Radiation Effects on Dopamine and Norepinephrine

J. P. Basly,[1,3] I. Longy,[1] and M. Bernard[2]

Received April 1, 1997; accepted June 12, 1997

Purpose. Radiation sterilization is becoming increasingly popular for the sterilization of many pharmaceutical products. We have investigated the gamma radiation induced effects on dopamine and norepineprine by ESR spectroscopy.

Results and Discussion. Equations to describe the evolution of the ESR curves versus doses and time of storage are presented. Linear regression is, for dopamine hydrochloride, applicable for doses ranging from 10 to 25 kGy. Since the radiation dose selected must always be based upon the bioburden of the products and the degree of sterility required, doses in the range 10–25 kGy could be investigated and linear regression would appear to be the least expensive route to follow and gives good results. The compartment of noradrenaline bitartrate is more complex and the use of linear regression would appear more hazardous especially for low doses. For doses higher than 25 kGy, a more general equation is required. Power function using only 2 parameters could give good results but must be validated. Decay kinetics for radicals versus storage were considered. Non-homogenous kinetics with time dependent rate constant and bi-exponential function appeared valid to reproduce the decay of radicals for, respectively, dopamine and norepinephrine.

Conclusions. 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.

KEY WORDS: dopamine; norepinephrine; radiation treatment; ESR spectroscopy; dosimetry; storage.

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 (1–4). The increased use of radiation processing for other industrial purposes (such as sterilization of medical devices) has led to the development of more efficient and economical irradiation equipment and processes. It may be the only way to sterilize many biologicals or biologically derived products because of their sensitivity to heat.

While the regulations governing the use of gamma radiation processing for pharmaceuticals may vary from country to country, all require that the use of the process be documented. With the publication of 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. Electron spin resonance (ESR) is one of the leading methods for identification of irradiated foodstuffs (5) and recently has proven to be an accurate and reliable technique for dosimetry analysis of irradiated pharmaceuticals (6–9). ESR yields both qualitative information (i.e. whether or not a sample has been irradiated) and quantitative results (i.e. the dose it received).

Following previous studies (10–12), the aim of the research reported here was to investigate by ESR spectroscopy the formation of free radicals in dopamine and norepinephrine after gamma irradiation.

MATERIALS AND METHODS

Reagents and Samples

Dopamine hydrochloride was kindly supplied by Institut de Recherche Pierre Fabre [Labège, France] and norepinephrine bitartrate was purchased from Fluka AG [Buchs, Switzerland]. Water was deionized and double distilled prior to use. All other reagents were of analytical grade and were used as received.

Irradiation

Samples were irradiated with gamma rays (^{60}Co) emitted by an IBL 460 [Faculté de Pharmacie, Limoges] with a dose rate of 1.350 kGy/h; the dose rate was preliminarily calibrated using the Fricke dosimeters (Ferrosulphate dosimetry). One unirradiated sample was kept as a reference.

Apparatus and Procedures

ESR spectra were recorded at room temperature using a BRUKER ESP 300 E spectrometer equipped with a variable temperature control apparatus and a data acquisition system (Table I). 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:

—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.

Numerical Simulations

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

RESULTS AND DISCUSSION

ESR

The key elements in establishing an ESR dosimetric method are:

[1] Laboratoire de Chimie Analytique et Bromatologie, UFR de Pharmacie, Université de Limoges, 2 rue du docteur Marcland, 87025 Limoges Cedex, France.

[2] Laboratoire de Biophysique Pharmaceutique, UFR de Pharmacie, Université de Limoges, 2 rue du docteur Marcland, 87025 Limoges Cedex, France.

[3] To whom correspondence should be addressed. (e-mail: Basly@alpha1.unilim.fr)

0724-8741/97/0900-1192$12.50/0

Table I. ESR Parameters

	ESR parameters		
	DOPAMINE		NOREPINEPHRINE
Sweep field	———	340–350 mT	———
Microwave frequency	———	9.65 GHz	———
Microwave power	———	0.4 mW	———
Modulation frequency	———	100 kHz	———
Modulation amplitude	———	0.2 mT	———
Time constant	———	163.84 ms	———
Sweep time	———	2.1 min	———
Amplification factor	10000		2500
Peak to peak	343.1 mT		343.0 mT
amplitude	344.7 mT		344.1 mT
	Limit of detection (kGy)		
	DOPAMINE		NOREPINEPHRINE
peak to peak amplitude	0.5		0.5
	Limit of quantification (kGy)		
	DOPAMINE		NOREPINEPHRINE
peak to peak amplitude	2.0		0.5

—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.

