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Dosimetric Investigation of Acetaminophen Drug Raw Materials by Electron Paramgangnetic Resonance Spectroscopy

Fırat AKBALIK
Akyurt Anatolian High Scholl, Ankara, Turkey

Acetaminophen İlaç Hammaddelerinin Elektron Paramanyetik Rezonans Spektroskopisi ile Dozimetrik İncelenmesi

Abstract

In this study, powdered crystals of paracetamol, a drug active ingredient known for its use in alleviating postoperative pain and as an adjuvant in chemotherapy for cancer patients, were exposed to gamma radiation. The paramagnetic defects induced by the radiation were thoroughly investigated using Electron Paramagnetic Resonance (EPR) spectroscopy. The suitability of the drug sample for use as a dosimetric material, radical extinction data, saturation information occurring at microwave power values and parameters related to dose-response data were investigated at room temperature. No EPR signal was observed in the sample which was not exposed to gamma radiation. Spectroscopic properties generated as a result of radiation were determined via spectrum simulation.

Keywords: Dosimetry, Gamma Radiation, Drug sample, EPR, Radical, Irradiation.

Öz

Bu çalışmada, ameliyat sonrası ağrıların hafifletilmesi ve kanser hastalarında kemoterapi tedavisinde destekleyici olarak kullanıldığı bilinen parasetamolün toz kristalleri gama radyasyonuna maruz bırakılmıştır. Radyasyon sonucu oluşan paramanyetik bozukluk, Elektron Paramanyetik Rezonans (EPR) spektroskopisi kullanılarak detaylı bir şekilde incelenmiştir. Örneğin dozimetrik malzeme olarak kullanıma uygunluğu, radikal sönüm bilgileri, mikrodalga güç değerlerinde doyum bilgileri ve doz-cevap eğrisi oda sıcaklığında araştırılmıştır. Gama radyasyonuna maruz bırakılmayan örnekte EPR sinyali gözlenmemiştir. Spektrum simülasyonu (benzetişimi) yapılarak ışınlama sonucunda ortaya çıkan spektroskopik özellikleri belirlenmiştir.

Anahtar Kelimeler: Dozimetri, Gama radyasyonu, İlaç örneği, EPR, Radikal, Işınlama.

Sorumlu Yazar/Corresponding Author:
F. Akbalık
E-mail: fakbalik@gmail.com

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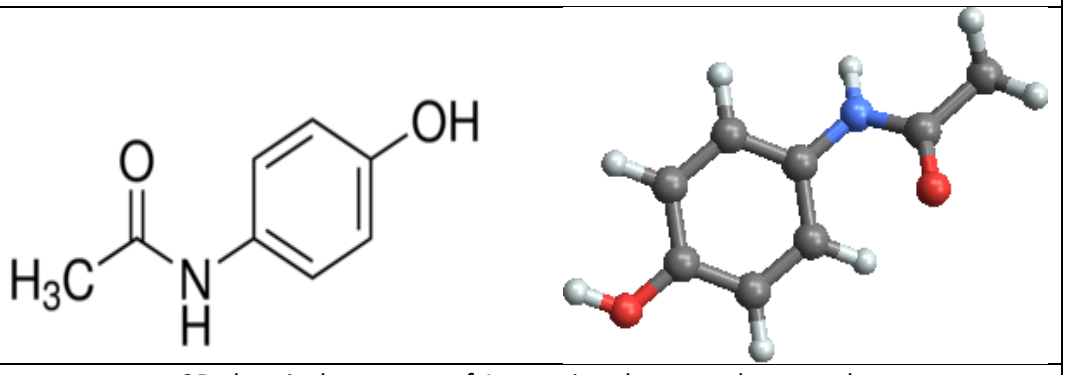
Introduction

