Radiation Sterilization of Formoterol

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

Results and Discussion. Numerical simulation of the evolution of the ESR signal versus dose was performed using linear regression, quadratic fit and power function. The shape of the dosimetric curve is linear in the range 5–30 kGy. Owing to the weak number of free radicals generated during the irradiation, the accuracy of measurements is low. For a dose of 25 kGy, discriminating irradiated from unirradiated samples is possible if the storage period is less than 250 days. The comparison between chromatographic profiles of irradiated and unirradiated samples showed minor differences.

Conclusions. From our preliminary results, radiosterilization of formoterol fumarate may be technically feasible. Estimation of the irradiation dose by ESR may be possible but, due to the weak number of free radicals generated during the irradiation, the accuracy of measurements appeared low.

KEY WORDS: formoterol; radiation treatment; ESR spectroscopy; dosimetry; storage; HPLC; degradation.

INTRODUCTION

Radiation sterilization and its application 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.

Radiosterilization, however, has the following problems:

—while gamma irradiation produces new radiolytic products, it is important to have analytical methods which will permit the determination of degradation products in very small amounts. This will aid in determining the method of degradation (oxidative or reductive) and permit the introduction, where warranted, of protective molecules. High performance liquid chromatography (HPLC) is the analytical method of choice for the majority of drug stability protocols (5). It is a very selective technique allowing the separation and possible measurements of degradation products;

—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. Recently, Electron spin resonance (ESR) has proven to be an efficient technique for radiosterilization dosimetry (7–10). 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 (11–13), the aim of the research reported here was to investigate the degradation of formoterol fumarate by ESR and HPLC after gamma irradiation.

MATERIALS AND METHODS

Reagents and Samples

Formoterol fumarate was kindly supplied by Ciba Geigy [Basle, 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 emitted by an IBL 460 (^{60}Co); the dose rate was 1.6 kGy/h. One unirradiated sample was kept as reference.

Apparatus

LC separations were achieved using an HPLC system consisting of a Bischoff M2200 isocratic pump and a Kratos 783 Spectroflow absorbance detector; peaks area were determined with an HP 3390A integrator. Sample introduction was via a Rheodyne model 7125 injection valve fitted with a 20 µl loop for direct injection and all analyses were carried out at 25°C with a mobile phase rate of 1ml/min. Separations were performed using three methods (Table I): ion pair chromatography (IPC), reversed-phase chromatography (RPLC) and micellar liquid chromatography (MLC). The samples used for IPC and RPLC analysis were dissolved in mobile phase modified by an increase of the methanol percentage (e.g. formoterol fumarate is fairly soluble in IPC and RPLC mobile phase)

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 II). BRUKER strong pitch was used as ESR standard. For the measurements, 10 mg of substance was weighed with an accuracy of 0.2 mg

Numerical Simulations

Numerical simulations were conducted using a Macintosh LC III supported by Mathematica 2.2 software program (Wolfram research).

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Table I. 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)
methanol percentage (v/v) : 35
flow: 1 ml/min
sample concentration : 1mg/ml in CH_3COOH 0.17 N-MeOH (50:50 v/v)

Method 2: RPLC
column: Merck RP Select B (125 × 4 mm)
λ : 280 nm
mobile phase: MeOH-KH_2PO_4 (0.05 M)
methanol percentage (v/v) : 23
flow : 1 ml/min
sample concentration : 1 mg/ml in KH_2PO_4 0.05M-MeOH (50:50 v/v)

Method 3: MLC
column : Merck RP Select B (125 × 4 mm)
λ : 280 nm
mobile phase : PrOH-SDS (0.05M)
propanol percentage (v/v) : 20
flow : 1 ml/min
sample concentration : 1mg/ml in mobile phase

RESULTS AND DISCUSSION

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 formoterol after irradiation is presented in Figure 1; the shape of the signal did not depend on dose. It is important to notice the weak number of free radicals generated during the irradiation.

