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Research Article

Electron Spin Resonance (ESR) Investigation of Gamma-Irradiated Antibiotic Azithromycin

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ABSTRACT
The use of ionizing radiation for sterilization of pharmaceutical is now a well established technology. Electron spin resonance (ESR) spectroscopy was used to investigate the degradation Gamma- damage in the microcrystalline powder from antibiotic Azithroomycin (AZ) before and after exposure to 5 up to 25 KGy absorbed dose. ESR measurements proved various stable paramagnetic species after irradiation and relative yielding of free radicals depends on the absorbed gamma dose. The thermal behavior and their assignments of (AZ) were studies by using (DTA, TG) and Infra red. Some spectroscopic and thermal properties and suggestions concerning possible structure of the radicals are discussed in this paper.

Keywords: Azithromycim, Gamma-irradiation, ESR, DTA,TG

1. INTRODUCTION

Azithromycin is a 15-memberd antibiotic with a broad spectrum of activity that has been used in treating a wide spectrum of activity of infections caused by susceptible organisms [1]. It has high tissue affinity, a feature that is thought to be due to the presence of two basic tertiary amine groups. This attributed results in much greater tissue or secretion drug concentrations compared to the simultaneous plasma concentration [2].

Azithromycin is used to treat or prevent certain bacterial infections, most often those causing middle ear infections, strep throat, pneumonia, typhoid, and sinusitis. In recent years, it has been used primarily to prevent bacterial infections in infants and those with weaker immune systems. It is also effective against certain sexually transmitted infections, such as non-gonococcal urethritis, chlamydia, and cervicitis. Recent studies have indicated it also to be effective against late-onset asthma, but these findings are controversial and not widely accepted[2,3].Azithromycin is used to treat many different infections including acute otitis media, streptococcal pharyngitis, gastrointestinal infections such as traveler's diarrhea, respiratory tract infections such as pneumonia, cellulitis, babesiosis, bartonella, chancroid cholera, donovanosis, leptospirosis, lyme disease, malaria, mycobacterium avium complex, neisseria meningitis, pelvic inflammatory disease, pertussis, scrub typhus, toxoplasmosis, and salmonella[4]. It has a similar antimicrobial spectrum as erythromycin, but is more effective against certain Gram-negative bacteria, in particular, Haemophilus influenzae[citation needed]. Azithromycin resistance has been described [5] and is endemic in many areas. Long-term use in treating Staphylococcus aureus infections with azithromycin may increase bacterial resistance to this and other macrolide antibiotics [6].

The most commonly employed technique for the determination of azithromycin in biological matrixes has been based on HPLC using electrochemical detection [7, 8]. The studies of the effect of high-energy ionizing radiations (gamma and X-rays, electron beams) on the medical devices and drugs are increasing due to applications in the medical sterilization [9].

Stress testing of the drug substance by gamma-irradiation can help to identify the degradation product, which can help to identify the degradation pathways and the intrinsic stability of molecule. The main problem of damage of ionizing radiations on the drugs is the possibility of chemical and physical alterations, which can lead to loss of their biological activity [10]. Electron paramagnetic resonance (EPR) spectroscopy is a very sensitive method for the detection of free radicals and can be used for studying the radiolysis mechanism [11].

2. Experimental

Materials: The drug Azithromycin (Az) substances was commercial product suitable for clinical use. Az was kindly supplied in powder in purity form (Pfizer-Egypt company) with 99.9 % purity.

2.1. Irradiation facility

A group of samples of Az were irradiated with different absorbed dose ranging from 5 kGy up to 25 kGy using a Cs 137 source. The absorbed dose rate, 0.4 cGy/h was measured using farmer dosimeter (S.No.Ne 2571) under the optimum conditions of irradiation, at 30 cm from the source in air, at National Institute for Standard – Egypt

2.2. Electro spin resonance (ESR)

Electron spin resonance spectra were instrumentation recorded at room temperature with a (Bruker) spectrometer, with the following parameter (Centerfield 3480.43 G, Sweep width 200, 00 G, Resolution 1024 pints, Microwave frequency 9.79 GHZ, Power 19.97 mw with field modulation 100 KHZ, amplitude 4.00 G , equipped with a computer acquisition system.

