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# EPR Analysis of Antioxidant Compounds

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Betül Çalışkan and Ali Cengiz Çalışkan

Additional information is available at the end of the chapter

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## Abstract

A free radical is a molecular species having an unpaired electron and it is a highly reactive entity and unstable. A free radical is a molecule with one or more unpaired electrons in its outer shell. Free radicals can be formed by chemical bond breakage from molecules or by redox reactions. When cells use oxygen, the oxidative stress occurs. The oxidative stress causes free radical formation. Free radicals can also be generated from ionizing radiations, ozone, heavy metal poisoning, cigarette smoking, and chronic alcohol intake. These free radicals are highly reactive and oxidize biomolecules leading to tissue injury and cell death. They also cause toxic effects and diseases. Antioxidants neutralize free radicals resulting from oxidative stress. Antioxidants play an important role in the treatment of diseases. The most suitable method for the analysis of free radicals is the electron paramagnetic resonance (EPR) spectroscopy method. The EPR method detects a paramagnetic center with a single electron. It gives information about the interactions with other nuclei around one electron. It provides information on the structure and environment of radicals.

**Keywords:** free radical, radiation damage center, antioxidant, electron paramagnetic resonance

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## 1. Introduction

Free radicals are an atom or a molecule that bears an unpaired electron and is extremely reactive, capable of engaging in a rapid change reaction that destabilizes other molecules and generates many more free radicals. In plants and animals, these free radicals are deactivated by antioxidants. These antioxidants act as an inhibitor of the process of oxidation, even at

relatively small concentrations, and thus have diverse physiological roles in the body. The body is constantly exposed to the negative and sometimes lethal effects of oxidants during normal physiological processes. The harmful free radicals such as hydroxyl, peroxy and the superoxide anion are constantly being produced as a result of metabolic reactions in living systems. On a daily basis, up to 5% of inhaled oxygen may be converted to reactive oxygen species (ROS). These ROS have the ability to bind to cellular structures and have been implicated in a number of pathological processes such as aging, inflammation, reoxygenation of ischemic tissues, atherosclerosis, cancer and even Parkinson's disease in men [1]. Two processes, which produce free radicals in vivo, have been identified and named the Fenton reaction and the Haber-Weiss reaction [2]. Antioxidants play an important role in animal health. Conventional antioxidants have been shown to improve animal performance during conditions characterized by increased tissue oxidant levels such as stress, injury and infections [3].

Free radicals can be classified as reactive oxygen species (ROS), reactive nitrogen species (RNS) and reactive sulfur species (RSS). Even though free radicals are mainly produced through regular metabolic routes, there are also some external factors that promote their production, including smoking, environmental pollutants, radiation and drugs, among others [4]. In healthy organisms, there is a delicate balance between the production and the removal of free radicals, which guarantees that they remain in adequate concentrations. However, when this balance is broken, these reactive species start producing chemical damages to proteins, lipids, DNA, RNA and sugars generating the so-called oxidative stress of body cells [4]. These processes have been associated with several diseases including cardiovascular, liver, neurological and renal disorders, as well as cancer, auto-immune deficiency and degenerative disorders associated with aging, diabetes, obesity, autism, Alzheimer's, Parkinson's and Huntington's diseases [4]. Although humans have developed different endogenous defense mechanisms to protect cells from the excess of free radicals and to avoid the oxidative stress, often these mechanisms are not enough. Therefore, to increase protection from oxidative damage, dietary supplements with antioxidants are recommended as a way to maintain the concentration of free radicals as low as possible [5].

Antioxidants destroy free radicals. Antioxidants neutralize the radical, and thus the radical-antioxidant association maintains itself in a stationary state. For all these situations, antioxidants must be in a structure that will quench the radical. Therefore, unpaired electrons in the structure of antioxidants must be either radical ions or free radicals. So, they must form a paramagnetic center. Paramagnetic structures are analyzed by electron paramagnetic resonance (EPR) spectroscopy. Thus, the EPR analysis of antioxidants is as valuable and important as the investigation of the paramagnetic centers of radicals. There are many studies done for this purpose. Among these studies, especially the works we have done recently are noteworthy.

Potassium hydroquinone monosulfonate (PHM), succinic anhydride and 3-nitroacetophenone compounds are antioxidants. Paramagnetic centers formed by gamma irradiation effect on single crystals of these materials were analyzed by the EPR method [6–8].

## 2. EPR studies of antioxidant compounds

### 2.1. Gamma-irradiated potassium hydroquinone monosulfonate single crystal

Free radicals or other odd electrons have often been postulated as intermediates in the decomposition of organic compounds by ionizing radiation. It is still important for formulating mechanisms in radiation chemistry to identify the radicals that are formed. If several organic compounds are irradiated and resulting radicals are identified, one may hope that generalizations can be drawn concerning modes of bond rupture.

The quinone structure is widespread in nature: for example, quinones play an integral role in many biological electron-transfer processes, particularly respiration and photosynthesis [9, 10]. Quinones have long been considered for their fungicide, antibacterial and anticancer properties [11]. The quinone derivatives are especially interesting because they have significant properties due to the ready reversibility of the quinone-semiquinone redox system [12].

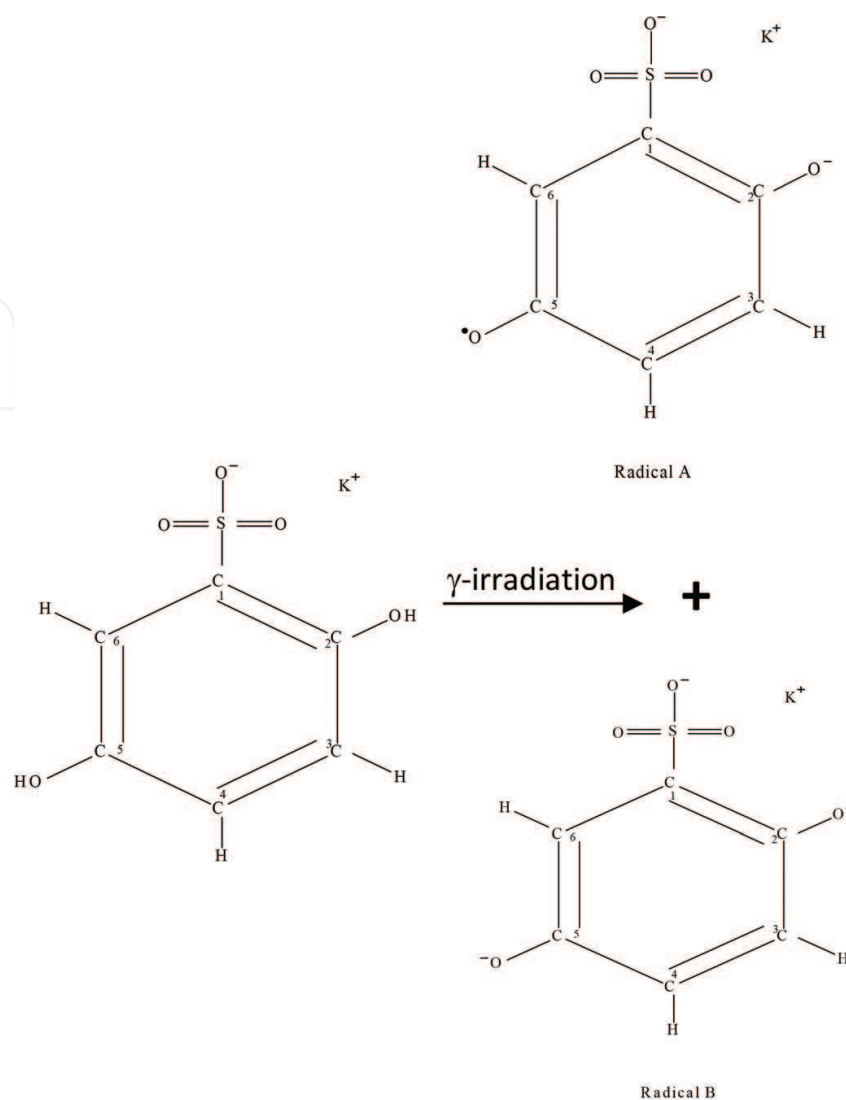
The single crystal of PHM was irradiated with gamma rays at room temperature and studied by the EPR technique at 125 K. The spectra were dependent on the orientation of H in the planes, which are perpendicular to each other. The paramagnetic centers formed from PHM are shown in **Figure 1** [6].

