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GAMMA RADIATION INDUCED EFFECTS ON CEFUROXIME AND CEFOTAXIME. INVESTIGATION ON DEGRADATION AND SYN-ANTI ISOMERIZATION

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ABSTRACT

The effects of gamma irradiation on cefuroxime and cefotaxime were studied. Degradation products and long-lived free radicals were investigated as a function of radiation dose. The formation of new impurities was evidenced by HPLC in both the compounds. A valuable increase of the pre-existent impurities, anti-isomer included, was found only in cefuroxime. ESR measurements put in evidence the presence of gamma radiation induced long-lived free radicals. Moreover, the influence of gamma irradiation on syn-anti isomerization was evaluated in comparison with the photoisomerization induced by ultraviolet light exposition on powder samples.

INTRODUCTION

In recent years, gamma irradiation has been frequently used for sterilization of pharmaceutical products. Ionizing radiation induced effects on the drug structure and physicochemical characteristics have been investigated (1-5). On this subject, our attention was focused on cephalosporins due to their susceptibility to degradation and likely sensitiveness to irradiation. A previous study on cephradine put in evidence the formation of foreign substances and long-lived free radicals, demonstrating the unfeasibility of the irradiation as sterilization method for this substance (6). On the line of that work, the present study is concerned with the effects of gamma irradiation on two oximino cephalosporins that is cefuroxime and cefotaxime sodium salts. Changes in structure, decrease in purity, increase in degradation products and production of free radicals were investigated.

The oximino cephalosporinic antibiotics may exist in two isomeric forms that are syn (Z) and anti (E) isomers. The active form has the syn configuration of the oximino group (7,8) that confers a greater resistance to the attack of β-lactamases secreted by the bacteria. Syn-anti α-oxo-oximes isomerization by ultraviolet (UV) light on their solutions has been described (9,10); the presence of the OC-C=N-O- moiety suggests a possible electronic excitation with radiation of $\lambda > 300$ nm (11). On this basis, it seemed interesting to investigate also eventual gamma radiation induced syn-anti isomerization and verify the above mentioned hypothesis (11), on the solid phase. With this aim, gamma and ultraviolet radiation effects on cefuroxime and cefotaxime, irradiated in solid phase, were compared.

MATERIALS

Syn (Z) and anti (E) cefuroxime sodium salts (sodium 3-[[(aminocarbonyl)oxy] methyl] -7-[[2-furanyl(methoxyimino)-acetyl] amino] -8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2--carboxylate) (I) (Fig.1) of pharmaceutical grade were kindly supplied by Firma, Firenze, Italy.

Syn (Z) and anti (E) cefotaxime sodium salts (sodium 3-[(acetyloxy)methyl]-7-[[(2-amino-4-thiazolyl)(methoxyimino)-acetyl] amino] -8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylate) (II) (Fig.1) of pharmaceutical grade and its related compounds were kindly supplied by Hoechst, Scoppito (L'Aquila), Italy.

Reagents for liquid chromatography were: HPLC-grade methanol (from Baker, Deventer, Holland), potassium dihydrogen phosphate (from BDH Limited Pole England), di-sodium hydrogen phosphate-12-hydrate (from Riedel-De Haën Seelze, Hannover) and water filtered through a 0.45 μm Nylon-66 membrane on a Millipore Milli-Q device.

METHODS

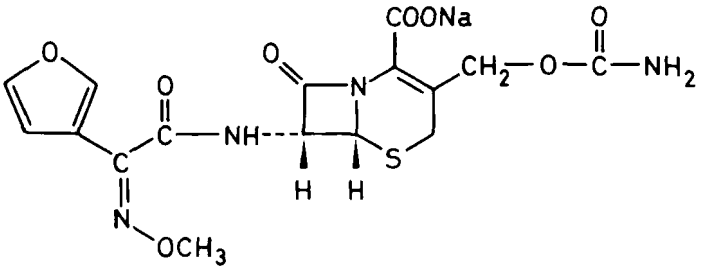
Sample Preparation

For gamma irradiation, powder samples I and II were put into glass (type I) tubes that were closed, in presence of air, with chlorobutyl closures.