2.3. Thermal analysis

TGA and DTA measurements are carried using shimadzu DT thermal analyzer with platinum crucible temperature programs was designed for AZ before and after gamma irradiation , different sample mass of AZ were used in the rising temperature runs at a heating rate 10 Co /min from 25-500Co.

2.4. IR absorption

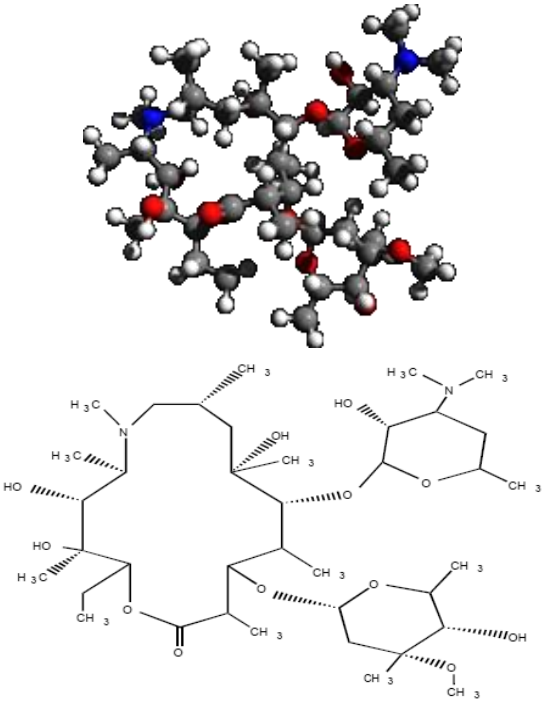
Are recorded using KBr pellet with Shimadzu (Japan) AZ is mixed with dry kBr in a 1:100 ratio before and after absorbed gamma dose. The mixture placed into dice and subjected to approximately 10000 psi of pressure for a period of time to produce a uniform and unbroken glass pellet.

3. RESULTS AND DISCUSSION

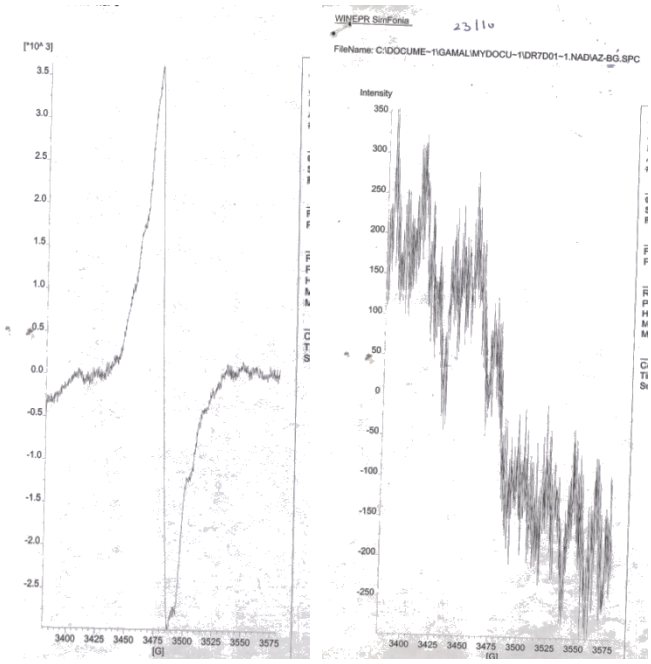
The radiostreilization however has the following two problems first gamma irradiation producer's new radioyltic products at a very low concentration in drugs. To prove the softy radiostreilization it is important to determine the radiolytic products and elucidate the mechanism of radiolysis of drugs. Second the regulations of radiostreilization of drugs are different among countries.

Thus it is desirable to establish a method to discriminate between irradiated and non-irradiated drugs and evaluate this dose of irradiation. Because electron spin resonance (ESR) is a very sensitive method or detection of radicals, ESR measurement can be used for detection and discrimination of irradiated drugs from non-irradiated ones [12].

