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RESEARCH ARTICLES

Gamma Radiation Studies on Sulfathiazole (Powder and Model-Ophthalmic Solution)

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Summary : *Gamma irradiation is an excellent technique for the sterilization of pharmaceutical raw materials and products [1,2]. One of the major possible disadvantages of radiosterilization is the production of new radiolytic intermediates during the irradiation process [3].*

The Radiosterilization was carried out for sulfathiazole (antibacterial product) powder and model-ophthalmic solution in this research. Irradiation at room temperature at radiation doses of 10, 25 and 40 kGy was investigated via different physical, chemical, microbiological and biological techniques both in normal and accelerated stability conditions (40±2°C and 75±5 % relative humidity, 3 months).

Changes in organoleptic features, pH, melting point, UV, IR, NMR, TLC, ESR, DSC characteristics, and microbiological activities of active compound at normal and accelerated stability test conditions were studied.

It was observed that UV, IR, and NMR characteristics of sulfathiazole did not change with the applied dose ranging between 10-40 kGy except λ_{max} values of irradiated solid samples dissolved in 0.1 N HCl solution. Some radicals were detected by ESR signal intensity of solid samples. Attempt for determination of radicals produced in irradiated model-ophthalmic solution by ESR technique failed due to the short life of these radicals in solution.

The results obtained under accelerated stability test conditions over a period of three months were observed to be consistent with the reference values and the activity of sulfathiazole remained unaffected even at the end of the test period.

Microbiological and biological properties both in normal and accelerated conditions were also investigated.

Based on the physical, chemical, microbological and biological results, the optimum radiation dose of 10 kGy can be applied for the sterilization of sulfathiazole powder and model-ophthalmic solution.

Keywords: *Sulfathiazole, Irradiation of Drugs, Radiosterilization, Gamma Irradiation, Stability.*

Sülfatiyazolün Gama Radyasyonu Üzerinde Çalışmalar (Toz ve Model Oftalmik Çözelti)

Özet: *Gama ışınlama, farmasötik ürün ve hammaddelerin sterilizasyonunda değerli bir tekniktir[1,2]. Radyosterilizasyonun muhtemel en büyük dezavantajı, ışınlama sırasında yeni radyolitik ara ürünlerin oluşmasıdır [3].*

Bu araştırma bir antibakteriyel olan sulfatiazol tozunun ve model-oftalmik çözeltisinin radyosterilizasyonu üzerinde sürdürülmüştür. Işınlama oda sıcaklığında; 10, 25 ve 40 kGy dozlarda yapılmış ve normal ve hızlandırılmış stabilite şartlarında (40°C ve %75 bağıl nemde, 3 ay süreyle), farklı fiziksel, kimyasal, mikrobiyolojik ve biyolojik tekniklerle araştırılmıştır.

Etkin maddenin organoleptik özelliklerindeki değişmeler, pH, e.d., UV, IR, NMR, ESR, DSC özellikleri, mikrobiyal aktivitesi normal ve hızlandırılmış stabilite şartlarında incelenmiştir.

10-40 kGy doz aralığında uygulanan ışınlamayla sulfatiyazolün UV, IR, NMR özellikleri, 0,1 N HCl 'de çözünen örneklerinin λ_{max}'ları dışında değişme olmamıştır. Katı örneklerin ESR sinyal şiddetiyle saptanan bazı radikaller oluşmuştur. Işınlanan model-oftalmik çözeltilerde oluşan radikallerin ömürlerinin kısalığı nedeniyle, ESR teknolojisiyle tayin başarılı olmamıştır.

Üç ay boyunca, hızlandırılmış stabilite şartlarında elde edilen sonuçlar referansla uyumlu bulunmuş ve test süresi sonunda bile sulfatiyazolün aktivitesi korunmuştur.

Normal ve hızlandırılmış şartlarda, mikrobiyolojik ve toksikolojik özelliklerde incelenmiştir.

Fiziksel, kimyasal, mikrobiyolojik ve biyolojik sonuçlara dayanarak Sulfatiyazol toz ve model-oftalmik çözeltisinin sterilizasyonu için optimum 10 kGy radyasyon dozu uygulanabileceği sonucuna varılmıştır.

Anahtar kelimeler: *Sulfatiyazol, İlaçların Işınlanması, Radyosterilizasyon, Gama Işınlama, Stabilite .*

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INTRODUCTION

Sterilization facilitates elimination or killing of the microorganisms present in the contaminated environment, and reduction of the initial contamination[3-5].

The sterilization methods are classifed into four groups:

- Heat sterilization
- Filtration
- Sterilization by ethylene oxide or formaldehyde (chemical sterilization)
- Radiosterilization

Radiosterilization is an interesting alternative particularly where conventional methods are inadequate. Certain molecules, depending on their thermolability, cannot be sterilized by humid heat, while use of ethylene oxide is less recommended for toxicity reasons.

Some studies have demonstrated that irradiation does not generate the same consequences because of the considerable therapeutical molecules. A difficulty, however, is that physical and chemical changes can accompany ionizing radiation[3-5]. It is necessary to describe the radicals formed by the radiosterilization process.

The major problem of radiosterilization is the production of new radiolytic products. Therefore, our intention was to determine and characterize these physical and chemical changes[6,7].

Sulfathiazole is widely used in treatment as an antibacterial agent[8,9]. The aim of this study was to investigate the gamma irradiation of sulfathiazole as raw material and model-ophthalmic solution as products. The effect of gamma irradiation was determined before and after the process.

A "sterile" product (like ophthalmic solution) was prepared as a model and the gamma irradiation effect was determined on this product. Additionally, the results of raw material and product were compared regarding gamma irradiation effect.

Samples of powder sulfathiazole and model-ophthalmic solution irradiated at room temperature at radiation doses of 10, 25 and 40 kGy were investigated by different physical, chemical and microbiological techniques under normal and accelerated stability conditions[6,7].

MATERIALS AND METHODS:

Solid sulfathiazole powder was provided from "YED", Yeni Ecza Deposu, Turkey.

