ESR Dosimetry of Irradiated Ascorbic Acid

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Purpose. 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 ascorbic acid. Several ESR data sets previously acquired in this laboratory were adopted to check the performance of the models.

Results. Limit of detection and limit of discrimination are respectively 0.5 kGy and 2 kGy for ascorbic acid. Linear regression is applicable for doses lower than 25 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 5–25 kGy could be investigated and linear regression would appear to be the least expensive route to follow and gives good results. Quadratic fit, power function, exponential function and bi-exponential functions are of more general applicability to predict irradiation dose. Decay kinetics for radicals versus storage were considered. Nonhomogeneous kinetics with time-dependent rate (diffusion-controlled second-order reaction) and bi-exponential function appeared valid to reproduce the experimental data. Discrimination between irradiated and unirradiated ascorbic acid is possible after a storage of 800 days.

Conclusions. It is worth noting that, at present, ESR is the only technique which proves 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.

KEY WORDS: radiosterilization; ascorbic acid; ESR; dosimetry; decay of radicals.

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 (1–4). 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 from country to country, all require that the use of the process be documented. With the publication of EN 552 (5) and ANSI/AAMI/ISO 11137 (6), 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. Electron Spin Resonance (ESR) is one of the leading methods for identification of irradiated foodstuffs (7) and recently has proven to be an accurate and reliable technique for dosimetry analysis of pharmaceuticals (8–13). 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 crystalline ascorbic acid. Dose-ESR response curves and decay versus storage time is highly dependent on the environment but as described by GOPAL (14), radiation sterilization is mainly applied to injectables, ophthalmic ointments, and raw materials. It's why our research is actually focused on raw materials. Several ESR data sets previously acquired in this laboratory were adopted to check the performance of the models.

The influence of irradiation on the impurity profile by HPLC was not mentioned because other authors have previously studied this part (15).

MATERIALS AND METHODS

Irradiation

Samples of crystalline ascorbic acid from Coopérative Pharmaceutique Française (Melun, France) were irradiated with gamma rays (^{60}Co) emitted by an IBL 460 (UFR de Pharmacie, Limoges, France); the dose rate was preliminarily calibrated using Fricke dosimetry (ferrosulphate dosimetry). An unirradiated sample was kept as a reference.

Apparatus and Procedures

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 the parameters described in Table I. A BRUKER strong pitch was used as ESR standard.

For the measurements, 15 mg of substance was weighed with an accuracy of 0.2 mg. The evolution of the ESR signal in the dose response curves was followed by calculating:

Table I. ESR Parameters

ESR parameters	
Sweep field (mT):	340–350
Microwave frequency (GHz):	9.65
Microwave power (mW):	0.4
Modulation frequency (kHz):	100
Modulation amplitude (mT):	0.2
Time constant (ms):	163.84
Sweep time (min):	2.1
Amplification factor:	3200
Peak to peak amplitude determination (mT):	345.3–345.7
Limit of detection	*Limit of quantification*
0.5 ± 0.5 kGy	2.0 ± 0.5 kGy

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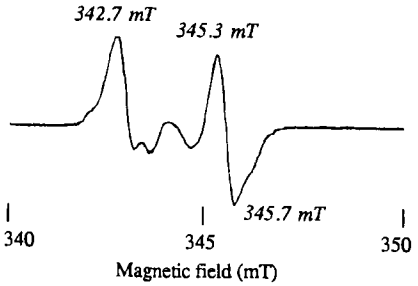

Fig. 1. ESR spectrum (25 kGy).

—the ratio (sample versus strong pitch) of the peak to peak amplitude;
—the ratio (sample versus strong pitch) of the second integral of the ESR spectra; the second integral is proportional to the spin concentration (16).

Multivariable Regression

Calculations were performed using Mathematica 2.2 (Wolfram Research Inc.) and EXCEL 4.0 (Microsoft) on a Macintosh LC III.

RESULTS AND DISCUSSION

ESR powder spectrum of ascorbic acid after gamma irradiation is presented in Figure 1; no paramagnetic centers were detected in unirradiated samples.

