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Influence of radiation treatment on theodrenaline: ESR and HPLC study

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Abstract

The use of ionizing radiation for sterilization of pharmaceuticals is now a well established technology. The purpose of the present work was to apply High performance liquid chromatography (HPLC) and Electron spin resonance (ESR) spectroscopy to study the degradation of theodrenaline after gamma radiation treatment. Numerical simulations of the free radicals dependence on dose at ambient temperature were performed using linear regression, quadratic fit and power function. Tests were carried out to investigate the effects of storage on the free radicals concentration. The decay was fit using bi-exponential regression. Degradation of theodrenaline was studied by ion pair chromatography (IPC) and reversed-phase chromatography (RPLC). The pre-existent impurities did not show a significant increase with dose but the initial amount of impurities (nearly 2%) caused the radiosterilization of this sample not to be technically feasible. © 1997 Elsevier Science B.V.

Keywords: Theodrenaline; Radiation treatment; ESR spectroscopy; Dosimetry; Storage; HPLC; Degradation

1. Introduction

The sterilization of thermolabile medical devices, such as catheters or syringues, 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; 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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Radiosterilization, however, has the following problems:

- gamma irradiation produces new radiolytic products; to prove the safety of radiosterilization, it is important to determine the radiolytic products and to elucidate the mechanism of radiolysis. High performance liquid chromatography (HPLC) is the analytical method of choice for the majority of drug stability protocols (Kagan, 1994). It is a very selective technique allowing the separation and measurement of degradation products.
- 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., 1994). ESR spectroscopy appears to be very suitable for the determination of free radical 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 investigate the degradation of theodrenaline by ESR and HPLC after gamma irradiation.

2. Materials and methods

2.1. Reagents and samples

Theodrenaline was kindly supplied by LIPHA [Lyon, France]. Water was desionized and double distilled prior to use. All other reagents were of analytical grade and were used as received.

2.2. Irradiation

Samples were irradiated with gamma rays emitted by an IBL 460 (^{60}Co); the dose rate was 1.6 kGy/h. One unirradiated sample was kept as reference.

Table 1
HPLC parameters

Method 1: IPC
Column: Waters μ-Bondapak (300 × 3.9 mm)
λ: 280 nm
Mobile phase: MeOH − CH_3COOH (1%) + Heptanesulfonic Acid Sodium Salt (5 mM)
Organic modifier percentage (v/v): 20
Flow: 1 ml/min
Sample concentration: 1 mg/ml in sodium metabisulfite (3%)
Method 2: RPLC
Column: Merck RP Select B (125 × 4 mm)
λ: 280 nm
Mobile phase: MeOH − $KH2PO_4$ (0.05 M)
Organic modifier percentage (v/v): 10
Flow: 1 ml/min
Sample concentration: 1 mg/ml in mobile phase

2.3. Apparatus

Isocratic analysis were carried out on a Bischoff M2200 HPLC pump equipped with a Kratos 783 Spectroflow absorbance detector and a Hewlett Packard 3390A integrator. Sample introduction was via a Rheodyne model 7125 injection valve fitted with a 20 μl loop for direct injection. Separations were carried out using two methods (Table 1): ion pair chromatography (IPC) and reversed-phase chromatography (RPLC).

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 2).

Table 2
ESR parameters

Sweep field (mT): 340.0–350.0
Frequency (GHz): 9.65
Microwave power (mW): 0.4
Modulation frequency (kHz): 100
Amplification factor: 8000
Modulation amplitude (mT): 0.2
Time constant (ms): 163.8
Sweep time (min): 2.1
Peak to peak height: 343.0–344.6 mT

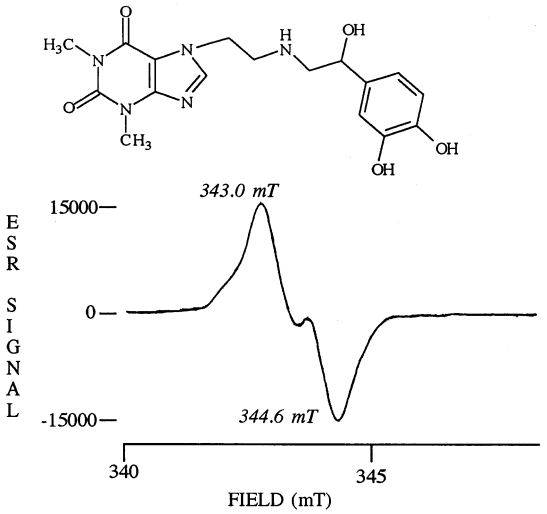

Fig. 1. ESR spectrum.