Table II. ESR Parameters

sweep field (mT) : 340.0–350.
frequency (GHz) : 9.65
microwave power (mW) : 0.4
modulation frequency (kHz) : 100
gain : 20 000
modulation amplitude (mT) : 0.2
time constant (ms) : 163.8
sweep time (min): 2.1
peak to peak height : 342.8 mT–344.2 mT

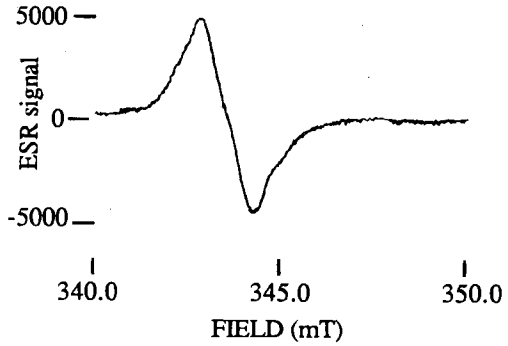

Fig. 1. ESR spectrum (25 kGy).

Dosimetry

Figure 2 shows plot of the evolution of the ESR signal versus dose; the results are the mean of single determination on three samples. This evolution was followed by calculating:

—the ratio (sample vs. strong pitch) of the peak to peak amplitude;
—the ratio (sample vs. strong pitch) of the second integration of the signals; this ratio is proportional to the spin concentration (14).

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, exponential function and bi-exponential function. Finally, evolution of the ESR signal was fit using linear regression, quadratic fit and power function (Table III). Exponential and bi-exponential functions were disused; they appeared actually too sophisticated to be used in further studies (e.g. post-irradiation).

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

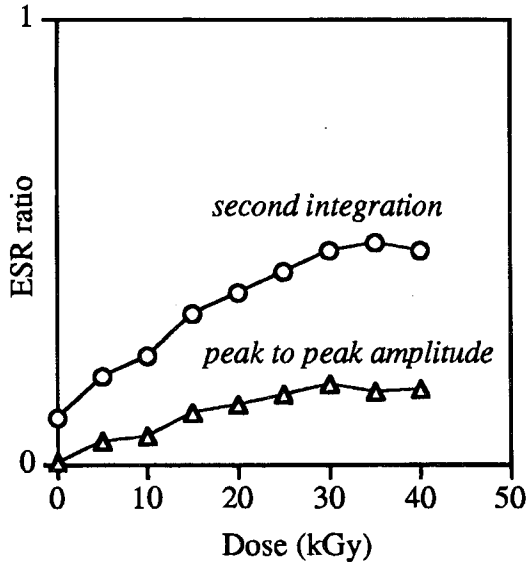

Fig. 2. Free radicals dependence on dose.

Table III. Functions and Coefficients Used in Numerical Simulations

peak to peak amplitude

linear regression (0–30 kGy)
ESR signal ratio = 0.019153 + 0.005448 D

quadratic fit (0–40 kGy)
ESR signal ratio = $-0.002964 + 0.009230D - 0.000125D^2$

power function (0–40 kGy)
ESR signal ratio = $0.0230D^{0.5657}$

second integration

linear regression (0–30 kGy)
ESR signal ratio = 0.147330 + 0.011429 D

quadratic fit (0–40 kGy)
ESR signal ratio = $0.103861 + 0.018098D - 0.000195D^2$

power function (0–40 kGy)
ESR signal ratio = $0.0970D^{0.4530}$

the S/N = 10 criterion, have been determined to be (1.5 ± 0.5) kGy and (5 ± 0.5) kGy respectively using the ratio of the peak to peak amplitude. On the basis of our observations, for a dose of 25 kGy, discriminating irradiated samples seems possible.

Validation of the Models

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

Fig. 2 shows that the shape of the dosimetric curves is linear only in the range 5–30 kGy; the utility of the linear regression is consequently reduced to doses lower than 30 kGy. For doses higher than 30 kGy, owing to the weak number of free radicals generated during the irradiation, the accuracy of measurements is low; consequently, the uncertainties on the irradiation doses, using quadratic fit and power function, are on an average 10–15%.

