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FEASIBILITY STUDIES ON RADIATION STERILIZATION OF CEPHRADINE

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ABSTRACT

A comprehensive study was carried out to examine and evaluate the feasibility of radiation sterilization of cephradine antibiotic in the solid dry state. Absorbed gamma ray doses in the range of 5-25 kGy caused a dose-dependent loss of bactericidal activity. Moreover, radiation caused a dose-dependent degradation of the antibiotic and decrease in cephradine content due to radiation conversion to cephalexin. The former deleterious effect was detected spectrophotometrically and confirmed by high-performance liquid chromatography (HPLC). The latter effect was detected by NMR spectroscopy and by HPLC. The study revealed a noticeable change in the tint of the irradiated powdered drug which is dose-dependent and clearly remarkable at high radiation doses.

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INTRODUCTION

Ionizing radiation is increasingly being employed for the sterilization of single use pre-packed medical and pharmaceutical products (1). However, the susceptibility of some pharmaceuticals such as antibiotics and proteins to radiation sterilization is still under extensive study (2-3). The occurence of partial decomposition, color and molecular changes of pharmaceuticals have been reported (4-5).

Consequently, it deemed necessary to investigate the effects of radiation sterilization on the bactericidal activity as well as any radiation-induced changes in the physicochemical properties of a pharmaceutical under study.

This work reports the effects of gamma irradiation in the range of 5-25 kGy dose on the bactericidal activity and the physicochemical characters of cephradine (Fig. 1) in the solid dry state.

FIGURE 1
Chemical Structure of Cephradine

MATERIALS AND METHODS

Chemicals

Pharmaceutical grades of cephradine (I), cephalexin (II) and amoxycillin (III) were kindly donated by local Jordanian Pharmaceutical Manufacturers. Other chemicals and reagents were of analytical grade. The mobile phase for HPLC was

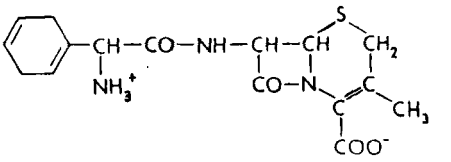

FIGURE 1

Chemical structure of cephradine

prepared by mixing 0.01 M sodium acetate solution, acetonitrile and methanol (HPLC grade) in a ratio 80: 10: 10 and adjusting the pH to 4.5 with glacial acetic acid. The mobile phase was filtered by passing it through a 0.45 u membrane filter (Millipore, Bedford, MA, USA) and thoroughly degassed before use.

Instruments

Spectroscopic analysis of irradiated and unirradiated samples were performed using UV/VIS spectrophotometer (Model UV 240), IR spectrophotometer (Shimadzu 435), and Bruker WP 80 pulse spectrometer (80 M Hz). HPLC analysis was carried out using a single piston pump (Model 114 M, Beckman) equipped with uv variable detector, an injector (100 ul) loop size and an integrator-plotter (SP 4270). The chromatographic separations were achieved using 5 u RP C-18 column.

Gamma Irradiation

Gamma irradiation of solid dry cephradine powder was conducted in aluminium foil utilizing the National Centre for Radiation and Research Technology (NCRRT) Industrial Co^{60}

facility, Egypt, manufactured by the Atomic Energy of Canada Limited, permitting a dose rate of about 2.5 Gys^{-1} at the time of the experiment. Cephradine samples were exposed to several radiation doses over the range of 5 to 25 KGy.

Procedures

HPLC assay

In separate disposable 1-ml culture tubes, different aliquots of the working standards (100 ug ml^{-1}) 100-400 ul (I), or 25-150 ul (II) were mixed with 60 ul of 1000 ug ml^{-1} (III). The solution in each tube was diluted to 1000 ul with the mobile phase and mixed on vortex mixer for 10 seconds. For irradiated cephradine samples, an aliquot of 400 ul was mixed with 60 ul of (III) and diluted to 1000 ul with the mobile phase and then mixed as previously mentioned. A 20 ul aliquot was injected onto the column and chromatographed under the forementioned chromatographic conditions.

Microbological assay

The agar diffusion assay for cephradine was performed using tryptone soya agar (Oxoid) and Staphylococcus aureus ATCC 29737 as recommended by USP XX1, (6). Seeded tryptone soya agar with S. aureus was poured into the plates and wells of 8 mm diameter were cut into the agar. The wells were individually filled with five dilutions, 5-25 ug ml^{-1}, of unirradiated and irradiated samples of cephradine in five replicates. Agar plates were incubated at 35°C for 24 hours and the resultant inhibition zones for the tested samples were plotted semilogarithmically. The relative potency of each irradiated sample was then calculated in comparison with the unirradiated cepharadine using two by two assay procedure (7).