Irradiation Process:

All irradiations were performed at room temperature (approximately 20°C) using a ^{60}Co source (Gamma Cell 220) providing a dose rate of 4 $kGy.h^{-1}$ in the sample located at Sarayköy Nuclear Research Centre of Turkish Atomic Energy Authority (TAEK) in Ankara. The dose rate was measured by Fricke dosimeter[6,7].

The samples were irradiated at doses of 10, 25 and 40 kGy[10].

Studies Conducted Under Normal Conditions

Unirradiated samples were used as controls to detect the changes related to the physicochemical and antimicrobial activities resulting from the action of ionizing radiation[6].

A-Physico-Chemical Properties

pH change measurements of the reference and irradiated solid samples were performed using 1 $mg.ml^{-1}$ aqueous solutions of these samples. Solubility changes in boiling water, acetone, hydrochloric acid, sodium hydroxide and potassium hydroxide; and changes in melting point, color and sediment, primary aromatic amine reaction, solution appearance, acidity, heavy metal, loss on drying, sulfated as and DSC (differential scanning calorimetry)

(General V2. 2A Du Pont 9900 Heating 10°C.min^{-1} at N_2 atm) were also tested.

Similarly, irradiated model-ophthalmic solution was evaluated for appearance, pH changes, homogeneity, foreign particles, organoleptic properties, color and odor[11].

Calorimetric evaluation is one of the best methods for characterizing the samples; therefore, sulfathiazole was also evaluated from this point of view.

B- Spectroscopic Methods and Techniques

Changes in spectral properties of control and irradiated solid samples were studied using IR, UV, NMR[12] and electron spin resonance (ESR) techniques[13]. IR spectra were obtained for control and irradiated powders in KBr matrix (instrument: IR Vector 22 Bruker Opus version 3)[11]. In UV analysis (Shimadzu UV-160A), determination of λ_{max} values was performed both in 0.1 N NaOH and 0.1 N HCl for powder and for model-ophthalmic solution.

NMR analysis was performed using proton NMR spectrometer with the dissolving of irradiated and unirradiated powders in DMSO-d_6. Tetramethylsilane was used as an internal standard[12].

The species and amount of the molecular fragments or radicals processing unpaired electrons created by radiation can best be detected by ESR spectroscopy, which is frequently used to measure the absorbed dose of an irradiated product[14,15].

ESR measurements were carried out using Varian E-19, X-Band ESR spectrometer USA equipped with a TE104 rectangular double cavity. All measurements were performed using a DPPH reference sample placed in the front cavity. The position of the reference sample in the cavity was not changed throughout the experiments to avoid any signal intensity measurements due to any possible changes in the cavity-filling factor. The spectra were double integrated over the magnetic field range of 3200-3320 mT to give a figure proportional to the radical numbers in the sample. Each spectrum was corrected for variation in the amount of material in the "active length" of the ESR tube, and in the spectrometer tuning conditions. Simulation studies based on possible radical species were also carried out[13].

Determination of radicals produced in irradiated model-ophthalmic solution samples by ESR technique failed due to the short life of these radicals; therefore, UV and TLC techniques were performed to determine degradation products.

C- Chromatographic Methods

Chromatographic (TLC) methods for identification of sulfathiazole have been given by European Pharmacopeia (EP)[16]. Sulfathiazole was detected by TLC using silica gel HR plates. Dimethylaminobenzaldehyde solution 1g.L^{-1} of ethanol was used as location reagent[17,18].

D- Antimicrobial Activity Studies

Antibacterial activities of irradiated and unirradiated preparations were determined by the microdilution method recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 1997).

According to this procedure, microorganism inoculum was first prepared, then antimicrobial activity was determined against these reference microorganisms: *Staphylococcus aureus* (*S. Aureus* ATCC 25923), *Escherichia coli* (*E. Coli*) (ATCC 25922), *Enterococcus faecalis* (*E. Faecalis*) (ATCC 29212), and *Pseudomonas Aeruginosa* (*Ps. Aeruginosa*) (ATCC 27853). The results were expressed as minimum inhibitory concentrations (MIC).

Preparation of microorganism inoculum: Before the test each microorganism was incubated in Mueller-Hinton broth for 2-5 hours at 35°C. Microorganism concentration was adjusted to 0.5 McFarland standard (0.5-1 x10^8 cfu.mL^{-1}) and final concentration was diluted to 5.5 x 10^5 cfu. mL^{-1} in the well of microtiter plates[19].

Microdilution broth method: 96 well u-shaped, microtiter plates were used in the test. Two-fold dilutions of irradiated and unirradiated preparations were prepared in Mueller-Hinton broth in the well of the plates. Each samples was diluted from 1-11 wells of the micro-titer trays ($^1/_4$ to 1/4096 dilutions). Previously prepared microorganism suspensions were added to each well and the plates were incubated for 18-24 hours at 35°C. Minimum inhibitory concentrations (MIC, mg. mL^{-1}) were defined as the lowest concentration (dilution) of the samples that inhibited visible growth of the microorganism.

E- Sterility

The sterility test of irradiated samples was performed according to USP XXII (United States Pharmacopoeia 1990)[20]. This ophthalmic solution was not sterilized as it was only a model. After irradiation of samples at 10, 25 and 40 kGy, sterility test was performed both in Soybean-casein digest medium (SCDM) and fluid thioglycollate medium (FTM) for determining both aerobic and anaerobic microorganisms. After 14 days incubation period of the dosage forms, samples were evaluated for microbial growth[6,7].

F- Sterility Assurance Level (SAL) Dose Determination

Since the type and the concentration of the microorganisms in production condition were unknown, *B. Pumilus* spores, which are resistant to gamma sterilization, were used as microbial contamination model.

Studies Carried Out Under Accelerated Conditions

In this part of the work, studies performed under accelerated conditions were repeated for samples in uncapped glass tubes at high temperature (40±2)°C and high relative humidity (75±5 %) conditions over a period of three months to investigate possible degradation mechanism and kinetics of irradiated powders and model-ophthalmic solution during shelf-life. Accelerated stability conditions were chosen according to the "guide for the stability of drugs" issued by the Ministry of Health in Turkey[21]. Samples were stored in the climate chamber continuously and aliquots were taken off for measurements at room temperature. Unirradiated samples were used as standard controls for comparison, and measurements were repeated (2nd week, 1st month, 2nd month and 3rd months)[22].

