



Electron Paramagnetic Resonance Investigation of Radiation Effect on Certain Types of Drug Powders

Şemsettin OSMANOĞLU^{1*}, Kerem SUTCU²

¹Dicle University, Faculty of Science, Department of Physics, Diyarbakır, TURKEY

²Dicle University, Faculty of Education, Department of Science Education, Diyarbakır, TURKEY

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Abstract: In this study, gamma rays were applied to the sulfadimethoxine, pyrazinamide, and chloramphenicol in the dose range of 1-15 kilogray, in order to detect radiosensitivity of paramagnetic species, which are formed after irradiation by electron paramagnetic resonance spectroscopy. Firstly, the intensities of electron paramagnetic resonance signals of the samples as a function of the microwave power were investigated at room temperature. While no signal was obtained from the unirradiated samples, the irradiated samples exhibited significant spectra. Additionally, the time dependency of the samples was analyzed to study the stability of the radicals.

Keywords: Electron Paramagnetic Resonance, Free radicals, Drug.

Bazı İlaç Tozlarında Radyasyon Etkisinin Elektron Paramanyetik Rezonans İncelenmesi

Özet: Bu çalışmada, elektron paramanyetik rezonans spektroskopisi ile ışınlama sonrası oluşan paramanyetik türlerin radyasyon duyarlılığını saptamak için sülfadimetoksin, pirazinamid ve kloramfenikol numunelerine 1-15 kilogray dozunda gama ışınları uygulandı. İlk olarak, numunelerin elektron paramanyetik rezonans sinyal şiddetleri, mikrodalga gücünün bir fonksiyonu olarak oda sıcaklığında incelendi. Işınlanmamış örneklerden herhangi bir sinyal elde edilmeyen, ışınlanmış numuneler belirgin spektrumlar sergiledi. Ek olarak, radikallerin kararlılıklarını incelemek amacıyla numunelerin zamana bağlılığı analiz edildi.

Anahtar Kelimeler: Elektron Paramanyetik Rezonans, Serbest radikaller, İlaç.

1. INTRODUCTION

Sterilization is a process of inactivating microorganisms that can be anywhere, anytime [1]. While determining the methods to be used in sterilization, properties such as type and structure of the material should be taken into account; because there is no ideal method of sterilization for each material. Currently, the sterilization method using radiation is often benefited in order to improve hygienic quality

of the food, drugs and pharmaceutical raw materials, disposable medical products as well as to prolong their shelf lives and prevent food borne diseases [2-4]. Because, thanks to the powerful penetration ability of gamma rays, sterilization method with radiation can be applied to the product to be sterilized even in the final packaging stage of the product and it doesn't leave any chemical residues behind [5]. In addition, the product sterilized with gamma rays doesn't display any radioactivity and its

* Corresponding author. Email address: sems@dicle.edu.tr
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temperature doesn't increase [6]. However, in addition to these advantages, gamma rays have also some disadvantages due to their high energies. Free radicals created in the product by gamma rays and the regulations vary from country to country in respect to method of irradiation are some of these disadvantages [7]. Therefore, the consumers would like to know whether the product, which they will consume, was exposed to any radiation application; and the amount of dose in kGy, if it was exposed to radiation [8]. As a result, it is necessary to investigate the radiation sensitivity of the raw materials of drugs to be exposed to radiosterilization. Electron Paramagnetic Resonance (EPR) spectroscopy is one of the leading methods used to investigate the radiation sensitivity of irradiated samples of food and drugs [9,10].

Gamma radiation effects on metronidazole, which is an antibacterial agent, were investigated by Basly et al.[11]. The free radicals evolution with dose was quasi-linear from 1 to 50 kGy. The variations of the heights of sulfatiazole, one of the commonly used sulfonamides as an antibacterial agent in drugs, with applied microwave power were studied [12]. The heights of the peak saturated with different rates as inhomogenously broadened resonance lines do. This behaviour of resonance peak indicated different radical species in the studied sample. γ -radiolysis of some antibiotics were studied by Miyazaki et. al.[13]. The radicals were observed by EPR even after storage time of the irradiated samples for ca. 150 days. (ca.: abbreviation of "circa", meaning approximately)

