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Radiosterilization of sulfonamides: I: determination of the effects of gamma irradiation on solid sulfonamides

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Abstract

Three antibiotics sulfafurazole (SFZ), sulfamethoxazole (SMZ) and sulfacetamide sodium (SSA-Na) belonging to sulfonamides were irradiated and changes in physico-chemical properties, pH, melting points, UV, IR, NMR, TLC, GC-MS, ESR characteristics, antimicrobial activities of active components were studied at normal and accelerated stability test conditions. Two radiolytic intermediates for both SFZ and SMZ and four radiolytic intermediates for SSA-Na were found in the irradiated solid samples after the GC-MS analysis. The results obtained under normal and accelerated stability test conditions were observed to be consistent with the unirradiated values.

Keywords: Sterilization; ESR; GC-MS; Sulfamethoxazole; Sulfacetamide sodium; Sulfafurazole

1. Introduction

The use of gamma rays for the sterilization of pharmaceutical raw materials and dosage forms is an alternative method for sterilization (Jacobs, 1995; Berk and Özer, 1999; Olguner, 2000). However, one of the major problem of the radiosterilization is the production of new radiolytic products during the irradiation process (Barbarin et al., 2001). Therefore, the principal problem in radiosterilization is to determine and to characterize these physical and chemical changes originating from high-energy radiation (Mathews and Sangster, 1965; Dziegielewski, 1975; Gopal, 1978; Tsuji et al., 1979; Hayes et al., 1980; Bussey et al., 1982; Gibella et al., 2000).

Sulfonamides are widely used as antibacterial agents in the treatment of urinary system infection and in meningococal meningitis profilaxis. Previous investigations on radiosterilization of sulfonamides have been rather devoted to the study of color change, loss of potency, acid and gas formation in irradiated solid and/or aqueous solution samples (Trigger and Caldwell, 1968; Philips et al., 1971; Fleurette et al., 1975). Our investigation is to re-examine in detail, quantitatively the extent and nature of physico-chemical and microbiological activity changes resulting from the action of ionizing radiation in the dose range of 5–50 kGy on the three important members of sulfonamide group antibacterial agents, namely sulfafurazole (SFZ), sulfamethoxazole (SMZ) and sulfacetamide sodium (SSA-Na) using various sensitive spectroscopic, chromatographic and microbiological test techniques beside simple pH, solubility and melting point measurements in order to provide reliable data for the evolution of the radiosterilization of sulfonamides. Therefore, the results relevant to unirradiated and irradiated solid sulfonamides under normal and accelerated stability test conditions will be presented.

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2. Materials and methods

SFZ powder was kindly provided from Adilna-Sanovel Corp. (Turkey), SMZ powder from Roche Corp. (Turkey), SSA-Na powder from Abdi Ýbrahim Corp. (Turkey). Solid samples were used without any further purification. The following procedure was adopted for the irradiation of solid sulfonamide samples.

2.1. Irradiation process

Irradiation was performed at room temperature using a ^{60}Co Gamma Cell 220 available at Turkish Atomic Energy Agency at a dose rate of 2.84 kGy h^{-1}. Solid samples in Type I glass vials were irradiated at the doses of 5, 10, 25 and 50 kGy. The actual doses received by samples were determined by measuring the change in absorbance of red perspex dosimeters (Harwell 4034) at 603 nm attached to the sample glass tubes during the irradiation period. The corresponding doses were obtained from a calibrating graph. Administered doses were accurate to $\pm 3\%$ (95% confidence).

2.2. Studies carried out under normal conditions

Unirradiated samples were used as controls to detect physico-chemical and antimicrobial activity changes resulting from the action of ionizing radiation on studied sulfonamides.

2.2.1. Physico-chemical properties

pH change measurements of the control (unirradiated) and irradiated solid samples were performed using 100 mg ml^{-1} aqueous solutions of these samples. Solubility changes in water, acetone, ether and chloroform and changes in melting points were also monitored.

2.2.2. Spectroscopic methods and techniques

Changes in spectral properties of control and irradiated solid samples were studied using IR, UV, NMR, ESR techniques. IR spectra were recorded for control and irradiated powders in KBr matrix. λ_{max} values in 0.1 N NaOH and 0.1 N HCl were determined for UV analysis.

NMR analysis was performed using proton NMR spectrometer on unirradiated and irradiated samples dissolved in DMSO-d_6. Tetramethylsilane was used as an internal standard.

