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Gama ile Işınlanmış Klorpropamid ve Prokainamid Hidroklorür İlaç Hammaddelerinde Oluşan Serbest Radikallerin EPR Spektroskopisi ile Tanımlanması

Kerem SÜTÇÜ[1*], Yunus Emre OSMANOĞLU[2]

Öne Çıkanlar:

- Klorpropamid ve prokainamid hidroklorürde gama ışınlaması ile oluşan serbest radikallerin yapısal özellikleri elektron paramanyetik rezonans spektroskopisi ile incelenmiştir.
- Radikal konsantrasyonları hesaplanmıştır.
- EPR parametreleri ve radikallerin yapısı belirlenmiştir.

Anahtar Kelimeler:

- Elektron paramanyetik rezonans
- Gama ışınlaması
- İlaç hammaddesi

ÖZET:

Bu çalışmada tıpta yaygın kullanılan klorpropamid ve prokainamid hidroklorür ilaç hammaddelerinde gama ile ışınlanarak oluşan serbest radikallerin yapısal özelliklerinin elektron paramanyetik rezonans spektroskopisi kullanılarak belirlenmesi amaçlanmıştır. Gama ile ışınlama sonucunda hem klorpropamid ve hem de prokainamid hidroklorür numunelerinde iki farklı radikal oluştuğu tespit edilmiştir. Klorpropamid ve prokainamid hidroklorür numunelerinde sırasıyla -NĊH-, -$NCH_2\dot{C}H_2$- ve –NĊH-, -NĊHCH- radikallerinin oluştuğu önerilmiştir. Toz formunda çalışılan örneklerin oda sıcaklığında kaydedilmiş deneysel spektrumlarına en yakın simüle spektrumları simülasyon yazılımı kullanılarak elde edilmiştir. Önerilen radikallerin deneysel spektrumlara olan katkıları hesaplanmıştır. Elde edilen radikallerin g değerleri ve eşlenmemiş elektronların aşırı ince yapı sabitleri hesaplanmıştır.

Identification of Free Radicals Formed in Gamma-Irradiated Chlorpropamide and Procainamide Hydrochloride Pharmaceutical Raw Materials by EPR Spectroscopy

Highlights:

- The structural properties of free radicals formed by gamma irradiation in chlorpropamide and procainamide hydrochloride were examined by electron paramagnetic resonance spectroscopy
- Radical concentrations were calculated.
- The EPR parameters and the structure of the radicals were identified

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Keywords:

- Electron paramagnetic resonance
- Gamma irradiation
- Pharmaceutical raw material

ABSTRACT:

In this study, it was aimed to determine the structural properties of free radicals formed by gamma irradiation in chlorpropamide and procainamide hydrochloride drug raw materials, which are widely used in medicine, by using electron paramagnetic resonance spectroscopy. As a result of gamma irradiation, it was determined that two different radicals were formed in both chlorpropamide and procainamide hydrochloride samples. It was suggested that –NĊH-, -$NCH_2\dot{C}H_2$- and –NĊH-, -$N\dot{C}HCH_2$- radicals were formed in chlorpropamide and procainamide hydrochloride samples, respectively. The simulated spectra closest to the experimental spectra recorded at room temperature of the samples studied in powder form were obtained using simulation software. The contributions of the proposed radicals to the experimental spectra were calculated. The g values of the obtained radicals and the hyperfine structure constants of the unpaired electrons were calculated.

