EPR Study of Gamma Induced Radicals in Amino and Iminodiacetic Acid Derivatives

Murat Aydin*
Faculty of Education, Adiyaman University, TR 02040-Adiyaman, Turkey

M. Halim Baskan
Faculty of Education, Dicle University, Diyarbakir, Turkey

Y. Emre Osmanoglu
Institute of Science, Dicle University, Diyarbakir, Turkey

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In this study, electron paramagnetic resonance spectroscopy was used to investigate free radicals formed in gamma irradiated L-glutamine hydrochloride, iminodiacetic acid hydrochloride and N-(2-hyroxyethyl) iminodiacetic acid powders. The free radicals produced in L-glutamine hydrochloride powders were attributed to the $CH_2\dot{C}HCOOH$ radical; and those in iminodiacetic acid hydrochloride and N-(2-hyroxyethyl) iminodiacetic acid powders to the $HNCHCH_2(COOH)_2$ and $HOCH_2CH_2N\dot{C}HCH_2(COOH)_2$, respectively. The g-values of the radicals and the hyperfine structure constants of the free electron with the environmental protons and ^{14}N nucleus were determined. The samples were not displayed before they were not irradiated. The free radicals were found stable at room temperature for more than six months. Some spectroscopic properties and suggestions concerning possible structure of the radicals are discussed in this paper.

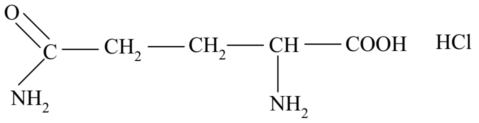
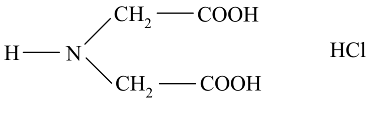
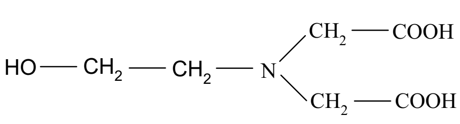
Keywords: EPR, Free radicals, Amino acid derivatives, Gamma-irradiation.

1. INTRODUCTION

Electron paramagnetic resonance (EPR) spectroscopy is a very sensitive method for the detection of free radicals. Moreover, the hyperfine structure of the EPR spectrum (when it is well resolved) provides more important information about the radical than the g-value, because most radicals detected are carbon or nitrogen centered radicals and the spectra positions are almost in the same magnetic field range [1]. The EPR technique has been widely used for long time in the identification of irradiation damage centers in many substances including drugs, amino acid and iminodiacetic acid derivatives [2-18]. L-glutamine and iminodiacetic acid powders were gamma-irradiated and studied by EPR at room temperature [19, 20]. The radiation damage centers were attributed to the $CH_2\dot{C}HCOOH$ and $HN\dot{C}HCH_2(COOH)_2$ radicals, respectively. Furthermore, L-glutamine hydrochloride and N-carbamoyl-L-glutamic acid single crystals are gamma-irradiated and studied at room temperature with EPR [21].

L-glutamine is a biologically very important amino acid which play an essential role in metabolic process. It occurs in the free state and as part of the protein structure in plants and animals. Further it has been found that the amide group in this molecule is more susceptible to hydrolysis and attack by nitrous acid than acetamide [22]. Iminodiacetic acid and N-(2-hyroxyethyl)iminodiacetic acid are a biologically important organic substances. Therefore, it is the aim of this work to give some information about the activities of selected compounds which have not been studied so far.

TABLE 1: List of the organic compounds studied with their commercial names, chemical formulas, molecular weights (g/mol) and the chemical structures.

No	Name, formula, molecular weight, chemical structure
1.	L-glutamine hydrochloride, $C_5H_{10}N_2O_3\cdot HCl$, 182.61 
2.	Iminodiacetic acid hydrochloride, $C_4H_7NO_4\cdot HCl$, 169.57 
3.	N-(2-Hyroxyethyl)iminodiacetic acid, $C_6H_{11}NO_5$, 177.16 

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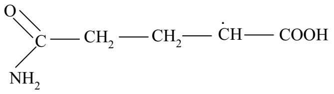
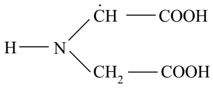
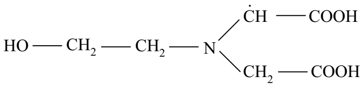
[*] Electronic address: `maydin@adiyaman.edu.tr`