ESR measurements were carried out using a Varian 9 E-L X-band ESR spectrometer equipped with a TE_{104} rectangular double cavity. Following spectrometer settings were adopted throughout the experiments: Central field, 326.0 mT; sweep width, 10 mT; microwave frequency, 9.1 GHz; microwave power, 10 mW; modulation frequency, 100 kHz; modulation amplitude, 0.2 mT; receiver gain, $2.5 \times 10^3 - 10 \times 10^3$; scan time, 240 s; time constant, 1 s and temperature, room. All measurements were performed using a DPPH reference sample placed in the front cavity. The position of the standard sample in the cavity was not changed throughout the experiments to avoid any possible changes in the cavity filling factor. The spectra were double integrated over the magnetic field range of 320.0–332.0 mT which gives a figure proportional to the numbers of radicals in the sample. Each spectrum was corrected for variation in the amount of material in the "active length" of the ESR tube. A simulation study based on possible radical species was also carried out.

2.2.3. Chromatographic methods

TLC experiments were performed using the technique proposed by Fister and Kajangovic for separation and identification of sulfonamides (Fister and Kajangovic, 1963a, b).

The volatile compounds were analyzed by gas chromatography with MS detection. A headspace sampling method was adopted. The use of static headspace sampling allows the determination of volatile compounds present in a nonvolatile matrix which could not be sampled (Barbarin et al., 1996). Following headspace conditions were adopted throughout the experiments: Auxiliary pressure, 1.4 bar; bath temperature, 45°C; sample vial pressurization 10 s; headspace vent opened for 2 s and injection time, 108 s.

As for GC analysis, it was performed with 3700 Varian chromatograph coupled with mass spectrum detector. The column used was a Pora Plot Q column of 25 cm × 320 μm. Helium purified with oxygen and moisture traps were used as gas carrier at a flow rate of 2 m s^{-1}, the injector and oven temperature were set at 250°C and 4°C, respectively.

2.2.4. Antimicrobial activity studies

Antimicrobial activities of irradiated and nonirradiated preparations were performed by the microdilution broth method recommended by National Committee for Clinical Laboratory Standards (NCCLS, 1997). According to this procedure, microorganism inoculum was prepared and 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), *Pseudomonas aeruginosa* (*P. 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 h at 35°C. Microorganism concentration was adjusted to 0.5 McFarland standard ($0.5–1 \times 10^8$ cfu ml^{-1}) and diluted to be 5.5×10^5 cfu ml^{-1} in each well.

Preparation of stock antibiotic solutions: All the active substances of sulfonamides were dissolved in 1/2 volume hot water and minimal amount of 2.5 mol l^{-1} NaOH. The total volume of stock solutions was achieved by adding appropriate amount of distilled water.

Microdilution process: In the test, 96 well, microtiter trays were used. Two-fold dilutions of irradiated and unirradiated preparations were prepared in Mueller–Hinton broth in the wells of the plates. Each sample was diluted from 1 to 11 wells of each row of microtiter plates (512–0.5 μg ml^{-1}). Previously prepared micro-organism suspensions were added to each well and the plates were incubated for 18–24 h at 35°C. MICs (μg ml^{-1}) were defined as the lowest concentrations (dilution) of the samples that inhibited visible growth of the microorganism.

2.3. Studies carried out under accelerated conditions

In this part of the work, studies performed under normal environmental conditions were repeated for samples stored in a climate chamber in open glass tubes at high temperature (40 ± 2)°C and high relative humidity (75 ± 5)% conditions over a period of 3 months to investigate possible degradation mechanism and kinetics of irradiated powders. Samples were stored in the chamber and aliquots were taken off for measurements at room temperature. Unirradiated samples were used as negative controls for comparison and measurements were repeated every week during the first month and then every month.

3. Results

3.1. Studies carried out under normal conditions

3.1.1. Physico-chemical properties

Although irradiation did not produce changes in color, solubility in water, acetone, ether and chloroform and melting point of samples, significant changes in pH of SSA-Na and SFZ were observed to occur. The results for pH are summarized in Table 1.

3.1.2. Spectroscopic methods and techniques

Unirradiated and irradiated solid samples were studied with IR, UV, NMR and ESR spectroscopic techniques. Analysis of IR spectra indicated that H_2N stretch bands of SSA-Na could not be seen at low radiation dose, while C=C stretch band of benzene ring and symmetric SO_2 stretch (only in 50 kGy) band could not be seen at high doses (25 and 50 kGy). As for SMZ and SFZ C=C stretch bands could not be seen at the high radiation doses, of SMZ although SO_2 stretch bands of SFZ could not be seen at low radiation doses.

λ_{max} values calculated from UV spectra of control and irradiated samples dissolved in 0.1 N NaOH and 0.1 N HCl are given in Table 2 for comparison. As seen from this table, λ_{max} values calculated for control and irradiated samples in both medium are in good agreement with the values given in the literature (Sunshine, 1981). Although, the variation with absorbed dose of the wavelength corresponding to maximum UV absorbance is not significant for samples dissolved in 0.1 N NaOH, a drastic decrease of it is observed for samples dissolved in 0.1 N HCl.