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INTRODUCTION

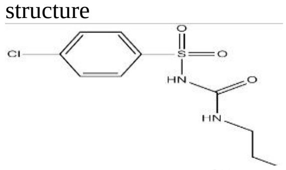
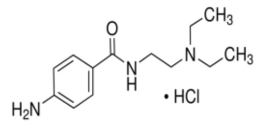
Chlorpropamide (CLP) is a long-acting oral hypoglycemic agent commonly used in type 2 diabetes (Bakare-Odunola et al., 2008). It is known that CLP is a sulfonamide derivative that is insoluble in water but soluble in alcohol (Iweala and Okeke, 2005). In addition, procainamide hydrochloride (PAH), an antiarrhythmic reagent, is used to treat cardiac arrhythmia in patients with heart disease (Al-Tamrah and Al-Abbad, 2015). Radiation sterilization of drugs, which is a non-contact method, is becoming increasingly important in today's world where the effect of the pandemic continues (Ambroz et al., 2002; Çolak and Korkmaz, 2004; Sütçü, 2019). However, as a result of radiation application, free radicals are formed in drugs and the identities of these free radicals should be identified. EPR spectroscopy is the most suitable method because of its high sensitivity in detecting free radicals (Murrieta et al., 1996).

In the study in which the EPR characterization of the Airfix drug was performed, it was determined that alkyl-type radical was formed as a result of irradiation (Ece et al., 2022). In the study, in which some drugs used in neurological and high blood pressure diseases were examined using EPR spectrometry after irradiation with gamma, g values of radicals and hyperfine structure constants of free electrons were determined by using simulation spectra (Köseoğlu et al., 2003). Damian examined the metoclopramide sample, which is in the antiemetic drug category, by EPR spectroscopy (Damian, 2003). It was stated that a single signal was recorded from the metoclopramide sample. The g and linewidth values of the single signal were calculated as g=2.0047 and ΔH=20 G, respectively. When the literature is examined, it is seen that EPR spectroscopy is frequently used in examining irradiated food and amino acid compounds as well as irradiated drugs (Desrosiers, 1996; Kasumov et al., 2001, Aydın et al., 2008; Aydın et al., 2009; Başkan et al., 2010; Sayın, 2013, Başkan et al., 2015; Zhang, 2015; Sezer et al.,2017, Tokatlı et al., 2018, Karakaş et al., 2018). In this study, it was aimed to determine the chemical structures of the free radicals formed after gamma irradiation of CLP and PAH samples, which have such important properties in medicine.

MATERIALS AND METHODS

CLP and PAH pharmaceutical raw materials in powder form were purchased from commercial sources and gamma irradiation was carried out at Turkish Energy Nuclear and Mineral Research Agency (TENMAK). The irradiation process was achieved at room temperature equivalent to 15 kGy using a 60Co gamma-ray source (Isotope, Ob-ServoSanguis) with a dose rate of 1785 Gy/h. The spectra of all samples were recorded using the E109C EPR X-band EPR spectrometer, which was operated at microwave frequency 9.821 GHz and microwave power 1.577 at room temperature after irradiation. The simulated spectra closest to the experimental spectra were performed using the EPR winsim2002 software program (Nih, 2012). Chemical formulas, molecular weights and molecular structures of CLP and PAH samples are given in Fig. 1.

Figure 1. Symbol, name, formula, molecular weight and chemical structure of CLP and PAH samples

Symbol	Name	Chemical Formula	Molecular Weight	Chemical structure
CLP	Chlorpropamide	$C_{10}H_{13}ClN_2O_3S$	276.74 g/mol	
PAH	Procainamide hydrochloride	$H_2NC_6H_4CONHCH_2CH_2N(C_2H_5)_2 \cdot HCl$	271.79 g/mol	

RESULTS AND DISCUSSION

Experimental spectra of CLP and PAH samples are given in Fig. 2a and Fig.3a respectively. When these spectra are examined, it is observed that hyperfine coupling constants cannot be obtained due to the poor resolution of the signals. Experimental EPR signals of drugs are difficult to interpret because of the large chemical structures of drug molecules and the poor resolution of EPR signals (Köseoğlu et al., 2003). In this context, the spectroscopic parameters of the samples were obtained by interpreting the simulations of the spectra.