The experimental spectrum in **Figure 2** has 1:1:1:2:1 intensity ratios and exhibits the superimposition due to the two PHM anion radicals. Because of the radical A, the spectra exhibit 2-lines with intensity ratios 1:1. Owing to the C6  $\beta$ -proton, the spectra exhibit a doublet (1:1). Because of the radical B, the spectra exhibit 3-lines with intensity ratios 1:2:1. Owing to the C3 and C4  $\beta$ -protons, the spectra exhibit a triplet (1:2:1). The spectrum in **Figure 3** has 1:2:2:1 intensity ratios and exhibits the superimposition due to the two PHM anion radicals.

The simulation values of the hyperfine coupling constants of the spectra in **Figures 2** and **3** are given in **Table 1**. The EPR parameters belonging to two semiquinone anion radicals observed in PHM are included in **Tables 2** and **3**.

The EPR measurements have shown the existence of two semiquinone anion radicals. The semiquinone anion radicals were stable. The observed semiquinone anion radicals were obtained from the oxidation of PHM single crystal.

The angular dependences of EPR spectra were obtained for different orientations of the static magnetic field with respect to the crystalline axes. For the radical A and radical B, the spectroscopic splitting factor and the hyperfine coupling constants are anisotropic. For the radical A, the average values of the g-factor and the hyperfine coupling constant were obtained as  $g_A = 2.01477$  and  $(a_{\text{CH}(\beta)})_A = 0.326$  mT, respectively. For the radical B, the average values of the g-factor and the hyperfine coupling constant were obtained as  $g_B = 2.01054$  and  $(a_{\text{C}_2\text{H}_2(\beta)})_B = 0.568$  mT, respectively.



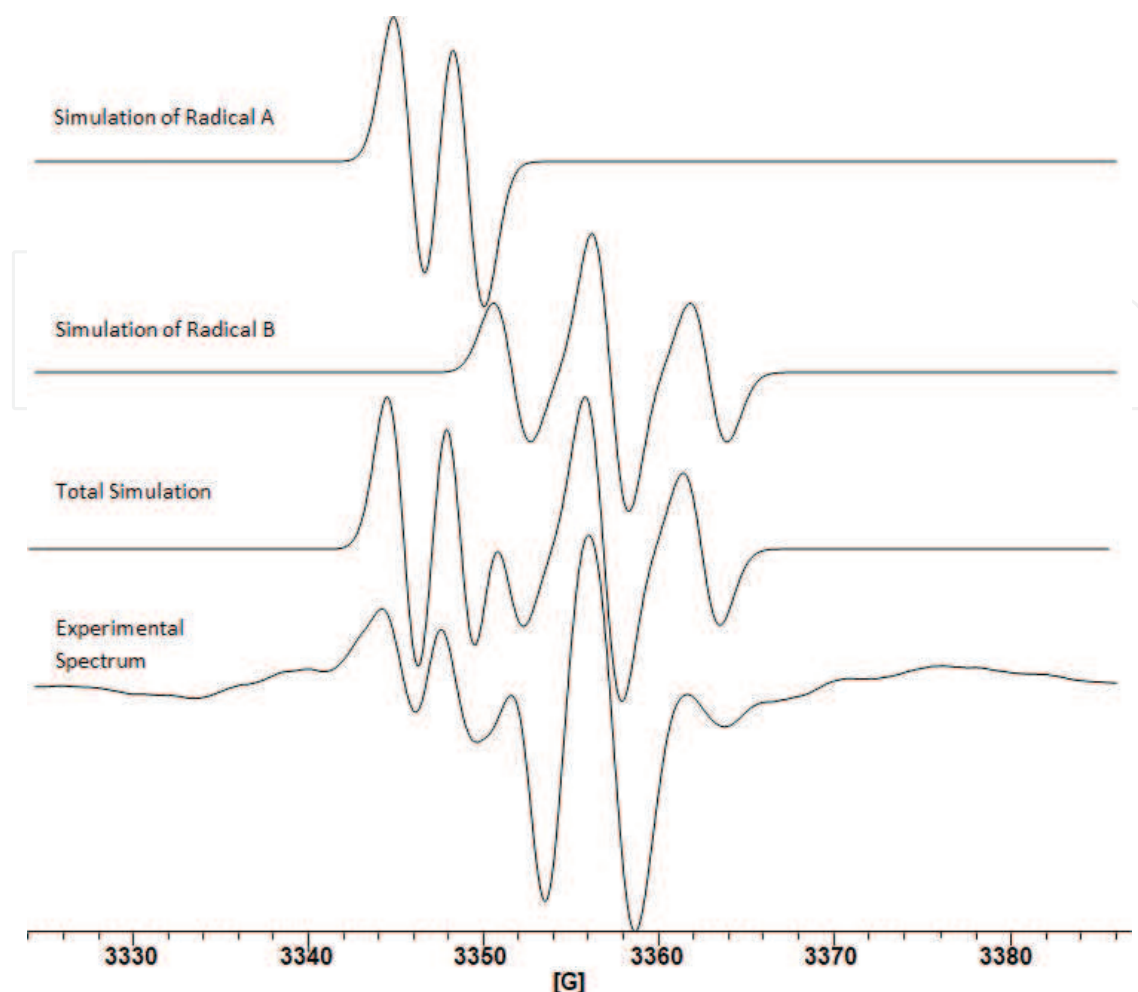
**Figure 1.** Structure of two semiquinone anion radicals observed in PHM single crystal.

## 2.2. Gamma-irradiated succinic anhydride single crystal

Succinate:quinone reductase (SQR) of complex II occupies a unique central point in the mitochondrial respiratory system as a major source of electrons driving reactive oxygen species (ROS) production. It is an ideal pharmaceutical target for modulating ROS levels in normal cells to prevent oxidative stress-induced damage or alternatively, increase ROS in cancer cells, inducing cell death [13].

The single crystals of succinic anhydride were irradiated gamma-rays at room temperature and studied by the EPR technique at 125 K [7]. The spectra were dependent on the orientation of H in the planes which are perpendicular to each other. The paramagnetic centers formed from succinic anhydride are shown in **Figure 4**.