RESULTS AND DISCUSSION

The bactericidal effect of cephradine samples in the solid state, irradiated with different doses of gamma rays in the range 5-25 kGy, on S. aureus ATCC 29737 was investigated. As shown in Table 1, a remarkable dose-dependent decrease in the bactericidal potency of irradiated cephradine was observed.

TABLE 1

Relative Bactericidal Potency of Gamma Irradiated Cephradine Samples Using Agar Diffusion Assay. *

Irradiation dose (kGy)	Relative potency ± S.D.
00	100 ± 00.00
05	093 ± 07.90
10	091 ± 05.52
15	086 ± 13.63
25	083 ± 08.72

* Average of five determinations.

In order to investigate whether the decrease in bactericidal activity of irradiated cephradine is accompanied by changes in the physicochemical properties of the antibiotic, irradiated and unirradiated cephradine samples were analyzed spectrophotometrically in the UV/VIS and IR regions of the spectrum. The examined spectra of the irradiated samples exhibited no differences from those of unirradiated samples even at high irradiation doses. However, the measured UV/VIS spectra at high concentrations (1 mg ml^{-1}) displayed a significant dose-dependent increase

in the intensity of the yellow color at 335 nm for the irradiated samples.

The radiation-induced increase in the color intensity may be attributed to a partial oxidation of the 1,3-cyclohexadiene ring in the side chain moiety of cephradine molecule to benzoquinone derivatives. This postulation was further confirmed by the appearance of a similar broad peak in the UV/VIS spectrum of, p-benzoquinone, but with slight shift towards a shorter wavelength range.

To further investigate the possible radiation-induced changes in the physicochemical properties of irradiated cephradine, analysis of samples using ^{1}H-NMR was carried out. The proton magnetic resonance spectra of unirradiated and irradiated (25 kGy) cephradine in D_2O were shown in Fig. 2 a, b. The olefinic protons of 1,3-cyclohexadiene ring at 3,4 positions splitted as double doublets (AB system) in the range 4.87-5.54 ppm. The small signal at 7.51 ppm was attributed to the aromatic protons and appeared in the spectrum as a result of original existance of cephalexin traces in the unirradiated cephradine samples and/or radiation-induced conversion into cephalexin in the irradiated samples. At the same time, the AB splitting of 1,3-cyclohexadiene protons at 3,4 positions was partially decreased due to ring aromatization. The chemical shifts of the remaining protons exhibited no changes in the irradiated and unirradiated samples. ^{1}H-NMR data clearly showed a dose-dependent conversion of cephradine into cephalexin.

In an attempt to confirm the ^{1}H-NMR results and to elucidate further radiation-induced changes in the physicochemical properties of cephradine, high performance