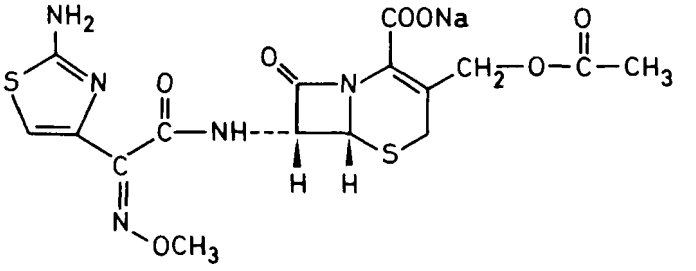
A thin layer of powder samples I and II, put between two quartz glasses (1 cm^2 surface) was irradiated with UV light. Solid samples, instead of liquid were used in order to compare the results from gamma and UV irradiation.

Irradiation

Gamma irradiation was performed at room temperature at the doses of 10, 25 and 50 KGy (samples G_{10-50}).



(I)



(II)

FIGURE 1
Chemical structure of syn-cefuroxime (I) and syn-cefotaxime (II) sodium salts.

A cobalt-60 plant, operating at the "Istituto Superiore di Sanità" (Roma, Italy), was utilized. The dose rate at sample location was 0.3 Gy/s with an uncertainty of about ±3%.

Ultraviolet irradiation was performed at room temperature with monochromatic light at 365 nm (±5) by using an Applied Photophysic photoirradiator equipped with a high pressure Xe, 2000 W lamp. Incident power was about 180 mW/m^2. The samples

were placed at 1 cm from the apparatus. The irradiation time was in the 15-60 minutes range.

Experimental Techniques

High performance liquid chromatography (HPLC) and electron spin resonance (ESR) spectroscopy were utilized for the evaluation of the radiolytic effects.

HPLC analyses were performed with an HP-1050 pumping system, an HP-1040A diode array detector (DAD) equipped with an HP-9000-300 computing integrator and a Rheodyne 7125 injection valve (20/ul loop). A LiChrospher 100 RP-18 , 5/um column, 125x4.0 mm (Merck, Darmstadt FRG) was utilized. A modified method from literature (14) was set up. The mobile phase for the isocratic elution was: pH 6.5 phosphate buffer-methanol (80:20 v/v). The phosphate buffer was prepared dissolving 7 g of potassium dihydrogen phosphate and 23.3 g of disodium hydrogen phosphate-12-hydrate in 2000 ml of water. The measurements were performed at room temperature, at a flow rate of 1.0 ml/min at 274 or 235 nm (absorption maxima of cefuroxime and cefotaxime respectively). A weighed amount of each sample was dissolved in the mobile phase in order to obtain 20/ug in the injection volume (20/ul). After the preparation, the solutions were immediately injected. I and II anti-isomer solutions were also prepared in the same way and properly diluted for identification purpose.

ESR measurements were performed at room temperature with a X band Bruker ESP 300 spectrometer equipped with a standard TE102 rectangular microwave cavity. Each sample was inserted on a quartz tube, completely filled up in order to make results independent on mass. The following parameter setting for the ESR signal detection was used: 2 gauss modulation amplitude, 0.6 mT/s scan rate, 80 ms time constant, 0.5 mW microwave power.

RESULTS AND DISCUSSION

The comparison between chromatographic profiles of unirradiated and gamma irradiated cefuroxime and cefotaxime showed a higher impurity level in both the irradiated compounds and, in a more marked way, for sample I. Mostly, new degradation products, not present in the unirradiated samples, were found. A radiation dose-dependent increase in the impurity level was observed for both the substances (Figures 2,3).

As concerns cefuroxime (Fig. 2), the chromatografic profile of the irradiated sample showed a negligible dose-dependent increase in the level of the originally present impurities, except for the anti-isomer compound that showed a high percent increase. Nevertheless, its absolute amount was very low, considering the negligible original quantity in the unirradiated sample (see next Table I). Moreover, four new unknown foreign substances were found in significant amounts (a_I,b_I,c_I,d_I; r.r.t. 0.23, 0.69, 1.18, 1.35, respectively; cefuroxime: r.t. 5.27). Their different ultraviolet spectra are reported in the inset of Figure 2.