Studies using EPR spectroscopy, a branch of atomic and molecular physics, can be used to detect free radicals formed in active pharmaceutical ingredients by ionising radiation and to obtain data on the dispersion of a single electron on the molecule (Abbar et al., 2011; Akbalık, 2016; Ghasemi & Bagheri, 2019; Basly & Bernand, 1997; Basly et al., 1997; Bhat et al., 2011; Damian, 2003; Fincur et al., 2021; Gibella et al., 1993; Jeon et al., 2021; Osawa et al., 2019; Polat & Korkmaz, 2006; Proelss et al., 1978; Smyth et al., 2006). The results of EPR spectroscopy studies of irradiated drug samples have shown EPR spectroscopy to be a highly effective method for characterising irradiation-induced free radicals (Basly & Bernand, 1997; Basly et al., 1997; Gibella et al., 1993; Damian, 2003; Polat & Korkmaz, 2006). The molecular formula of Acetaminophen drug is $CH_3CONHC_6H_4OH$. Its chemical formula is N-acetyl-4-aminophenol and its molecular weight is 151.163 g/mol. Table 1 shows the information of Acetaminophen drug sample.

Table 1. Chemical structure, ring formula, molecular weight and chemical structure of Acetaminophen drug sample

	
2D chemical structure of Acetaminophen powder crystals	
Ring Formula	$CH_3CONHC_6H_4OH$
Molecular Weight	151.163 g/mol
Chemical Name	Acetaminophen

The maximum radiation dose that can be used for drug samples accepted by Pharmacopeia (USP XXII, BP 1993) is selected as 25 kGy. During the experimental studies, the Acetaminophen drug sample was irradiated at doses of 1, 5, 10, 15, and 20 kGy and their spectra were recorded. The current study aims to determine the spectral properties of free radicals formed in the Acetaminophen drug sample irradiated with γ-rays and to determine whether they can be used as an example for normal and/or accidental dosimetry by creating dose-response curves as a result of EPR examination at room temperature. In addition, EPR is also used in accident dosimetry.

Material and Method

Irradiations at all doses were carried out with a Cobalt-60 gamma source having an activity of 370.000 curies and a dose rate of 2 kGy/hr at the Industrial Irradiation Facility of Sarayköy Nuclear Research Center SANAEM, now known as TENMAK, located in Kahramankazan district affiliated to the Turkish Atomic Energy Authority. The products to be irradiated in the gamma irradiation facilities in the experimental field are processed in sample packages dosimeters are placed in the product boxes and process control is performed. The samples used in the current study were prepared in a 2 ml Eppendorf tube, placed in the unit, and irradiated. Six prepared samples were irradiated at doses of 1 kGy, 5 kGy, 10 kGy, 15 kGy, and 20 kGy respectively on different dates. Even though Acetaminophen is generally used in the treatment of moderate pain, it is also known to be used to reduce the severity of post operative pain and for chemotherapy in cancer patients. The powder samples of the active pharmaceutical ingredient were placed in an Eppendorf tube with no treatment, and EPR spectra were recorded under various spectrometer operating conditions (Bruker e-scan X-band EPR spectrometer: center magnetic field 349 mT, scan range 10 mT, microwave power ~0.1 mW, microwave frequency 9.8 GH, receiver gain $3.17x10^2$, modulation frequency 86 kHz, modulation amplitude 2 G). All spectra measurements were recorded at room temperature.

Results

Unirradiated and irradiated samples

Except for gamma irradiation, no other physical or chemical treatment was performed on the Acetaminophen drug sample used in the present study. EPR spectra were recorded after the Acetaminophen drug sample was irradiated with γ-rays at certain dose levels. The untreated Acetaminophen sample was irradiated and EPR spectra were recorded. While no EPR signal was observed in the unirradiated Acetaminophen sample, it was recorded that the irradiated Acetaminophen sample gave an EPR spectrum. At this stage, no resonance signal was observed in the Acetaminophen sample before irradiation, while the observation of a significant resonance signal after irradiation gave information about the formation of unpaired electrons in the sample by irradiation. The spectrum shows an EPR spectrum dispersed on an area of 20 mT. Through the simulation program, the excess fine structure constant was calculated as a=1.1494 mT and the line width as 3.26 mT. The simulation of irradiated Acetaminophen was obtained using Mc Kelvey software. It was observed that no EPR signal was obtained in the unirradiated sample. Figure 1(a) shows the resonance signals in the irradiated samples from low to high doses that become more significant and stronger depending on the dose value. When Figure 1(a) was examined, the EPR spectrum is seen with an approximate *g* value of 2.0040. Figure 1(b) shows the spectrum irradiated at a dose of 20 kGy. Figure 1(c) shows the simulation spectrum of the sample irradiated at 20 kGy dose.