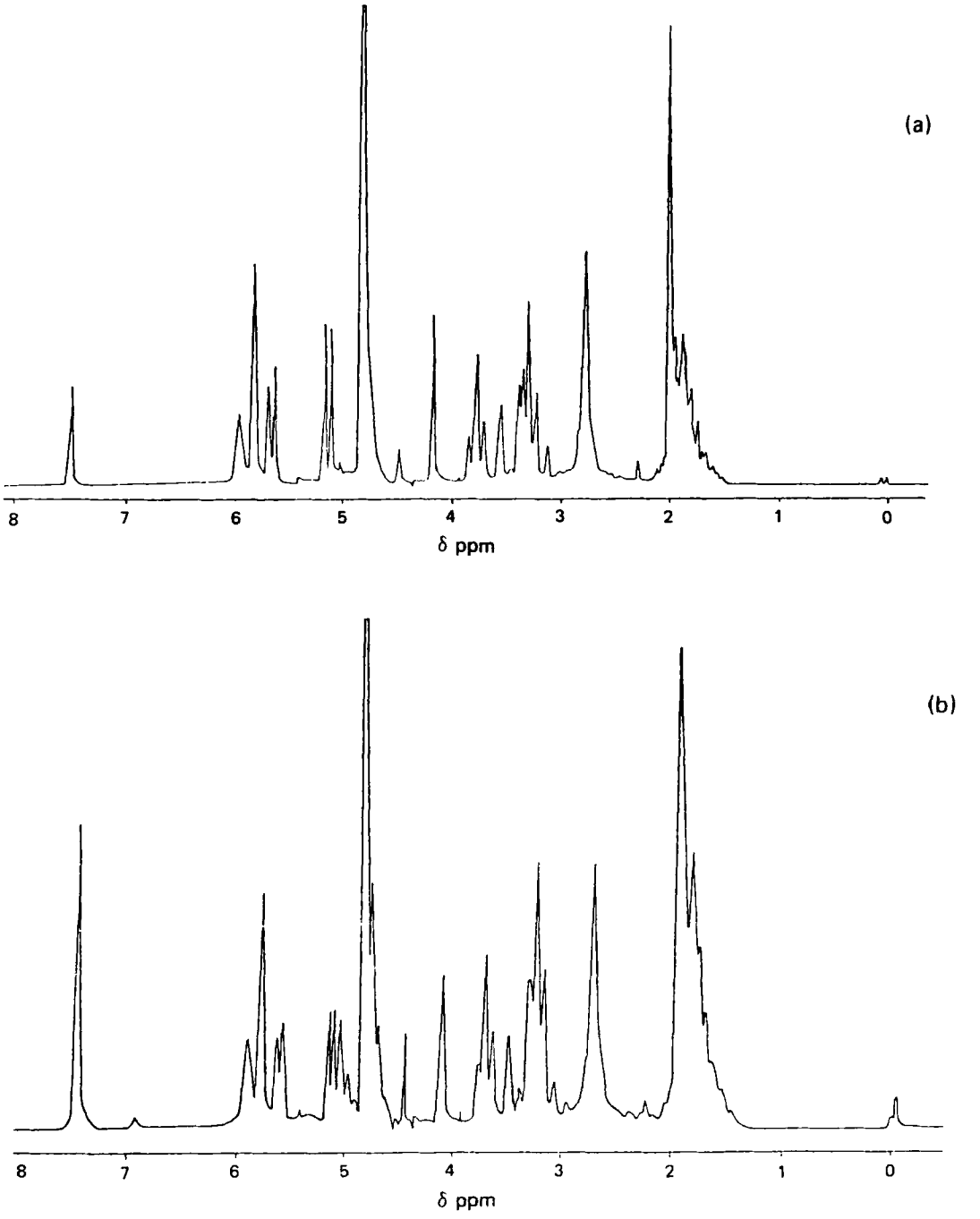

FIGURE 2

^{1}H-NMR spectra of (a) unirradiated cephradine and (b) irradiated cephradine (25 kGy).

liquid chromatographic analysis (HPLC) was adopted. Preliminary studies were conducted to select the most suitable chromatographic conditions. A mobile phase consisting of 0.01M sodium acetate solution, acetonitrile and methanol in a ratio of 80: 10: 10 with a pH of 4.5 gave optimum resolution of cephradine (Rt 5.9 min) and the internal standard amoxycillin (Rt 2.0 min).

Injection of unirradiated or irradiated cephradine samples onto the HPLC column showed a well-defined peak at Rt 4.5 min. This peak was proposed to be due to cephalexin originally present in the unirradiated cephradine or as a result of conversion of cephradine into cephalexin in the irradiated state. The intensity of this peak was significantly noticeable in the irradiated powder. Cephalexin samples injected onto the column under similar chromatographic conditions showed a single peak at the same retention time. The results were assured by standard addition of a known concentration of standard cephalexin solution to the unirradiated cephradine, where a significant increase in the cephalexin peak at Rt 4.5 min was observed. (Fig. 3 a,b). Furthermore HPLC chromatogram of a 100 ul aliquot of standard irradiated cephradine samples exhibited a slightly developed peak at Rt 2.3 min which was assumed to be due to radiation-induced degradation product. Such a peak was not noticed in unirradiated cephradine samples (Fig 4 a,b). In addition, the HPLC chromatogram of irradiated cephradine samples showed a dose-dependent decrease in the intensity of cephradine peak accompanied with a dose-dependent increase in the intensity of cephalexin peak.

Quantification of the remaining cephradine and the radiation-induced cephalexin in the various irradiated samples, was achieved using the peak height ratio of each

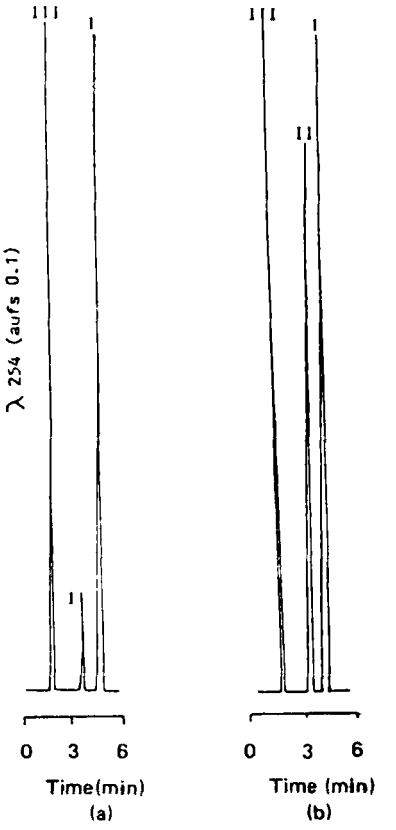

FIGURE 3

HPLC chromatogram of 20 ul aliquot of (a) unirradiated cephradine (I) 40 ug ml^{-1} and (b) a mixture of cephradine (I) 40 ug ml^{-1} and cephalexin (II) 12.5 ug ml^{-1}.