Proton NMR spectra of the samples dissolved in DMSO-d_6 containing tetramethylsilane as an internal reference, consist of different chemical shifts which vary from 1.63 to 11.1 depending on the chemical environment of the related protons. The latter data are in good agreement with those reported previously by Turzcan and Medwicch (1972). Irradiation of solid samples in the dose range of 5–50 kGy did not produce any significant effects on the chemical shifts of sulfonamide protons as shown in Table 3.

ESR spectra of control and irradiated solid samples were also investigated. Although control samples exhibit no ESR signal, irradiated samples are found to present a singlet resonance line centered at $g = 2.0075$ even at the lowest applied dose (5 kGy). Increase in irradiated dose caused an increase in the signal intensity as shown in

Table 1
Measured pH values for control and irradiated solid sulfanomides ($n = 6$)

Sample	PH					
	Reference values	Applied dose (kGy)				
		Unirradiated	5	10	25	50
SSA-Na	8.00–9.35	8.35 ± 0.04	8.35 ± 0.02	8.04 ± 0.04	8.09 ± 0.03	8.28 ± 0.05
SMZ	4.00–5.00	5.73 ± 0.03	5.74 ± 0.03	5.71 ± 0.03	5.71 ± 0.01	5.70 ± 0.06
SFZ	—	4.66 ± 0.02	4.65 ± 0.04	4.62 ± 0.05	4.35 ± 0.02	4.30 ± 0.04

Significance of the effect is $p < 0.05$.

Table 2
λ_{max} values calculated from UV spectra of control and irradiated solid samples dissolved in 0.1 N NaOH and 0.1 N HCl in the 200–400 nm wavelength range ($n = 6$)

Sample	Medium	Unirradiated	Applied dose (kGy)			
			5	10	25	50
SSA-Na	0.1 N NaOH	257.4±0.30	256.8±0.20	256.2±0.20	257.0±0.20	255.6±0.20
	0.1 N HCl	271.8±0.30	265.2±0.20	270.0±0.20	269.8±0.20	202.0±0.20
SMZ	0.1 N NaOH	256.4±0.20	256.0±0.20	256.6±0.20	256.2±0.2	256.0±0.20
	0.1 N HCl	265.2±0.20	265.2±0.20	201.4±0.20	204.2±0.20	209.0±0.20
SFZ	0.1 N NaOH	253.0±0.30	253.8±0.20	253.4±0.20	252.6±0.20	253.2±0.20
	0.1 N HCl	267.0±0.20	202.2±0.20	216.6±0.20	216.2±0.20	203.8±0.20

Significance of the effect $p < 0.3$.

Table 3
Calculated proton chemical shift values for control and irradiated solid samples

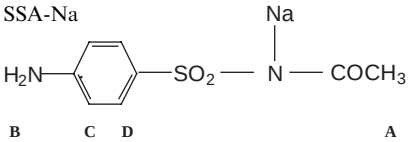
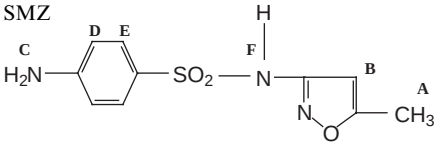
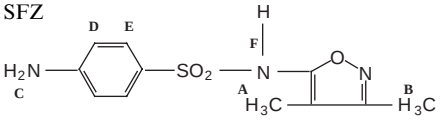
Sample	Related proton(s)	Unirradiated	Applied dose (kGy)			
			5	10	25	50
SSA-Na 	A	1.60	1.50	1.60	1.60	1.60
	B	2.10	1.90	2.20	2.10	2.10
	C	6.10	6.10	6.10	6.10	6.10
	D	6.50	6.60	6.60	6.60	6.60
SMZ 	A	2.20	2.20	2.20	2.20	2.20
	B	6.07	6.03	6.03	6.10	6.07
	C	6.10	6.10	6.10	6.06	6.10
	D	6.60	6.60	6.60	6.50	6.60
	E	7.40	7.50	7.50	7.50	7.50
	F	10.9	10.90	10.90	10.90	10.90
SFZ 	A	1.60	1.50	1.60	1.60	1.60
	B	2.10	1.90	2.20	2.10	2.10
	C	6.10	6.10	6.10	6.10	6.10
	D	6.50	6.60	6.60	6.60	6.60
	E	7.40	7.40	7.40	7.40	7.40
	F	10.50	10.50	10.40	10.50	10.40

Fig. 1. The increases in the peak-to-peak heights with applied dose were found to follow a linear curve in the 0–50 kGy dose range with G values smaller than 0.1 for all studied solid sulfonamide samples as given in Fig. 3. Simulation calculations have shown that four different radicals for SSA-Na and two different radicals for SFZ are responsible for the induced ESR spectra. Characteristic parameters calculated for the radicals and theoretical spectra obtained using these parameters are given in Table 4 and Fig. 2, respectively.