Dosimetry

Figure 2 shows plot of the evolution of the dose—ESR response curve after radiosterilization; the results are the mean of single determination on five samples (RSD < 3.9%). The limit of detection (LOD), predicted by the S/N = 3 criterion and the limit of quantification (LOQ) and predicted by the S/N = 10 criterion have been determined and are summarized in Table I. Since 25 kGy was established and accepted by many regulatory authorities (5,6) discrimination from irradiated and unirradiated samples (crystalline ascorbic acid) is possible just after irradiation.

Five functions have been tried to fit the data:

—linear regression (function currently used in food irradiation);
—quadratic fit; the quadratic term was introduced as correction to take into account the non-linear shape of the dosimetric curves.
—power function;
—exponential (17) described by Poisson statistics and double exponential function.

The functions used in numerical simulations are given below:

peak to peak amplitude	***second integration***
equation 1 (0 − 25 kGy)	
ESR ratio = 0.1619 + 0.0502D (r^2 = 0.9494)	ESR ratio = 0.4205 + 0.0874D (r^2 = 0.9052)
equation 2 (0 − 35 kGy)	
ESR ratio = 0.0719 + 0.0753D $-0.00098D^2$ (r^2 = 0.9903)	ESR ratio = 0.2458 + 0.1303D $-0.0017D^2$ (r^2 = 0.9717)
equation 3 (0 − 40 kGy)	
ESR ratio = $0.2368D^{0.5230}$ (r^2 = 0.9929)	ESR ratio = $0.5243D^{0.4660}$ (r^2 = 0.9950)
equation 4 (0 − 40 kGy)	
ESR ratio = 1.7259[1-exp (−0.0587D)] (r^2 = 0.9965)	ESR ratio = 3.0047[1-exp(−0.0675D)] (r^2 = 0.9767)
equation 5 (0 − 40 kGy)	
ESR ratio = −1.3290 exp (−0.0729D) +1.3475 exp (0.0051D) (r^2 = 0.9955)	ESR ratio = −1.5933 exp (−0.1602D) +1.6264 exp (0.0149D) (r^2 = 0.9842)

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

Validation of the Models

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 (Figure 3 and 4). 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.

The following statements can be established:

—equation 1 (linear regression) is applicable for doses lower than 25 kGy. Since the radiation dose selected must always be based upon the bioburden of the products

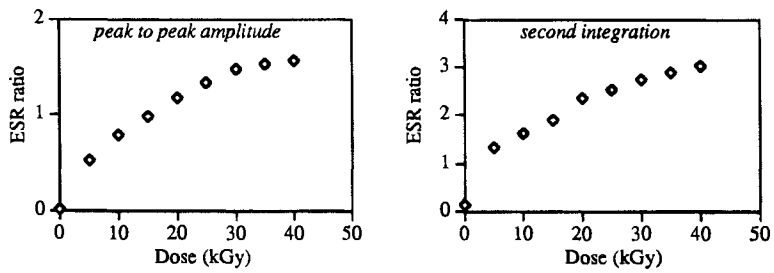

Fig. 2. Dose—ESR responses curves.