antibiotic to the internal standard (amoxycillin). Standard curves for cephradine and cephalexin were constructed over the range of 10-40 ug ml^{-1} and 5-15 ug ml^{-1} respectively. Linear regression analysis gave the following linear equations:

$$Y = 0.103 + 0.022\ X \quad \text{(Cephradine)}$$

$$(r = 0.9917)$$

$$Y = 0.012 + 0.054\ X \quad \text{(Cephalexin)}$$

$$(r = 0.9928)$$

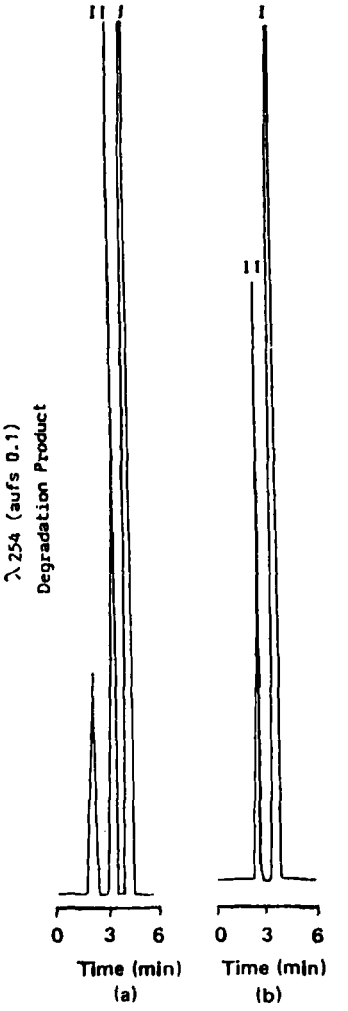

FIGURE 4

HPLC chromatogram of 100 ul aliquot of (a) irradiated cephradine (I,25 kGy) 100 ug ml^{-1} and (b) unirradiated cephradine (I) 100 ug ml^{-1}.

Recovery studies were carried out to determine the percentages of cephradine remaining at various radiation doses. The obtained results were sumarized in Table 2. As indicated from the Table, the loss in cephradine content was

TABLES 2

Recovery Studies of Cephradine Remaining and Cephalexin Originally Present and or Radiation - Induced at Various Gamma Ray Doses Using HPLC.*

Dose (kGy)	% of Cephradine remaining ± S.D.	% of Cephalexin originally present and/or radiation-induced ± S.D.
00	95.56 ± 0.63	04.63 ± 0.95
05	81.21 ± 3.72	08.22 ± 0.58
10	67.60 ± 1.83	08.17 ± 0.20
15	60.20 ± 1.20	09.20 ± 0.06
25	55.47 ± 1.37	10.39 ± 0.02

* **Average of five determinations.**

not only due to radiation-induction of cephalexin, but also due to cephradine degradation. It is obvious that for cephradine samples irradiated with a dose of 25 kGy, about 40% loss in cephradine content is observed. About 6% of this loss is due to cephradine conversion into cephalexin.

Accordingly, a mechanism for cephradine degradation may be postulated on the basis of initial oxidation of hydrated cephradine to the intermediate, cephalexin, under the influence of both direct irradiation by gamma photons and indirect irradiation, mediated by water-induced free radicals. This intermediate is subsequently partially oxidized into benzoquinone derivatives.

Comparing the HPLC results with the microbiological data (Table 1,2), it is apparent that the percentage decrease in the bactericidal activity of cephradine is less than that as

determined by HPLC assay. This may be attributed to both cephradine conversion to cephalexin which has very similar antibacterial activity as cephradine (8) and the possible bactericidal activity of the other radiolytic degradation products.

Research is going on to separate and identify the radiation-induced radiolytic products using preparative HPLC. Further studies to test the physicochemical, bactericidal, and toxic properties of these products seem fruitful.

The results obtained so far suggest that radiosterilization of cephradine in the solid dry state may not prove to be technically practicable especially at high doses where losses in the bactericidal activity and the antibiotic content are deleterious.

Acknowledgements

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