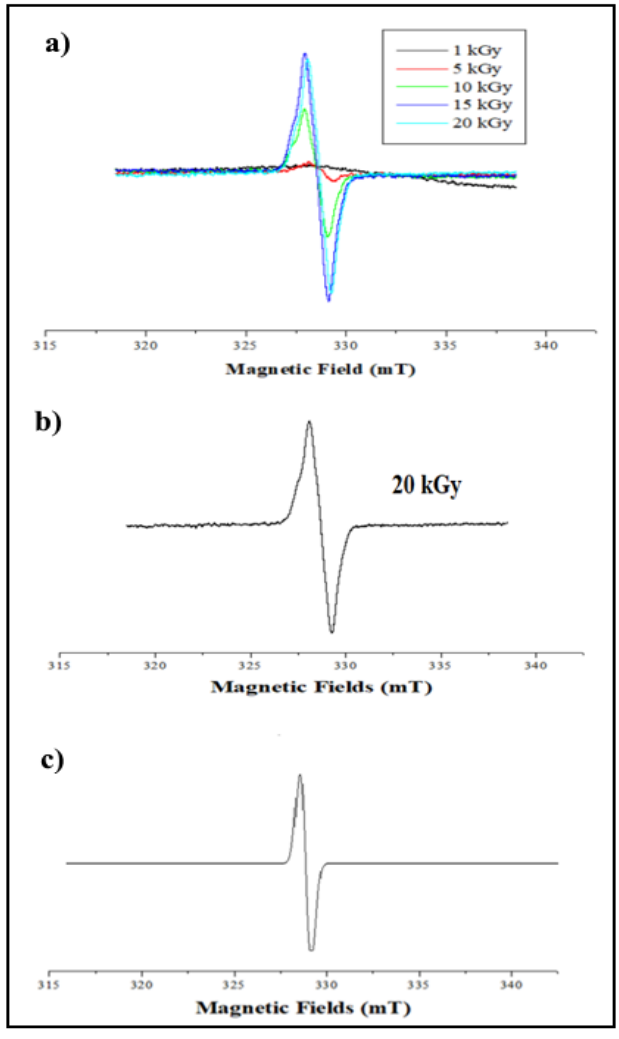


Figure 1a. Spectra obtained from irradiated Acetaminophen drug sample. **1b.** EPR spectrum of acetaminophen drug sample at 20 kGy dose. **1c.** The closest simulation spectrum of the Acetaminophen drug sample obtained at a dose of 20 kGy

Change of EPR signal intensity with microwave power

In this section of the study, EPR spectra of Acetaminophen drug samples irradiated at 10 kGy dose value were recorded at different microwave power values in the range of 0.01-0.1-2-3-4-6-8, and 15 mW. Table 2 shows the microwave power $P^{1/2}(mW)^{1/2}$ and the resonance signal intensity. Origin 6.0 program was used to calculate the resonance signal intensity and the signal intensities were calculated by determining the strong peaks from peak to peak in the spectrum produced using numerical values with the program. Accordingly, the corresponding resonant signal intensity deviates from linearity and reaches saturation at approximately 8 mW power value.

Table 2 Microwave Power $P^{1/2}(mW)^{1/2}$ and resonance signal intensity.
Values of the resonance signal intensity of the Acetaminophen sample depending on the square root of the applied microwave power.