ESR powder spectra of dopamine and noradrenaline after irradiation are presented in Figure 1; the shape of the ESR spectra did not depend on dose. The concentrations of radiation free radicals, evaluated by double integration of the ESR spectra, were $510^{16} - 510^{17}$ spin/g for dopamine and $410^{17} - 410^{18}$ spin/g for norepinephrine.

Dosimetry

Figure 2 shows the plot of the evolution of the dose-ESR response after radiosterilization; the results are the mean of single determination on three samples. 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 I. An important step in the development of irradiation dosimetry of pharmaceuticals has been the choice of functions to fit the data. Five functions have been tried:

—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 function (this function derives from those described in dispersive kinetics) (13) and bi-exponential function (equation used in the calibration curve of alanine/ESR dosimetric system for industrial radiation processing (14).

The coefficients of numerical simulations are given in Table II. It should be noted that no attempt has been made to force the regression through zero and background ESR signal was subtracted in the simulations.

To be useful, the models described in Table II must be capable of predicting the irradiation dose. In order to verify

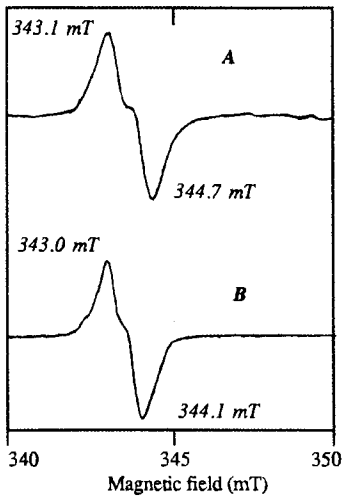

Fig. 1. ESR spectra (25 kGy).

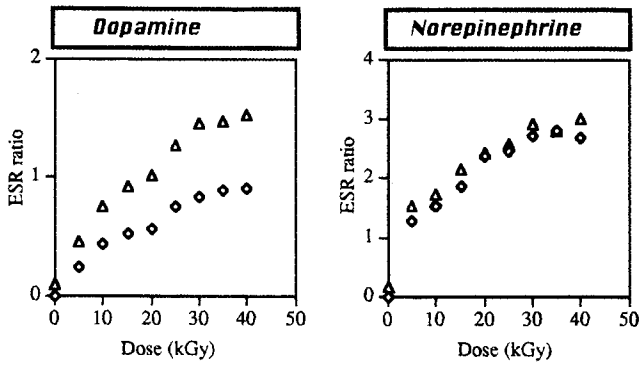

Fig. 2. Dose-ESR response curves.

Table II. Coefficients of Numerical Simulations

DOPAMINE	
peak to peak amplitude	
equation 1 (0–25 kGy)	ESR ratio = 0.0857 + 0.0271D (r^2 = 0.946)
equation 2 (0–40 kGy)	ESR ratio = 0.0428 + 0.0387D − 0.0004 D^2 (r^2 = 0.986)
equation 3 (0–40 kGy)	ESR ratio = 0.1060 $D^{0.5925}$ (r^2 = 0.988)
equation 4 (0–40 kGy)	ESR ratio = 1.0874 [1 − exp(−0.0456D)] (r^2 = 0.986)
equation 5 (0–40 kGy)	ESR ratio = −0.6391 exp(−0.0709D) + 0.6577 exp (0.0096D) (r^2 = 0.988)
second integration	
equation 1 (0–25 kGy)	ESR ratio = 0.0976 + 0.0445D (r^2 = 0.983)
equation 2 (0–40 kGy)	ESR ratio = 0.0292 + 0.0634D − 0.0007 D^2 (r^2 = 0.992)
equation 3 (0–40 kGy)	ESR ratio = 0.1490 $D^{0.6266}$ (r^2 = 0.987)
equation 4 (0–40 kGy)	ESR ratio = 1.7942 [1 − exp(−0.0415D)] (r^2 = 0.992)
equation 5 (0–40 kGy)	ESR ratio = −0.9649 exp(−0.0692D) + 0.9705 exp(0.0123D) (r^2 = 0.991)
NOREPINEPHRINE	
peak to peak amplitude	
equation 1 (0–25 kGy)	ESR ratio = 0.4538 + 0.0910D (r^2 = 0.889)
equation 2 (0–40 kGy)	ESR ratio = 0.2412 + 0.1492D − 0.0022 D^2 (r^2 = 0.969)
equation 3 (0–40 kGy)	ESR ratio = 0.6673 $D^{0.3987}$ (r^2 = 0.981)
equation 4 (0–40 kGy)	ESR ratio = 2.8522 [1 − exp(−0.0855D)] (r^2 = 0.977)
equation 5 (0–40 kGy)	ESR ratio = −2.3020 exp(−0.1017D) + 2.3831 exp(0.0044D) (r^2 = 0.979)
second integration	
equation 1 (0–25 kGy)	ESR ratio = 0.5143 + 0.0863D (r^2 = 0.850)
equation 2 (0–40 kGy)	ESR ratio = 0.3073 + 0.1407D − 0.0020 D^2 (r^2 = 0.950)
equation 3 (0–40 kGy)	ESR ratio = 0.7116 $D^{0.3773}$ (r^2 = 0.990)
equation 4 (0–40 kGy)	ESR ratio = 2.7832 [1 − exp(−0.0917D)] (r^2 = 0.970)
equation 5 (0–40 kGy)	ESR ratio = −1.7405 exp(−0.1932D) + 1.7688 exp(0.0121D) (r^2 = 0.983)