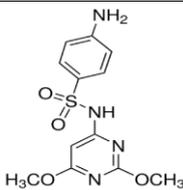
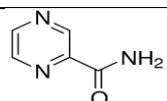
Sulfadimethoxine (SD), Pyrazinamide (PA) and Chloramphenicol (CP) are biologically very important antibiotics. SD is a sulfonamide that is widely used to treat many types of coccidiosis [14]. Sulfonamides are chemotherapeutic drugs used for the treatment of bacterial infections in human. PA, which is poorly water soluble, white and in the form of crystalline powder, is used in the treatment of

tuberculosis [15]. CP is a broad spectrum antibiotic used for the treatment of typhoid, dysentery and ocular bacterial infections [16]. Due to the fact that irradiation at low doses has the advantage of minimizing the damage to the products, [17] up to 15 kGy of irradiated SD, PA, and CP samples were used in this study. It has been aimed to determine radiation sensitivities of free radicals formed in the structure of SD, PA and CP drug samples using EPR technique after getting irradiated by certain doses of gamma rays.

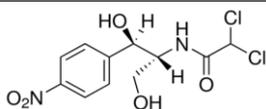
2. MATERIALS and METHODS

For the study purpose, SD, PA and CP samples were supplied from commercial sources in powder form. The chemical formulas and molecular weight of SD, PA and CP were given in Table 1. The samples were irradiated with ^{60}Co γ -ray source (Isotope, Ob-Servo Sanguis) to 15 kGy at room temperature and EPR measurements were taken over a period of several months in order to follow the stability of the radicals formed after irradiation. The EPR measurements were conducted in a Varian E-109 Line Century Series EPR spectrometer operating at microwave power 1 mW, microwave frequency of 9.2 GHz. The g factors were calibrated by comparison with a DPPH sample ($g=2.0036$). Additionally, SEM analysis was done using a FEI FEG 250 electron microscope.

Table 1. Chemical list of SD, PA, and CP samples.

Symbol Formula Molecular Weight (g/mol)	Chemical structure
SD $\text{C}_{12}\text{H}_{14}\text{N}_4\text{O}_4\text{S}$ 310.33	
PA $\text{C}_5\text{H}_5\text{N}_3\text{O}$ 123.11	

CP
 $C_{11}H_{12}Cl_2N_2O_5$
 323.13



3. RESULTS and DISCUSSION

3.1 Microwave Studies

It is important to analyse the changes occurring in EPR signal intensities depending on the microwave power in terms of conducting experiments within the range of power values under microwave power saturation values [18]. In this regard, firstly the changes in signal intensities depending on microwave power of three drug samples, which are irradiated by gamma rays within a dose of 15 kGy, were investigated. Microwave power saturation changes of irradiated SD, PA and CP are given in Figs. 1-3, respectively. In the calculations made by examining the spectra obtained from the SD and PA samples, it was determined that the EPR signal intensity of the SD represents the best compatibility with polynomial function, however, PA represents the best compatibility with the logarithmic function. Besides, the curve obtained from the signal labelled with I_1 showed a logarithmic development, whereas curves labelled with I_2 and I_3 showed linear development. This shows that the origin of free radicals induced in gamma-irradiated CP is different [19].

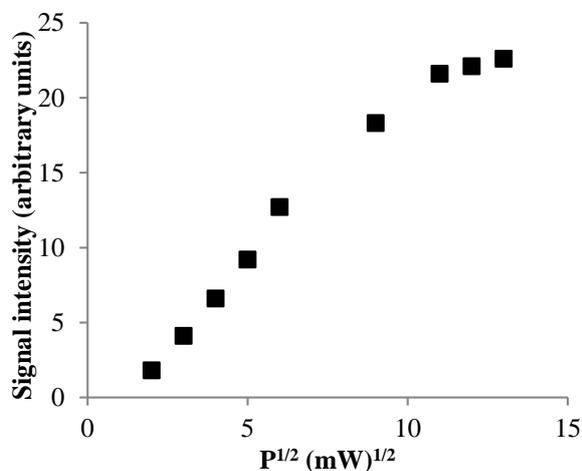


Figure 1. Variations of the EPR peak intensities of 15 kGy irradiated SD sample with square root of applied microwave power (P).