3.1.3. Chromatographic methods

TLC experiments performed using the technique proposed by Fister and Kajangovic (1963a, b) for separation and identification of sulfonamides have given similar R_f values for control and irradiated samples in the applied dose range 15–50 kGy. All TLC spots were found to exhibit blue–violet color when they are illuminated with UV light.

The GC-MS chromatograms of irradiated samples were compared with those obtained for unirradiated

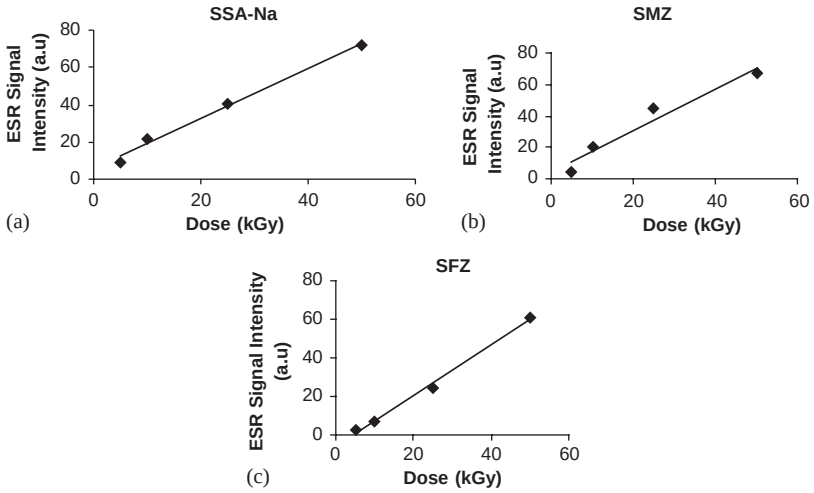


Fig. 1. Variation of ESR signal intensities with applied dose ((a) SSA-Na, (b) SMZ, and (c) SFZ).

Table 4
Characterization of radicals detected by ESR technique

Sample	Radical	I^a (a.u)	Γ^b (G)	g^c	A^d (g)
SSA-Na	$^{\bullet}N$	72.73	5.34	2.0021	17.31
	$^{\bullet}CH_3$	75.54	3.41	2.0046	$A(H) = 0.58$
	$^{\bullet}C$	121.90	1.83	2.0083	—
	$^{\bullet}NNa$	4.32	1.24	2.0040	$A(N) = 22.55$
					$A(Na) = 3.62$
SFZ	$^{\bullet}NH_2$	47.49	1.78	2.0073	$A(N) = 2.46$
	$^{\bullet}C$	296.96	2.56	2.0055	$A(H) = 0.17$
	$^{\bullet}C$	193.52	6.56	2.0058	

[a] Contributing weight.
[b] Half-height at half width.
[c] Lande g factor.
[d] Hiperfine splitting constant.

samples. Four radiolytic products for SSA-Na and two radiolytic products for both SMZ and SFZ were determined to be produced in the irradiated solid samples prepared by headspace method after the GC-MS analysis. The identification of the chromatographic peaks was made according to NIST/EPA/NHI mass spectral database (Stein, 1999). The identity of the detected GC-MS peaks is summarized in Table 5.

3.1.4. Antimicrobial activity study

In the irradiated samples, negligible activity loss was observed. MIC values of irradiated and unirradiated samples were given in Table 6. From these data it is concluded that irradiation of sulfonamide powders did not produce any changes in the antimicrobial activities toward studied standard strains.