RESULTS

Studies Carried Out Under Normal Conditions

A) Dose Mapping of Irradiation Source

The actual doses received by samples were determined by measuring the changes in absorbance. The corresponding doses were obtained from a calibrating graph (Figs 1 and 2), (Tables 1,2). Administration doses were 2.8 $kGy.h^{-1}$ for centre and 3.05 $kGy.h^{-1}$ for the wall of gamma-cell[10].

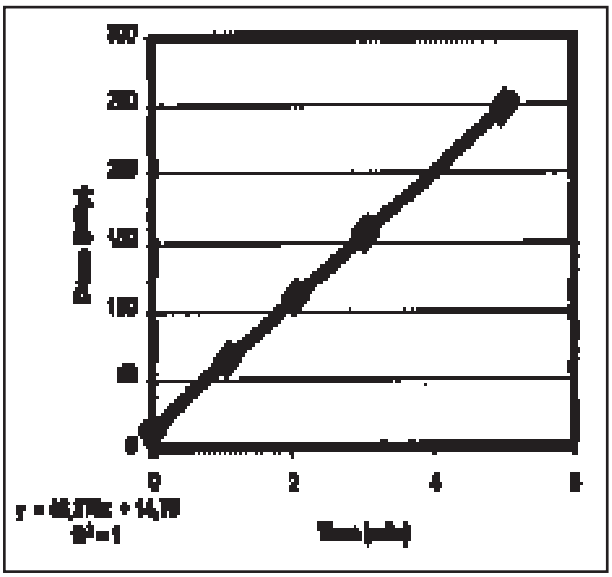


Figure 1: Calibration curve of dosimeter in the centre of gamma-cell.

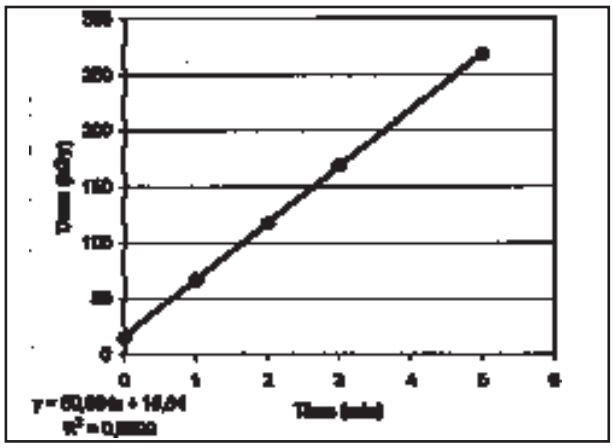


Figure 2: Calibration curve of dosimeter in the centre of gamma-cell wall.

Table 1: Doses received by samples in centre of gamma cell

Time (min)	Absorbance
Un irrad	0.045
Un irrad	0.045
0	0.096
0	0.100
1	0.264
1	0.267
2	0.435
2	0.428
3	0.599
3	0.597
5	0.937
5	0.928
Time (min)	Dose (Gy)
0	14.73
1	61.30
2	107.45
3	153.73
5	246.73

Table 2: Doses received by samples near the gamma cell wall

Time (min)	Absorbance
0	98
0	102
1	280
1	290
2	459
2	477
3	648
3	660
5	993
5	1027
Time (min)	Dose (Gy)
0	15.29
1	66.72
2	117.59
3	169.30
5	268.27

B) Physico-Chemical Properties

Gamma irradiation of sulfathiazole caused a slight change in color[23]. Irradiation did not produce changes, however in solubility in different solutions (such as boiling water, acetone, hydrochloric acid, sodium hydroxide and potassium hydroxide) (Table 3), melting point (Table 4), color and sedimentation (Table 5), primary aromatic amine reaction (Table 6), solution appearance (Table 7), acidity (Table 8), heavy metal (Table 9), loss on drying (Table 10) and sulfated ash (Table 11). No significant change was observed in pH for sulfathiazole powder and model-ophthalmic solution (Table 12).

Table 3: Solubility results of sulfathiazole powder before and after irradiation.

Solvents	Solubility ($mg.ml^{-1}$)				
	Ref	Control	Applied dose (kGy)		
			10	25	40
Acetone	1/10	+	+	+	+
Hydrochloric acid	1/10	+	+	+	+
Sodium hydroxide	1/10	+	+	+	+
Potassium hydroxide	1/10	+	+	+	+
Boiling water	1/40	+	+	+	+

(+: conforms)

Table 4: Melting point results of sulfathiazole powder before and after irradiation.

Sample	Melting point (°C)				
	Reference	Control	Applied dose (kGy)		
			10	25	40
Sulfathiazole	200-203	199±0.00	199±0.00	199.2±0.08	199.4±0.05

Table 5: Color and sedimentation results of sulfathiazole powder before and after irradiation.

Sample	Color and sedimentation				
	Reference	Control	Applied dose (kGy)		
			10	25	40
Sulfathiazole	Color: blue grey and sediment	+	+	+	+

(+: conforms)

Table 6: Primary aromatic amine reaction results of sulfathiazole powder before and after irradiation

Sample	Primary aromatic amine reaction				
	Reference	Control	Applied dose (kGy)		
			10	25	40
Sulfathiazole	Red orange color and sediment	+	+	+	+

(+: conforms)

Table 7: Solution appearence results of sulfathiazole powder before and after irradiation

Sample	Solution appearance				
	Reference	Control	Applied dose (kGy)		
			10	25	40
Sulfathiazole	Not more color than reference	+	+	+	+

(+: conforms)

Table 8: Acidity test results of sulfathiazole powder before and after irradiation

Sample	Acidity (ml)				
	Reference	Control	Applied dose (kGy)		
			10	25	40
Sulfathiazole	≤0.1	0.062±0.077	0.062±0.053	0.064±0.042	0.063±0.023

Table 9: Heavy metal test results of sulfathiazole powder before and after irradiation