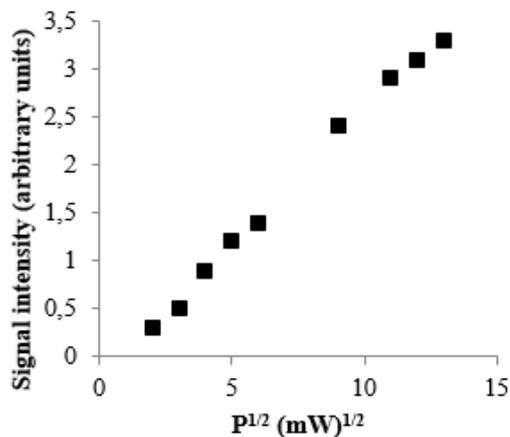


Figure 2. Variations of the EPR peak intensities of 15 kGy irradiated PA sample with square root of applied microwave power (P).

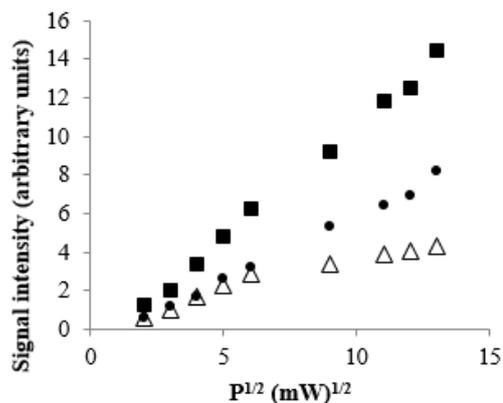


Figure 3. Variations of the EPR peak intensities of 15 kGy irradiated CP sample with square root of applied microwave power (P). Signal I_1 (Δ), Signal I_2 (\blacksquare), Signal I_3 (\bullet).

3.2 Dose-Response Curves

Aside from qualitative detection EPR can be used for dose estimation [18,20]. Therefore, the effect of increasing radiation dose on the spectra of drugs was studied. Despite the increasing dose of radiation, no change was observed in the form of spectra, but signal intensities appeared to increase significantly. The increases in the peak-to-peak distance of the EPR spectra signals reveal that the number

of the free radicals increases in the samples after the irradiation [21,22]. When each spectrum was examined separately, a significant change in g and ΔH values was not determined despite the increased dose of radiation. The EPR spectra of SD, PA, and CP recorded at room temperature are shown in Figs. 4-6, respectively. The measured values of g -factors of SD, PA and CP are 2.0022 ± 0.0005 , 2.0003 ± 0.0005 and 2.0093 ± 0.0005 , respectively. The g values of these samples seem to be in agreement with literature [23-25].

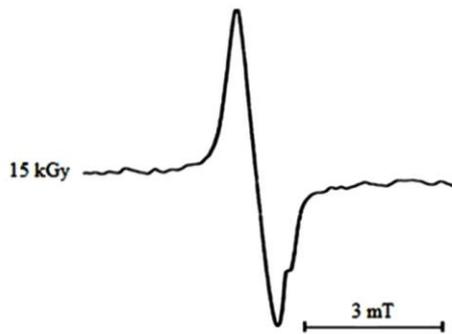


Figure 4. The EPR spectrum of 15 kGy irradiated SD sample.

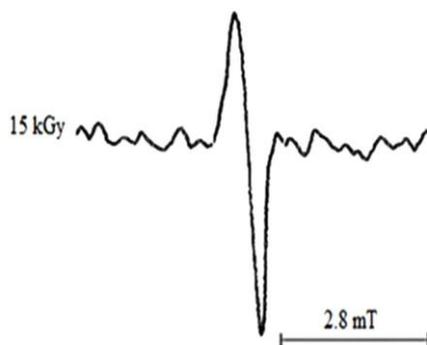


Figure 5. The EPR spectrum of 15 kGy irradiated PA sample.