As concerns cefotaxime (Fig. 3), the comparison between chromatographic profiles of irradiated and unirradiated samples evidenced minor differences than those described for cefuroxime. In particular, even if three new impurities (a_{II},b_{II},c_{II}) were observed, only one of them (a_{II}, r.r.t. 0.52, max absorption 256 nm; cefotaxime r.t. 5.71) was found in a valuable amount; the other two were present in traces. The pre-existent impurities (nos. 1-7), five of which (1,2,3,6,7) were identified, (see figure caption), did not show a significant increase with dose. In addition, the cefotaxime anti-isomer amount remained almost

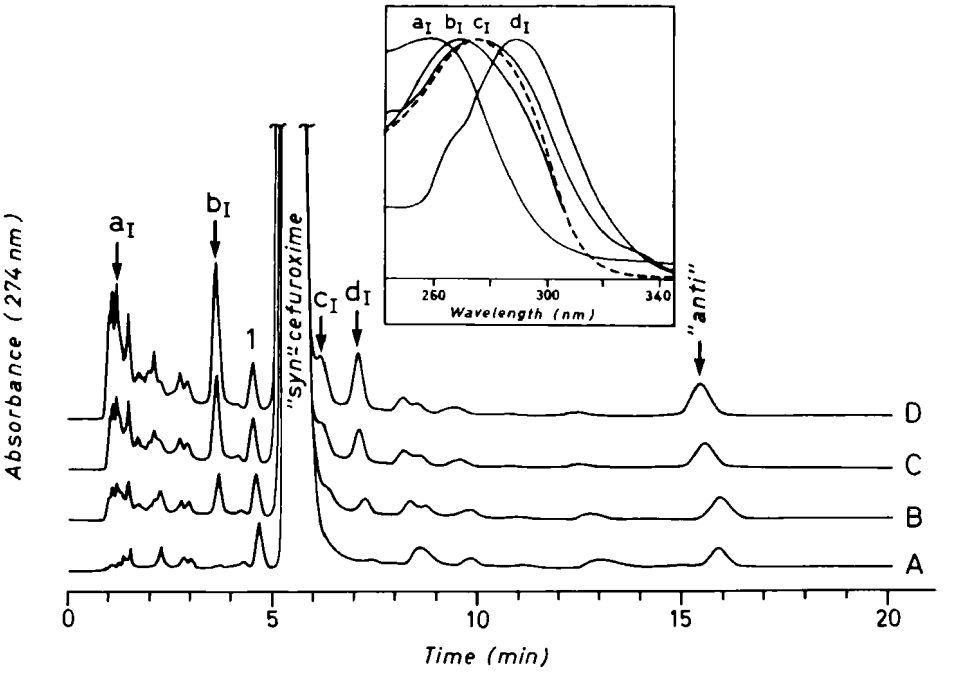


FIGURE 2
Cefuroxime degradation after gamma irradiation. HPLC analysis at 274 nm; sensitivity: 0.05 AUFS. A: unirradiated sample; B, C, D: 10,25,50 KGy irradiated samples respectively; a_I, b_I, c_I, d_I: gamma radiation induced impurities; 1: unknown pre-existent impurity.
Inset: U.V. spectrophotometric profiles (DAD) of cefuroxime (---) and a_I,b_I, c_I,d_I impurities (——).

constant even at the highest radiation dose (50 KGy), in contrast with that found for cefuroxime.

In the assumption that Z/E isomerization is favoured by conjugative intra-molecular interaction (10), the difference in the behaviour of the two substances (I,II) could be an evidence that the 2-aminothiazol-4-yl ring is less conjugated to the methoxyimino group than the furane ring.

The HPLC quantitative data related to cefuroxime, cefotaxime and radiation induced impurities are reported

TABLE 1

Syn-Cefuroxime and -Cefotaxime Decrease and their Impurity Increase in Gamma Irradiated Samples, at Different Doses. HPLC: 274 nm and 235 nm respectively.