BRUKER strong pitch was used as ESR standard.

For the measurements, 10 mg of substance was weighted with an accuracy of 0.2 mg and all simulations were performed using Mathematica software.

3. Results and discussion

3.1. ESR

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.

ESR powder spectrum of theodrenaline after irradiation is presented in Fig. 1. The shape of the signal did not depend on dose and it is important to notice the large number of free radicals generated during the irradiation.

3.1.1. Dosimetry

Fig. 2 shows the plot of the evolution of the ESR signal versus dose. This evolution was followed by calculating:

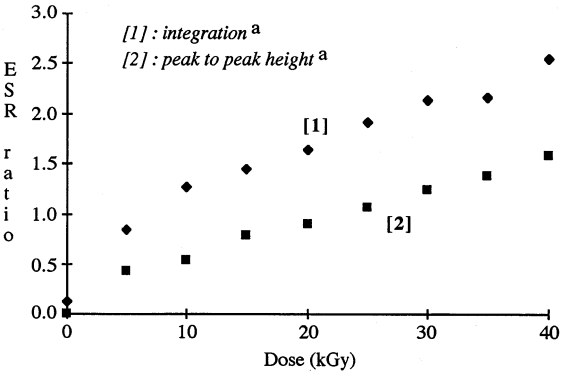

Fig. 2. Free radicals dependence on dose.

- the ratio (sample vs. strong pitch) of the peak to peak intensities;
- the ratio (sample vs. strong pitch) of the double integration of the signals; this ratio is proportional to the spin concentration (Yordanov and Ivanova, 1994).

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, quadratic fit, power function, Michaelis function and Hill function. Finally, evolution of the ESR signal was fit using linear regression, quadratic fit and power function (Table 3):

linear regression: ESR signal ratio $= a + bD$

quadratic fit: ESR signal ratio $= c + dD - eD^2$

eD^2 was introduced as a corrective term.

Table 3
Coefficients of functions used in numerical simulations

Peak to peak height
Linear regression (0–40 kGy)
ESR signal ratio $= 0.157978 + 0.036057$ D
Quadratic fit (0–40 kGy)
ESR signal ratio $= 0.095018 + 0.04685D - 0.000270D^2$
Power function (0–40 kGy)
ESR signal ratio $= 0.1163$ $D^{0.6980}$
Double integration
Linear regression (0–40 kGy)
ESR signal ratio $= 0.509467 + 0.052793$ D
Quadratic fit (0–40 kGy)
ESR signal ratio $= 0.292952 + 0.089739D - 0.000924D^2$
Power function (0–40
ESR signal ratio $= 0.3648$ $D^{0.5159}$

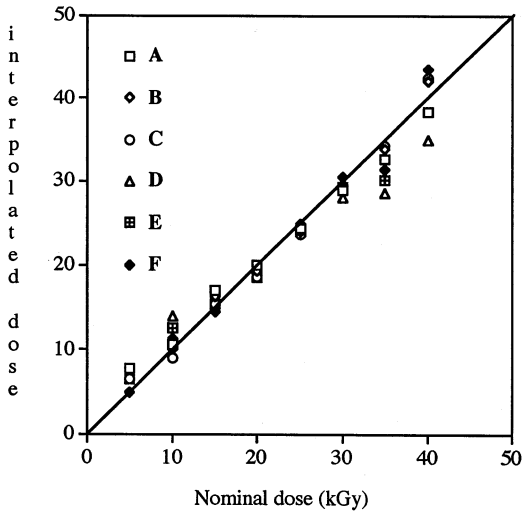

Fig. 3. Interpolated dose versus nominal dose.

power function: ESR signal ratio = fD^g

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

Michaelis and Hill functions were disused; they appeared too sophisticated to be used in further studies (e.g. post-irradiation).