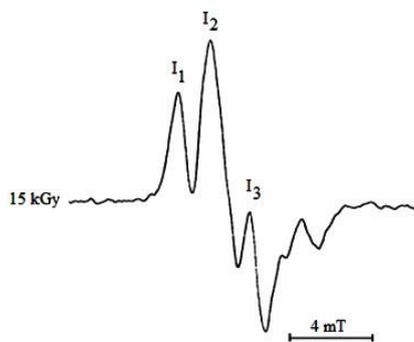


Figure 6. The EPR spectrum of 15 kGy irradiated CP sample.

EPR signal intensities of SD and PA samples depending on radiation dose applied are given in Figs. 7 and 8, respectively; while the change graph of 3 resonance peaks of CP sample obtained after irradiation depending on the dose of radiation applied is displayed in Fig. 9.

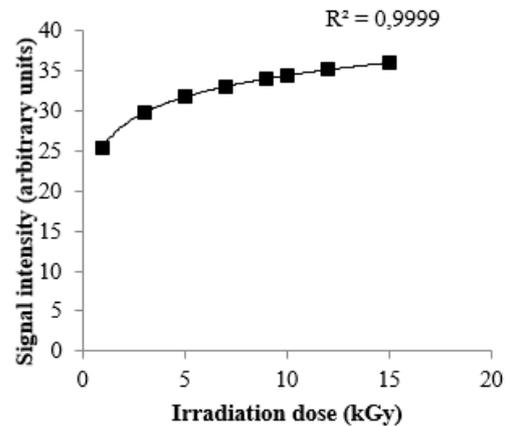


Figure 7. Dose-response curve of SD sample.

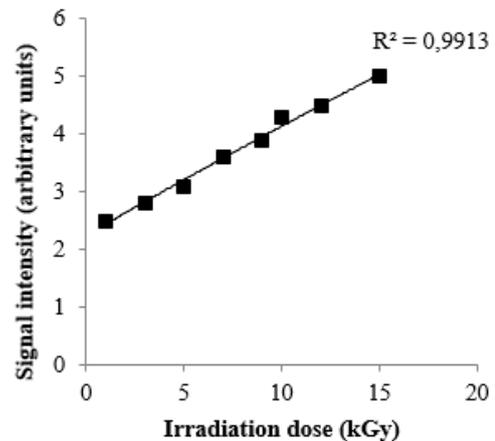


Figure 8. Dose-response curve of PA sample.

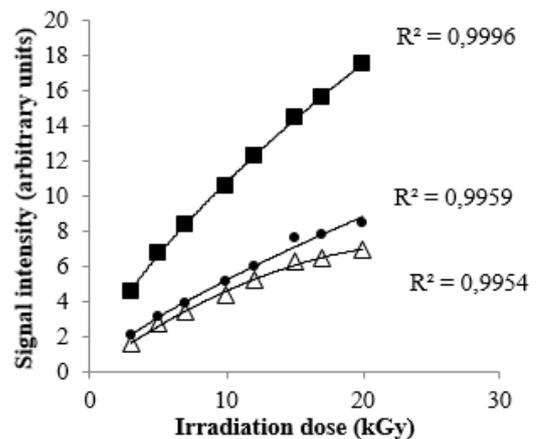


Figure 9. Dose-response curve of CP sample. Signal I₁(Δ), Signal I₂(\blacksquare), Signal I₃(\circ).

In the graphs; ■, Δ and ● signs represent experimental results; while curves connecting them represent the closest mathematical functions to experimental results. The choice of mathematical functions is quite important to determine the amount of absorbed radiation dose by measuring peak to peak signal intensities of the samples. However, there is no single ideal method for this selection. Mathematical equations that show the best fit of the samples and correlation coefficient (R^2) are

given in Table 2. This comment agrees with results obtained for various kind of drugs studied before [26,27]. In these functions, I and D are used for EPR signal intensity and treated irradiation dose in kGy, respectively and other parameters are constants to be determined. Considering Table 2; the best fit of SD sample seems to be with logarithmic function and the best fit of PA sample is with linear function, respectively.

Table 2. Mathematical functions calculated for dose-response curve of gamma irradiated SD, PA, and CP samples.