Sample	% Content (•) (C.V.) (Sodium salt)										
	Cefuroxime (°)						Cefotaxime (°)				
	Syn	Impurities					Syn	Impurities			
		anti	a_I	b_I	c_I	d_I		anti	a_{II}	b_{II}	c_{II}
Unirradiated	98.71 (0.75)	0.19 (1.35)	-	-	-	-	96.95 (0.59)	-	-	-	-
G_{10}	98.02 (1.01)	0.24 (1.68)	0.02 (2.01)	0.22 (1.32)	0.07 (1.91)	0.16 (1.34)	96.61 (0.88)	-	0.18 (1.71)	traces	traces
G_{25}	96.67 (0.89)	0.29 (1.42)	0.05 (1.81)	0.44 (1.15)	0.15 (1.68)	0.30 (1.52)	95.85 (0.71)	traces	0.35 (1.62)	0.05 (2.02)	0.10 (1.85)
G_{50}	95.36 (0.93)	0.36 (1.39)	0.09 (1.75)	0.70 (1.45)	0.21 (1.71)	0.47 (1.61)	94.09 (0.81)	0.09 (1.98)	0.62 (1.35)	0.12 (1.41)	0.29 (1.33)

(•) mean of five replications
(°) U.S.P. and Eur. Ph. limits: 90-105% (12) and 96-101% (13), respectively

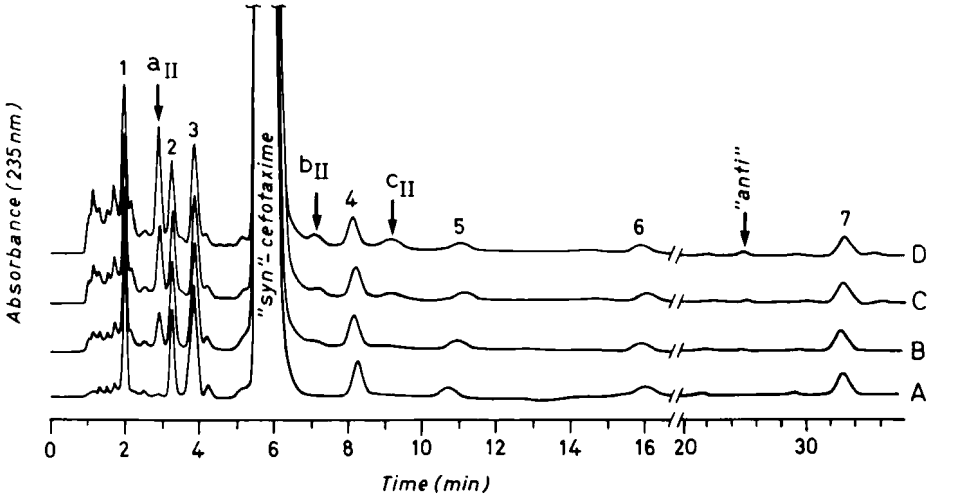


FIGURE 3
Cefotaxime degradation after gamma irradiation. HPLC analysis at 235 nm; sensitivity: 0.05 AUFS. A: unirradiated sample; B,C,D: 10,25,50 KGy irradiated samples respectively; a_{II}, b_{II}, c_{II},: gamma radiation induced impurities; 1: desacetyl--cefotaxime, 2: desacetoxy-cefotaxime, 3: cefotaxime-lacton, 4: unknown impurity, 5: unknown impurity, 6: N-formyl-cefotaxime, 7: cefotaxime dimer.

in Table I for different doses. The negligible and not significant content variations of the pre-existent impurities are not reported in the table. As it is shown, the cefuroxime and cefotaxime content decreased of about 3% at 50 KGy. At the dose usually adopted for sterilization purposes (25 KGy) the cefuroxime and cefotaxime decreased of 2% and 1%, respectively.

The effects of ultraviolet rays in relation to isomeric transformation are evident in Figures 4 and 5 for samples I and II, respectively. They show the chromatographic profiles at different irradiation times (15, 30 and 60 minutes) of samples I and II, irradiated at 365 nm, indicated