2. EXPERIMENTAL

The list of the samples studied with their chemical formulas and commercial names are presented table 1. The powders were irradiated at room temperature with a 60Co gamma-ray source of 20 kGy. After irradiation, all samples were kept in plastic bags at room temperature in the dark. For the EPR measurements the samples were introduced in quartz tubes with internal and external diameters of 4.75 and 5.75 mm, respectively. The EPR spectroscopy was carried out in a Varian model X-band E-109C EPR spectrometer at room temperature. The modulation amplitude was below 5.10^{-2} mT and the microwave power was 2 mW. The g factors were found by comparison with a dipenhylpicrylhydrazyl (DPPH) sample with of $g = 2.0036$. The hyperfine coupling constant (a) values arise by magnetic interactions between the unpaired electron dipole and nearby magnetic nuclei such as H, ^{14}N and ^{2}D [23]. The hyperfine coupling constant of a nucleus is directly related to splitting of lines in an EPR spectrum. The spectra were simulated using the McKelvey's programs [24].

TABLE 2: Precursor number, EPR parameters and proposed structure of irradiation- produced radical species.

Precursor no	EPR parameters, proposed radical structure
1.	$g = 2.0035 \pm 0.0005$, $a_{CH} = 2.87$ mT, $a_{CH2} = 1.08$ mT, $\Delta H = 0.35$ mT. 
2.	$g = 2.0028 \pm 0.0005$, $a_{CH} = 1.23$ mT, $a_{CH2} = 0.89$ mT, $a_{NH} = 0.48$ mT, $a_N = 0.57$ mT, $\Delta H = 0.30$ mT. 
3.	$g = 2.0030 \pm 0.0005$, $a_{CH} = 1.45$ mT, $a_{CH2} = 0.76$ mT, $a_N = 0.50$ mT, $\Delta H = 0.30$ mT. 

3. RESULTS AND DISCUSSION

No EPR signal could be observed from unirradiated L-glutamine hydrochloride (LGHCl), iminodiacetic acid hydrochloride (IDAHCl) and N-(2-hyroxyethyl)iminodiacetic acid (NHIDA).

TABLE 3: The EPR parameters of some amine radicals.

Amine	Radical	Hyperfine coupling constant (mT)						
		a_α^H	a_β^H	a_γ^H	a^N	$a_{NH_2}^H$	g values	Ref.
Dimethylamine	$\dot{C}H_2NHCH_3$	1.09	–	0.42	0.58	0.63	2.0028	[28]
Trimethylamine	$(CH_3)_2N\dot{C}H_2$	1.34	–	0.37	0.70	–	2.0028	[29]
n-Propylamine	$CH_3CH_2\dot{C}HNH_2$	1.46	1.64	–	0.48	0.48	2.0026	[29]
Triethylamine	$(C_2H_5)_2N\dot{C}HCH_3$	1.37	1.96	0.23	0.52	–	2.0031	[29]
Iminodiacetic acid (Aqueous solutions)	$^{-}OOCCH_2NH\dot{C}HCOO^{-}$	1.28	–	0.56	0.68	0.51	2.0033	[28]
Iminodiacetic acid (Powders)	$HN\dot{C}HCH_2(COOH)_2$	1.40	–	1.40	0.39	0.58	2.0031	[20]
Iminodiacetic acid (Single crystal)	$HN\dot{C}HCH_2(COOH)_2$	1.83	–	1.83	0.38	0.97	2.0028	[30]
IDAHCl	$HN\dot{C}HCH_2(COOH)_2$	1.23	–	0.89	0.57	0.48	2.0028	This work
NHIDA	$HOC_2H_4N\dot{C}HCH_2(COOH)_2$	1.45	–	0.76	0.50	–	2.0030	This work