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. This decay could be simulated using a bi-exponential regression (15):

Free radicals (%) = 72.41 exp (−0.0096 t) + 27.59 exp (−0.7689t) [*peak to peak height*] (r = 0.9870)

Free radicals (%) = 62.67 exp (−0.0001t) + 37.33 exp (−0.0679t) [*integration ratio*] (r = 0.9834) where t is the time of storage in days.

As described in previous works (13), this decay can be divided in two phases: A first corresponding to a fast decay (0–5 days) (*0–40 days for integration ratio*) and a second corresponding to a "quasi-linear" decay (over 5 days of storage) (*over 40 days of storage for integration ratio*). After 30 and 63 days of storage, the losses of free radicals are respectively 50 and 58% for peak to peak ratio (*36% and 41% for integration ratio*). In commercial market of drugs, radicals should be detected up to two years after irradiation (9). On the basis of these observations, for a dose of 25 kGy, discriminating irradiated from unirradiated samples is possible if the storage period is less than 250 days.

3.2 HPLC

The impurity profiles were recorded using ion pair chromatography (IPC), reversed phase chromatography (RPLC) and micellar liquid chromatography (MLC). Recently, micellar liquid chromatography (MLC) has expanded the scope of LC. Liquid chromatographic secondary chemical equilibria, e.g. solute-micelle association, provide an additional mechanism to

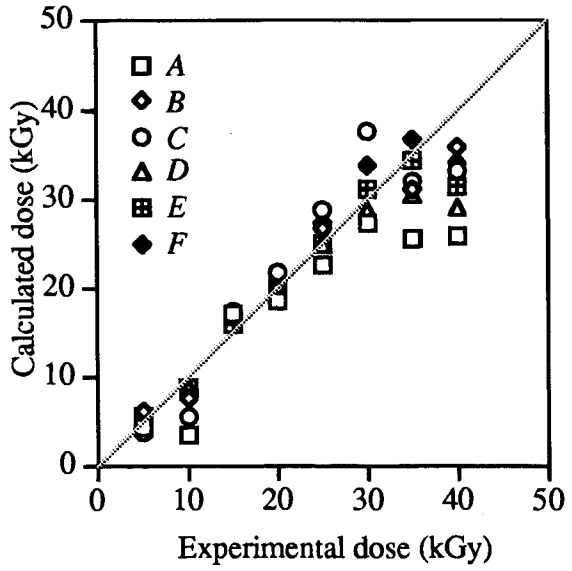

Fig. 3. Calculated dose versus experimental dose.

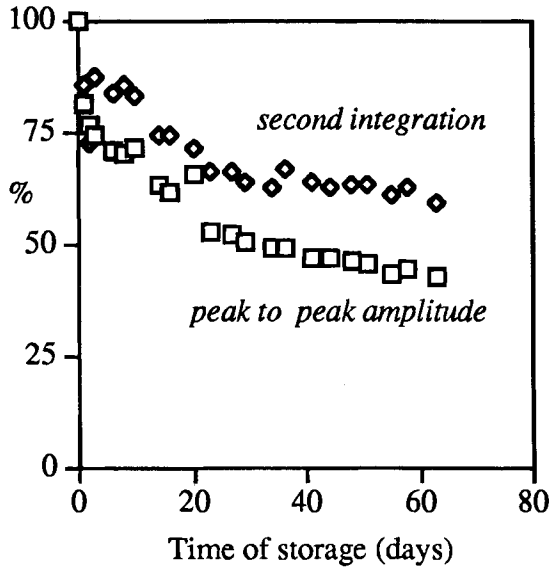

Fig. 4. Decay of radicals upon storage.

control retention and selectivity (16,17); MLC has also the capability of simultaneous separation of ionic and non-ionic compounds. It also offers the advantages gradient elution with electrochemical detectors, enhancement of fluorescence and phosphorescence detection and low cost and toxicity (18). The chromatograms of irradiated samples (40 kGy) are shown in Fig. 5. Other 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 formoterol at 280 nm). 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 (Fig. 6); the results are the mean of single determination on three samples.