Sample	Heavy metal				
	Reference	Control	Applied dose (kGy)		
			10	25	40
Sulfathiazole	The reference has more color than the blank solution of 10 ppm or pbt	+	+	+	+

(+: conforms)

Table 10: Loss on drying test results of sulfathiazole powder before and after irradiation

Sample	Loss on drying (%)				
	Reference	Control	Applied dose (kGy)		
			10	25	40
Sulfathiazole	≤0.5	0.405±0.032	0.411±0.024	0.401±0.420	0.403±0.220

Table 11: Sulphated ash test results of sulfathiazole powder before and after irradiation

Sample	Sulphated ash (%)				
	Reference	Control	Applied dose (kGy)		
			10	25	40
Sulfathiazole	≤0.1	0.070±0.003	0.072±0.024	0.069±0.004	0.068±0.002

Table 12: pH values for control and irradiated powder and model-ophthalmic solution samples before and after irradiation

Sample	pH				
	Reference Values	Control	Applied dose (kGy)		
			10	25	40
Powder	-	8.101±0.047	8.104±0.036	8.111±0.025	8.108±0.012
MOS*	-	8.053±0.047	8.068±0.024	8.101±0.032	8.101±0.051

* MOS: Model-ophthalmic solution

Gamma irradiation of sulfathiazole powder did not produce changes in DSC results (Table 13) (Fig. 3).

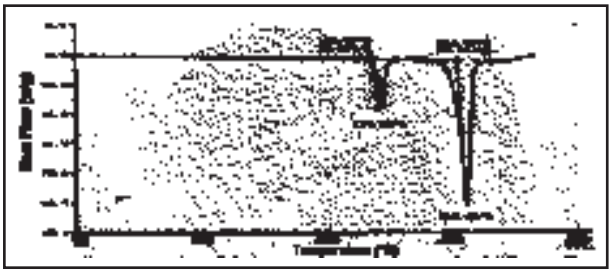


Figure 3: DSC thermogram of sulfathiazole powder before irradiation

Table 13: DSC results of sulfathiazole powder before and after irradiation

Sample	DSC (°C)				
	Reference Values	Control	Applied dose (kGy)		
			10	25	40
Powder	200-203	205.88	205.60	204.25	204.86

C) Spectroscopic Methods and Techniques

Control and irradiated solid samples were studied for evaluation from the spectroscopic point of view such as IR, UV, NMR and ESR[24].

In the IR analysis at the lower radiation doses of sulfathiazole H_2N stretch bands, C=C stretch in benzene ring and SO_2 stretch bands could be seen, for all doses (10, 25 and 40 kGy) (Fig. 4) (Table 14).

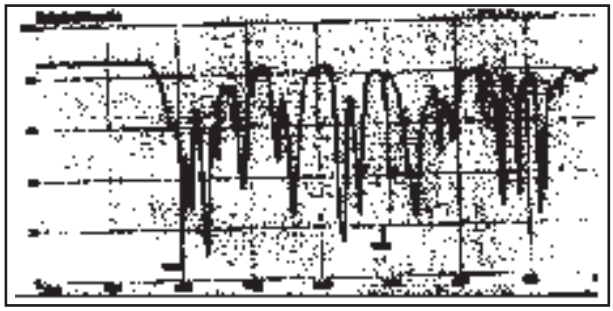


Figure 4: IR spectrum of sulfathiazole before irradiation

Table 14: IR results of sulfathiazole powder before and after irradiation

Sample	Characteristic Peak	Peak (cm-1)				
		Reference	Control	Applied dose (kGy)		
				10	25	40
	NH_2 group and		3277.8-	3276.5-	3276.5-	3276.4-
	N-H bonds	3100-350	3319.9	3319.2	3319.0	3318.9
	NH_2 vibration	1601	1595.2	1595.5	1595.8	1595.9
	C-H single bonded	2853-2962	2920.9	2930.0	2931.2	2926.3
	C=C aromatic	1500-1595	1495.2-1573.7	1494.8-1595.5	1494.6-1573.6	1494.6-1573.6
	Asymmetric SO_2	1303-1313	1281.5	1281.0	1280.6	1280.5
	Symmetric SO_2	1143-1155	1138.5	1135.9	1133.3	1133.0
	-H	820-840	821.0	820.0	819.6	819.6

For comparison, λ_{max} values calculated for control and irradiated powders in both media were in good agreement with the values given in the literature. Although the variation with absorbed dose of the wave number corresponding to maximum UV absorbance was not significant for samples dissolved in 0.1N NaOH, a significant decrease was observed for samples dissolved in 0.1N HCl (Table 15). No significant change for model-ophthalmic solution was observed (Table 16).

Table 15: λ_{max} values calculated from UV spectra of sulfathiazole powder before and after irradiation

Sample	λ_{max} (nm)				
	Medium	Control	Applied dose (kGy)		
			10	25	40
NaOH 0,1N	256.00	256.00±0.01	256.20±0.04	256.74±0.03	256.65±0.03
HCl 0.1 N	257.00	257.00±0.00	264.80±0.02	267.00±0.01	270.01±0.01

Table 16: λ_{max} values calculated from UV spectra of model-ophthalmic solution before and after irradiation

Sample	λ_{max} (nm)				
	Medium	Control	Applied dose (kGy)		
			10	25	40
MOS*	257.00	257.00±0.00	257.10±0.01	257.6±0.05	257.8 ±0.04

* MOS: Model-ophthalmic solution

Proton NMR spectra of sulfathiazole in dimethyl sulfoxide DMSO-d_6 containing tetramethylsilane as internal reference consisted of different chemical shifts which varied from two to 10 depending on the chemical environment of the related protons. The latter data are in good agreement with those reported previously by Turczan and Medwick[12] in the literature regarding identification of sulfonamides by NMR spectroscopy[12]. In the case of sulfathiazole, in the region either containing singlets arising from methoxyl, methyl, or methylene proton resonance, the aromatic region is distinctive and permits identification (Fig. 5).

