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Influence of radiation treatment on two antibacterial agents and four antiprotozoal agents: ESR dosimetry

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Abstract

Radiation sterilization is becoming increasingly popular for the sterilization of many pharmaceutical products. We have investigated the gamma radiation-induced effects on two antibacterial agents and four antiprotozoal agents by ESR spectroscopy. Numerical simulation of the evolution of the ESR signal versus dose was performed using linear regression, quadratic fit, power function and Hill function. Estimation of the irradiation dose is possible using Hill function or power function; the use of linear regression may be technically feasible for doses ranging from 5–30 kGy. For a dose of 25 kGy (dose usually recommended for radiosterilization), discrimination from irradiated and unirradiated samples is possible. Tests were carried out to investigate whether storage has an effect on the free radicals concentration. The decay could be simulated using a bi-exponential regression. © 1997 Elsevier Science B.V.

Keywords: Antibacterial agents; Antiprotozoal agents; Radiation treatment; ESR spectroscopy; Dosimetry; Storage

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 radiations (Jacobs, 1995; Reid, 1995; Tilquin and Rollmann, 1996; Boess and Böegl, 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.

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The regulations governing radiosterilization vary from one country to another. In the international market of the future, there will be a number of drugs that will be irradiated by gamma rays. Thus, it is desirable to establish a method of discrimination between irradiated and unirradiated drugs and to evaluate the dose of irradiation. Recently, Electron spin resonance (ESR) has proven to be an efficient technique for radiosterilisation dosimetry (Gibella et al., 1993; Ciranni Signoretti et al., 1993, 1994; Miyazaki et al., 1994a,b). ESR spectroscopy appears to be very suitable for the determination of free radicals concentration in complex media. ESR measurements can also be used to detect and distinguish irradiated drugs from unirradiated ones.

Following previous studies (Basly et al., 1996) the aim of the research reported here was to apply the ESR spectroscopy to the irradiation dosimetry of six drugs: two antibacterial agents (chloramphenicol [1], furaltadone [2]) and four antiprotozoal agents (furazolidone [3], secnidazole [4], tinidazole [5], metronidazole [6]).

Secnidazole and tinidazole were, respectively, supplied by Rhone Poulenc Rorer (Vitry Alforville, France) and Pfizer (Orsay, France). Furazolidone, metronidazole, furaltadone and chloramphenicol were purchased from Coopérative Pharmaceutique Française (Melun, France).

Samples (30 mg) were irradiated with gamma rays emitted by an IBL 460 (^{60}Co); the dose rate was 1.6 kGy/h. 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 using the parameters previously described (Basly et al., 1996). Numerical simulation of the results was performed using Mathematica 2.2 software (Wolfram Research). For ESR measurements, 10 mg of substance was weighted with an accuracy of 0.2 mg.

The key elements in establishing an ESR dosimetric method are:

the radicals are quite stable with regard to the maximum time of storage;

the relative signals are clearly distinguishable from the ones of the reference samples;

the signal is strictly constant if we also require an estimation of the initial dose.

Fig. 1 shows plot of the evolution of the ESR signal versus dose. This evolution was followed by monitoring the maximum height (peak to peak) of the spectra.

An important step in the development of irradiation dosimetry of pharmaceuticals has been the choice of functions to fit the data. Four functions have been tried: linear regression, quadratic fit, power function and Hill function (Table 1).

It should be noted that no attempt has been made to force the regression through zero.

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:

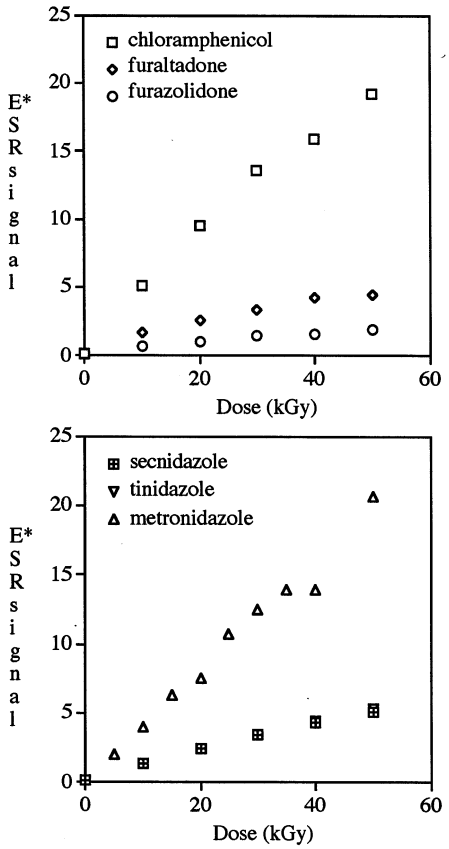

Fig. 1. Free radicals evolution with dose.

Table 1
General equations and coefficients of numerical simulations

	Coefficients					
	[1]	[2]	[3]	[4]	[5]	[6]
a	172.7	353.6	137.0	116.9	168.1	146.2
b	461.4	108.8	45.25	109.9	112.9	405.3
c	32.14	179.6	98.71	−6.392	160.9	33.20
d	537.3	153.7	57.91	136.8	125.2	465.2
e	3.223	1.391	0.454	0.756	0.504	2.420
f	906.6	432.8	144.9	218.1	205.1	601.9
g	0.782	0.600	0.658	0.806	0.831	0.874
h	48076	12427	6553	16430	19646	65119
i	74.60	100.9	150.4	111.9	137.4	102.3
n	1.061	0.807	0.822	1.010	0.9978	1.194

General equations:
1. Linear regression: ESR signal $= a+bD$.
2. Quadratic fit: ESR signal $= c+dD-eD^2$.
3. Power function: ESR signal $= fD^g$.
4. Hill function: ESR signal $= (h)/[1+(i/D)^n]$.

[1]–[6] are chloramphenicol, furaltadone, furazolidone, secnidazole, tinidazole, and metronidazole, respectively.

[1]: 0.5, 2 kGy; [2]: 2, 5 kGy; [3]: 4, 10 kGy; [4]: 2, 8 kGy; [5]: 2, 7 kGy; [6]: 0.5, 1.5 kGy. Since the dose usually recommended for radiosterilization is 25 kGy, discrimination from irradiated and unirradiated samples seems possible.

To be useful, the models described in Table 1 must be able to predict the irradiation dose especially for dose of 25 kGy. In order to verify the utility of the equation obtained, we have calculated the interpolated doses (Fig. 2). Briefly, the interpolated (back-calculated) doses were obtained by entering the measured response [ESR signal] in the models described above.

From this back-calculated doses, we have calculated the mean errors between back-calculated and nominal doses using the equation:

$$r^2 = \sum_{5\ \mathrm{kGy}}^{40\ \mathrm{kGy}} \frac{(\text{interpolated dose} - \text{nominal dose})^2}{(\text{nominaldose})^2}$$

The best fit between interpolated and nominal doses were obtained with:

Hill function for chloramphenicol ($r = 0.028$);
Power function or Hill function for furazolidone ($r = 0.060$);
Hill function for furaltadone ($r = 0.061$);
Hill function for secnidazole ($r = 0.013$);
Power function for tinidazole ($r = 0.015$);
Linear function for metronidazole ($r = 0.072$).

Since the linear regression is currently used in food irradiation, we have assumed that the shape of the dosimetric curves is linear in the range 5–30 kGy. The uncertainty on the irradiation doses are 3% for chloramphenicol, 10% for furazolidone, 6% for furaltadone, 6% for secnidazole,