From (Fig -1a) for non-irradiated and stress-tested polycrystalline samples Azitheromyc(AZ) exhibits very weak signal showing the presence of detectable amount of free radicals ,probably due to sunlight radiation and stress condition [13].

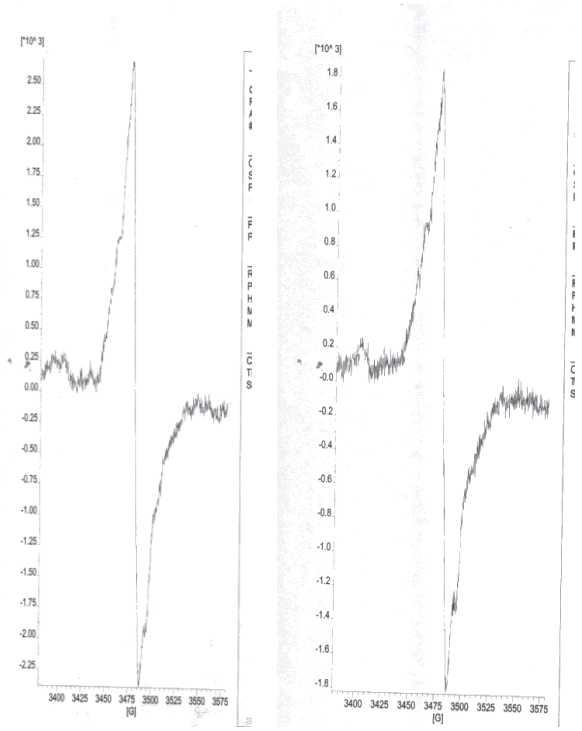


Scheme (1) the structure of Azithromycin



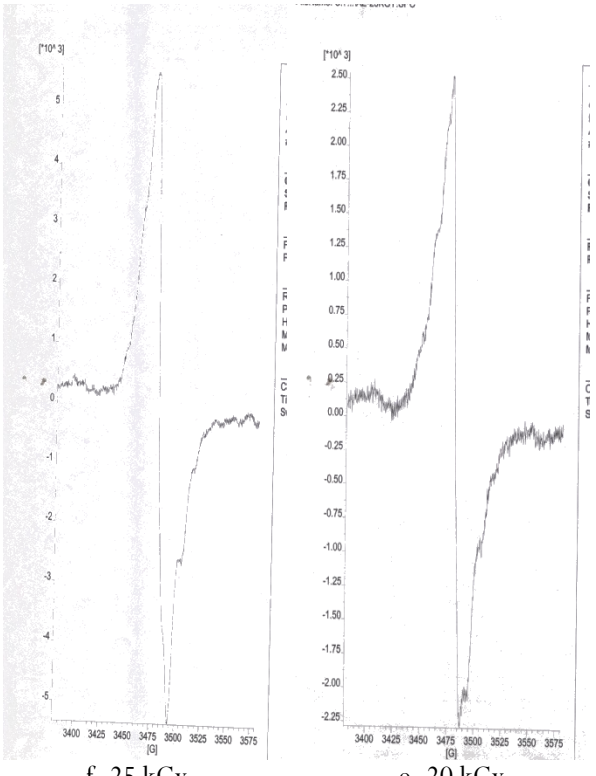
b-5kGy a-0kGy

Fig.1 ESR signal for 0 and 5 kGY γ- absorbed dose of Azithromy cin



d-15 kGy c- 10 kGy

Fig. 2 ESR signal for 10 and 15 kGY γ- absorbed dose of Azithromycin



f- 25 kGy e- 20 kGy

Fig.3 ESR signal for 20 and 25 kGY γ- absorbed dose of Azithromycin

Atypical ESR powder spectrum after irradiation at 5up to25 kGy is presented in (Fig.1b-3f) using the accumulation scans method. The presence of hyperfine structure of ESR spectra of Gamma irradiation (AZ) is due to its more complex chemical structure.