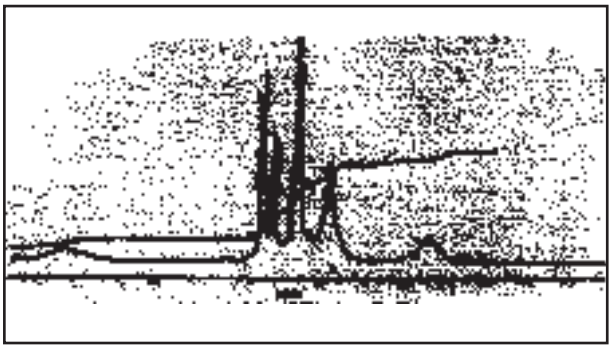
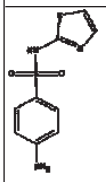


Figure 5: NMR of sulfathiazole before irradiation.

Irradiation of solid samples in the dose range of 10-40 kGy did not produce any significant effect on the

chemical shifts of sulfathiazole protons as shown in Table 17.

Table 17: Calculated proton chemical shift values for control and irradiated solid samples before and after irradiation

Sample	NMR					
	Related Proton (s)	Reference	Control	Applied dose (kGy)		
				10	25	40
	A	5.80	5.7	5.7	5.8	5.2
	B	6.64	6.5	6.5	6.5	6.5
	C	6.73	6.6	6.7	6.8	6.8
	D	7.18	7.1	7.1	7.2	7.4
	E	7.50	7.4	7.5	7.5	7.5
	F	12.00	12.1	12.3	12.4	12.1

ESR spectra of control and irradiated solid samples were also investigated[25]. In the characterization study of radicals formed by irradiation of sulfathiazole, peak height against magnetic field value was measured in the experimental ESR spectra. According to molecular structure of sulfathiazole (Fig. 6), possible radicals and their types and structure were estimated, and experimental ESR was plotted. The possible degradation pathways are given in Figure 6. Depending on possible radicals, mathematical models were developed and simulation studies were carried out. As a result of these studies, it is thought that four different possible radicals (A, B, C and D radicals) were formed by irradiation of sulfathiazole (Table 18).

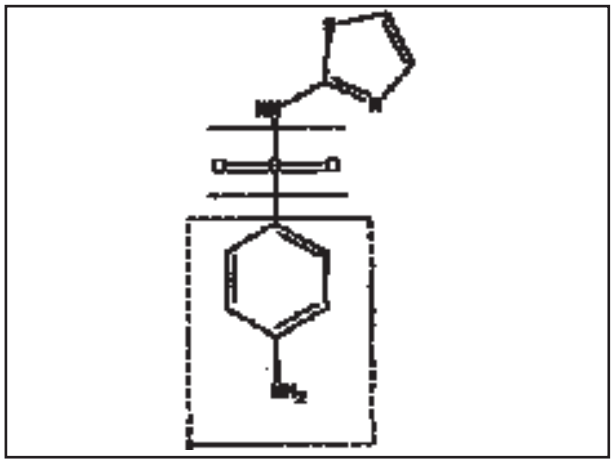
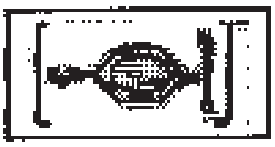
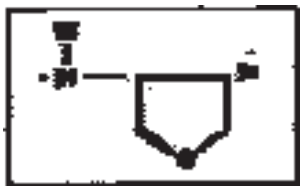
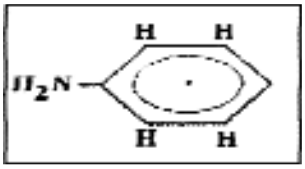


Figure 6: Chemical structure of irradiated sulfathiazole and the possible decomposition pathways.

Table 18: ESR results of irradiated at 40 kGy sulfathiazole powder

Radicals	Developed formula
A	$(SO_2)^-$
B	
C	
D	

An ionic radical has isotropic "g" value because this radical is free and it can move fast. Movement of B radical is limited because of the large group bonded to sulfur atom, which is why, the B radical has nonisotropic "g" value. C radical is formed by break of S-N bond. The unpaired electron is placed onto nitrogen N atoms. Movement of C radical is limited as with B radicals because of the large group bonded to nitrogen atoms. Thus, C radical has nonisotropic "g" value. D radical is formed by the breaking of nitrogen and bonded hydrogen. C radical has extremely thin structure because of hydrogen atoms bonded to phenyl ring. In experimental ESR of bond between phenyl and sulphur, unpaired electron placed onto phenyl ring and this radical has isotropic "g" value. Because of neutralization between unpaired electrons, spectrum was obtained by using these four different radicals and simulation studies were carried out. According to simulation studies, possible radicals proposed were determined and spectroscopic parameters of these radicals were calculated. They are given in Table 19.

Table 19: ESR "g" value result of irradiated at 40 kGy sulfathiazole powder before and after irradiation

Radicals	Intensity	Half-band	Extremely thin structure	G value
A (1 lined, isotropic)	65.83900	1.2660	--------	G =2,0047
B (1 lined, anisotropic)	4.46130	1.3457	--------	G_{I} =2,0098 G_{II}=2,0098
C (6 lined, anisotropic)	0.19576	0.7925	6.5816 (for n) 2.6788 (for h)	G_{I} =2,0086 G_{II}=1,9960
D (1 lined, isotropic)	75.20100	7.5453	3.5262 (for h)	G =2,0034

Theoretical ESR spectra were plotted by using these spectroscopic parameters. Theoretical and experimental ESR spectra are given together in Figure 7. It was found that there was a good correlation between theoretical and experimental spectra. These results showed that radicals formed by irradiation of sulfathiazole samples are the same for A, B, C and D radicals proposed in simulation studies[25-28].