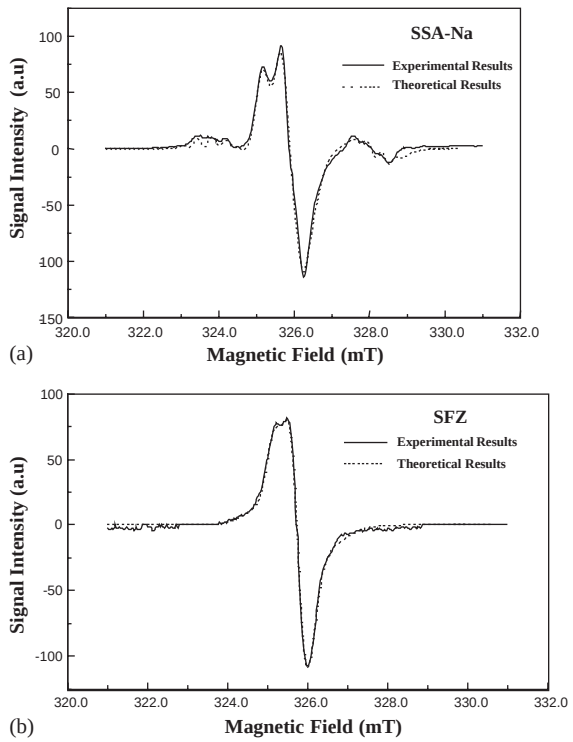


Fig. 2. Theoretical and experimental spectra obtained for irradiated solid sulfonamide.

3.2. Studies carried out under accelerated conditions

Experimental results showed that organoleptic features such as color, smell, solubility in water, acetone, ether, chloroform and melting points of control and irradiated solid samples did not change under accelerated stability

Table 5
GC-MS chromatography

(a) *Volatile impurities found in unirradiated samples*

Identified product	Active substance		
	SSA-Na	SMZ	SFZ
Methane	–	+	+
Acetonitrile	+	+	+
Acetone	+	+	+
Chloroform	+	+	+
Styrene	+	+	+

(b) *Volatile compounds found in irradiated samples*

Identified product	SSA-Na				SMZ				SFZ			
	Applied dose (kGy)				Applied dose (kGy)				Applied dose (kGy)			
	5	10	25	50	5	10	25	50	5	10	25	50
Methane	+	+	+	+	–	–	–	–	+	+	–	–
tert-butyl methyl ether	+	+	+	+	–	–	–	–	–	–	–	–
Carbon oxide sulfide	+	+	+	+	–	–	+	+	–	+	+	+
Acetaldehyde	–	–	+	+	+	+	+	+	–	–	–	–

(+) present, (–) not present.

Table 6
MIC values of irradiated and unirradiated samples

Sample	Bacteria	MIC ($\mu g.ml^{-1}$)				
		Unirradiated	Applied dose (kGy)			
			5	10	25	50
SSA-Na	*S. aureus*	256	256	128	512	512
	E. coli	512	512	512	512	512
	E. faecalis	512	512	512	512	⩾512
	P. aeruginosa	⩾512	⩾512	⩾512	⩾512	⩾512
SMZ	*S. aureus*	32	32	32	32	256
	E. coli	16	16	16	32	64
	E. faecalis	16	32	64	64	64
	P. aeruginosa	512	⩾512	⩾512	⩾512	⩾512
SFZ	*S. aureus*	64	64	64	64	64
	E. coli	128	128	128	128	128
	E. faecalis	128	128	128	128	128
	P. aeruginosa	512	⩾512	⩾512	⩾512	⩾512

test conditions; however, pH values did change as shown in Table 7.

Although λ_{max} values of unirradiated and irradiated powders dissolved in 0.1 N NaOH were found to exhibit no changes all over the stability studies, that of control samples dissolved in 0.1 N HCl experienced a significant decrease after the first week, then it stayed approximately constant. However, the changes in λ_{max} values of irradiated samples were less pronounced. IR and NMR spectra of control and irradiated samples stored for 3 months under the stability test conditions, were found to exhibit characteristic features of the spectra obtained for samples stored at normal environmental conditions.

As is emphasized in previous sections of the present work, unirradiated samples do not exhibit any ESR signal. Storing of these samples at stability test conditions, that is, at high temperature and at high relative humidity, did not create any changes in this feature. However, storing irradiated samples in the accelerated test conditions was observed to cause rapid decreases in the ESR signal intensities of the samples due to the decay of the radiolytic intermediates created during the irradiation at room temperature. The results obtained for studied sulfonamide samples irradiated at a dose of 25 kGy are given in Fig. 3. As is seen from this figure, ESR signal intensity decay curves exhibit biphasic character; At the beginning of the storage period (0–12 day) the unstable radiolytic products decay completely, then the more stable ones dominate the decay curve. However, at the end of the storing period (90th day) all the radiolytic intermediates decay almost completely.

R_f values determined by TLC method for control and irradiated solid samples stored at accelerated stability test conditions were found to be independent of storage time, that is, they were calculated to be similar to those obtained just at the beginning of the test period.