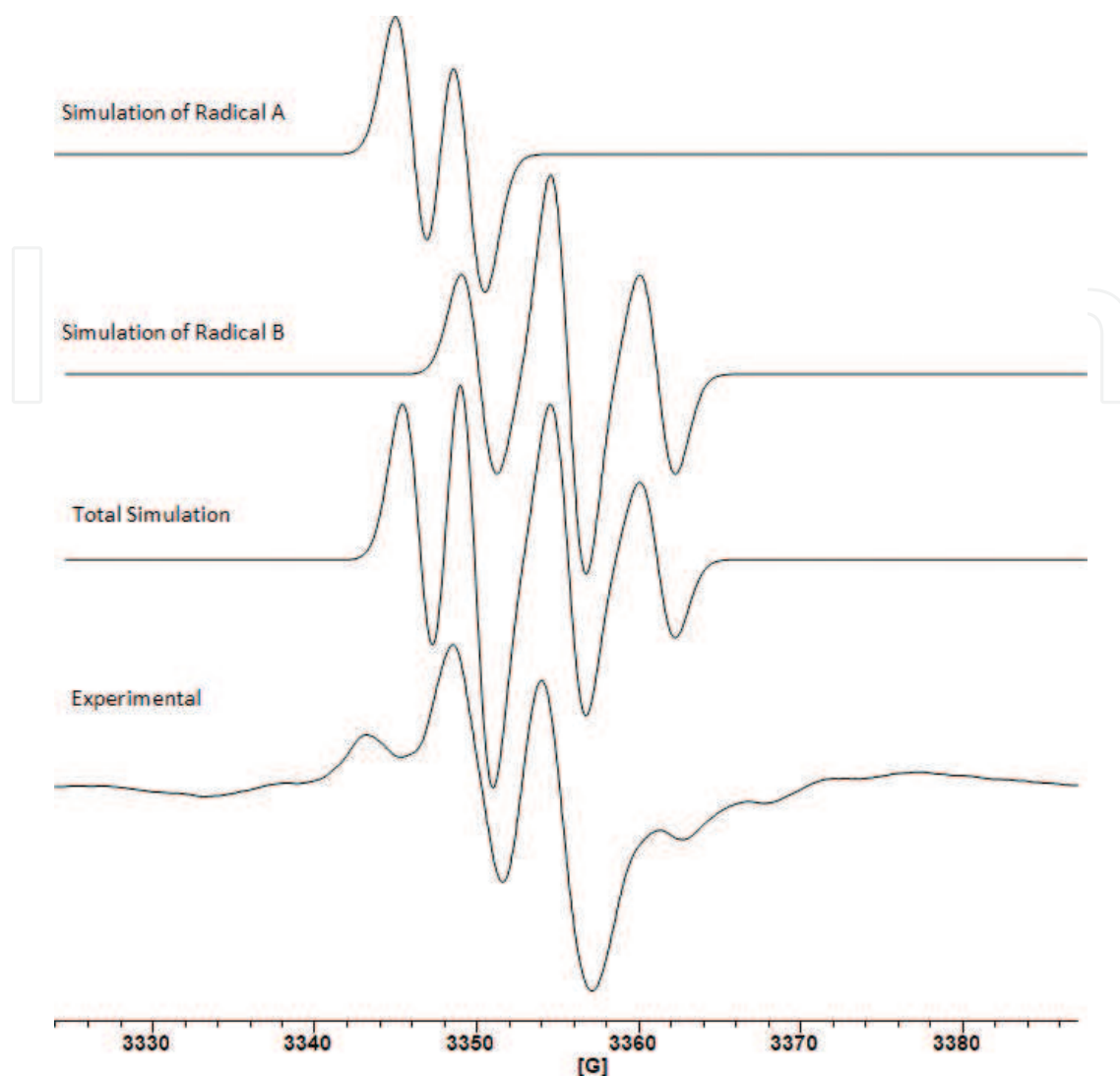
For only a few angles, the spectra have 1:2:1:1:2:1:2:4:2:2:4:2:1:2:1:1:2:1 intensity ratios. For many angles, the spectra have 1:1:1:1:1:1:1:2:2:2:2:2:2:2:1:1:1:1:1:1:1 intensity ratios. The experimental spectrum in **Figure 5** has 1:2:1:1:2:1:2:4:2:2:4:2:1:2:1:1:2:1 intensity ratios and



**Figure 2.** EPR spectrum of gamma-irradiated PHM single crystal at 125 K when the magnetic field is in the ab plane at an angle  $0^\circ$  toward the axis.

exhibits the superimposition due to the two  $\text{CH}_2\text{CH}_2$  atom groups. Because of the radical A, the spectra exhibit 9-lines with intensity ratios 1:2:1:2:4:2:1:2:1. Owing to the  $\beta$ -protons, the spectra exhibit a triplet (1:2:1). Each of the lines (1:2:1) splits into triplets (1:2:1) due to the two equivalent  $\gamma$ -protons. Because of the radical B, the spectra exhibit 9-lines with intensity ratios 1:2:1:2:4:2:1:2:1. Owing to the  $\beta$ -protons, the spectra exhibit a triplet (1:2:1). Each of the lines (1:2:1) splits into triplets (1:2:1) due to the two equivalent  $\gamma$ -protons. Total 18-lines are observed due to the two anion radicals. The spectrum in **Figure 6** has 1:1:1:1:1:1:1:2:2:2:2:2:2:2:1:1:1:1:1:1:1 intensity ratios and exhibits the superimposition due to the two  $(\text{CH}_2)_\beta\text{CH}_{\gamma_1}\text{H}_{\gamma_2}$  atom groups. Because of the radical A, the spectra exhibit 12-lines with intensity ratios 1:1:1:1:2:2:2:2:1:1:1:1. Owing to the  $\beta$ -protons, the spectra exhibit a triplet (1:2:1). Each of the lines (1:2:1) splits into doublets (1:1) due to the  $\gamma_1$ -proton. Each of the lines (1:1:2:2:1:1) splits into doublets (1:1) due to the  $\gamma_2$ -proton. The hyperfine structure splittings in the radical A are the same as that of the radical B. The total 24-lines are observed due to the two anion radicals.

We have obtained the computer simulations of the spectra that give the best agreement with experimental values. The simulations of the EPR spectra have been carried out using the Bruker's WINEPR software.



**Figure 3.** EPR spectrum of gamma-irradiated PHM single crystal at 125 K when the magnetic field is in the ac plane at an angle  $70^\circ$  toward the axis.

|                 | Radical A  | Radical B  |
|-----------------|--|--|
| <b>Figure 2</b> | $(a_{\text{CH}(\beta)})_A = 0.328 \text{ mT}$<br>Center field = 335.5 mT<br>$\nu = 9.38 \text{ GHz}$<br>Line width = 0.2 mT  | $(a_{\text{CH}_2(\beta)})_B = (a_{\text{H}\beta})_B = 0.572 \text{ mT}$<br>Center field = 335.5 mT<br>$\nu = 9.408 \text{ GHz}$<br>Line width = 0.215 mT |
| <b>Figure 3</b> | $(a_{\text{CH}(\beta)})_A = 0.327 \text{ mT}$<br>Center field = 335.5 mT<br>$\nu = 9.38 \text{ GHz}$<br>Line width = 0.22 mT | $(a_{\text{CH}_2(\beta)})_B = (a_{\text{H}\beta})_B = 0.55 \text{ mT}$<br>Center field = 335.5 mT<br>$\nu = 9.401 \text{ GHz}$<br>Line width = 0.22 mT   |

**Table 1.** EPR parameters of simulated spectra.

|                                 | Principal values   | Direction cosines |           |           |
|---------------------------------|--------------------|-------------------|-----------|-----------|
| $(A_{\text{CH}(\beta)})_A$ (mT) | $A_{xx} = 0.347$   | 0.807090          | 0.452748  | -0.378980 |
|                                 | $A_{yy} = 0.324$   | -0.198271         | 0.812426  | 0.548318  |
|                                 | $A_{zz} = 0.307$   | 0.556143          | -0.367402 | 0.745467  |
|                                 | $a_{av} = 0.326$   |                   |           |           |
| $g_A$                           | $g_{xx} = 2.01690$ | 0.745218          | 0.366680  | -0.556952 |
|                                 | $g_{yy} = 2.01479$ | 0.353981          | 0.490298  | 0.796643  |
|                                 | $g_{zz} = 2.01263$ | 0.565109          | -0.790667 | 0.235582  |
|                                 | $g_{av} = 2.01477$ |                   |           |           |

Note: The errors are estimated to be  $\pm 0.00005$  and  $\pm 0.005$  mT for all the calculated g- and A-values, respectively.