In general the complex molecular structure in the stress condition changed its conformational structure. In case of (AZ), the most probable change is due to reorientation of dim thyl amine group versus (C5H5O) and breaking the bond between carbon and nitrogen. The fact that by increasing the irradiation due the different ESR lines do not very the same way prove the presence in the irradiated sample, simulate easily by of radicals with different magnetic parameters. In order to clarify how the paramagnetic centers responsible for the observed structure are generated by the damaging radiation, we investigated the dependence of their intensity, or the integral of the ESR absorption spectrum is proportional to the number of spins in the sample [14].

Samples of AZ irradiated to dose of 5,10,15,20 and 25 kGy were used to generate dose-response curve for the radicals associated with the integral ESR absorption spectrum.
In(Fig-4) the yields of paramagnetic centers has an exponential growth for low gamma doses up to 25 kGy and tend to an asymptotic value at higher doses (saturation kinetics). Dose-response curves, was obtained by best least squares fit, to the parameters given by the following relationships I(D)= 337.68+1221.748[1- exp 0.2402]

Within these dose limits,the relative integral of the ESR absorption spectra intensities of AZ i.e dose response parameters curves, was obtained by least squeres fit, to the parameters given by the following relationships:

$$I(D)= 337.68+1221.748[1- \exp 0.2402]$$

Where I is the double integral of ESR spectrum and D the absorbed dose in kGy $A(D)= A0+ Asat.[1-\exp(-KA)]$

In which A0 is the concentration of radicals of non-irradiated samples, Asat. the limiting value corresponding to the steady-state concentration of the radicals and K is the rate constant of destroying the radicals by the radiations.
A (D), A0 and Asat. (represent the relative integral of ESR absorption spectrum and D the irradiation dose).

Within these dose limits, the relative integral of the ESR absorption spectra intensities of (AZ) i.e dose response curves, was obtained by beast least squires fit, to the parameters given by the following relationships: I(D)= 337.68+1221.748(1-exp0.2402D),where I is the double integral of ESR spectrum and D the absorbed dose in kGy.

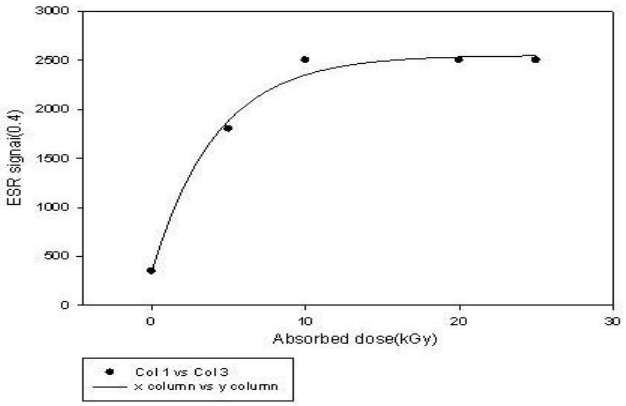


Fig.4 The relative yields of radicals in γ-irradiated AZ as a function of absorbed dose

3.1. Infrared absorption (IR)

(Fig.5) display the IR spectra of (AZ) before (0kGy) and after (5-25 kGy) absorbed dose. IR peaks of (AZ) before irradiation were assigned of follow 3600-3300 cm-1 (NH, OH) 3200-2800 (CH st.), 1800-1500 (C=O)

The results of IR spectra measurements indicate that no change or disappearance in the position of characteristics bonds. This indicates that the material still preserves its identity.