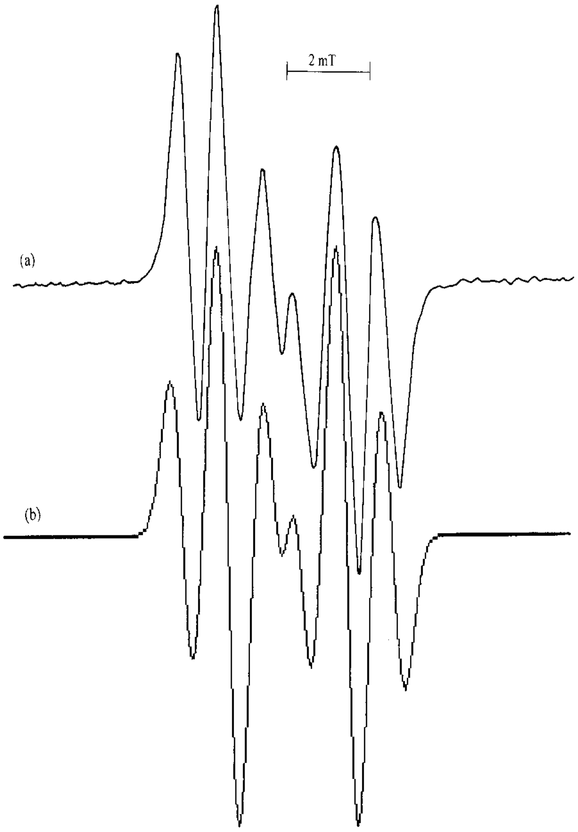


FIG. 1: (a) The EPR spectrum of gamma- irradiated LGHCl powder, (b) simulation of the spectrum.

The EPR spectrum of gamma-irradiated LGHCl powders at room temperature is shown in figure 1a. The spectra of EPR of the sample of LGHCl showed carbon-centered free radical. This spectrum exhibits an intensity distribution at a rating of 1 : 2 : 1 : 1 : 2 : 1, and can be attributed to the radical shown in table 2. It can be seen that it consist of a doublet with a spacing 2.87 mT. Then each line of doublet is further subdivided into three lines of spacing with 1.08 mT and on intensity distribution of 1 : 2 : 1. These inferences indicate the hyperfine coupling of the free electron with α-proton and two methylene protons. The measured value of the g factor is $g = 2.0035 \pm 0.0005$. These values agree well with those derived from the $CH_2\dot{C}HCOOH$ radical in L-glutamine powders [19]. The spectrum simulated with these hyperfine

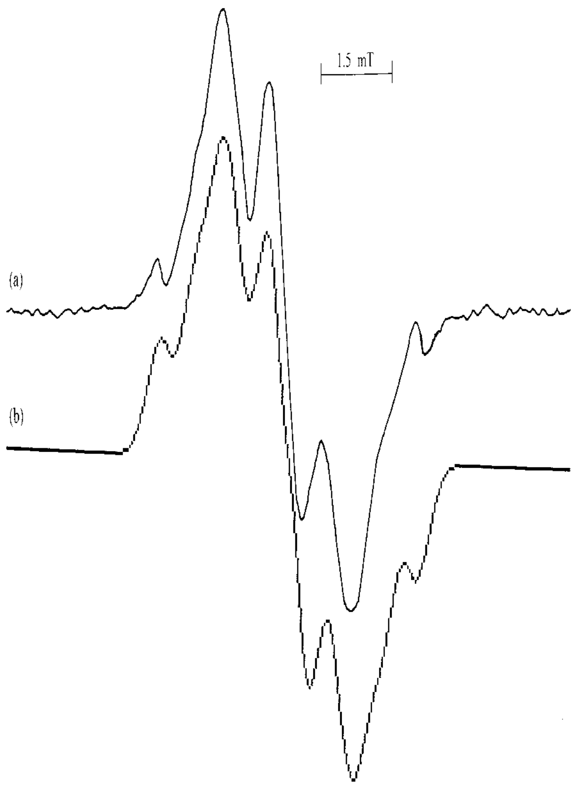


FIG. 2: The EPR spectrum of gamma- irradiated IDAHCl powder, (b) simulation of the spectrum.

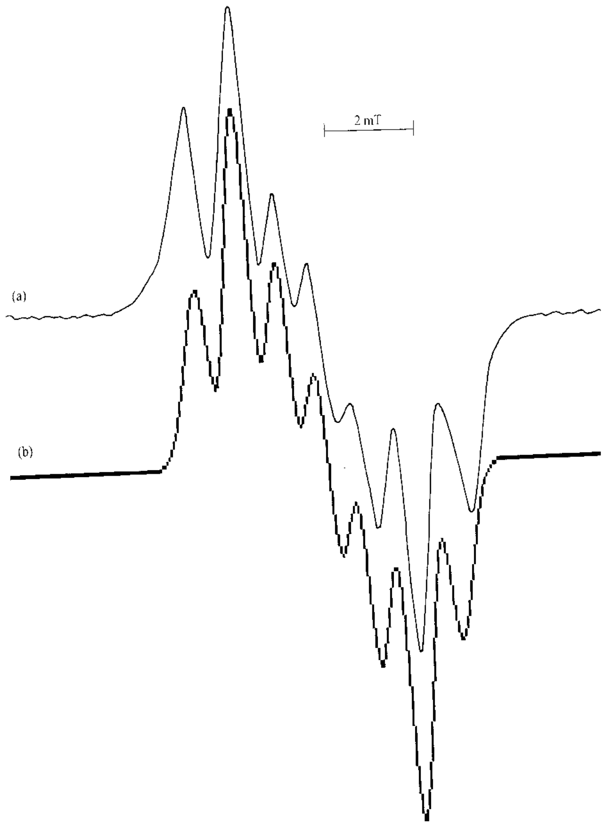


FIG. 3: (a) The EPR spectrum of gamma- irradiated NHIDA powder, (b) simulation of the spectrum.