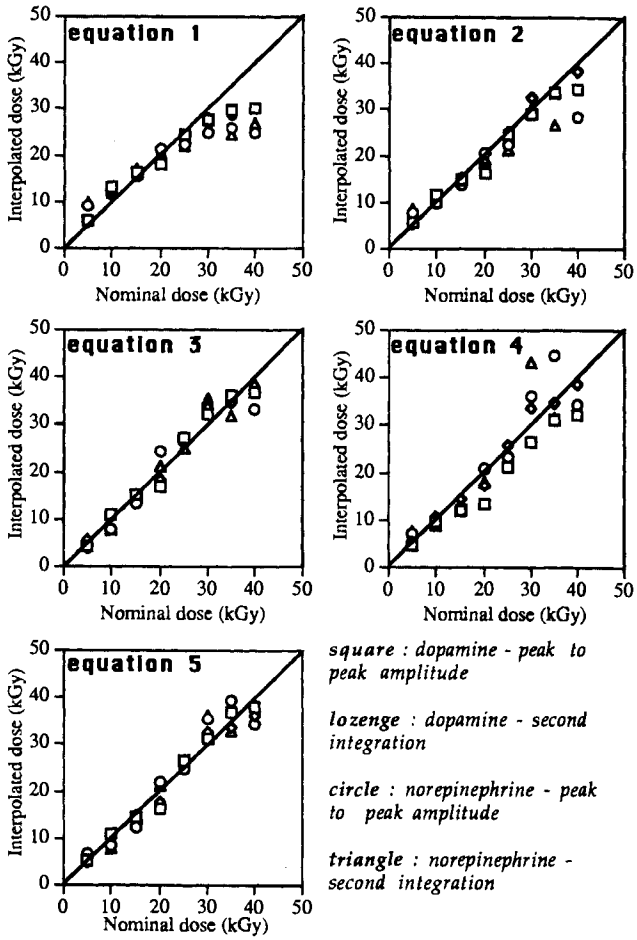

Fig. 3. Interpolated doses versus nominal doses.

the utility of the equation obtained, we have calculated the interpolated doses (Fig. 3). Briefly, the interpolated (back-calculated) doses were obtained by entering the measured response [ESR signal ratio] in the models described above. Regression statistics were applied and the results are given below:

equation 1 (0–25 kGy)
Dopamine-slope: 0.851; intercept: 2.74; r^2: 0.963; 10 pts
Norepinephrine-slope: 0.677; intercept: 5.95; r^2: 0.967; 10 pts
equation 2 (0–40 kGy)
Dopamine-slope: 0.909; intercept: 1.34; r^2: 0.974; 16 pts
Norepinephrine-slope: 0.686; intercept: 4.83; r^2: 0.915; 14 pts
equation 3 (0–40 kGy)
Dopamine-slope: 0.990; intercept: 0.24; r^2: 0.971; 16 pts
Norepinephrine-slope: 0.972; intercept: 0.75; r^2: 0.937; 16 pts
equation 4 (0–40 kGy)
Dopamine-slope: 0.915; intercept: 0.16; r^2: 0.941; 16 pts
Norepinephrine-slope: 1.059; intercept: −0.44; r^2: 0.844; 15 pts
equation 5 (0–40 kGy)
Dopamine-slope: 0.963; intercept: 0.59; r^2: 0.972; 16 pts
Norepinephrine-slope: 1.001; intercept: 0.33; r^2: 0.937; 16 pts