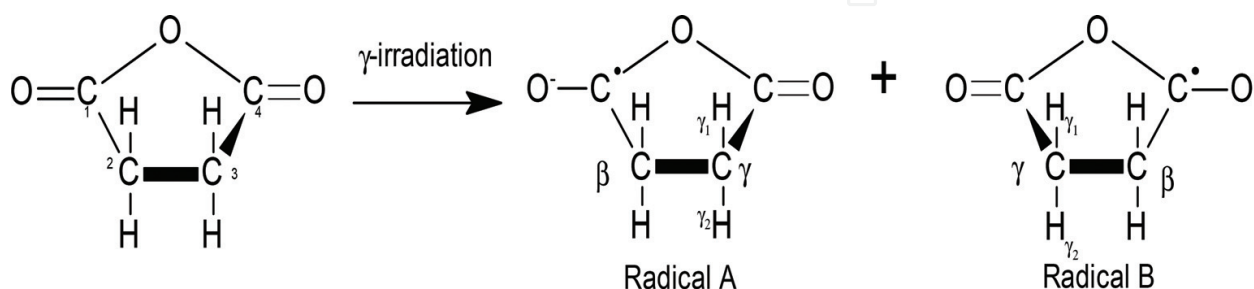
**Table 2.** The EPR parameters of radical A observed in PHM at 125 K.

|                                       | Principal values   | Direction cosines |           |           |
|---------------------------------------|--------------------|-------------------|-----------|-----------|
| $(A_{\text{C}_2\text{H}_2(\beta)})_B$ | $A_{xx} = 0.662$   | 0.618669          | 0.457813  | 0.638480  |
|                                       | $A_{yy} = 0.56$    | -0.736026         | 0.053485  | 0.674838  |
|                                       | $A_{zz} = 0.483$   | 0.274800          | -0.887439 | 0.370051  |
|                                       | $a_{av} = 0.568$   |                   |           |           |
| $g_B$                                 | $g_{xx} = 2.01119$ | 0.506864          | -0.262535 | 0.821075  |
|                                       | $g_{yy} = 2.01056$ | 0.787949          | 0.527402  | -0.317780 |
|                                       | $g_{zz} = 2.00986$ | -0.349608         | 0.808037  | 0.474185  |
|                                       | $g_{av} = 2.01054$ |                   |           |           |

Note: The errors are estimated to be  $\pm 0.00005$  and  $\pm 0.005$  mT for all the calculated g- and A-values, respectively.

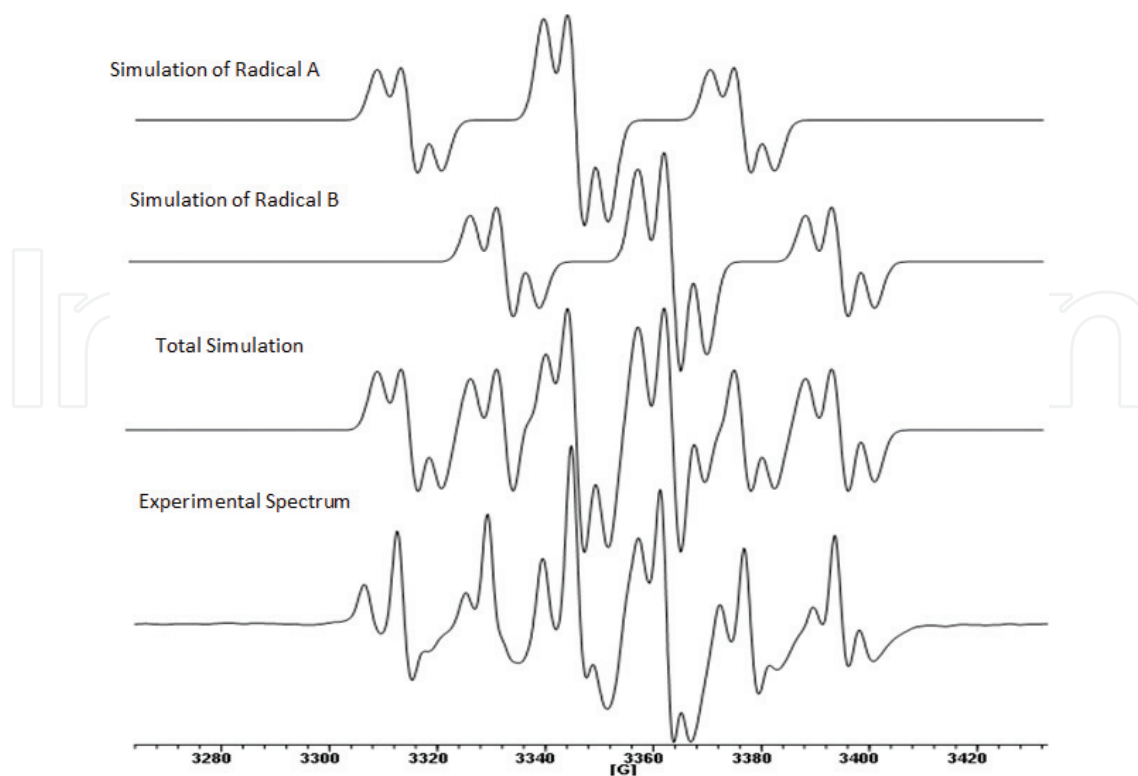
**Table 3.** The EPR parameters of radical B observed in PHM at 125 K.

The simulation values of the hyperfine coupling constants of the spectra in **Figures 5** and **6** are given in **Table 4**. The EPR parameters belonging to two succinic anhydride anion radicals observed in succinic anhydride are included in **Tables 5** and **6**.