Microwave Power $P^{1/2}(mW)^{1/2}$	0.1	0.15	2	3	4	6	8	15
Central Resonance Signal Intensity (a.u.)	17.02	28.167	43.464	46.8	48.44	48.74	50	49.38

Figure 2 shows the variation of the microwave power of Acetaminophen drug depending on the signal intensity, and Figure 3 shows the spectra of the irradiated Acetaminophen drug sample obtained at 0.1-...-15 mW microwave area values.

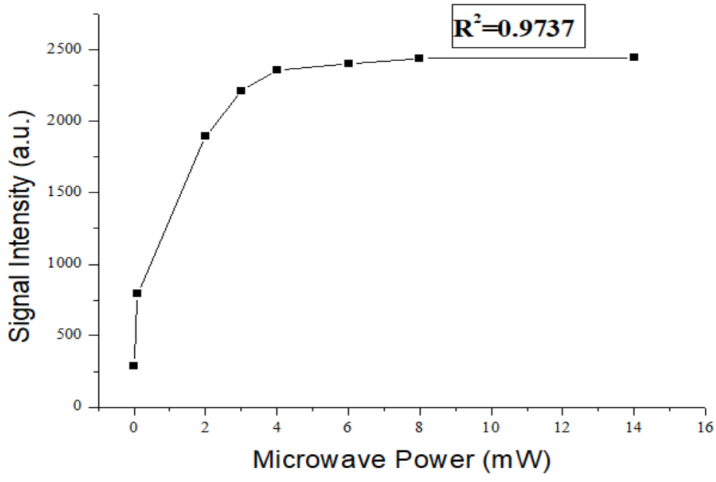


Figure 2. Variation of microwave power of Acetaminophen drug with signal intensity.

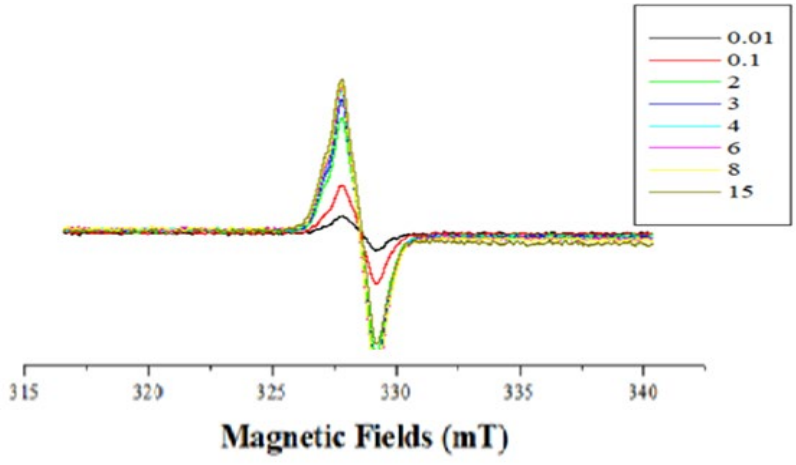


Figure 3. Spectra of irradiated Acetaminophen drug sample obtained different microwave power

Dose-response curve of Acetaminophen sample

In this section, examinations were conducted to determine the dosimetric potential of the Acetaminophen drug sample. The most appropriate mathematical functions that can describe the dose-dependent variations of the resonance signal intensity results were determined by the mathematical functions given in the graph. Figure 4 shows the dose-dependent variations in the resonance signal intensity of the irradiated samples.