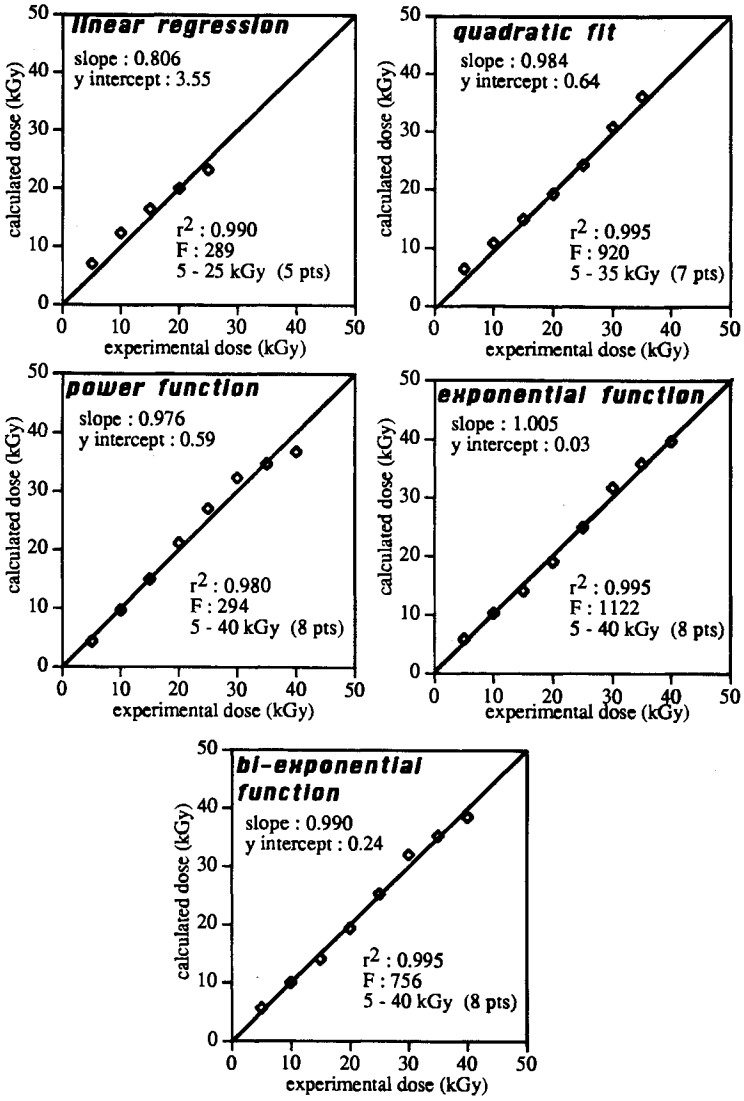

Fig. 3. Calculated dose versus experimental dose—peak to peak amplitude.

and the Degree of Sterility required (ANSI/AAMI/ISO 11137), 25 kGy could no longer be accepted as a "routine" dose for sterilizing a pharmaceutical. Doses in the range 5–25 kGy could be investigated and linear regression would appeared the least expensive route to follow and gives good results;

—equation 2 (quadratic fit) is of more general applicability to predict irradiation dose than equation 1 but intercepts and slopes of the straight lines are generally not close to zero and unity, respectively, which is a good indication of the validity of the models.

—The best results, for peak to peak amplitude or second integral, are obtained with equation 3 (power function) and equation 5 (bi-exponential function); intercepts and slopes are close to zero and unity, respectively.

Comparison with Other Data Sets

Several ESR data sets previously acquired in this laboratory (18–25) on sympathomimetics (formoterol, dopamine, norepinephrine, theodrenaline, fenoterol, orciprenaline, ritodrine, terbutaline, albuterol, isoprenaline) and antibacterial agents (metronidazole, secnidazole, tinidazole, chloramphenicol, furaltadone, furazolidone) were adopted to check the performance of the models described above (Figure 5). All the experimental points in the above data sets were used for building the models.

The following statements can be established:

—equation 1 (linear regression) is applicable for doses lower than 25 kGy. Linear regression appeared the least expensive route to follow and gives good results.

—As described above for ascorbic acid is of more general applicability to predict irradiation dose than equation 1 and the best results are obtained with equation 3 and equation 5.

Nevertheless, some questions still exist:

—for doses ranging from 0 to 10 kGy, considerable errors on the calculated dose could appear due to the scattering of the points (Fig. 5);