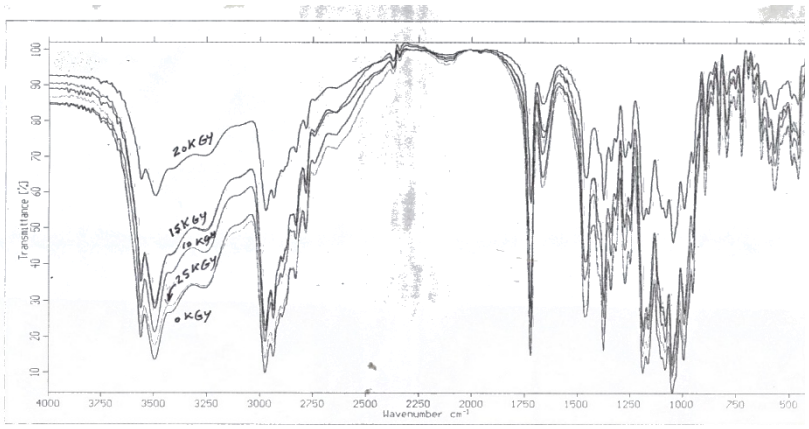


Fig: (5) IR-Spectra for unirradiated(0 kGY) and irradiated (5-25 kGy) Azithromycin

3.2. Thermal studies (TGA and DTA)

Fig. (6) and table(1) shows a collection of thermal analysis (TGA, DTA) of Az before(6-a) and after (b-f) absorbed gamma dose. Table (1) shows that some thermal parameters as melting point temperature, percentage of weight loss and heat enthalpy ΔH for (AZ), at different doss were measured. Fig (6a) shows that thermal behavior belong to the pure structure of (AZ) . After irradiation dose (5 up to 25 kGy) fig.(6b-f) it can be easily seen that the behavior of various gamma-irradiation sill preserve its identity, and gamma-irradiation stabilizes it with the same slight change into the shape of endothermic and exothermic due to some kind of lattice rearrangement.