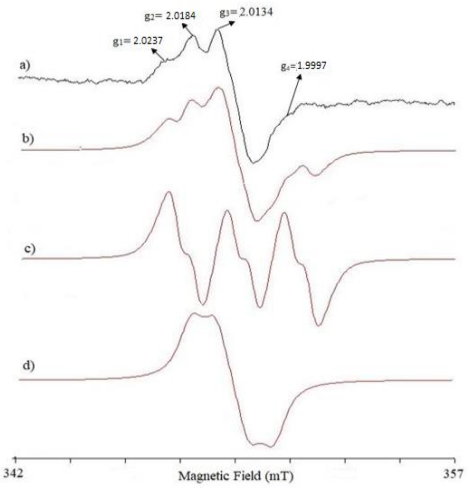


Figure 2. a) The EPR spectrum of 15 kGy irradiated CLP sample
b) Simulation of the spectrum
c) Simulation of radical (radical I)
d) Simulation of radical (radical II)

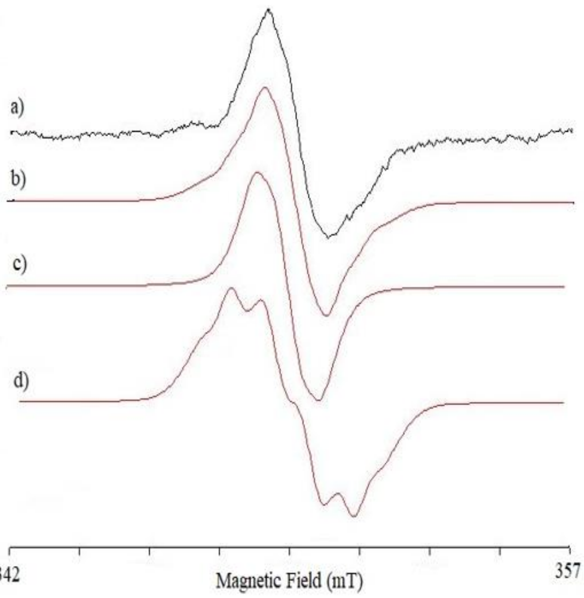


Figure 3. a) The EPR spectrum of 15 kGy irradiated PAH sample
b) Simulation of the spectrum
c) Simulation of radical (radical I)
d) Simulation of radical (radical II)

When the EPR spectrum of CLP irradiated with gamma at 15 kGy was examined at room temperature, four signals with g values of g_{I_1}= 2.0237, g_{I_2}= 2.0184, g_{I_3}=2.0134 and g_{I_4}= 1.9997 were calculated, respectively (Fig.2a). Additionally the spectrum centered at g = 2.0128 with a spacing of 5.3

mT. It was found that gamma irradiation caused the generation of -NĊH- (radical I) and -$NCH_2\dot{C}H_2$- (radical II) radicals in the CLP sample. The simulation spectrum of radical I given in Fig. 2c was obtained using a_N = 1.95 mT, a_{CH} = 0.66 mT and linewidth ΔH = 0.52 mT. The g value of radical I was calculated as g = 2.0100. In the spectrum with a g value of g= 2.0100, three signals are seen due to the interaction of the unpaired electron with the ^{14}N nucleus. Since the hyperfine coupling constant of the a_{CH} is approximately equal to the linewith of the spectrum, it has been determined that it does not make a significant contribution to the spectrum given in Fig. 2c. The contribution of the -NĊH- radical to the experimental spectrum was calculated as 26%. The 6 mT broadening spectrum of radical II, whose contribution to the experimental spectrum was calculated as 74%, is given in Fig 2d. As a result of irradiation, it was thought that -$NCH_2\dot{C}H_2$- radical was formed with the abstraction of a hydrogen from the methyl group in the structure. In a study in which EPR analysis of gamma-irradiated progesterone molecule was performed, it was determined that -$CH_2\dot{C}H_2CH$- radical was formed in the structure as a result of irradiation (Sütçü, 2018). The unpaired electron interacts with two magnetically identical methylene protons with a 1:2:1 intensity distribution. Since the hyperfine coupling constants of both magnetically identical protons bonded to the adjacent carbon and the ^{14}N nucleus are smaller than the linewidth (ΔH = 0.45 mT) value of the spectrum, they do not cause splits in the simulated spectrum. These interactions only contributed to the broadening of the lines (Osmanoğlu et al., 2017). The simulated spectrum of the radical II, whose g value was measured as 2.0108, was obtained using $a^1_\alpha=a^2_\alpha$= 0.9 mT, $a^1_\beta=a^2_\beta$= 0.25 mT and a_N= 0.33 mT values. These a_N values agree well with those derived from the $CH_2\dot{C}HNHCNHNH$ radical in Nα-carbamyl-L-arginine (Zincircioglu et al., 2006).