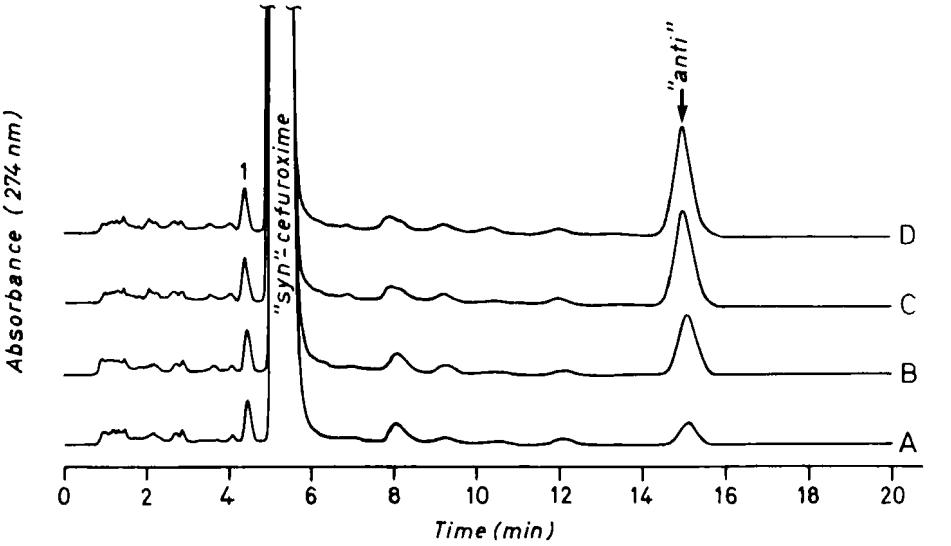


FIGURE 4
Cefuroxime syn-anti isomerization after ultraviolet (365 nm) irradiation. HPLC analysis at 274 nm; sensitivity: 0.05 AUFS. A: unirradiated sample; B,C,D: 15',30',60' irradiated samples respectively; 1: unknown pre-existent impurity.

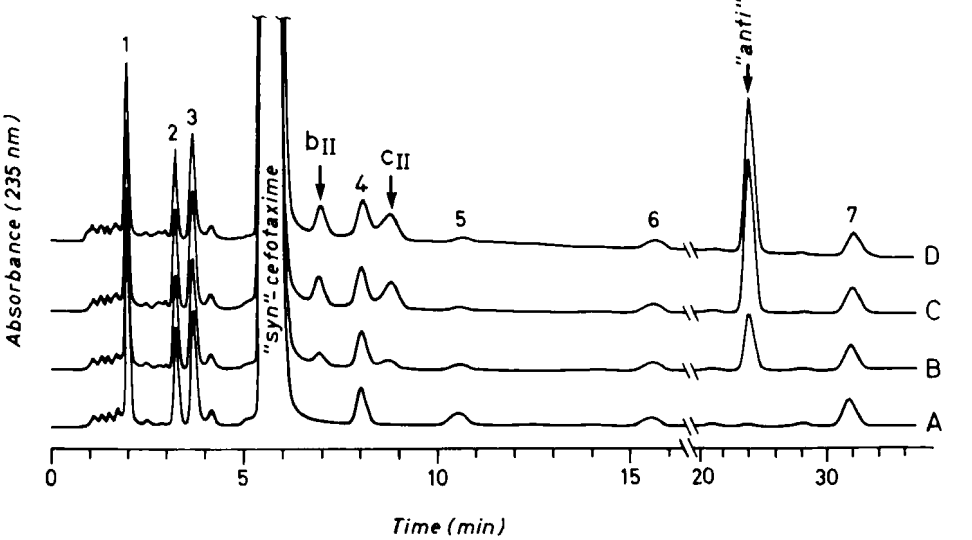


FIGURE 5
Cefotaxime syn-anti isomerization after ultraviolet (365 nm) irradiation. HPLC analysis at 235 nm; sensitivity: 0.05 AUFS. A: unirradiated sample; B,C,D: 15',30',60' irradiated samples respectively; b_{II},c_{II}: ultraviolet radiation induced impurities; 1: desacetyl-cefotaxime, 2: desacetoxy-cefotaxime, 3: cefotaxime-lacton, 4: unknown impurity, 5: unknown impurity, 6: N-formyl-cefotaxime, 7: cefotaxime dimer.

in the literature as the most effective wavelength for the isomerization (11).

As concerns cefuroxime, a noticeable amount of anti--isomer compound was found even after a short U.V. exposition (15'). Its amount remarkably increased during irradiation time. The isomerization occurred in a more evident way than that observed for the gamma irradiated sample. On the contrary, no new degradation impurity was observed (Fig. 4).