The following statements can be established:

—equation 1 (linear regression) is, for dopamine hydrochloride, applicable for doses ranging 10 to 25 kGy. Since the radiation dose selected must always be based upon the bioburden of the products and the degree of sterility required (EN 552 (15) and ANSI/AAMI/ISO 11137 (16)), 25 kGy could no longer be accepted as a "routine" dose for sterilizing a pharmaceutical. Doses in the range 10–25 kGy could be investigated and linear regression would appeared the least expensive route to follow and gives good results. The comportment of noradrenaline bitartrate is more complex and the use of linear regression would appeared more hazardous especially for low doses.

—The introduction of a quadratic term to take into account the non-linear shape of the dosimetric curve is of more general applicability than equation 1 but the intercepts and the slope of the straight lines (Figure 3) are, especially for noradrenaline, not very close to zero and unity respectively which is a good indication of the validity of the models.

—Equation 3 (power function), equation 4 (exponential function) and equation 5 (bi-exponential function) are of more general applicability to predict irradiation dose than equation 2 (quadratic fit); intercept and slopes of the straight lines are close to zero and unity respectively, contrary to quadratic fit. In the case of equation 3, model apparently the easier to use (2 variables to determine), a sudy of the power factor on several ESR data sets previously acquired in this laboratory (8 sympathomimetics and 6 antibacterial agents) gives 0.671 ± 0.135. The results obtained for dopamine hydrochloride (0.592 for peak to peak ratio and 0.627 for integration ratio) are in this range.

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 (63 days) was performed. Fig. 4 plots the evolution of the percentage of free radicals versus storage. The behaviour of the two sympathomimetics is slightly different:

In the case of norepinephrine, 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 nonhomogenous kinetics with time dependent rate constant, that has been successfully applied to many systems with reactivity distribution (13). The relation for this model is:

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

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 homogenous kinetics. This model, applied to the data plotted in Figure 4 gives the following results:

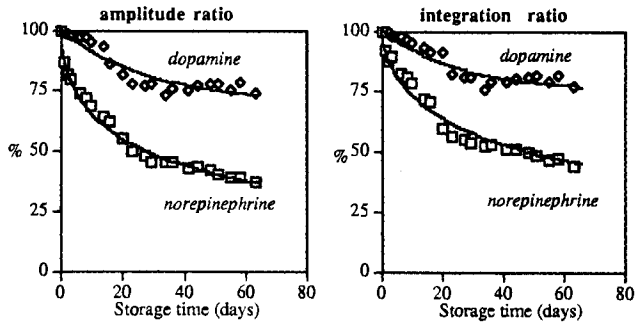

Fig. 4. Decay of radicals upon storage.

Norepinephrine – peak to peak amplitude

$$[\text{free radicals } (\%)] = \frac{100}{1 + 0.1185t^{0.6402}}$$

$$r^2 = 0.987$$

Norepinephrine – integration

$$[\text{free radicals } (\%)] = \frac{100}{1 + 0.0792t^{0.6641}}$$

$$r^2 = 0.971$$

where t was the storage time in days. Simulated curves are plotted in figure 4. After 30 and 63 days of storage, the losses of free radicals were respectively 53 and 63% for peak to peak ratio (*46% and 56% for integration ratio*). In commercial market of drugs, radicals should be detected up to two years after irradiation (8). The kinetic decrease causes discrimination between irradiated and unirradiated samples possible after a storage lower than 350 days.

The comportment of dopamine hydrochloride was slightly different. The free radicals concentration decreased during 25 days and remains constant (bi-exponential reaction).

Dopamine-peak to peak amplitude

Free radicals (%) = 29.69 exp (−0.0375t) + 71.05 exp (−0.0001t) $r^2 = 0.914$

Dopamine-second integration

Free radicals (%) = 25.56 exp (−0.0417t) + 75.88 exp (−0.0001t) $r^2 = 0.915$

Simulated curves are plotted in figure 4.

During this time, about 25% of free radicals disappeared; radicals should probably be detected up to two years after irradiation.