Analyses using MLC showed a better reproducibility and regularity of the retention behavior than in IPC but a loss of efficiency as compared to RPLC; this loss of efficiency is thought to be the slow mass transfer of solutes from the surfactant-modified stationary phase.

CONCLUSIONS

This preliminary work shows the interest of the ESR spectroscopy in radiosterilization dosimetry.

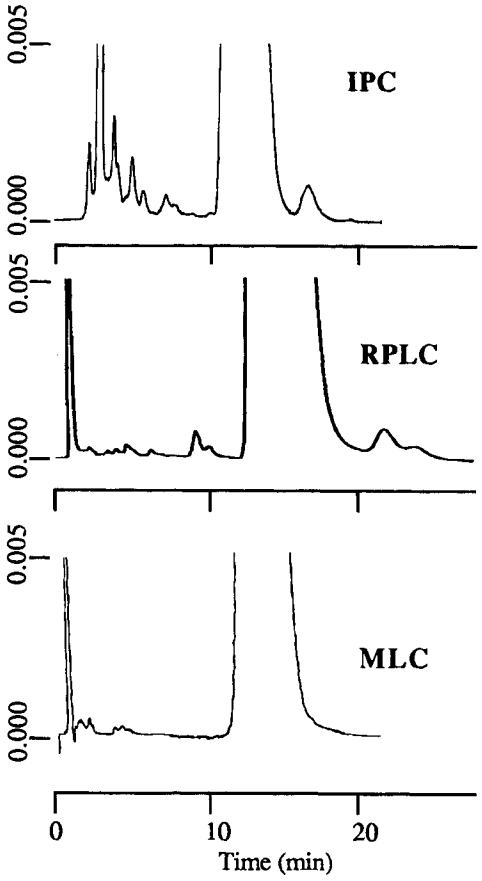

Fig. 5. HPLC chromatograms (40 kGy).

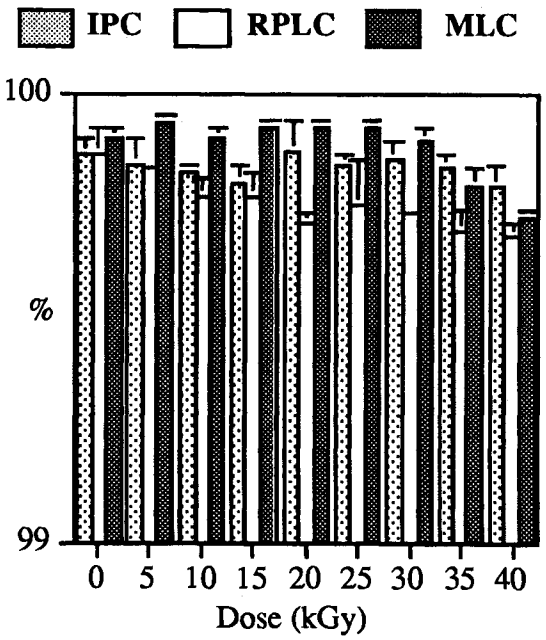

Fig. 6. Purity versus irradiation dose.

Estimation of the irradiation dose could be possible if:

—tests are carried out to investigate whether storage has an effect on the free radicals concentration;
—the free radicals dependence on dose is measured.

On the basis of these observations, for a dose of 25 kGy, discriminating irradiated from unirradiated samples is possible if the storage is less than 250 days. Estimation of the irradiation dose is possible but, due to the weak number of free radicals generated during the irradiation, the accuracy of measurements appeared low.

Formoterol fumarate showed little degradation after gamma irradiation. From our initial results, dose of 25 kGy slightly affect the amount of impurities; radiosterilization of formoterol may be technically feasible.

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