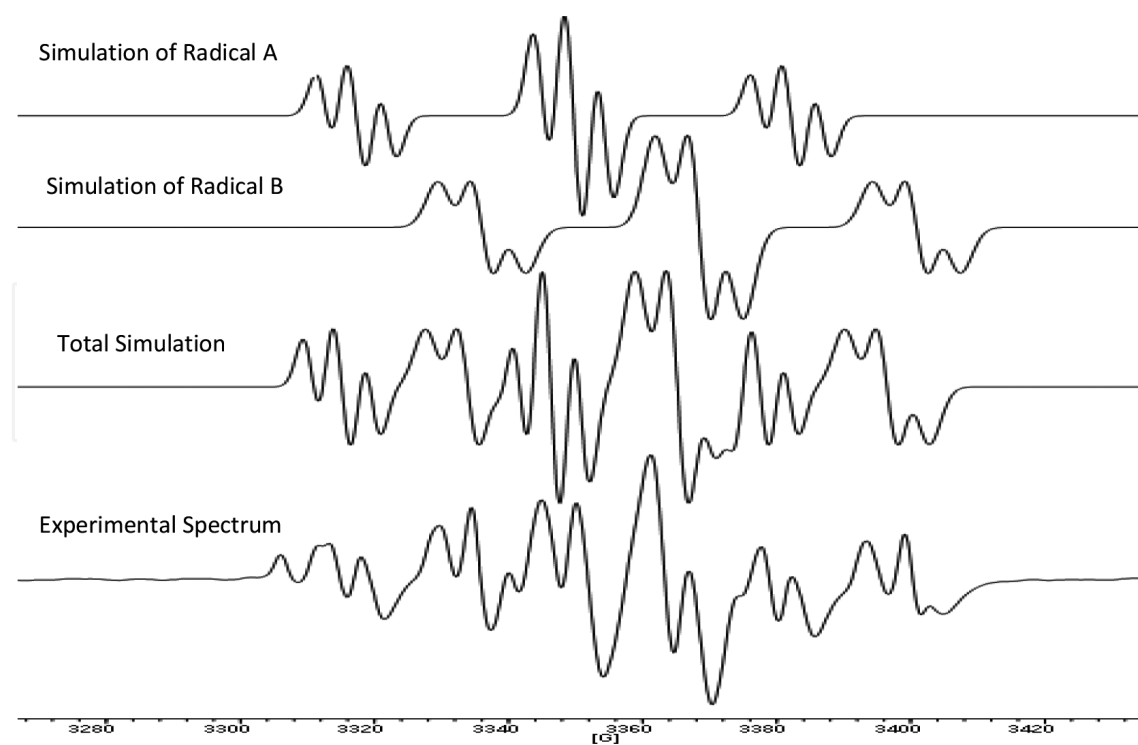


**Figure 4.** Structure of two succinic anhydride anion radicals observed in succinic anhydride single crystal.





**Figure 5.** Experimental and simulated EPR spectra of gamma-irradiated succinic anhydride single crystal at 125 K when the magnetic field is in the bc plane at an angle  $90^\circ$  toward the axis.



**Figure 6.** Experimental and simulated EPR spectra of gamma-irradiated succinic anhydride single crystal at 125 K when the magnetic field is in the ac plane at an angle  $110^\circ$  toward the axis.

|                 | Radical A  | Radical B   |
|-----------------|--|---|
| <b>Figure 5</b> | $(a_{CH_2(\beta)})_A = (a_{H\beta})_A = 3.26 \text{ mT}$<br>$(a_{CH_{\gamma_1}H_{\gamma_2}}^{H_{\gamma_1}})_A = (a_{H_{\gamma_1}})_A = 0.47 \text{ mT}$<br>$(a_{CH_{\gamma_1}H_{\gamma_2}}^{H_{\gamma_2}})_A = (a_{H_{\gamma_2}})_A = 0.43 \text{ mT}$<br>Center field = 335.576 mT<br>$\nu = 9.368 \text{ GHz}$<br>Line width = 0.4 mT  | $(a_{CH_2(\beta)})_B = (a_{H\beta})_B = 3.281 \text{ mT}$<br>$(a_{CH_{\gamma_1}H_{\gamma_2}}^{H_{\gamma_1}})_B = (a_{H_{\gamma_1}})_B = 0.505 \text{ mT}$<br>$(a_{CH_{\gamma_1}H_{\gamma_2}}^{H_{\gamma_2}})_B = (a_{H_{\gamma_2}})_B = 0.47 \text{ mT}$<br>Center field = 335.576 mT<br>$\nu = 9.423 \text{ GHz}$<br>Line width = 0.4 mT |
| <b>Figure 6</b> | $(a_{CH_2(\beta)})_A = (a_{H\beta})_A = 3.245 \text{ mT}$<br>$(a_{CH_{\gamma_1}H_{\gamma_2}}^{H_{\gamma_1}})_A = (a_{H_{\gamma_1}})_A = 0.39 \text{ mT}$<br>$(a_{CH_{\gamma_1}H_{\gamma_2}}^{H_{\gamma_2}})_A = (a_{H_{\gamma_2}})_A = 0.52 \text{ mT}$<br>Center field = 335.576 mT<br>$\nu = 9.368 \text{ GHz}$<br>Line width = 0.3 mT | $(a_{CH_2(\beta)})_B = (a_{H\beta})_B = 3.253 \text{ mT}$<br>$(a_{CH_{\gamma_1}H_{\gamma_2}}^{H_{\gamma_1}})_B = (a_{H_{\gamma_1}})_B = 0.44 \text{ mT}$<br>$(a_{CH_{\gamma_1}H_{\gamma_2}}^{H_{\gamma_2}})_B = (a_{H_{\gamma_2}})_B = 0.5 \text{ mT}$<br>Center field = 335.576 mT<br>$\nu = 9.423 \text{ GHz}$<br>Line width = 0.42 mT  |

**Table 4.** EPR parameters of simulated spectra.

| EPR parameters   | Principal values  | Direction cosines |           |           |
|--|---|-------------------|-----------|-----------|
| $(A_{CH_2(\beta)})_A = (A_{H\beta})_A \text{ (mT)}$                                    | $A_{xx} = 3.381$<br>$A_{yy} = 3.285$<br>$A_{zz} = 3.227$<br>$a_{iso} = 3.298$         | 0.194135          | 0.945231  | 0.262393  |
|  |   | -0.723568         | -0.042637 | 0.688935  |
|  |   | 0.662390          | -0.323606 | 0.675662  |
| $(A_{CH_{\gamma_1}H_{\gamma_2}}^{H_{\gamma_1}})_A = (A_{H_{\gamma_1}})_A \text{ (mT)}$ | $A_{xx} = 0.492$<br>$A_{yy} = 0.44$<br>$A_{zz} = 0.37$<br>$a_{iso} = 0.434$           | 0.818223          | 0.479980  | -0.316433 |
|  |   | 0.016324          | 0.530794  | 0.847344  |
|  |   | 0.574669          | -0.698482 | 0.426473  |
| $(A_{CH_{\gamma_1}H_{\gamma_2}}^{H_{\gamma_2}})_A = (A_{H_{\gamma_2}})_A \text{ (mT)}$ | $A_{xx} = 0.62$<br>$A_{yy} = 0.494$<br>$A_{zz} = 0.414$<br>$a_{iso} = 0.509$          | 0.325533          | -0.489384 | 0.809031  |
|  |   | 0.775337          | 0.627894  | 0.067839  |
|  |   | -0.541185         | 0.605188  | 0.583838  |
| $g_A$  | $g_{xx} = 2.01753$<br>$g_{yy} = 2.01513$<br>$g_{zz} = 2.01428$<br>$g_{iso} = 2.01565$ | 0.998258          | 0.021593  | 0.054902  |
|  |   | -0.054529         | 0.692912  | 0.718957  |
|  |   | -0.022518         | -0.720698 | 0.692883  |