parameters is presented in figure 1b. The experimental and simulated EPR spectra were found to agree well with each other. The hyperfine constants and the g-value of the radical discussed here seem to agree well with the literature data [19, 25, 26]. Single crystal of the gamma-irradiated 2,2-dimethyl succinic acid have been studied between 120 and 300 K [27]. The radiation damage center of this sample was attributed to the $CHCH_2$ radical. The reported average values of the hyperfine constants are a CH = 2.44 mT and aCH$_2$=0.82 mT. This radical and hyperfine values are similar to radical obtained in LGHCl. Consequently, it can be stated that gamma-irradiation produced free radicals in LGHCl by loss of a NH_2 group from the carbon atom bound to the -COOH group.

The EPR spectrum of irradiated IDAHCl powders and the most probable radical producing are shown in figure 2a, and table 2, respectively. The hyperfine interactions of the unpaired electron with α-proton, two methylene protons, one NH proton and ^{14}N nucleus can be taken as a_{CH} =1.23 mT, a_{CH2} =0.89 mT, $a_{NH} = 0.48$ mT and $a_N = 0.57$ mT. The g-value of the radical is g= 2.0028 ± 0.0005. The spectrum simulated with these hyperfine parameters is presented in figure 2b. The agreement between the experimental and simulated EPR spectra is good. The hyperfine constant and g-values are relevance with the literature data in table 3 [20, 28-30]. Powders methyliminodiacetic acid have been investigated at room temperature and the reported average values of the hyperfine constants and g factor are $a_{CH} = 1.34$ mT, $a_{CH2} = 0.89$ mT, $a_{CH3} = 0.68$ mT, $a_N = 0.53$ mT and g= 2.0030 [20]. Moreover, an irradiated aqueous solution of iminodiacetic acid was studied and the hyperfine constants and g factor values were reported as $a_{CH} = 1.28$ mT, $a_{CH2} = 0.56$ mT, $a_{NH} = 0.51$ mT, $a_N = 0.68$ mT and g= 2.0033 [28]. Two common mechanisms by which unpaired electron and nuclei interact are Fermi contact interaction and by dipolar interaction. In liquid, only isotropic interaction (Fermi contact) is observed, because dipolar interaction due to anisotropic contribution to hyperfine interaction are generally averaged to zero by high-frequency molecular reorientations [31]. Also, in some aqueous solution of amine compounds (isopropylamine, tert-butylamine, tetramethylammoniumhydroxide, acetylalanine and aminomalonamide) no spectra could be observed to be due to the large number of splittings which divide the intensity among many lines and as a result reduce the line intensities to near or below the noise level. Another possible reason for the absence of lines from acid solutions is the chemical exchange of the protons of the ammonium group. In contrast, spectra were detected when the amino group was in a position further away from the unpaired electron or when the amino group was in the basic form [28]. Powders solids, of course, exhibit statistical average of anisotropic contribution to hyperfine interaction [32]. Thus, the hyperfine constant of aqueous solution of iminodiacetic acid are similar to in the IDAHCl. We can state that the gamma-irradiation breaks the bond of the hydrogen atom from the carbon atom bond to the ^{14}N nucleus and the –COOH group in IDAHCl.

After irradiation of NHIDA the EPR spectra recorded, is shown in figure 3a. The spectrum consists of 7 lines and the radical for this spectrum is thought to result from the abstraction of one hydrogen atom from the α-carbon atom (table 2). The free electron interacts with α-proton, two methylene protons and 14N nucleus. The hyperfine constants obtained by the simulation are $a_{CH} = 1.45$ mT, $a_{CH2} = 0.76$ mT and $a_N = 0.50$ mT. The g value of spectrum was measured as g= 2.0030 ± 0.0005. These measured values of the radical are in good agreement with previous work (table 3). Powders of gamma-irradiated pergolid mesylate (Permax) were investigated at room temperature [33]. The radiation damage center of this sample was attributed to the $N\dot{C}HCH_2CH_3$ radical. The hyperfine constants and g factor values were reported as $a_{CH} = 1.45$ mT, $a^{1,2}_{CH2} = 2.00$-1.60 mT, $a_N = 0.40$ mT and g = 2.0033. These determined values are similar to radical obtained in NHIDA. The hyperfine constants and g- value of radical discussed here seem to be in agreement with the literature data for their analogs and various other amine radicals [29]. The values of the g factor in amino alkyl radicals vary between 2.0027-2.0031 [29] and are consistent with g-value reported in this study.