Fig. 3 shows the good adequacy between interpolated and nominal doses using linear regression, quadratic fit and power function. The interpolated (back-calculated) doses were obtained by entering the measured response [ESR signal ratio] in the models described above.

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 kGy and 1.5 kGy respectively. Since the dose currently used for radiosterilization is 25 kGy, discrimination from irradiated and unirradiated samples and quantification of free radicals generated during the irradiation seem possible.

Estimation of the irradiation dose could be performed:

- by entering the response (ESR ratio) in the three functions described above;
- by post-irradiation. Analysing Fig. 3, the use of linear regression to fit the ratio of peak to intensities could be the easier.

3.1.2. Decay of radicals upon storage

Tests were carried out to investigate wether storage had an effect on the free radicals concentration. Storage at ambient temperature in a sealed quartz tube over several weeks (58 days) was performed. Fig. 4 plots the evolution of the percentage of free radicals versus storage. This decay could be simulated using a bi-exponential regression:

Free radicals $(\%) = 69.32 \exp(-0.0026t) + 30.68 \exp(-0.0792t)$ where t is the time of storage in days.

As described in previous works (Basly et al., 1996), this decay can be divided in two phases: A first corresponding to a fast decay (0–40 days) and a second corresponding to a 'quasi-linear' decay (over 40 days of storage). After 30 and 58 days of storage, the losses of free radicals are respectively 36 and 40%. In commercial market of drugs, radicals should be detected up to two years after irradiation (Miyazaki et al, 1994). In spite of the bi-exponential decay, the large number of free radicals generated during the irradiation causes discrimination between irradiated and unirradiated samples possible even after a storage up to 2 years.

3.2. HPLC

The impurity profiles were recorded using IPC and RPLC. The chromatograms of irradiated samples (40 kGy) are shown in Fig. 5. Other

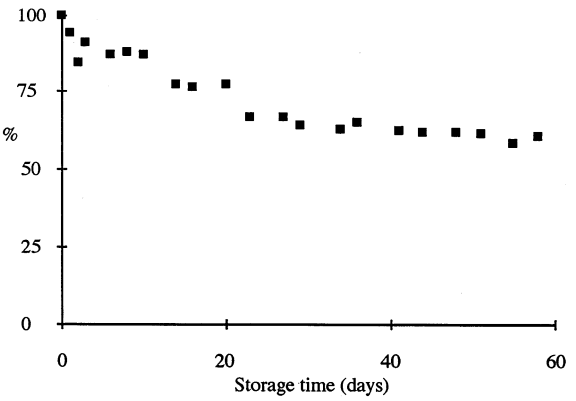

Fig. 4. Decay of radicals upon storage.

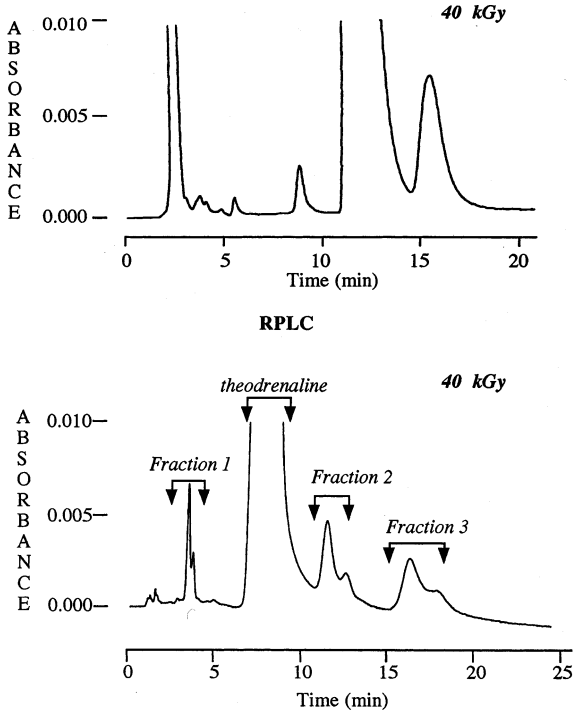

Fig. 5. HPLC chromatograms after gamma irradiation.

samples (irradiated and unirradiated) were examined and found to be similar in their impurity profiles. The amount of impurities was determined at 280 nm assuming that the relative molar response factor (RRF) for an impurity was equal to one (i.e. the molar response factor of impurities at 280 nm were equal to the molar response factor of theodrenaline at 280 nm). The comparison between chromatographic profiles of irradiated and unirradiated samples evidenced minor differences. The pre-existent impurities did not show a significant increase with dose (Fig. 6) but the initial amount of impurities (nearly 2%) caused the radiosterilization of this sample not to be technically feasible.