**Table 5.** The EPR parameters of radical A observed in succinic anhydride at 125 K.

| EPR parameters   | Principal values    | Direction cosines |           |           |
|--|---------------------|-------------------|-----------|-----------|
| $(A_{CH_2(\beta)})_B = (A_{H_\beta})_B$ (mT)                                   | $A_{xx} = 3.36$     | 0.021180          | -0.990260 | -0.137614 |
|  | $A_{yy} = 3.283$    | 0.829840          | -0.059355 | 0.554835  |
|  | $A_{zz} = 3.251$    | -0.557599         | -0.125949 | 0.820500  |
|  | $a_{iso} = 3.298$   |                   |           |           |
| $(A_{CH_{\gamma_1}H_{\gamma_2}}^{H_{\gamma_1}})_B = (A_{H_{\gamma_1}})_B$ (mT) | $A_{xx} = 0.586$    | 0.066077          | 0.942855  | 0.326586  |
|  | $A_{yy} = 0.485$    | -0.896800         | -0.087383 | 0.433722  |
|  | $A_{zz} = 0.379$    | 0.437475          | -0.321541 | 0.839778  |
|  | $a_{iso} = 0.483$   |                   |           |           |
| $(A_{CH_{\gamma_1}H_{\gamma_2}}^{H_{\gamma_2}})_B = (A_{H_{\gamma_2}})_B$ (mT) | $A_{xx} = 0.612$    | 0.759274          | -0.102410 | 0.642663  |
|  | $A_{yy} = 0.464$    | -0.632874         | 0.113810  | 0.765844  |
|  | $A_{zz} = 0.288$    | -0.151571         | -0.988210 | 0.021600  |
|  | $a_{iso} = 0.455$   |                   |           |           |
| $g_B$  | $g_{xx} = 2.00660$  | 0.977193          | 0.210889  | -0.024888 |
|  | $g_{yy} = 2.00410$  | -0.096452         | 0.545205  | 0.832736  |
|  | $g_{zz} = 2.00300$  | 0.189184          | -0.811343 | 0.553111  |
|  | $g_{iso} = 2.00457$ |                   |           |           |

**Table 6.** The EPR parameters of the radical B observed in succinic anhydride at 125 K.

The radical formation mechanism in the present work is the same as that of potassium hydroquinone monosulfonate [6]. The angular dependences of EPR spectra were obtained for different orientations of the static magnetic field with respect to the crystalline axes. For the radical A and radical B, the spectroscopic splitting factor and the hyperfine coupling constants of the  $H_\beta$ ,  $H_{\gamma_1}$  and  $H_{\gamma_2}$  protons are anisotropic. For the radical A, the average values of the g-factor and the hyperfine coupling constants were obtained as  $g_A = 2.01565$ ,  $(a_{H_\beta})_A = 3.298$  mT,  $(a_{H_{\gamma_1}})_A = 0.434$  mT,  $(a_{H_{\gamma_2}})_A = 0.509$  mT, respectively. For the radical B, the average values of the g-factor and the hyperfine coupling constants were obtained as  $g_B = 2.00457$  and  $(a_{H_\beta})_B = 3.298$  mT,  $(a_{H_{\gamma_1}})_B = 0.483$  mT,  $(a_{H_{\gamma_2}})_B = 0.455$  mT, respectively.

### 2.3. Gamma-irradiated 3-nitroacetophenone single crystal

Nitroaromatic compounds have widespread actual or potential use in medicine and cancer therapy [14]. Nitroaromatic compounds have been extensively studied and reviewed mainly because of their interesting biological activities as well as their extended use in the chemical industry [15].

The radical in 3NAP identified as 3-nitroacetophenone anion radical is shown in **Figure 7**. The spectra were found to be temperature dependent. When we examined the EPR spectra at a

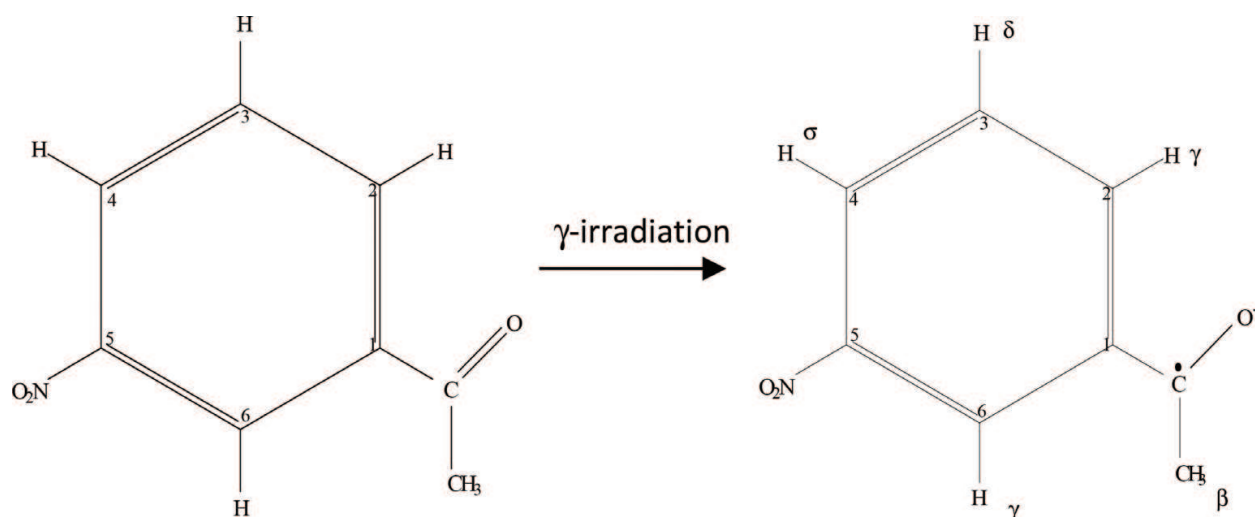


Figure 7. Structure of the 3NAP anion radical observed in 3NAP.

temperature range of 120–360 K, we decided that the radiation damage center in the sample was due to the break of the pi bond in the carbon-oxygen double bond. It was seen that the unpaired electron interacted with all the protons in the molecular structure [8].

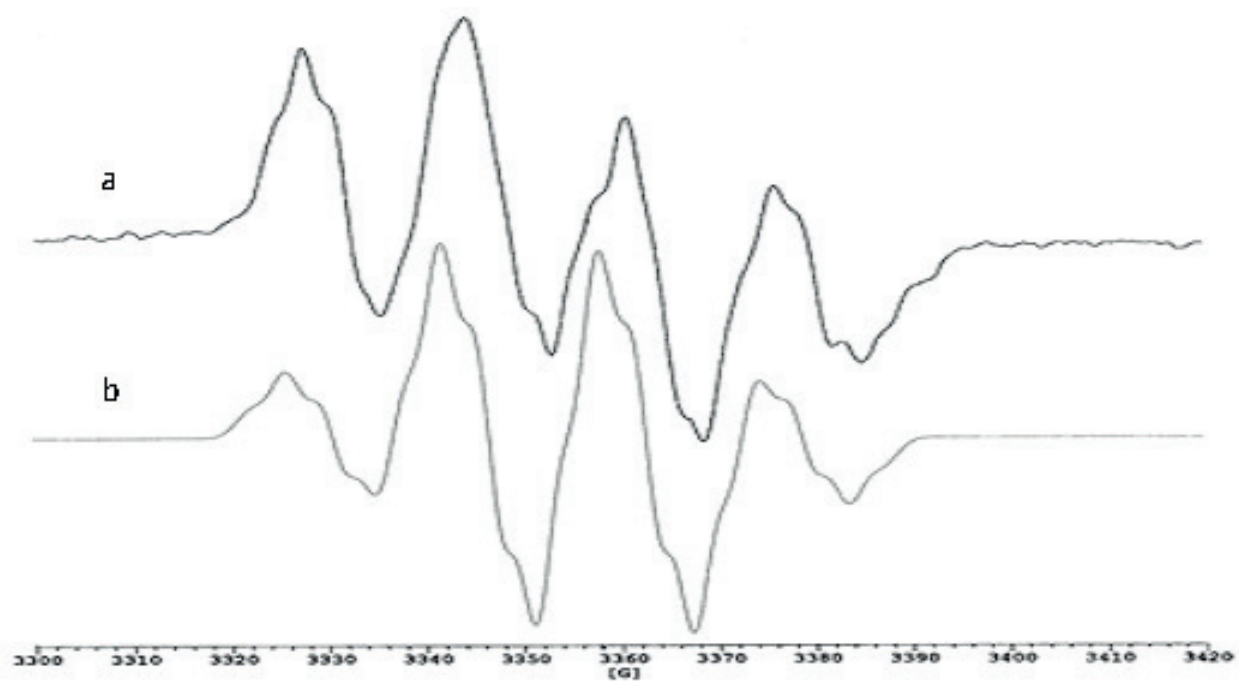
In the EPR spectra, especially the impact of the methyl protons was felt. The hyperfine coupling constants of the methyl protons were measured at 300 K. The angular variations of EPR parameters of the 3NAP only at 300 K could be investigated. The spectra were clear only in a few angles, at 120 K. Therefore, only the simulations of these spectra were made, at 120 K. In addition, the simulation was also made for a single angle, at 300 K.