Also in the case of ultraviolet irradiated cefotaxime a high quantity of anti-isomer was found (Fig. 5). In addition, two impurities (b_{II}, c_{II}) present in a small amount also in gamma irradiated compound, were found in a considerable quantity.

The HPLC data related to I and II ultraviolet irradiated compounds have not to be considered quantitative, but only indicative of the trend. In fact, due to the low ultraviolet ray penetrating power, the solid samples did not result homogeneously irradiated, even if the layer of the powder was thin. Within the limits of the used experimental approach, the anti-isomer amount in the two irradiated samples approximatively ranged from 0.5-1% to 1-3%, after irradiation times of 15' and 60' respectively. Considering the above mentioned low reproducibility of the data, the different results found for the two substances cannot be certainly ascribed to their different behaviour.

The presence of gamma radiation induced free radicals was put in evidence by ESR measurements.

No paramagnetic centers were detected in unirradiated cefuroxime and cefotaxime powder while both the samples presented ESR signals after gamma irradiation. In the former (Fig. 6) the signal is a superposition of a singlet and a multiplet, mostly hidden by the singlet. Their different dependence on the microwave power led to the hypothesis that

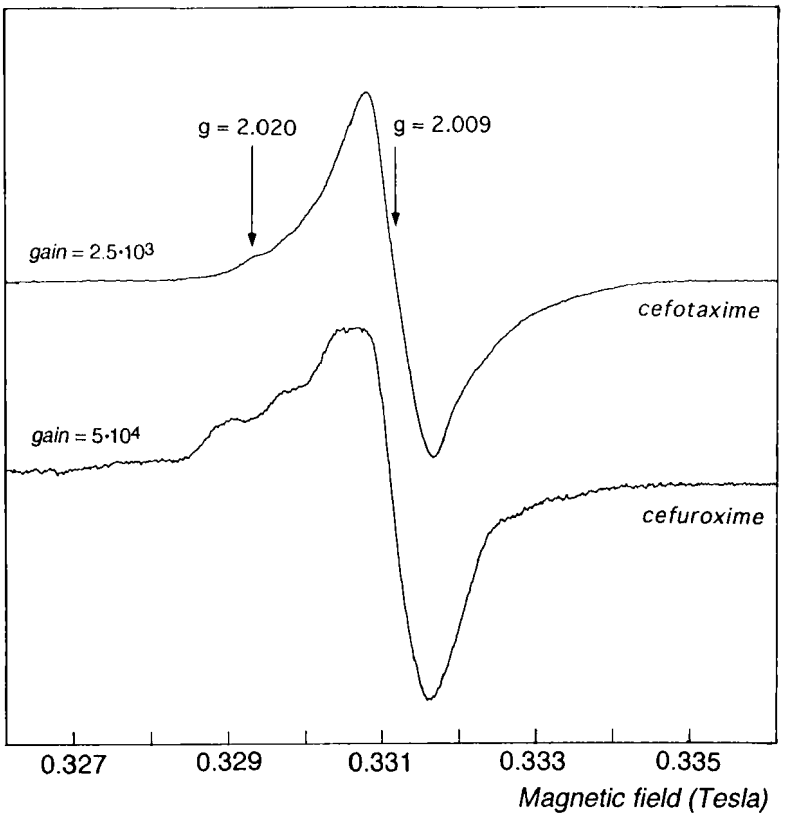


FIGURE 6
ESR spectra of cefuroxime and cefotaxime powders irradiated to 50 kGy.

the two signals are probably originated from two different paramagnetic centers. Cefotaxime sample presented a single line signal (Fig. 6), whose amplitude was about 20 times higher than the one obtained in the cefuroxime irradiated at the same dose. In the g = 2.020 region a shoulder can be seen, suggesting the presence of a different signal that from a more detailed analysis resulted to be a triplet.

The triplet and the singlet lines could be originated by nitrogen centered and carbon centered

paramagnetic radicals, respectively. This hypothesis might be consistent with the interaction of the unpaired electron with the nitrogen or carbon atom nucleus following the cleavage of bonds N-OCH_3 (triplet) and CO-NH_2 (singlet, for cefuroxime) or CO-CH_3 (singlet, for cefotaxime). The difference in the intensity ratio of the singlet and the triplet between the two samples can be attributed to the different energy of CO-NH_2 and CO-CH_3 bonds.