Functions	Symbol	SD sample	PA sample	CP sample		
				I ₁	I ₂	I ₃
I = aD+b	a	0.6834	0.1858	0.3796	0.8125	0.4707
	b	27.204	2.2723	0.6434	2.475	0.7204
	R ²	0.8612	0.9910	0.9951	0.9968	0.9970
I = -aD ² +bD+c	a	0.0583	0.0007	0.0041	0.0106	0.0061
	b	1.6001	0.1966	0.4536	1.0028	0.5800
	c	24.718	2.2432	0.3823	1.8037	0.3349
	R ²	0.9757	0.9913	0.9966	0.9989	0.9991
I = a ln(D)+b	a	3.8926	0.9104	2.8148	6.0249	3.4864
	b	25.519	2.0798	-1.766	-2.683	-2.259
	R ²	0.9999	0.8512	0.9678	0.9694	0.9673
I = aD ^b	a	25.717	2.2668	0.6713	2.1268	0.8052
	b	0.1275	0.2610	0.8271	0.7056	0.8346
	R ²	0.9968	0.9039	0.9950	0.9994	0.9995
I = ae ^{bD}	a	27.268	2.4297	1.4249	4.014	1.7132
	b	0.0220	0.0514	0.1064	0.0916	0.1079
	R ²	0.8256	0.9813	0.9312	0.9517	0.9442

Considering changes in the signals labelled with I₁, I₂ and I₃ of CP sample depending on increased radiation dose; similar to microwave changes, I₁ signal is determined to behave differently from I₂ and I₃ signals. It has been determined that the best fit of I₁ signal is the function of I = aD²+bD+c ($R^2=0.9966$), whereas the best fit for I₂ and I₃ signals is the function of I = aD^b ($R^2=0.9994$, $R^2=0.9995$). According to this result, in parallel with microwave change, it can be stated that at least two types of free radical are

formed in the sample of CP after irradiation. In addition, SEM images of the samples are obtained in order to see whether gamma radiation has any effect on the surfaces of the samples either non-irradiated or irradiated by gamma rays in 15 kGy. In Figs. 10a-10f SEM images of the irradiated and non-irradiated SD, PA and CP samples are presented, respectively. Considering SEM images of the samples, irradiation didn't cause any changes on the surfaces of the samples.

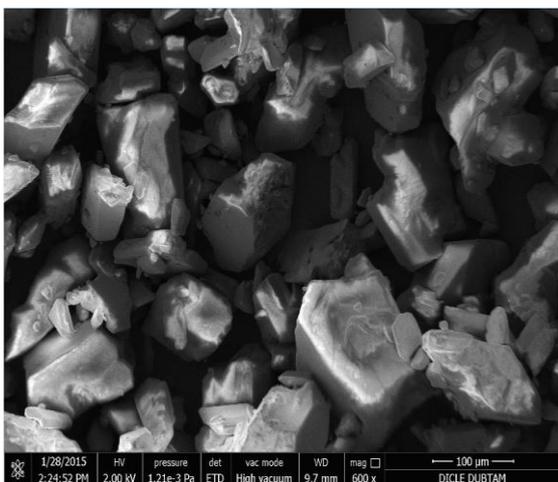


Figure 10a. SEM image of unirradiated SD sample.

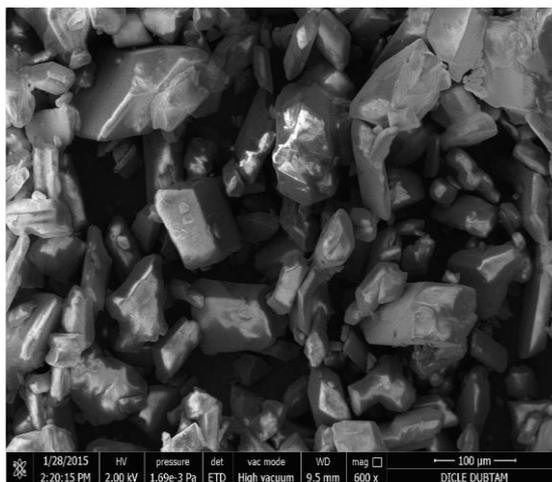


Figure 10b. SEM image of irradiated SD sample.



Figure 10c. SEM image of unirradiated PA.