The impurity profiles were recorded using ion pair chromatography (IPC) and micellar liquid chromatography (MLC).

Table III. Radiolytic Degradation[a] (%) Versus Irradiation Dose

Dose (kGy)	DOPAMINE		NOREPINEPHRINE	
	IPC[b]	MLC[c]	IPC	MLC
5	0.00 ± 0.06	0.00 ± 0.04	0.04 ± 0.05	0.01 ± 0.01
10	0.03 ± 0.01	0.08 ± 0.04	0.00 ± 0.08	0.00 ± 0.01
15	0.03 ± 0.03	0.14 ± 0.07	0.00 ± 0.02	0.00 ± 0.02
20	0.00 ± 0.01	0.09 ± 0.03	0.03 ± 0.04	0.00 ± 0.04
25	0.04 ± 0.02	0.02 ± 0.08	0.03 ± 0.02	0.02 ± 0.02
30	0.02 ± 0.02	0.15 ± 0.06	0.08 ± 0.02	0.06 ± 0.04
35	0.02 ± 0.01	0.08 ± 0.06	0.03 ± 0.06	0.22 ± 0.02
40	0.00 ± 0.01	0.08 ± 0.08	0.19 ± 0.01	0.30 ± 0.04

[a] Assay—HPLC determination (unirradiated sample) Dopamine: IPC (99.89 ± 0.02%) – MLC (99.84 ± 0.02%) Norepinephrine: IPC (99.72 ± 0.04%) – MLC (99.87 ± 0.02%).

[b] IPC conditions column Waters μ-Bondapak C18 (300 × 3.9 mm); mobile phase: CH_3COOH (1%) + heptanesulfonic acid salt (5 mM)—MeOH [95-5]; λ: 280 nm; 1 ml/min; 1 mg/ml.

[c] MLC conditions column Merck RP Select B (125 × 4 mm); mobile phase: SDS 0.05 M—PrOH [94-6 (dopamine) and 96-4 (norepinephrine)]; λ: 280 nm; 1 ml/min; 1 mg/ml.

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 (Table III).

CONCLUSIONS

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.

REFERENCES

1. G. P. Jacobs. *J. Biomed. Appl.* **10**:59–96 (1995).
2. B. D. Reid. *PDA J. Pharm. Sci. Technol.* **49**:83–89 (1995).
3. B. Tilquin and B. Rollmann. *J. Chim. Phys.* **93**:224–230 (1996).
4. C. Boess and K. W. Böegl. *Drug Dev. Ind. Pharm.* **22**:495–526 (1996).
5. M. Gibella, A. S. Crucq, and B. Tilquin. *J. Chim. Phys.* **90**:1041–1053 (1993).
6. E. Ciranni Signoretti, S. Onori, L. Valvo, P. Fattibene, A. L. Savella, C. De Sena, and S. Alimonti. *Drug Dev. Ind. Pharm.* **19**:1693–1708 (1993).
7. E. Ciranni Signoretti, L. Valvo, P. Fattibene, S. Onori, and M. Pantaloni. *Drug Dev. Ind. Pharm.* **20**:2493–2508 (1994).
8. T. Miyazaki, T. Kaneko, T. Yoshimura, A. S. Crucq, and B. Tilquin. *J. Pharm. Sci.* **83**:68–71 (1994).
9. T. Miyazaki, J. Arai, T. Kaneko, K. Yamamoto, M. Gibella, and B. Tilquin. *J. Pharm. Sci.* **83**:1643–1644 (1994).
10. J. P. Basly, J. L. Duroux, and M. Bernard. *Int. J. Pharm.* **139**:219–221 (1996).
11. J. P. Basly, J. L. Duroux, and M. Bernard. *Int. J. Pharm.* **142**:125–128 (1996).
12. J. P. Basly, J. L. Duroux, and M. Bernard. *Int. J. Pharm.* **142**:247–249 (1996).
13. A. Plonka. *Prog. Reaction Kinetics.* **16**:157–333 (1991).
14. F. Balestic. *Rayonnements et traitements ionisants*, Masson Physique fondamentale et appliquée, Paris, 1995.
15. *EN 552, Sterilization of Medical Devices—Validation and routine control of sterilization irradiation*, CEN, European committee for standardization, Brussels, Belgium, 1994.
16. *ISO 11137, Sterilization of Health Care Products—Requirements for Validation and Routine control—Radiation Sterilization*, International Organization for Standardization, Geneva, Switzerland, 1995.