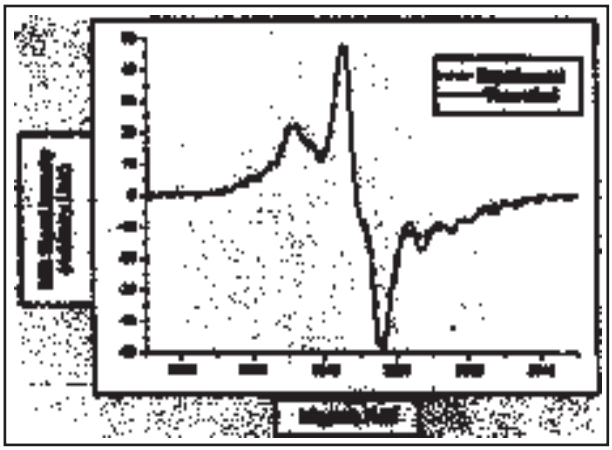


Figure 7: ESR simulation curves of sulfothiazole powder irradiated at 40 kGy

D) Chromatographic Methods

TLC experiments were performed using the technique proposed by EP 1997[16]. For identification of sulfathiazole, a mixture of ammoniac and butanol was used as solvent and p-dimethylaminobenzaldehyde R solution as location reagent[17, 28]. This reagent produces bright yellow spots in spraying with many compounds, but heating at 100°C is necessary with some of the compounds before the spots are visible. R_f values for unirradiated (control), irradiated samples and model-ophthalmic solution in the applied dose range (10-40 kGy) were found significantly different. The R_f values are given in Table 20 for sulfathiazole powder and model-ophthalmic solution.

Table 20: TLC results of sulfathiazole powder before and after irradiation

Reference		Rf							
Sample		Powder				Model-ophthalmic solution			
		Applied dose (kGy)				Applied dose (kGy)			
		0	10	25	40	0	10	25	40
	The spot obtained by								
Sulfathiazole	sulfathiazole was not more intense than spot of sulfanilamide and no second spot appeared	0.300 ± 0.012	0.385 ± 0.013	0.392 ± 0.011	0.395 ± 0.023	0.320 ± 0.047	0.380 ± 0.017	0.388 ± 0.021	0.420 ± 0.011

E) Antimicrobial Activity Studies

In the irradiated samples no activity loss was observed for sulfathiazole powder and model-ophthalmic solution .

F) Sterility

We did not observe any microbial growth in sterility test for all radiation doses (10, 25 and 40 kGy). Anaerobic and aerobic microorganisms did not generate in the studied media.

Sterility Assurance Level (SAL) Dose Determination

SAL test performed on B. Pumilus spores (10^6 cfu. mL^{-1}) infected samples did not work.

Studies Carried Out Under Accelerated Stability Conditions

Experimental results showed that physico-chemical properties such as color, odor, solubility in different solvents (boiling water, acetone, hydrochloric acid, sodium hydroxide and potassium hydroxide) (Table 21), melting point (Table 22), color and sedimentation, primary aromatic amine reaction, solution appearance, acidity (Table 23), heavy metal, loss on

drying (Table 24), sulphated ash (Table 25) and DSC (Table 26), of control and irradiated solid and model-ophthalmic solutions did not change under accelerated stability test conditions, however ph values did, as shown in Table 27.

Table 21: Solubity results of sulfathiazole powder under accelerated conditions

Time (day)	Solvent	Solubility (ml) Ref	Applied dose (kGy) 10	25	40
0			+	+	+
14	Acetone	1/10	+	+	+
28			+	+	+
60			+	+	+
90			+	+	+
0			+	+	+
14	Hydro chloric acid	1/10	+	+	+
28			+	+	+
60			+	+	+
90			+	+	+
0			+	+	+
14	Sodium hydroxide	1/10	+	+	+
28			+	+	+
60			+	+	+
90			+	+	+
0			+	+	+
14	Potassium hydroxide	1/10	+	+	+
28			+	+	+
60			+	+	+
90			+	+	+
0			+	+	+
14	Boiling water	1/40	+	+	+
28			+	+	+
60			+	+	+
90			+	+	+
(+: conforms)					

Table 22: Melting point results of sulfathiazole powder under accelerated conditions

Time (day)	Melting point (°C) Control	Applied dose (kGy) 10	25	40
0	199	199.00±0.00	199.20±0.08	199.40±0.05
14	199	198.08±0.18	198.25±0.38	198.16±0.23
28	199	198.41±0.18	198.25±0.38	198.16±0.23
60	199	200.60±0.48	199.20±0.74	199.00±0.63
90	199	199.81±0.40	199.20±0.74	199.80±0.74

Table 23: Acidity results of sulfathiazole powder under accelerated conditions

Time (day)	Acidity (ml) Control	Applied dose (kGy) 10	25	40
0	0.062±0.077	0.062±0.053	0.064±0.042	0.063±0.023
14	0.062±0.077	0.063±0.031	0.067±0.001	0.067±0.001
28	0.062±0.077	0.066±0.007	0.062±0.005	0.065±0.005
60	0.062±0.077	0.064±0.001	0.069±0.009	0.069±0.001
90	0.062±0.077	0.063±0.001	0.068±0.001	0.070±0.001

Table 24: Loss on drying results of sulfathiazole powder under accelerated conditions

Time (day)	Loss on drying (%) Ref	Control	Applied dose (kGy) 10	25	40
0		0.405±0.032	0.411±0.024	0.401±0.042	0.403±0.22
14		0.410±0.022	0.385±0.029	0.401±0.024	0.420±0.036
28	≤0.5	0.409±0.011	0.401±0.012	0.399±0.004	0.399±0.005
60		0.415±0.002	0.401±0.019	0.423±0.036	0.411±0.024
90		0.411±0.021	0.400±0.003	0.400±0.001	0.417±0.036

Table 25: Sulphated ash results of sulfathiazole powder under accelerated conditions

Time (day)	Sulphated ash (%) Ref	Control	Applied dose (kGy) 10	25	40
0		0.070±0.003	0.072±0.024	0.069±0.004	0.068±0.002
14		0.073±0.001	0.069±0.002	0.069±0.007	0.069±0.001
28	≤ 0.1	0.077±0.005	0.069±0.005	0.059±0.004	0.069±0.004
60		0.077±0.010	0.069±0.004	0.069±0.006	0.060±0.001
90		0.072±0.001	0.068±0.004	0.069±0.004	0.068±0.004