At the beginning of the storage period under stability test conditions, MIC values against *E. coli* were found to

Table 7
Measured pH values for control and irradiated solid sulfonamides stored at high temperature and at high relative humidity in a climate chamber ($n = 6$)

Time (day)	PH																			
	SSA-Na					SMZ					SFZ									
	Unird	5	10	25	50	Unird	5	10	25	50	Unird	5	10	25	50					
0	8.35±0.04	8.35±0.02	8.35±0.04	8.35±0.03	8.35±0.05	5.73±0.03	5.80±0.03	5.71±0.06	5.77±0.07	5.70±0.06	4.80±0.02	4.65±0.04	4.62±0.05	4.65±0.02	4.30±0.04					
7	8.15±0.05	8.3±0.05	8.00±0.07	7.95±0.08	8.63±0.02	4.75±0.04	5.62±0.03	5.45±0.09	5.47±0.09	5.76±0.04	4.61±0.03	4.09±0.01	4.58±0.06	4.20±0.05	3.23±0.02					
14	8.03±0.01	8.32±0.01	7.72±0.08	7.60±0.09	8.51±0.10	5.31±0.01	5.60±0.08	5.33±0.06	5.33±0.10	5.76±0.10	4.59±0.02	4.04±0.05	4.52±0.04	4.10±0.07	2.99±0.08					
21	8.00±0.06	8.43±0.04	8.34±0.05	8.55±0.02	8.52±0.02	5.25±0.05	5.25±0.05	5.08±0.10	4.83±0.05	5.60±0.02	4.58±0.03	3.91±0.08	3.77±0.06	3.66±0.04	2.94±0.10					
28	8.32±0.04	8.34±0.05	7.92±0.04	8.21±0.01	8.48±0.03	5.42±0.01	4.52±0.08	5.69±0.02	5.28±0.04	5.06±0.06	4.24±0.10	3.06±0.04	3.25±0.10	3.16±0.09	3.06±0.03					
60	8.50±0.06	8.38±0.04	7.41±0.05	8.20±0.04	8.32±0.04	4.74±0.03	4.57±0.05	4.52±0.03	4.52±0.03	4.57±0.05	4.52±0.03	3.07±0.06	3.52±0.03	3.12±0.04	2.97±0.06					
90	7.19±0.05	8.50±0.01	8.42±0.07	8.47±0.08	8.40±0.04	4.48±0.04	4.89±0.06	4.58±0.04	4.58±0.04	4.90±0.03	3.28±0.02	3.20±0.02	3.23±0.03	3.20±0.02	3.20±0.07					

Significance of the effect is $p < 0.05$.

be the same for all applied doses; then they decreased with increasing storage time up to 4 weeks. However, at the end of this period they started to increase. As for MIC values against *E. faecalis*, they increased with increasing storage time, while MIC values against *P. aeruginosa* strain stayed always bigger than 512 μg ml^{-1}.

4. Discussion

4.1. Studies carried out under normal conditions

Color change in the irradiated substances is the simplest and helpful observation to get information about possible radiolytical intermediates produced in these substances upon irradiation. As no color change was observed in irradiated solid sulfonamides in the applied dose region of 5–50 kGy, it can be concluded that either radiolytical intermediates are not produced by irradiation in studied samples or created intermediates do not exhibit any absorption in the visible region. The negative result in color change of the present work is consistent with the results reported in the literature for similar compounds (Philips et al., 1971). Experimental results concerning the decrease in pH value with increasing applied dose (Table 1) indicate acid formation in irradiated samples. Similar pH decreases were also observed in irradiated cephalosporin group of antibacterial agents (Jacobs, 1983) and ampicillin (Jacobs, 1984). Radiation did not cause any change in the solubility of studied sulfonamides in water, acetone, ether and chloroform and the melting point of these antibacterial agents.

UV spectra of control sulfonamides in acidic and basic media exhibit two λ_{max} values at about 253–256 and 264–271 nm, respectively (Table 2). λ_{max} observed in acidic medium at about 253–256 nm and in basic medium at about 264–271 nm originate from basic sulfonamide group and substitution groups, respectively. Observation of λ_{max} appearing at about 253 nm at nearly same wavelength even after irradiation indicates that sulfonamide group 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 3, shows that gamma radiation did not produce significant changes of the electronic environment of the protons of sulfonamide molecules.

