal} = 3.77$
	+	+
	$0.829 D_{nom}$	$0.749 D_{nom}$
	$r^2 = 0.990$, $F = 203$	$r^2 = 0.965$, $F = 56$
Eq. (2)	$D_{cal} = 2.90$	$D_{cal} = 2.10$
	+	+
	$0.864 D_{nom}$	$0.892 D_{nom}$
	$r^2 = 0.986$, $F = 430$	$r^2 = 0.979$, $F = 290$
Eq. (3)	$D_{cal} = -0.17$	$D_{cal} = 0.28$
	+	+
	$1.057 D_{nom}$	$0.988 D_{nom}$
	$r^2 = 0.983$, $F = 361$	$r^2 = 0.986$, $F = 429$
Eq. (4)	$D_{cal} = 0.21$	$D_{cal} = -1.39$
	+	+
	$1.008 D_{nom}$	$1.089 D_{nom}$
	$r^2 = 0.983$, $F = 342$	$r^2 = 0.953$, $F = 122$
Eq. (5)	$D_{cal} = -0.26$	$D_{cal} = -0.06$
	+	+
	$1.041 D_{nom}$	$1.006 D_{nom}$
	$r^2 = 0.988$, $F = 513$	$r^2 = 0.988$, $F = 486$

The following statements can be established:

Since the radiation dose selected must always be based upon the bioburden of the products and the degree of sterility required, 25 kGy can 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. The best results, for peak to peak amplitude or second integral, are obtained with Eq. (5) (bi-exponential function); intercepts and slopes are close to zero and unity, respectively.

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 first derivative curve is necessary. Estimation of the number of free radicals by comparison of the second integral from radiosterilized dobutamine and DPPH standard on the linear part of the curve gives $6 \pm 2 \times 10^{15}$ spin/g/kGy. From this result, the G value (number of radicals/100 eV) could be estimated to 0.1 ± 0.04.

3.2. Decay of radicals upon storage

Tests were carried out to investigate whether storage has an effect on the free radical 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. Classical homogenous kinetics (first-order reaction and second-order reaction) fail to reproduce the experimental data. For a quantitative description of the decay we have chosen the nonhomogeneous kinetics with a time-dependent rate constant, that has been successfully applied to many systems with

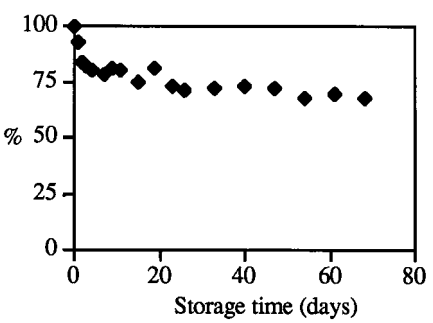

Fig. 4. Decay of radicals upon storage.

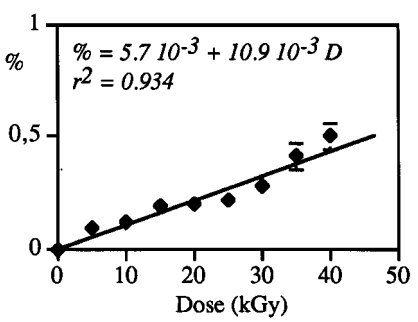

Fig. 5. Degradation (%) versus irradiation dose.

reactivity distribution (Plonka, 1991). The relation for this model is:

$$[\text{free radicals (\%)}] = \frac{100}{1 + (Bt^{\alpha}/\alpha)}$$

The parameter α is interpreted as a measure of non-homogeneity of reactivity in the system. The lower α, the more the reaction deviates from homogeneous kinetics. This model, applied to the data plotted in Fig. 4 gives the following results:

$$[\text{free radicals (\%)}] = \frac{100}{1 + 0.1412t^{0.2834}} \; r^2 = 0.959$$

where t is the storage time in days.

After 33 and 68 days of storage, the losses of free radicals were, respectively, 27.1 and 31.4%. In the commercial drug market, radicals should be detected up to 2 years after irradiation (Miyazaki et al., 1994); the limit of detection of free radicals ($3 \times$ unirradiated sample signal) after irradiation at 25 kGy is longer than 2 years.

3.3. Impurities profile

The impurity profiles were recorded using ion pair chromatography (IPC). The amount of impurities was determined at 280 nm, assuming that the relative molar response factor (RRF) for an impurity was equal to 1. The comparison between chromatographic profiles of irradiated and unirradiated samples evidenced minor differences. The pre-existent impurities and the radiolytic degradation did not show a significant increase with dose. The data (mean of single determination on three samples), are plotted in Fig. 5, which also shows the smooth linear function which modelled this curve.

4. Conclusion

The results obtained so far suggest that ESR spectroscopy is a suitable technique for identification and dosimetric purposes in radiosterilized dobutamine.

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