Table 26: DSC results of sulfathiazole powder under accelerated conditions

Time (day)	DSC (°C) Ref	Control	Applied dose (kGy) 10	25	40
0		205.88	205.60	204.25	204.86
28	200-203	205.88	205.60	203.25	203.66
60		205.88	202.96	203.56	203.62
90		205.88	204.41	204.37	203.37

Table 27: pH values for control and irradiated solids

stored at accelerated conditions

Time (day)	PH							
	Powder				Model-ophthalmic solution			
	Applied dose (kGy)				Applied dose (kGy)			
	0	10	25	40	0	10	25	40
0	8.101	8.104	8.111	8.108	8.053	8.068	8.101	8.101
	±0.047	±0.036	±0.025	±0.012	±0.047	±0.024	±0.032	±0.051
14	8.1	7.678	7.728	7.801	8.051	8.223	8.19	8.183
	±0.02	±0.029	±0.051	±0.01	±0.02	±0.012	±0.005	±0.004
28	8.00	8.006	8.013	8.676	8.052	8.216	8.183	8.178
	±0.02	±0.016	±0.007	±0.03	±0.04	±0.013	±0.012	±0.014
60	8.011	8.273	8.265	8.263	8.052	8.316	8.166	8.033
	±0.03	±0.007	±0.007	±0.004	±0.03	±0.068	±0.047	±0.074
90	8.001	8.028	8.05	8.035	8.053	8.25	8.283	8.3
	±0.02	±0.014	±0.016	±0.012	±0.04	±0.095	±0.089	±0.115

Although λ_{max} values of control and irradiated solid powders dissolved in 0.1 N NAOH were found to exhibit no changes overall the stability studies, that of control samples dissolved in 0.1 N HCI experienced a meaningful increase (Table 28) in the first week storage period, then stayed approximately constant[6]. However, the changes in lmax values of irradiated samples were less prounouced. λ_{max} values of control and irradiated model-ophthalmic solution were found to exhibit no changes throughout the stability studies. The amounts of sulfathiazole are shown in Table 29.

Table 28: λ_{max} values of sulfathiazole powder under accelerated conditions

Solvent	Time (day)	λ_{max} (nm)			
		Control	Applied dose (kGy)		
			10	25	40
NaOH 0.1N	0	256.00±0.01	256.20±0.04	256.74±0.03	256.65±0.03
	14	256.01±0.01	256.00±0.04	240.00±0.03	256.20±0.04
	28	256.01±0.05	257.00±0.02	256.00±0.03	240.00±0.08
	60	256.04±0.03	258.40±0.01	256.50±0.01	256.10±0.02
	90	256.01±0.01	239.90±0.07	257.60±0.07	265.50±0.05
HCl 0.1N	0	257.00±0.00	264.80±0.02	267.00±0.01	270.01±0.01
	14	257.01±0.01	279.10±0.01	264.80±0.08	277.40±0.02
	28	257.00±0.05	280.00±0.03	279.00±0.06	265.80±0.01
	60	257.02±0.04	278.60±0.02	281.00±0.01	279.00±0.02
	90	257.00±0.01	267.20±0.04	277.80±0.03	280.50±0.07

Table 29: Sulfathiazole determination of model-ophthalmic solution under accelerated conditions.

Time (Day)	Amount of sulfathiazole (mg. mL^{-1})			
	Control	Applied dose (kGy)		
		10	25	40
0	0.042±0.013	0.046±0.022	0.047±0.019	0.045±0.019
14	0.044±0.020	0.042±0.018	0.045±0.013	0.043±0.024
28	0.042±0.017	0.048±0.014	0.044±0.010	0.040±0.018
60	0.041±0.019	0.044±0.011	0.042±0.017	0.041±0.014
90	0.044±0.022	0.044±0.024	0.046±0.020	0.046±0.019

FT-IR (Table 30) and NMR (Table 31) spectra of control and irradiated samples stored for three months under stability test conditions were found to exhibit characteristic features of the spectra obtained for samples stored at normal environmental conditions.

Table 30: Amount of sulfathiazole powder under accelerated conditions.

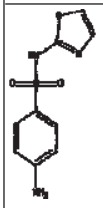
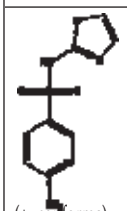
Sample	Characteristic	Peak (cm^{-1})															
	Peak	Reference	Applied dose (kGy)														
			10					25					40				
			0	14	28	60	90	0	14	28	60	90	0	14	28	60	90
	NH_2 group and																
	N-H bonds	3100-3500	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	NH_2 vibration	1601	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	C-H single bonded	2853-2962	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	C=C aromatic	1500-1595	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Asymmetric SO_2	1303-1313	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Symmetric SO_2	1143-1155	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	-H	820-840	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
(+: conforms)																	

Table 31: NMR results of sulfathiazole powder under accelerated conditions

Sample	Related	NMR															
	Proton	Reference	Applied dose (kGy)														
	(s)		10					25					40				
			0	14	28	60	90	0	14	28	60	90	0	14	28	60	90
	A	~5.80	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B	~6.64	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	C	~6.73	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	D	~7.18	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	E	~7.50	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	F	~12.00	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
(+: conforms)																	

As emphasized in the previous section of the pre-

sent work, unirradiated (control) solid samples do not exhibit any ESR signal. Storing of these samples at stability test conditions, i.e., at high temperature and high relative humidity, does not create any changes in this feature. However, storing irradiated samples in the same conditions have been observed to cause a decrease in the ESR signal intensities of the samples due to the decay of radiolytic intermediates created during the irradiation. The possible degradation pathways are given in Figure 6. The results obtained for solid sulfathiazole at the dose of 40 kGy are given in Figure 7. As can be seen, ESR signal intensity decay curve exhibits biphasic character just at the beginning, unstable radiolytic product decay completely, than the more stable ones dominate on the decay curve. However, at the end of the storing period (90th day) all the radiolytic intermediates decay almost completely.

Rf values determined by TLC method of control and irradiated solid and model-ophthalmic solution samples stored at stability test conditions are found to be independent of storage time, have a meaningful increase in the second week of storage period, and then stay approximately constant within the experimental error limits (Table 32).