The presence of ESR signal in irradiated but not in control sample points out definitively the production of radiolytic intermediates in solid samples upon irradiation. The ESR spectra of irradiated samples consist of a single resonance line with a shoulder at low magnetic field and it is distinguishable from noise even at the lowest applied dose (5 kGy). However, contributing

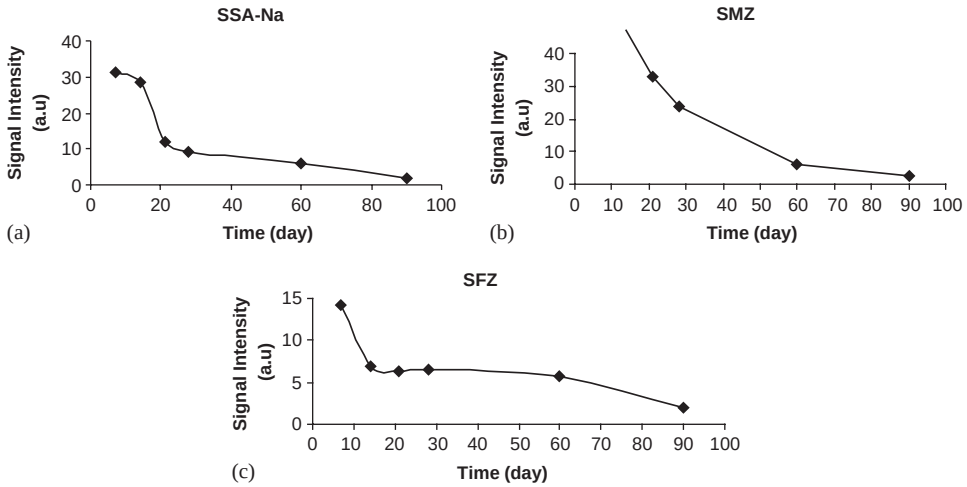


Fig. 3. The decay of ESR signal intensities for samples irradiated at a dose of 25 kGy and stored under accelerated test conditions (high temperature and high relative humidity) in a climate chamber: (a) SSA-Na, (b) SMZ, and (c) SFZ.

radicals were observed to have long-life decay characteristics. Calculated relatively small G value (<0.1) which represents radiation yield of the studied samples shows that sulfonamides are fairly resistant to gamma irradiation.

Although Philips et al. (1971) have reported four different radiolytic intermediates in their TLC experiment on irradiated sulfamerazine sodium, sulfadiazine and sulfametazine, our TLC results on solid sulfonamides irradiated up to 50 kGy revealed only one single component whose R_f value falls into the range reported for intact sulfonamide molecules (Philips et al., 1971). The inefficiency of the TLC technique used in the present work is believed to be at the origin of failure of detecting the missing intermediates.

GC-MS analysis of sulfonamides irradiated up to 50 kGy shows that four different radiolytic intermediates were produced by the radiation in SSA-Na (methane, carbon oxide sulfide, acetaldehyde and *tert*-butyl methyl ether); two in SMZ (methane and acetaldehyde) and two others in SFZ (methane and carbon oxide sulfide). However, the amounts of these intermediates are very low compared with those obtained in similar works (Barbarin et al., 1996). They could either come from the molecular degradation of studied sulfonamides or they could also originate from the residual solvent already present in the unirradiated drugs.

The decrease in SO_2 symmetric and antisymmetric stretching IR line intensities, the decrease of pH values at high radiation doses and the fact that gamma irradiated solid SFZ and SMZ; which have very similar chemical structure give rise to some radiolytic intermediates support this conclusion.

As it is emphasized in the experimental results section, solid samples irradiated up to 50 kGy do not loose their antimicrobial activities against *E. coli*, *E. faecalis*, *P. aeruginosa* and *S. aureus*. This feature of solid sulfonamides points out that the drugs containing these three sulfonamides (SSA-Na, SMZ and SFZ) as active components can be potential candidates for sterilization by gamma radiation.

4.2. Studies carried out under accelerated conditions

Physico-chemical properties of solid sulfonamides were observed not to change under accelerated test conditions except the increase in the water content of the samples originating from hygroscopic properties of sulfonamides. The fact that solubility and melting point of unirradiated and irradiated solid samples did not change and pH values of the latter decreased with increase in storage time demonstrate that accelerated stability test conditions have similar effects on unirradiated and irradiated samples.

λ_{max} of irradiated solid samples dissolved in basic medium stayed constant but its decrease in acidic media with increase in applied dose and storage time shows the difference in characteristic properties of the complexes formed by intact sulfonamide molecules and/or radiolytic intermediates in acidic and basic media. Observation of all characteristic IR bands even throughout the stability test experiments can be taken as an indication of stability of intact and radiolytic intermediates under accelerated stability test conditions.

The biphasic character of ESR signal intensity decay curve of irradiated solid samples under accelerated stability test conditions (Fig. 3) reflects the existence of at least two radical species of different decay characteristics. Although the g factors and corresponding line shapes of these radicals are similar, their other

spectroscopic features are different. The decay rates of the radicals are expected to be increased under stability test conditions, because the larger the water content of the sample, the faster the decay rate of the radicals. In fact, the decay rates of the radicals under normal conditions are found to be slower than the decay rate of the radicals under stability test conditions where the water content of the samples is higher due to the diffusion of water molecules into the sample. SO_2^- ionic radical is expected to be the dominant radical species governing the decay features of irradiated sulfonamides.