Fraction 1, theodrenaline, fraction 2 and fraction 3 were collected and analyzed by UV spectrophotometry using an HP 8450A DAD spectrophotometer; the maxima of absorption are respectively 272 nm, 278 274 nm and 275 nm. At present, only few informations have been obtained on the impurities.

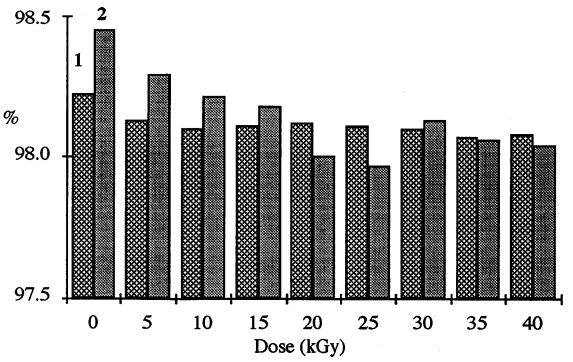

Fig. 6. Purity versus dose.

4. Conclusion

This work shows the applicability of ESR spectrometry to the radiosterilization dosimetry. Estimation of the irradiation dose for theodrenaline could be performed but the decay of radicals upon storage must be known. The comparison between chromatographic profiles of irradiated and unirradiated samples evidenced minor differences but the initial amount of impurities (nearly 2%) caused the radiosterilization of theodrenaline not to be technically feasible.

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References

Basly, J.P., Duroux, J.L. and Bernard, M., Gamma irradiation sterilisation of orciprenaline and fenoterol. *Int. J. Pharm.*, 142 (1996) 125–128.

Boess, C. and Böegl, K.W., Influence of radiation treatment on pharmaceuticals – a review: alkaloids, morphines derivatives and antibiotics. *Drug Dev. Ind. Pharm.*, 22 (1996) 495–526.

Ciranni Signoretti, E., Onori, S., Valvo, L., Fattibene, P., Savella, A.L., De Sena, C. and Alimonti, S., Ionizing radiation induced effets on cephradine. Influence of sample moisture content, irradiation dose and storage conditions. *Drug Dev. Ind. Pharm.*, 19 (1993) 1693–1708.

Ciranni Signoretti, E., Valvo, L., Fattibene, P., Onori, S. and Pantaloni, M., Gamma radiation induced effects on ce-

furoxime and cefotaxime. Investigation on degradation and syn-anti isomerization. *Drug Dev. Ind. Pharm.*, 20 (1994) 2493–2508.

Gibella, M., Crucq, A.S. and Tilquin, B., Détection RPE de l'irradiation des médicaments. *J. Chim. Phys.*, 90 (1993) 1041–1053.

Jacobs, G.P., A review of the effects of gamma radiation on pharmaceuticals materials. *J. Biomed. Appl.*, 10 (1995) 59–96.

Kagan, R.L., High performance liquid chromatography for small-scale study of drug stabilities. *Am. J. Hosp. Pharm.*, 51 (1994) 2162–2175.

Miyazaki, T., Kaneko, T., Yoshimura, T., Crucq, A.S. and Tilquin, B. Electron spin resonance study of radiosterilization of antibiotics: Ceftazidime. *J. Pharm. Sci.*, 83 (1994) 68–71.

Reid, B.D., Gamma processing technology: an alternativr technology for terminal sterilization of parenterals. *PDA J. Pharm. Sci. Technol.*, 49 (1995) 83–89.

Tilquin, B. and Rollmann, B., Recherches à conseiller pour l'application de la stérilisation ionisante des médicaments. *J. Chim. Phys.*, 93 (1996) 224–230.

Yordanov, N.D. and Ivanova, M., The present stata of quantitative EPR spectrometry: the results from an international experiment. *Appl. Magn. Reson.*, 6 (1994) 333–340.

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