The EPR spectra of irradiated 3-nitroacetophenone single crystals consist of four hyperfine structure lines with a 1:3:3:1 intensity ratio, which is caused by the interaction of the unpaired electron with the three equivalent protons of the methyl group, at most orientations of the crystal in the magnetic field, at 300 K. The H(2) and the H(6) protons are also considered approximately equivalent.

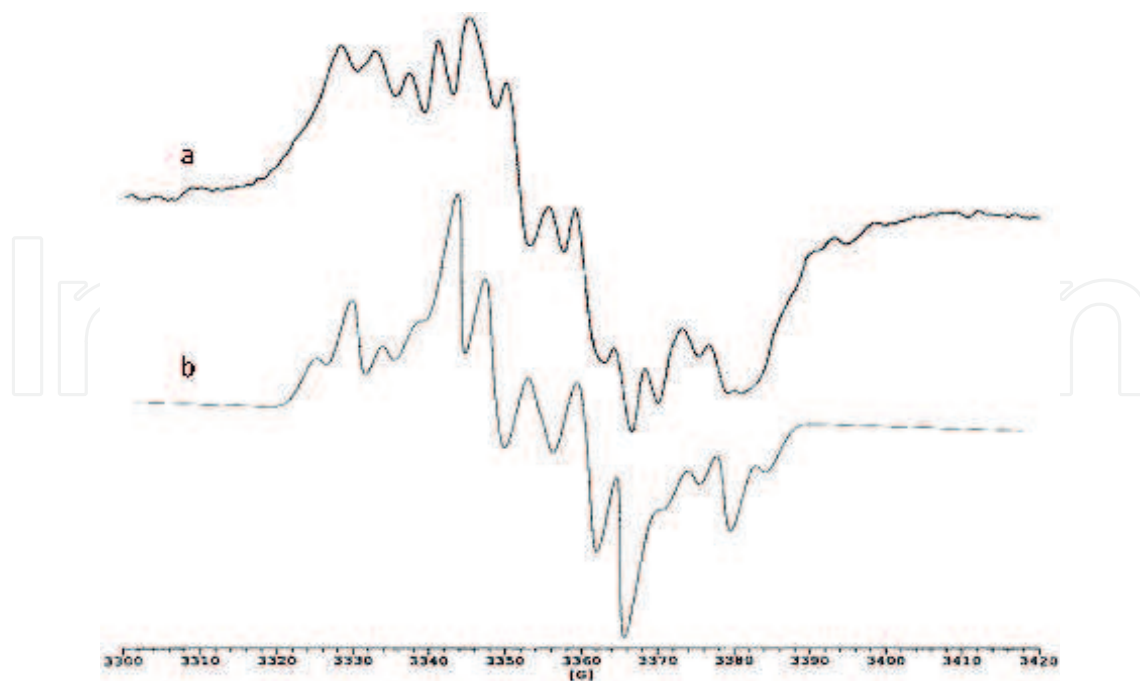
In **Figures 8–11**, the experimental spectra of the gamma-irradiated 3NAP single crystals and their simulation spectra were compared.

The simulation values of the hyperfine coupling constants of the spectra in **Figures 8–11** are given in **Table 7**. The EPR parameters of the 3-nitroacetophenone anion radical are given in **Table 8**.

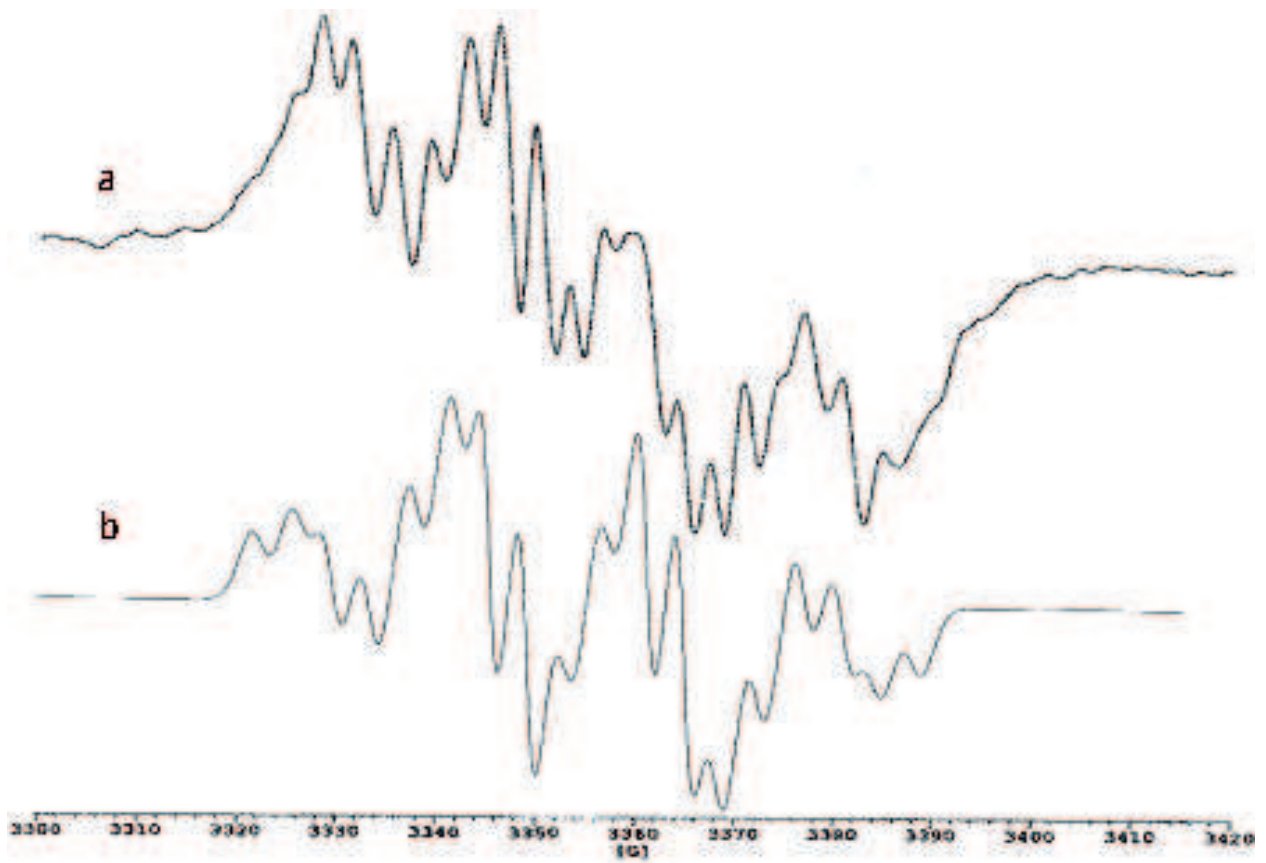
In the EPR spectra, especially the impact of the methyl protons was felt. Only the hyperfine coupling constants of the methyl protons could be experimentally measured at 300 K. The EPR spectra of irradiated 3-nitroacetophenone single crystals consist of four hyperfine structure lines with a 1:3:3:1 intensity ratio, which is caused by interaction of the unpaired electron with the three equivalent protons of the methyl group, at most orientations of the crystal in the magnetic field, at 300 K. The H(2) and the H(6) protons are also considered approximately equivalent. The hyperfine coupling for the CH<sub>3</sub> group is anisotropic and their average values being  $a_{CH_3} = 1.402$  mT.