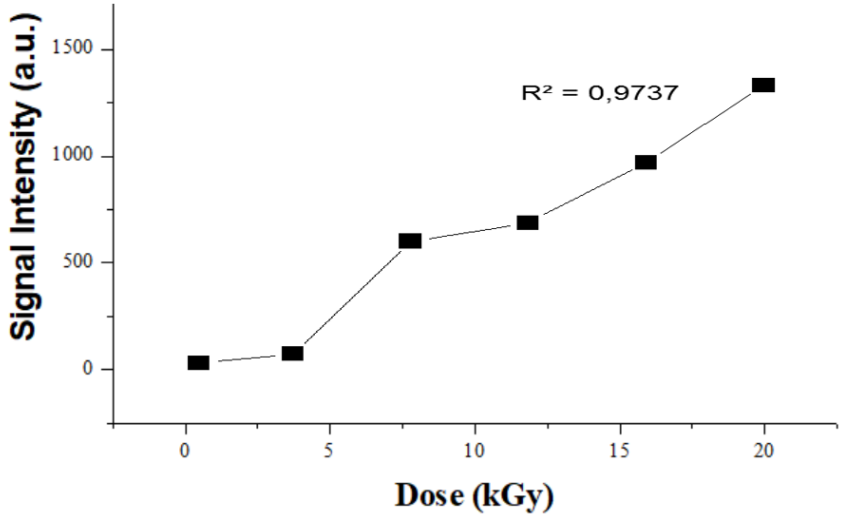


Figure 4. Variation of the signal intensity of irradiated Acetaminophen sample depending on the radiation dose applied

Table 3. Parameter values and coefficients of concordance calculated for three different mathematical functions tested using resonance signal intensity (I) values.

Function	Parameters	I
I = a+Dln(x) (Logarithmic)	A	5.172
	B	41.396
	C	……
	R^2	**0.9671**
I = a+bD+cD^2 (Polynomial)	A	13.977
	B	66.077
	C	0.304
	R^2	**0.9737**
I= aD^b (Exponential)	A	96.09
	B	0.8749
	C	…..
	R^2	**0.9039**

Allthough the radiation dose applied to the sample increased, no significant change was observed in the g value and EPR spectrum form of the sample. However, as shown in Figure 4, as the amount of radiation absorbed by the sample increased, there is a significant increase in the intensity of the EPR resonance signal obtained. Where I is the resonance signal intensity measured from the EPR spectrum of the sample and D is the radiation dose applied to the sample. The values shown as squares in Figure 4 show the experimental results. The mathematical functions closest to the experimental results were tested. The most appropriate mathematical functions to describe the dose-dependent changes of the resonance signal intensity findings were determined by trying the mathematical functions given in Table 3. For example, it was found that the dose-response curve was compatible with logarithmic, polynomial, and exponential functions. The best fit of the

experimental results was determined by the biggest value of R^2=0.9737. One of the necessary conditions for a material to be used dosimetrically is that the dose-response curve should be linear. When the table was analyzed, it was determined that the curve obtained showed the best fit with the polynomial function $I=a+bD+cD^2$. As the acetaminophen sample showed linearity, it is considered that it can be used as a dosimetric material.

Extinction findings of gamma irradiated Acetaminophen drug

After acetaminophen was irradiated with 20 kGy, the EPR spectra were recorded at regular intervals in a dark environment at room temperature and kept airtight. Figure 5 shows the variation of the central resonance signal intensity calculated from the spectra obtained over a period of 145 days after gamma irradiation of the sample depending on the dwell time. The regions with a black square in the figure show the peak-to-peak signal values exposed in the spectrum. When the EPR spectra were examined during this process, a decrease in the resonance signal of the sample was observed proportional to the elapsed time, and when the change in the peak-to-peak signal values was examined, it was found that the decrease in the extinction kinetics was 18% on the 30th day, the extinction kinetics reached 30% on the 53rd day, and the decrease in the extinction kinetics was 70% in the measurement taken on the 145th day.