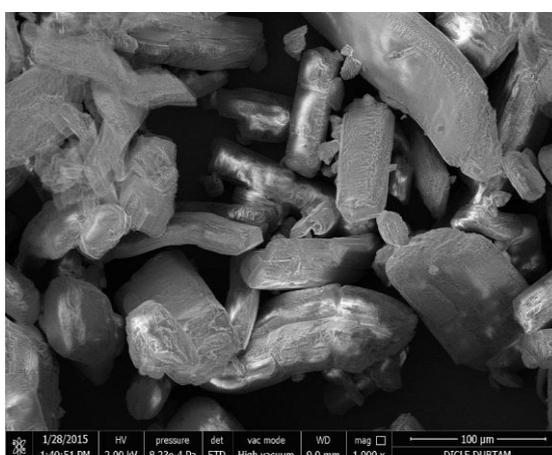


Figure 10d. SEM image of irradiated PA sample.

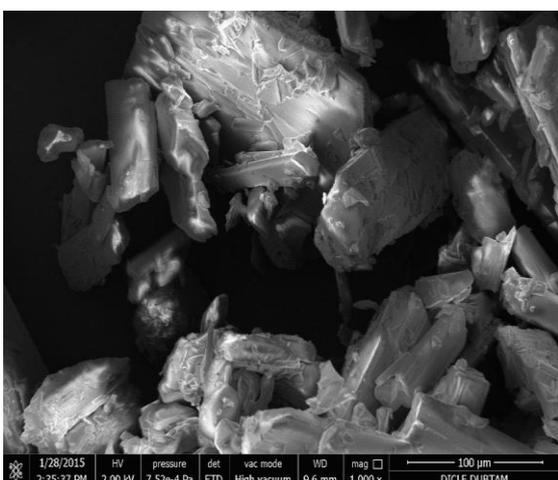


Figure 10e. SEM image of unirradiated CP sample.

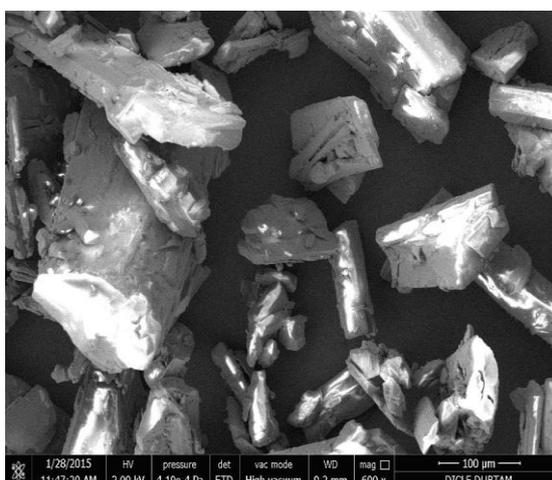


Figure 10f. SEM image of irradiated CP sample.

3.3 Radical Decays at Room Temperature

The signals created depending on irradiation of drugs should be capable of being tested during their shelf lives [28]. EPR spectrums of all three samples were recorded at room temperature

within regular intervals in the daily process of 260 days. In the spectra obtained, no significant changes in the shape of EPR signals and g values were observed, while significant decrease was identified in the signal intensities. Decreased signal intensities indicate that the number of free radicals formed after irradiation also reduced. Throughout the process, samples were stored in an airtight and dark environment at room temperature. Decay curves of SD and PA samples irradiated by 15 kGy are given in Figs. 11 and 12, respectively.

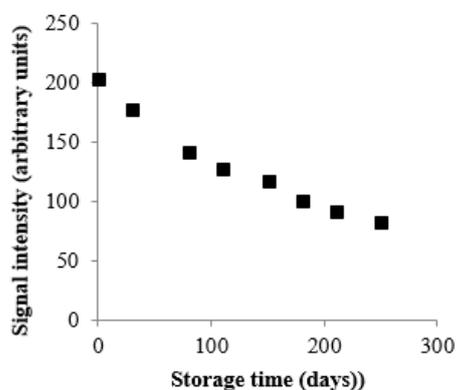


Figure 11. EPR signal intensity of SD sample irradiated at a dose of 15 kGy as a function of time.