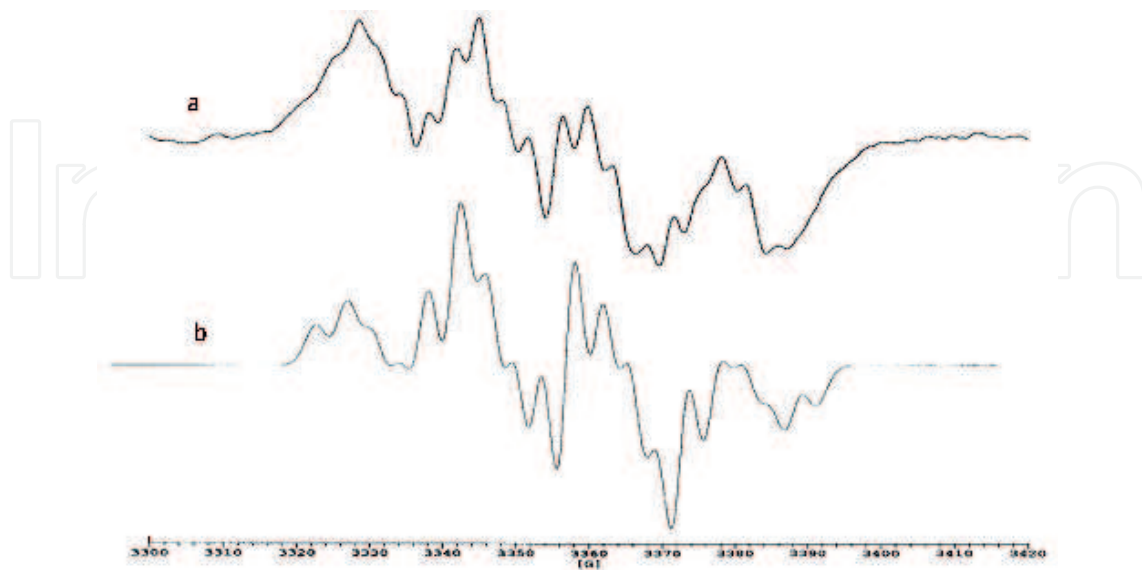
**Figure 8.** EPR spectra of  $^{60}\text{Co}$ - $\gamma$  irradiated 3NAP single crystal at 300 K when (a) the magnetic field is in the  $a^*b$ -plane and is away  $20^\circ$  from the  $a^*$ -axis and (b) simulation of the spectrum; line width is 0.34 mT.



**Figure 9.** EPR spectra of  $^{60}\text{Co}$ - $\gamma$  irradiated 3NAP single crystal at 120 K when (a) the magnetic field is in the  $a^*c$ -plane and is away  $140^\circ$  from the  $a^*$ -axis and (b) simulation of the spectrum; line width is 0.38 mT.



**Figure 10.** EPR spectra of  $^{60}\text{Co}$ - $\gamma$  irradiated 3NAP single crystal at 120 K when (a) the magnetic field is in the  $a^*c$ -plane and is away  $150^\circ$  from the  $a^*$ -axis and (b) simulation of the spectrum; line width is 0.32 mT.



**Figure 11.** EPR spectra of  $^{60}\text{Co}$ - $\gamma$  irradiated 3NAP single crystal at 120 K when (a) the magnetic field is in the  $a^*c$ -plane and is away  $165^\circ$  from the  $a^*$ -axis and (b) simulation of the spectrum; line width is 0.34 mT.

|   | Figure 8 | Figure 9 | Figure 10 | Figure 11 |
|---|----------|----------|-----------|-----------|
| $a_{CH_3(\beta)}$ mT                              | 1.55     | 1.36     | 1.68      | 1.585     |
| $a_{H(2)} \cong a_{H(6)} = a_{C_2H_2(\gamma)}$ mT | 0.38     | 0.43     | 0.45      | 0.46      |
| $a_{H(3)} = a_{H(\delta)}$ mT                     | 0.35     | 0.68     | 0.71      | 0.68      |
| $a_{H(4)} = a_{H(\sigma)}$ mT                     | 0.25     | 0.39     | 0.265     | 0.29      |

**Table 7.** Coupling constants values of simulated spectra.

|                        | Principal values     | Direction cosines |           |           |
|------------------------|----------------------|-------------------|-----------|-----------|
| $a_{CH_3(\beta)}$ (mT) | $A_{xx} = 1.743$     | 0.673208          | -0.717129 | -0.180325 |
|                        | $A_{yy} = 1.447$     | 0.572490          | 0.659816  | -0.486721 |
|                        | $A_{zz} = 1.015$     | 0.468023          | 0.224430  | 0.854743  |
|                        | $a_{iso} = 1.402$    |                   |           |           |
|                        | $\rho = 0.52$        |                   |           |           |
| g                      | $g_{xx} = 2.006443$  | 0.732137          | 0.415100  | -0.540063 |
|                        | $g_{yy} = 2.006110$  | 0.299886          | 0.515457  | 0.802728  |
|                        | $g_{zz} = 2.004067$  | 0.611591          | -0.749664 | 0.252902  |
|                        | $g_{iso} = 2.005540$ |                   |           |           |

**Table 8.** The EPR parameters of the 3-nitroacetophenone anion radical observed in 3-nitroacetophenone at 300 K.

The simulations of the EPR spectra were carried out using the Win-EPR software.

### 3. Conclusion

There are many factors that trigger the formation of free radicals. One of these is the radiation effect. The various compounds that undergo radiation effects have the radiation damage centers as a result of breaking bonds in their structures. The radiation damage center is a paramagnetic center, and the detection and motion of these centers are investigated in detail by EPR spectroscopy.

Antioxidants also show paramagnetic structure under radiation effect. EPR analysis of antioxidants is crucial to remove the effect of free radicals that cause many diseases. Potassium hydroquinone monosulfonate (PHM), succinic anhydride and 3-nitroacetophenone compounds show antioxidant properties. When EPR analyses of these materials were made, it was seen that their radical mechanisms were similar to each other. The anion radicals were observed in all three compounds. When the resonance structure and the formation mechanisms are carefully examined, it is seen that the oxygen atom takes an electron and forms a stable anion structure.

## Author details

Betül Çalışkan<sup>1\*</sup> and Ali Cengiz Çalışkan<sup>2</sup>

\*Address all correspondence to: [bcaliska@gmail.com](mailto:bcaliska@gmail.com)

1 Department of Physics, Faculty of Arts and Science, Pamukkale University, Denizli, Turkey

2 Department of Chemistry, Faculty of Science, Gazi University, Ankara, Turkey

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