5. Conclusion

The sulfonamide group antibacterial agents are being used in drug industry in parenteral, ophthalmic and enteric forms, and as for the sterilization aseptic filtration and autoclave methods are used. If the sterilization process is altered, the product is accepted as a new (innovate) drug by the pharmacopeias, and this necessitates showing the suitability of both the active substance and the pharmaceutical dosage form to the pharmacopeial criteria.

This study aims at the effective usage of gamma radiation because of many advantages like low cost effectivity, not requiring a separate area or any specific equipment as in aseptic filtration, the possibility of being used for the sterilization of packaged drugs, and the probability of serving to many different industrial branches (drug industry, cosmetic industry, chemical industry and food industry) with only a single radiation unit. With this study the researchers, via a sample physibility study, intent to form a reference for further industrial studies.

When the chemical properties of the studied sulfonamides are analyzed, it is observed that these three substances are affected by irradiation. SSA-Na was found to be the most sensitive antibacterial agent among studied drugs, likely due to the polar groups existing in its structure, because changes are expected to occur in these groups after the absorption of high-energy gamma rays.

The destruction of aromatic structure of SSA-Na at low radiation doses revealed by spectral investigations together with the decrease of pH values at high radiation doses support these results. Moreover, the presence of $(SO_2)^-$ ionic, carbon and nitrogen centered free radical species in the irradiated SSS-Na revealed by ESR spectra simulation calculations shows that both sulfanilamide group and the S–N bond of the structure were affected by the radiation (Fig. 4).

As for SFZ and SMZ which have similar chemical structure, SFZ is found to be more resistant to radiation than SMZ. Productions of methane at low and carbon oxide sulfide at high radiation doses, indicate that

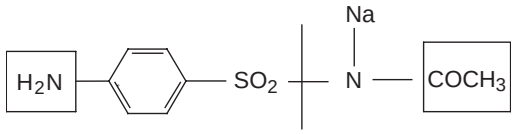


Fig. 4. Chemical structure of SSA-Na and the possible degradation pathways.

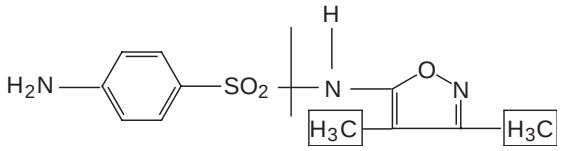


Fig. 5. Chemical structure of SFZ and the possible degradation pathways.

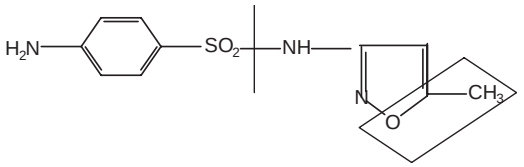


Fig. 6. Chemical structure of SMZ and the possible degradation pathways.

substituted CH_3 group in isoxasole ring is separated from the structure and possibly sulfonilamide structure is destroyed at these radiation dose levels, respectively. The absence of SO_2 IR-stretch bands in high-dose irradiated SFZ support this finding. Significant decreases in the pH values above 10 kGy witness the production of acid at high doses. ESR spectra simulation calculations confirms the production of SO_2^- ionic radical in gamma irradiated SFZ due to direct effect of radiation on the bonds S–N and S-benzene ring. Possible degradation pathways of SFZ are given in Fig. 5.

Similarly acetaldehyde and carbon oxide sulfide possibly produced via the degradation of substituted CH_3 group to isoxasole ring and by destruction of sulfanilamide group, respectively, were found as radiolytic products from GC-MS analysis of irradiated SMZ. Possible degradative pathways of irradiated SMZ is given in Fig. 6.

While evaluating the effect of irradiation on the antimicrobial activities of irradiated samples, negligible activity loss was observed with the increase in radiation dose.

Three-month stability test results show that ESR signal intensity decreases very rapidly under the accelerated stability test conditions. These decrease present biphasic character which is considered to indicate the presence of radical species of different

decay characteristics as revealed by ESR spectra simulation calculation.

Observation of similar effects both in unirradiated and irradiated samples during the stability period supports that in the accelerated test conditions, irradiated samples are not affected more by the irradiation than the unirradiated samples.

Although radiolytic intermediates are produced in studied solid sulfonamides, these substances are not very sensitive to gamma radiation. Small amount of radiolytical intermediates produced in these antibacterial agents decay very rapidly especially at accelerated test conditions; therefore, it is concluded that SSA-Na, SFZ and SMZ can be safely sterilized by gamma radiation.

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