The concentration of radiation induced free radicals can be evaluated by double integration of ESR spectra. In both the products the overall free radical concentration was in the approximate 10^{17}-10^{18} spin/g range, undetectable by HPLC.

The dose effect study did not put in evidence any variation in the shape of the signal but in the amplitude of the most intense central peak, which was considered as the important parameter for dose evaluation. Its variation with dose was linear in the 10-50 kGy range (Fig. 7).

The fading study showed a very different decay rate for the two substances. The radiation induced free radicals are more stable in cefotaxime than in cefuroxime. Indeed after 150 days of storage at 4°C and darkness, the amplitude of the main peak faded of about 30% for cefotaxime and 70% for cefuroxime. Moreover, after about 90 days the cefuroxime signal reached a plateau while the cefotaxime signal was still decreasing. Fading did not show any dose dependence.

CONCLUSIONS

Gamma and UV irradiation affected the degradation process of both cefuroxime and cefotaxime. The main radiation induced effects are here summarized.

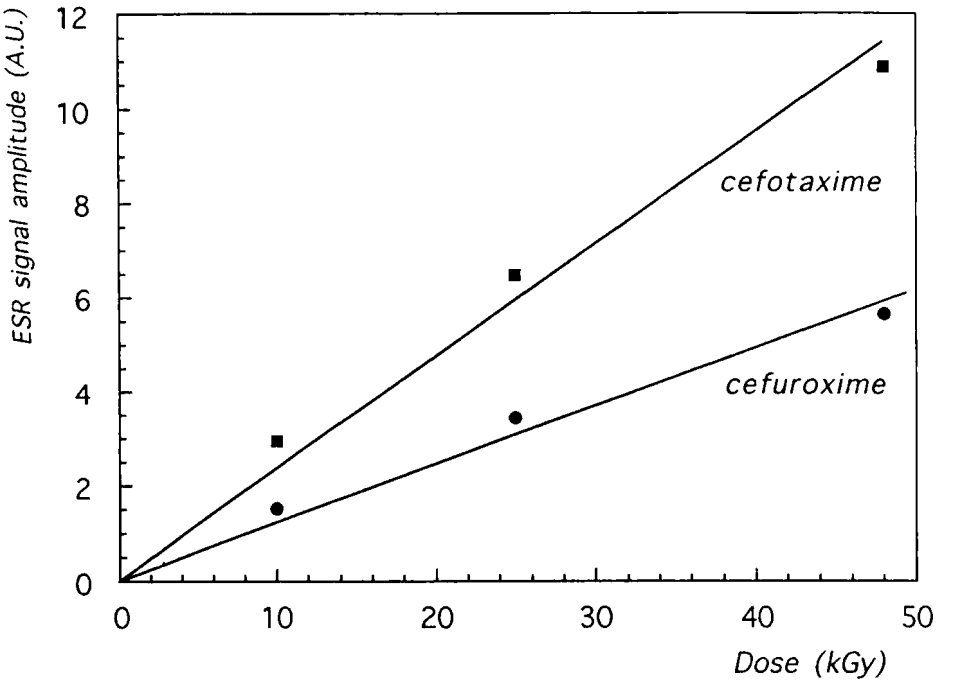


FIGURE 7
Dose-effect behaviour of cefuroxime and cefotaxime. The amplitude of the most intense peak (g = 2.009) was considered as the dose dependent parameter. The straight lines were obtained by a linear regression analysis.

a) Cefuroxime was sensitive to gamma radiation as concerns the formation of new impurities and the increase of the pre-existent foreign substances. In particular, a high per-cent increase in the anti-isomer compound was found. In gamma irradiated cefotaxime no valuable variation of the pre-existent impurities, anti-isomer included, was put in evidence, while new degradation products, even if in a small amount, were found. The observed degradative processes depended on radiation dose.

b) ESR measurements put in evidence the presence of gamma

radiation induced long-lived free radicals in both the substances.

c) UV light was more effective than gamma radiation in inducing syn-anti isomerization of the two products.

At the usually adopted dose for sterilization purposes (25 KGy), the title of the two irradiated substances (~96%) fell within the prescribed official limits. Nevertheless, the presence of unknown radiation induced impurities and free radicals gives rise to doubts on their safety.

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