Table 32: TLC results of sulfathiazole powder and model-ophthalmic solution under accelerated conditions.

Time	R_f							
(Day)	Powder				Model-ophthalmic solution			
	Applied dose (kGy)				Applied dose (kGy)			
	0	10	25	40	0	10	25	40
0	0.320	0.380	0.388	0.420	0.300	0.385	0.392	0.395
	±0.047	±0.017	±0.021	±0.011	±0.012	±0.013	±0.011	±0.023
14	0.420	0.479	0.444	0.444	0.322	0.516	0.515	0.493
	±0.003	±0.005	±0.004	±0.004	±0.004	±0.007	±0.003	±0.005
28	0.426	0.507	0.515	0.515	0.334	0.528	0.536	0.537
	±0.008	±0.002	±0.003	±0.003	±0.010	±0.003	±0.005	±0.012
60	0.435	0.523	0.526	0.526	0.327	0.578	0.562	0.544
	±0.002	±0.003	±0.003	±0.003	±0.014	±0.002	±0.003	±0.003
90	0.421	0.534	0.533	0.533	0.338	0.509	0.513	0.527
	±0.005	±0.06	±0.003	±0.003	±0.012	±0.09	±0.009	±0.009

During the stability period, the sterility test was found to be the same for all applied doses at the beginning and after the storage period.

DISCUSSION

Studies Carried Out Under Normal Conditions

Color change in the irradiated substances is the simplest and most helpful observation to obtein information about possible radiolytical intermediates produced in these substances upon irradiation[29,30]. Based on the fact that color change was observed in irradiated samples in the applied dose region of 10-40 kGy, it can be concluded that radiolytical intermediates are produced by irradiation of powders (Fig. 7)[4]. Gamma radiation transfers its energy indirectly to the target in the solution. Radicals produced by the direct action of radiation on water molecules are the principal elements in the degradation of aqueous solutions[31]. In its direct action, gamma radiation ejects electrons from water molecules. Positively charged water molecules react in their turn, react with unchanged water molecules and radicals; mainly OH^- is produced[32].

The latter is very strong oxidants and they play principal role in the degradation of aqueous systems. This feature of water molecules makes the aqueous systems more sensitive to radiolysis[7]. When evaluating of experimental results concerning pH, it was found that no change was observed in the irradiated solid and model-ophthalmic solution samples[8]. Radiation did not cause any change in solubility of sulfathiazole powder in boiling water, acetone, hydrochloric acid, sodium hydroxide and potassium hydroxide nor in its melting point.

UV spectra of control sulfathiazole in acidic and basic media exhibit two λ_{max} values at about 240-256 nm and 256-281 nm, respectively (Table 28). Observation of λ_{max} appearing at nearly the same wavelentgth even after irradiation indicates that sulfathiazole is conserved in the irradiated samples; however, the same is not true for substitution rings. Namely, this ring is affected, to a large extent, by gamma radiation. Comparison of the proton chemical

shifts of control and irradiated samples given in Table 10 shows that gamma radiation cannot produce significant changes in the electronic environment of the protons of sulfathiazole molecules.

The presence of ESR signal in the irradiated but not in the control sample definitively points out the production of radiolytic intermediates in solid samples upon irradiation. The ESR spectra of irradiated samples consist of a signal resonance line with a shoulder at low magnetic field and it is distinguishable from noise even at the lowest applied dose (10 kGy). However, the short life radicals decay immediately after the second of irradiation and therefore the recorded experimental spectra are due to the long-life radicals. G value which represents radiation yield of solid sulfathiazole.

Studies Carried Out Under Accelerated Conditions

Physico-chemical properties of solid sulfathiazole and model-ophthalmic solution were observed as not changing in the stability test experiments. The fact that solubility and melting point of unirradiated (control) and irradiated solid samples did not change. The fact that pH values did not significantly change for sulfathiazole powder and model-ophthalmic solution, demonstrates that accelerated stability test conditions have similar effects on unirradiated and irradiated samples.

UV spectra of control (unirradiated) sulfathiazole in acidic and basic media exhibited two λ_{max} values at about 257 nm and 256 nm, respectively, under accelerated conditions (Table 28). λ_{max} of irradiated solid samples dissolved in basic medium did not change, but increased in acidic medium in applied dose and in storage time. Observation of λ_{max} appearing at nearly the same wavelength even after irradiation indicates that sulfathiazole is conserved in the irradiated samples; however, the same is not true for substitution rings. Namely, this ring is affected to a large extent from gamma radiation. Comparison of the proton chemical shifts of control and irradiated samples given in Table 17 shows that gamma radiation cannot produce significant changes in the electronic environment of the protons of sulfonamide molecules, λ_{max} of irradiated model-ophthalmic solution staying constant.

The biphasic character of ESR signal intensity decay curve of irradiated solid samples under accelerated stability test conditions reflects the existance of four radicals of different decay characteristics. Although the g factors and corresponding line shapes of these radicals are similar, they have different features.

It is concluded that irradiation of sulfathiazole powder and model-ophthalmic solution did not produce any changes in the antimicrobial activities. Because of the solubility problems of sulfathiazole formulations, it was not possible to count the number of the micro-organisms (bioburden). Therefore, sal could not be determined preciously. The dose of 10 kGy could be applied to our powder and model-ophthalmic solution of sulfathiazole, and this is a lower dose level than the one mentioned (25 kGy) in EP[11].

CONCLUSION

When the physico-chemical properties of the irradiated substances are analyzed, it is observed that sulfathiazole powder and model-ophthalmic solution are not affected by the irradiation. While evaluating the effect of irradiation on the antimicrobial activities of irradiated samples, no activity loss was observed with the increase in radiation dose. Negative result of the tests concerning of B. Pumilus in uninfected and infected dosage forms by the spores of B. Pumilus indicates that radiation dose of 10 kGy can be applied to our model-ophthalmic solution of sulfathiazole without any changes.

The three month stability test showing that free radicals formed in irradiated samples during the stability period supports that in the accelerated conditions irradiated samples are not very affected by the irradiation when compared to the unirradiated samples.

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