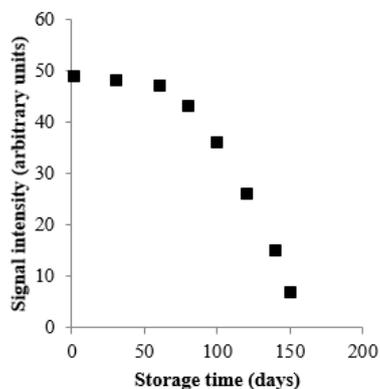


Figure 12. EPR signal intensity of PA sample irradiated at a dose of 15 kGy as a function of time.

Considering Fig. 11, according to spectra of SD sample recorded at the end of 160th day after irradiation, peak to peak signal intensity was reduced by 57%, whereas it was reduced by 60% at the end of the 250th day, respectively. These data show that even at the end of the 250th day, SD sample can be easily distinguished from non-irradiated sample by EPR technique.

On the other hand, the decay rate of signal intensity of PA sample seems rather slower in the first three months after irradiation; however, the decrease in the signal intensity of PA sample seems faster after three months (Fig. 12). In the EPR spectra recorded at the end of the 80th day, the central signal intensity was reduced by 12% compared to the signal intensity recorded right after irradiation. Furthermore, at the end of the 150th day, only 14% of the signal intensity is observed. At the end of the 250th day, no signal intensity was observed in the EPR spectrum of PA sample.

In Fig. 13, decay rates of signals labelled with I_1 , I_2 and I_3 of CP sample are presented. In the first 80 days, the reduction in the I_2 signal was calculated as 3%, whereas it was 23% and 15% for I_1 and I_3 signals, respectively. According to calculations based on spectra taken at the end of the 150th day, I_1 , I_2 , and I_3 signals have preserved 56%, 68% and 51% of their signal intensities, respectively. Since all three signals are visible despite weakly in the spectrum of irradiated CP sample recorded at the end of the 250th day, it has been shown that irradiated CP sample can be easily distinguished from non-irradiated sample by using EPR spectrometry method even at the end of the 250th day.

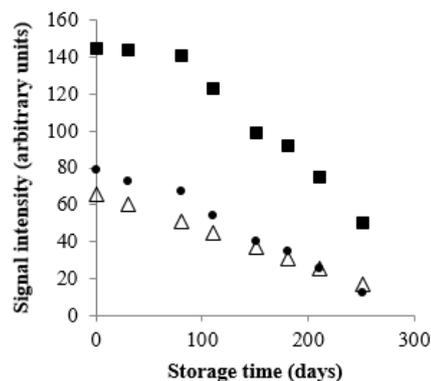


Figure 13. EPR signal intensity of CP sample irradiated at a dose of 15 kGy as a function of time. Signal I_1 (Δ), Signal I_2 (\blacksquare), Signal I_3 (\bullet).

4. CONCLUSION

Since no EPR signal is observed in the non-irradiated samples and signals are clearly seen

in all samples after irradiation, it is very obvious that free radicals are created in all the samples after irradiation. It has been concluded that at least two types of free radicals are formed in CP samples since peak to peak signal intensity of I_1 showed different development compared to I_2 and I_3 signals as microwave power and radiation dose were increased. Increasing irradiation dose up to 15 kGy has increased the signal intensities of the samples; however, no significant change was observed in their g spectroscopic splitting factors. Obtaining visible signals in the spectrums of samples, which were stored at room temperature, even after 200 days of gamma irradiation shows that stable radicals are formed in these samples after irradiation process. This result shows that samples are not suitable for sterilization by radiation. Considering SEM images of all samples either non-irradiated or irradiated by 15 kGy; since irradiation process didn't create any significant difference on the surfaces of samples, it has been determined that SEM screening method cannot be used to distinguish irradiated and non-irradiated samples from each other.

According to the analyses conducted, EPR spectroscopy can be used to distinguish irradiated and non-irradiated drug samples. The high correlation between experimental results of the samples on the dose-response curves and mathematical equations allows us to calculate the signal intensity of a sample obtained in any dose value.

In this dosimetric study conducted with EPR spectroscopy, radiation sensitivities of all three drugs were determined in addition to EPR parameters of the radicals formed in the samples after irradiation.

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