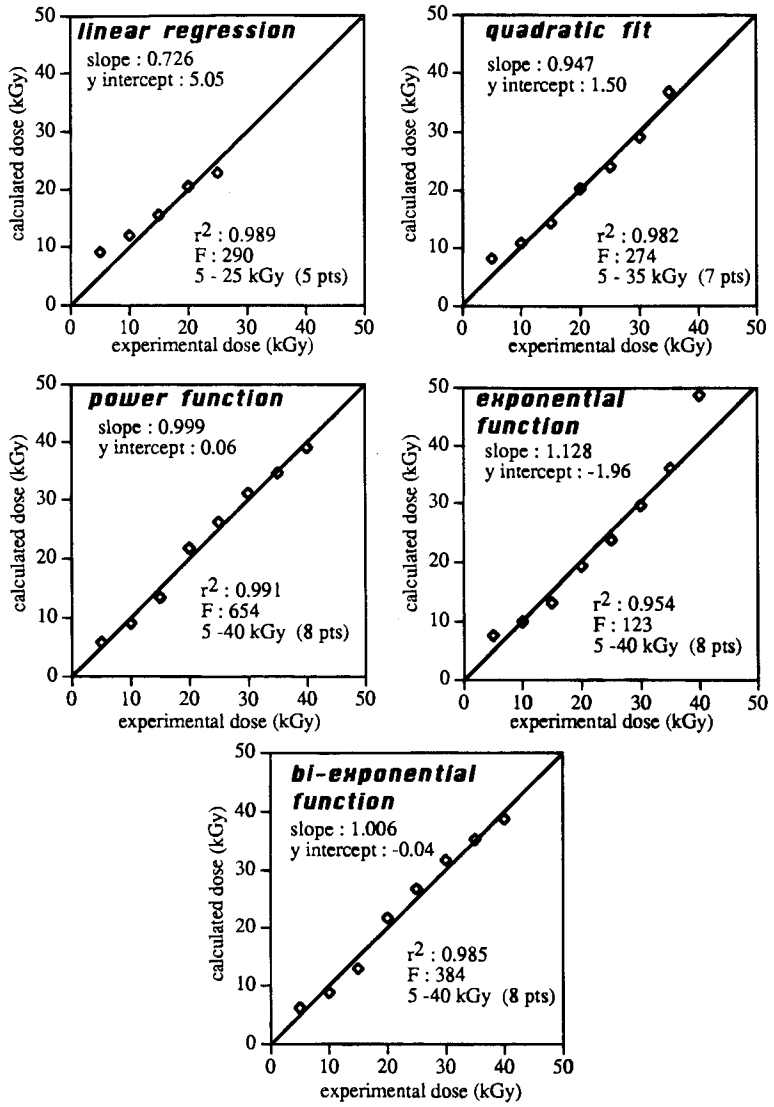

Fig. 4. Calculated dose versus experimental dose—integration.

—estimation of the irradiation dose using post-irradiation and second integration curves require, firstly, integration of the background.

Decay of Radicals Upon Storage—Ascorbic Acid

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 (63 days) was performed. Figure 6 plots the evolution of the percentage of free radicals versus storage. For a quantitative description of the decay, four possible decay kinetics for mobile radicals were considered; first-order reaction, second-order reaction, bi-exponential function and nonhomogenous kinetics with time-dependent rate constant (17).

Classical homogenous kinetics (first-order reaction and second-order reaction) fail to reproduce the experimental data; bi-exponential function and nonhomogenous kinetics with time-dependent rate constant appeared valid and gives the following results:

peak to peak amplitude

bi-exponential function

$$\text{Free radicals (\%)} = 9.07 \exp(-0.0835\ t) + 90.06 \exp(-0.0026\ t) \quad (r^2 = 0.9848)$$

nonhomogenous kinetics with time-dependent rate constant

$$[\text{Free radicals (\%)}] = 100/(1 + 0.0203\ t^{0.6575}) \quad (r^2 = 0.9861)$$

second integration

bi-exponential function

$$\text{Free radicals (\%)} = 15.75 \exp(-0.0688\ t) + 82.36 \exp(-0.0026\ t) \quad (r^2 = 0.9497)$$

nonhomogenous kinetics with time-dependent rate constant

$$[\text{Free radicals (\%)}] = 100/(1 + 0.0207\ t^{0.7606}) \quad (r^2 = 0.9740)$$

where t was the time of storage in days.