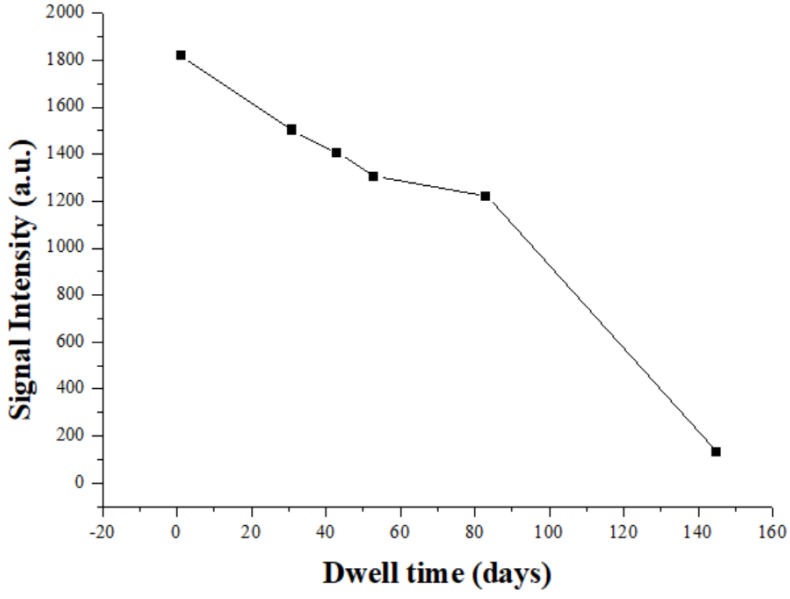


Figure 5. The central resonance signal intensity was calculated from the spectra obtained over 145 days.

The resonance signal obtained from irradiation in irradiated drugs should be testable throughout the shelf life of the sample. Although the resonance signal can be measured weakly, in the spectrum taken at the end of the 145th day in the Acetaminophen sample, it showed that even at the end of the 145th day, the irradiated Acetaminophen sample can be differentiated from the unirradiated sample using the EPR spectrometry method. Also, it was found that there was no significant change in the *g*-value measurements during this period. Figure 5 shows the variation of the resonance signal intensity of the irradiated Acetaminophen sample depending on the dwell time.

Conclusion

Acetaminophen, which is widely used in the USA, Canada, Japan, South Korea, and Colombia, is a medication commonly used to reduce the severity of post-operative pain and in anthracycline chemotherapy for cancer patients, although it is also used to treat moderate pain. Anthracyclines, used in cancer treatment, are known for their wide application in the treatment of various types of cancer, including leukemia, breast cancer, uterine cancer, and lung cancer (Ghasemi & Bagheri, 2019). The EPR spectrum of Acetaminophen drug sample irradiated with gamma rays was observed. It was determined that the form revealed in the spectrum did not change depending on the applied dose variation, and the signal strength increased in the spectrum. In Acetaminophen sample, it was observed that at approximately 8 mW power, the resonance signal intensity deviated from linearity and saturated. The mathematical functions closest to the experimental results were tested with the dose response study. The most appropriate mathematical functions that can describe the dose-dependent variations of experimental resonance signal intensity findings were tried. For example, it was determined that the dose-response curve was highly compatible with linear, exponential, and polynomial functions. The smallest value of the best fit of the experimental results was determined with R^2=0.9737. Acetaminophen spectrum simulation was performed by evaluating the extinction results at room temperature. It was concluded that the theoretical spectrum created by using the calculated theoretical spectroscopic parameter values was in good fit with the experimental spectrum and was appropriate and successful in terms of evaluating the results.

The absence of EPR signal in the Acetaminophen drug sample before irradiation and the dose-response curve showing linearity in a wide range are positive results in dosimetric terms. When the EPR spectra were examined in the extinction spectra, a decrease in the resonance signal of the sample proportional to the elapsed time was observed, and when the change in peak-to-peak signal values was examined by origin 6.0 program, it was observed that the decrease in extinction kinetics on the 30th day was 18%, the extinction kinetics reached 30% on the 53rd day, and the decrease in extinction kinetics in the measurement taken on the 145th day was 70%. According to these results, it can be said that if the Acetaminophen drug sample irradiated at a dose of 20 kGy is used for dosimetric purposes, no disadvantage can be stated when the measurement is made within two days following the irradiation process.

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