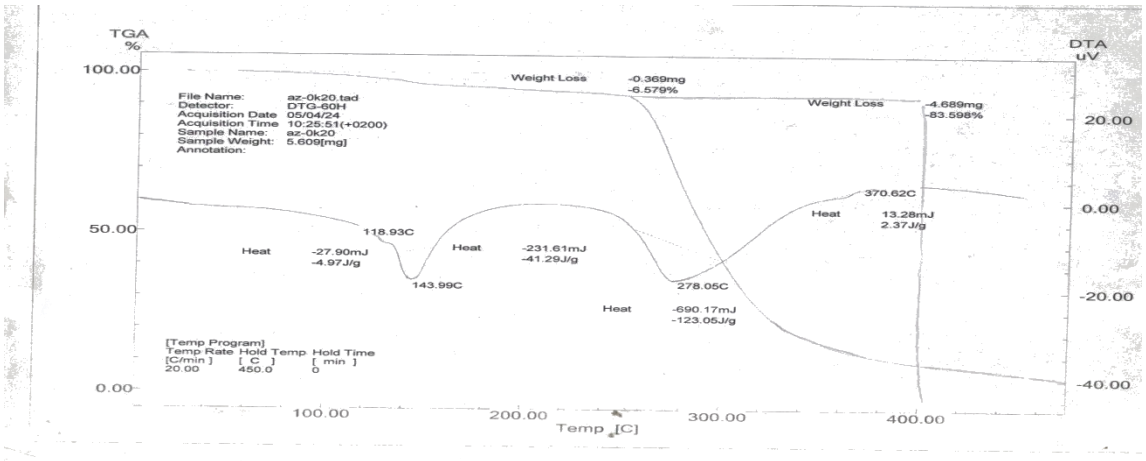


Fig: (6-a) Thermal analysis (DTA, TG) for unirradiated Azithromycin

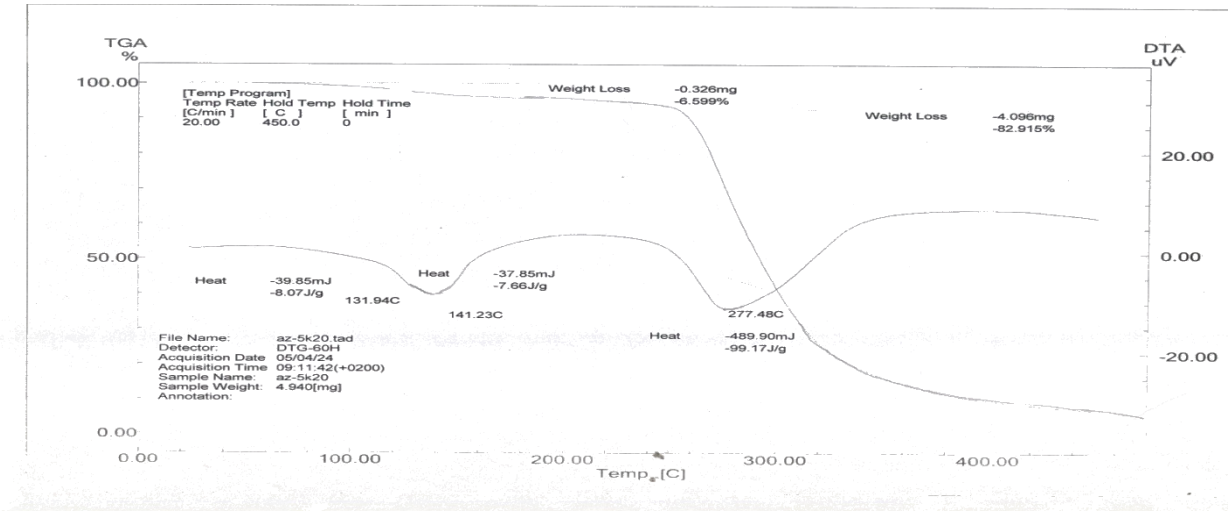


Fig: (6-b) Thermal analysis (DTA, TG) for 5kGy γ- irradiated Azithromycin

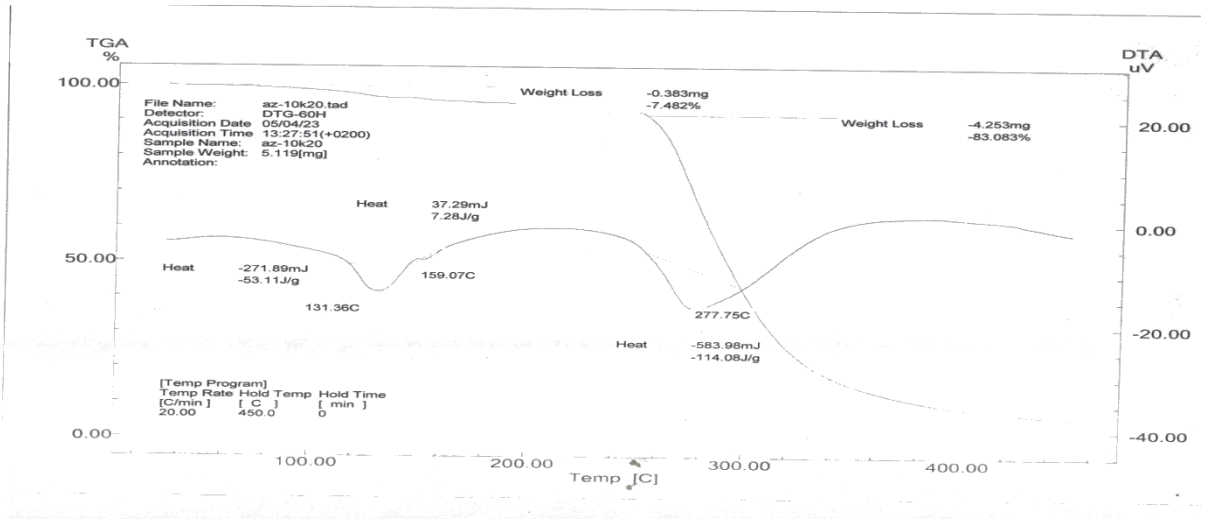


Fig:(6-c) Thermal analysis (DTA,TG) for 10 kGy γ- irradiated Azithromycin

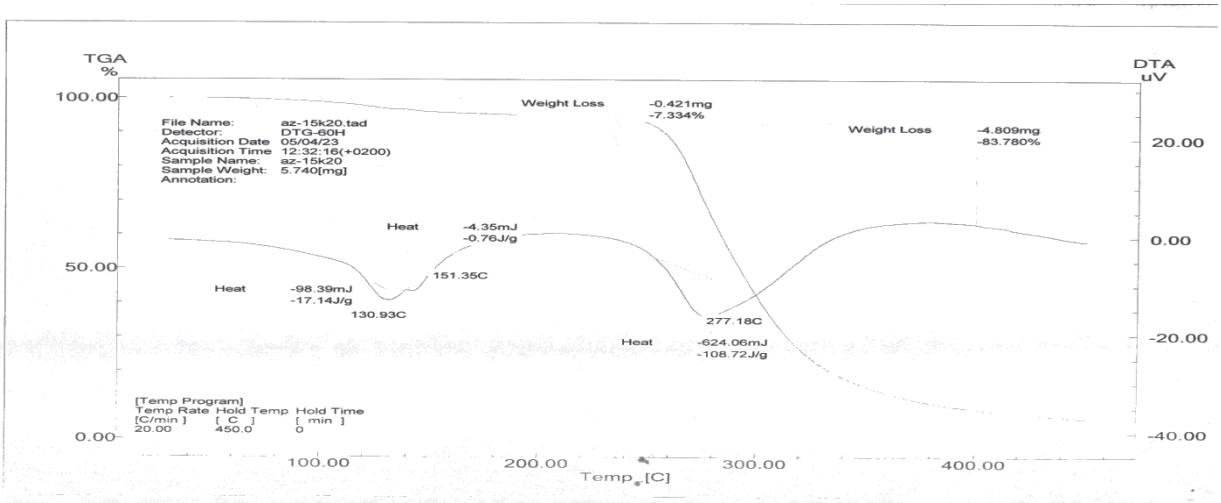


Fig. (6-d) Thermal analysis (DTA, TG) for 15 kGy γ- irradiated Azithromycin

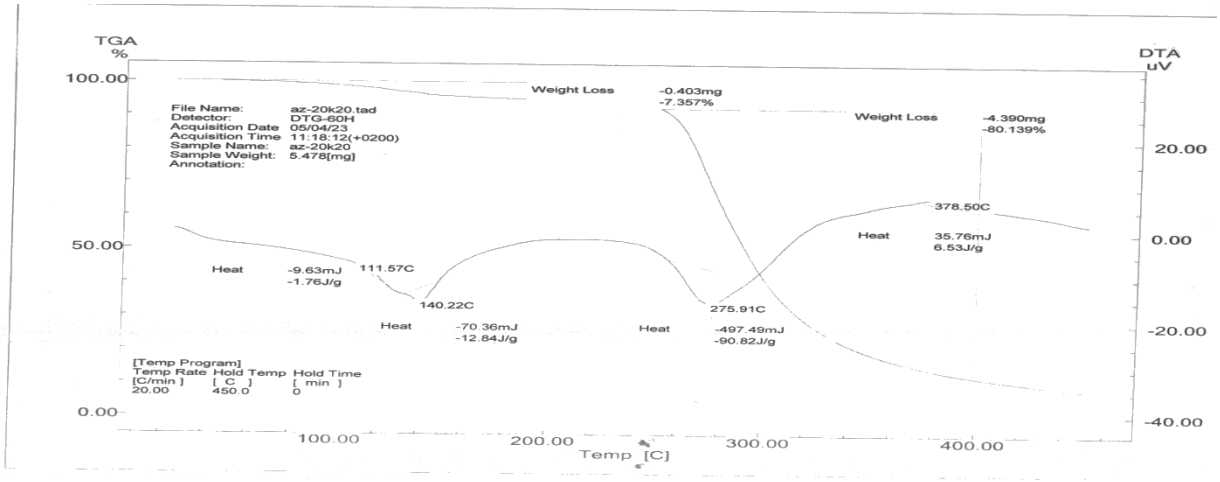


Fig: (6-e) Thermal analysis (DTA, TG) for 20 kGy γ- irradiated Azithromycin

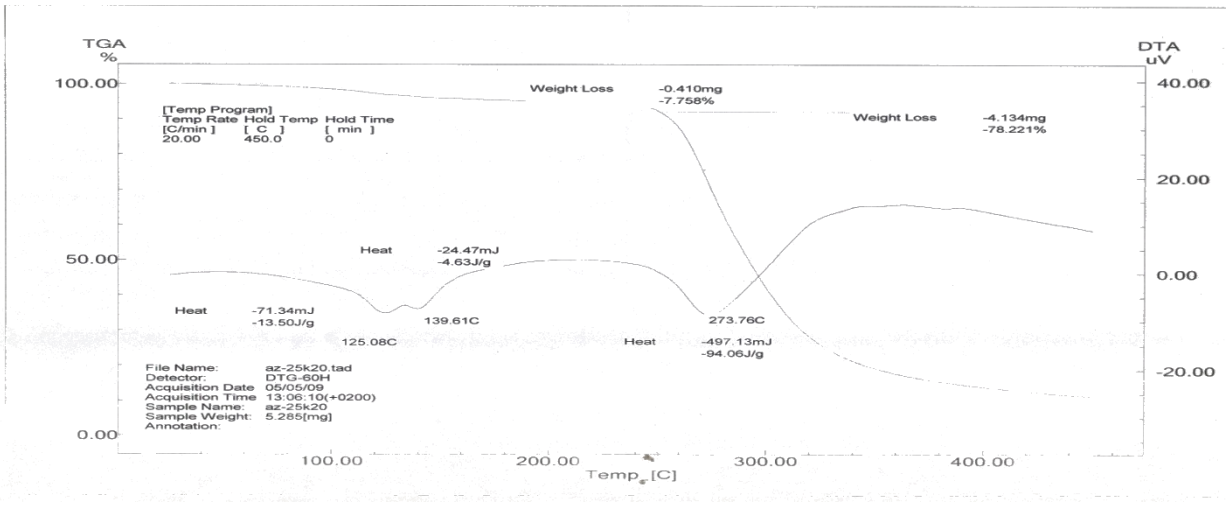


Fig: (6-f) Thermal analysis (DTA, TG) for 25 kGy γ- irradiated Azithromycin