The experimental and simulated spectra of the gamma-irradiated PAH sample are given in Fig. 3a and Fig. 3b., respectively. The formation of the experimental spectrum in singlet structure with g value g = 2.0087 is caused by the interaction of two radicals. It is seen that this calculated g value is quite compatible with the literature (Ambroz et al., 2000; Varshney and Dodke, 2004; Çolak et al., 2006). The simulated spectrum of radical I, one of the radicals contributing to the experimental spectrum, is given in Fig. 3c. It has been determined that the α-proton and ^{14}N nucleus contribute to the simulated spectrum which g value and linewidth were calculated as g = 2.0092 and ΔH = 0.54 mT, respectively. These a_N values agree well with those derived from the $CH_2\dot{C}(NH_2)COOH$ radical in N-carbamoyl-L-glutamic acid single crystals (Osmanoğlu et al., 2005)

In this context, the paramagnetic species originating in Fig. 3c have been attributed to the -NĊH- radical. The simulation spectrum were obtained by using hyperfine coupling constants a_{CH} = 0.58 mT and a_N = 0.42 mT. Since the measured hyperfine coupling constants are very close to the linewidth value, a singlet signal is obtained. Another paramagnetic species formed in the gamma-irradiated PAH sample was determined as the -$N\dot{C}HCH_2$- (radical II) radical. The g-value and linewidth of the simulated spectrum (Fig. 3d) extending to a region of 7.3 mT were measured as 2.0103 and ΔH = 5.3 mT, respectively. The $N\dot{C}HCH_2$ radical was formed by the removal of a hydrogen from the methylene group as a result of irradiation. The unpaired electron interacts with one α-proton, two magnetically identical β-protons and one ^{14}N nucleus. The simulated spectrum contributed by radical II was obtained by using a_α = 1.19 mT, $a^1_\beta = a^2_\beta$ = 0.78 mT and a_N = 0.87 mT hyperfine coupling constants. Similar values of the hyperfine coupling constants of α- protons have been found in the gamma-irradiated powders of ethyl 2-methyl-4-(2,6 dichlorophenyl)-5-oxo-7-phenyl-1,4,5,6,7,8 hexahydroquinoline-3-carboxylate at ambient temperature as 1.24 mT (Şimşek et al., 2017). It has been calculated by the simulation software that radical I contributes 52% and radical II contributes 48% to the experimental simulation exhibited in Fig. 3b.

CONCLUSION

In this study, the structures of free radicals formed in 15 kGy irradiated CLP and PAH drug raw materials were reported using EPR spectroscopy. The idea of having two different radicals in both samples as a result of irradiation has matured, with highly compatible simulations being obtained. Analysis of the experimental and simulated spectra revealed that -NĊH- and -$NCH_2\dot{C}H_2$-radicals were formed in the CLP sample, and -NĊH- and -$N\dot{C}HCH_2$- radicals were formed in the PAH sample. In the pharmaceutical industry; EPR spectroscopy will yield very productive results in determining the structures of free radicals that will occur in the chemical structures of drugs as a result of the destructive effect of radiation used to sterilize drugs. As a result, similar studies including EPR spectroscopy examinations of drug samples will be helpful in examining similar radical structures.

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Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

Kerem SÜTÇÜ: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data Curation, Writing- Original Draft, Writing- Review & Editing, Visualization, Supervision.

Yunus Emre OSMANOĞLU: Conceptualization, Methodology, Resources, Data Curation, Writing- Original Draft, Writing- Review & Editing, Visualization, Supervision.

Both authors read and approved the final manuscript.

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