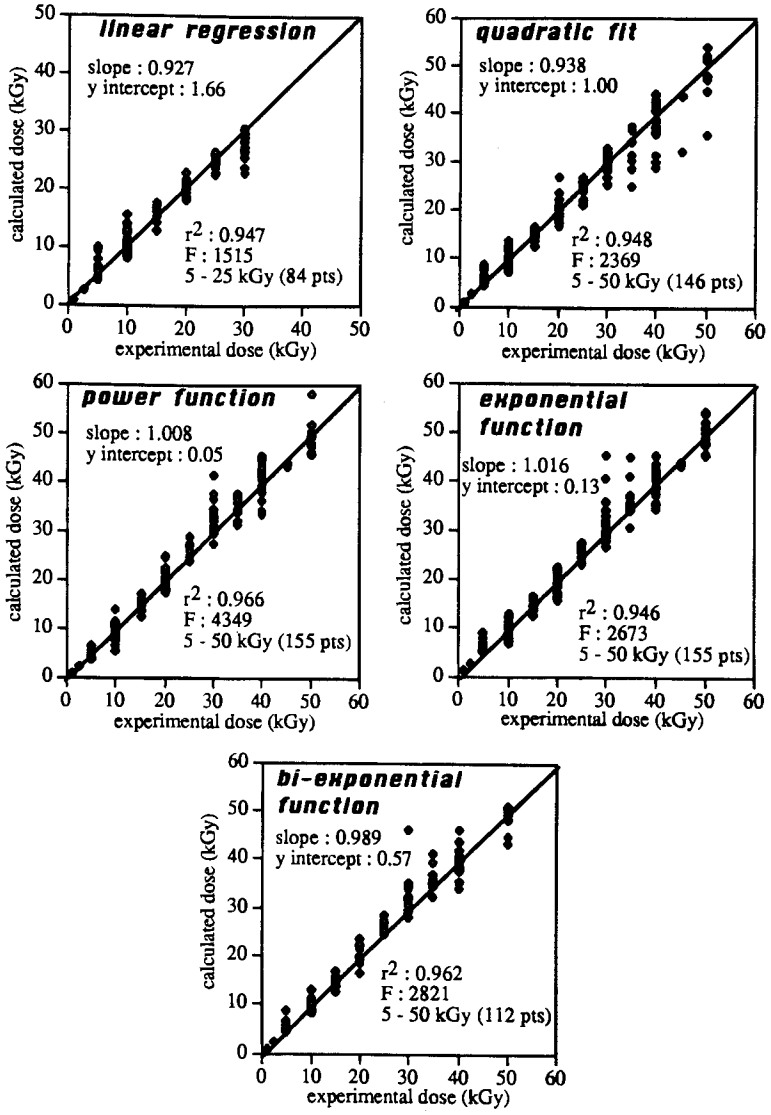

Fig. 5. Calculated dose versus experimental dose:sympathomimetics—antibacterial agents.

The parameters 0.6575 and 0.7606 are interpreted as a measure of non-homogeneity of reactivity in the system. The terms ($0.0203\ t^{0.6575}$) and ($0.0207\ t^{0.7606}$) can be replaced by ($0.0256\ t^{0.5} + 0.0018\ t$) and ($0.0208\ t^{0.5} + 0.0051\ t$), respectively; in this case, decay kinetic is a diffusion-controlled second-order reaction.

After 30 days and 63 days of storage, the losses of free radicals were, respectively, 16 and 24% for peak to peak amplitude (16 and 30% for second integration). In commercial market of drugs, radicals should be detected up to two years after irradiation (11). The stability of the radicals allows the detection of irradiated ascorbic acid after 800 days (2.2 years).

CONCLUSIONS

It is worth noting that, at present, ESR is the only technique which proves to be suitable for identification and quantification

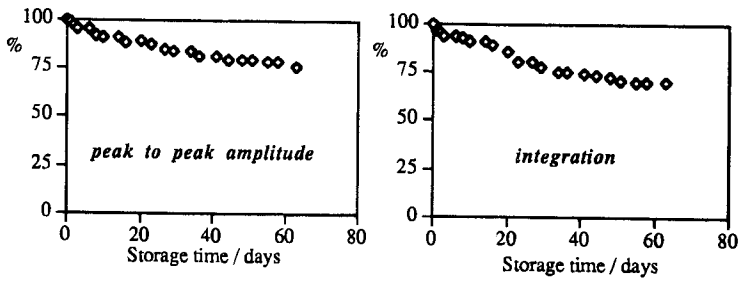

Fig. 6. Decay of radicals upon storage.

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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