Table 1 Thermal behavior (TGA, DTA) and their assignment for Azitheromycin before and after γ- irradiation.

γ-dose	TGA data Temp.range	Weight loss	DTA-dataAssignment	Temp.range	ΔH J/g
Before - γ-irradiation	200	-6.57%	Endothermic peak due to melting	108.9-143.9	-41.9
	260-400	83.59	Dissociate of oxidation product	278	-123.05
After γ- absorbed 5 KGy	240	-6.59%	Endothermic peak due to melting	131.9-141.2	-7.66
	250-400	-82.9%	Endothermic peak (dissociate of oxidation product	277.4	-99.1
After γ-absorbed 10 KGy	200	-7.28%	Endothermic peak due to melting	131.3-159	-53.1
	240-400	83.08%	Dissociate of oxidation product	277.7	-114.08
After γ-absorbed 15 KGy	200	-7.33%	Endothermic peak due to melting	130.9-151.3	-17.1
	260-400	83.7%	Endothermic peak (dissociate of oxidation product	277.1	-108.7
After γ-absorbed 20 KGy	200	-7.35%	Endothermic peak due to melting	140.2	-12.84
	280-400	80.13%	Endothermic peak (dissociate of oxidation product	275.9	-90.8
After γ-absorbed 25 KGy	200	-7.58%	Endothermic peak due to melting	139.6	-13.5
	240-400	-78.22%	Dissociate of (dissociate of oxidation product)	273.7	-94.06

4. CONCLUSION

From the analysis of ESR signal dependance on the absorbed dose, it can be concluded that gamma-irradiation causes an increases in the amount of this radical in samples. Gamma-irradiation produces free radicals which appear to be relatively stable, ESR discrimiation of irradiated drugs. Alternatively gamma-absorbed dose 25 kGy could be used safly for sterilization Azthiromycin antibiotic.

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