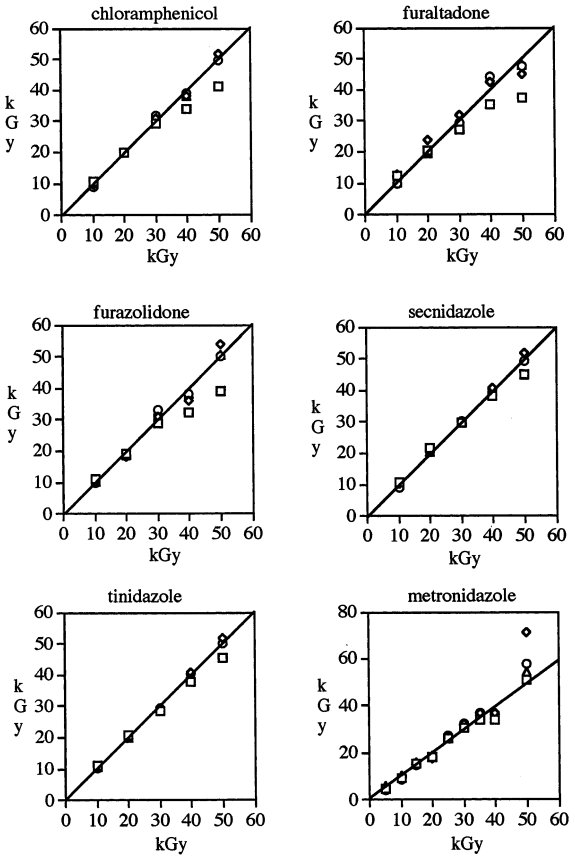

Fig. 2. Interpolated (back-calculated) dose versus nominal dose.

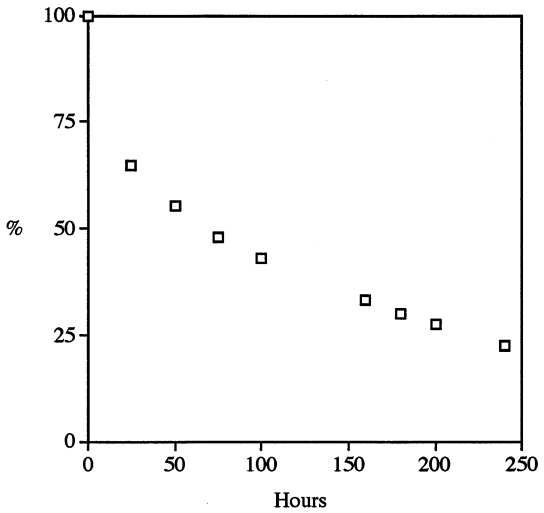

Fig. 3. Decay of radicals upon storage.

5% for tinidazole and 5% for metronidazole. From this results, the use of linear regression may be technically feasible for doses ranging from 5–30 kGy.

This four models were applied to results previously obtained on ceftazidime (Miyazaki et al., 1994a), ampicillin (Miyazaki et al., 1994b) and cephradine (Ciranni Signoretti et al., 1993). The best fit were obtained with Hill function.

Tests were carried out to investigate whether storage has an effect on the free radicals concentration. Chloramphenicol, for which radiosterilization has been approved by Health Authorities in the United Kingdom, Norway and India (Jacobs, 1995) was chosen. Storage at ambient temperature in a sealed quartz tube over several days was performed. Fig. 3 plots the evolution of the percentage of free radicals versus storage; this decay could be simulated using a bi-exponential regression:

$$\text{Free radicals}(\%) = 68.15 \exp(-0.0046t) + 31.85 \exp(-0.0893t)$$

where t was the storage time in hours.

This decay can be divided in two phases:

A fast exponential decay (coefficients 31.85 and 0.0893);

A 'quasi-linear' decay (coefficients 68.15 and 0.0046).

After 30 h (1.25 day) of storage, the exponential component became negligible and the decay appeared linear; during this time, 38% of free radicals disappeared. This decay was very fast compared to those obtained for metronidazole (53% of free radicals staying after 30 days of storage), ornidazole (30% of free radicals staying after 30 days of storage), ceftazidime (55% of free radicals staying after 30 days of storage) and ampicillin (75% of free radicals staying after 30 days of storage)

In conclusion, this preliminary work shows the interest of the ESR spectroscopy in radiosterilization dosimetry. Estimation of the irradiation dose could be possible if:

(1) Tests are carried out to investigate whether storage has an effect on the free radicals concentration;

(2) The free radicals dependence on dose is measured.

Estimation of the irradiation dose is possible using Hill function or power function; the use of linear regression may be technically feasible for doses ranging from 5–30 kGy.

This study doesn't prejudge of the technical feasibility of the gamma radiosterilization; to prove the safety of radiosterilization, it is important to determine the radiolytic products and to elucidate the mechanism of radiolysis.

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