4. CONCLUSION

Gamma-irradiation produces some very stable alkyl and amine type free radicals in some amino and iminodiacetic acids derivatives. The gamma-irradiated LGHCl, IDAHCL and NHIDA samples indicated the inducement of $CH_2\dot{C}HCOOH$, $HN\dot{C}HCH_2(COOH)_2$ and $HOCH_2CH_2N\dot{C}HCH_2(COOH)_2$ radicals respectively.

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[1] G. Damian, Talanta, **60**, 923 (2003).

[2] S. Talbi, J. Raffi, S. Arena, J. Colombani, P. Piccerelle, P. Prindere and J.M. Dolo, Spetrochim. Acta Part A, **60,** 1335 (2004).

[3] S.L. Cogo, A.M. Brinatti, S.C. Saab, M.L. Simoes, L. Martin-Neto, J.A. Rosa and Y.P. Mascarenhas, Braz. J. Phys., **39,** 31 (2009).

[4] N.D. Yordanov, O. Lagunov and K. Dimov, Radiat. Phys. Chem., **78,** 277 (2009).

[5] F. Chen, C.F.O. Graeff and O. Baffa, Nucl. Instr. and Meth., Phys. Research B, **264,** 277 (2007).

[6] N.D. Yordanov and K. Aleksieva, Radiat. Phys. Chem., **76,** 1084 (2007).

[7] E. Bozkurt, I. Kartal, F. Köksal and B. Karabulut, Radiat. Phys. Chem., **77,** 571 (2008).

[8] S.B. Zincircioğlu, N. Canoruç, S.. Osmanoğlu, M.H. Baskan, I.Y. Dicle and M. Aydın, Z. Naturforsch., **61a,** 577 (2006).

[9] M.R.R. de Oliveira and N. L. del Mastro, Radiat. Phys. Chem., **76,** 1459 (2007).

[10] O. Cozar, V. Chis, L. David, G. Damian and I. Barbur, J. Radioanalyt. Nucl. Chem., **220,** 241 (1997).

[11] A.M. Maghraby and M. A. Ali, Radiat. Phys. Chem., **76,** 1600 (2007).

[12] N.D. Yordanov and B. Mladenova, Radiat. Phys. Chem., **60,** 191 (2001).

[13] M. Gibella, A.S. Crucq, B. Tilquin, P. Stocker, G. Lesgards and J. Raffi, Radiat. Phys. Chem., **58,** 69 (2000).

[14] F. Köksal and S. Osmanoğlu, J. Chem. Res. **(S),** 84 (1993).

[15] A. Kinoshita, L. Figuty and O. Baffa, Braz. J. Phys., **36,** 93 (2006).

[16] F. Chen, P. Nicolucci and O. Baffa, Radiat. Measurements., **43,** 467 (2008).

[17] M. Tabak, D. de Sousa Neto and C.E.G. Salmon, Braz. J. Phys., **36,** 83 (2006).

[18] E. Di Mauro, C.L.B. Guedes and M.T. Piccinato, Appl. Magn. Reson., **32**, 303 (2007).

[19] M.H. Baskan, Radiat. Eff. Defect. Solids, **163,** 35 (2008).

[20] M. Aydin, Y.E. Osmanoğlu and M.H. Baskan, Radiat. Eff. Defects Solids, **163,** 47 (2008).

[21] S. Osmanoğlu, M. Aydin and M.H. Bakan, Z. Naturforsch., **60a,** 549 (2005).

[22] N. Shamala and K. Venkatesan, Cryst. Struct. Comm., **1,** 227 (1972).

[23] B. Mile, Current Organic Chem., **4,** 55 (2000).

[24] R.D. McKelvey, J. Chem. Educ., **64,** 497 (1987).

[25] D. Pooley and D.H. Whiffen, Mol. Phys., **4,** 81 (1961).

[26] H. Muto, T. Inoue and M. Iwasaki, J. Chem. Phys., **57,** 3220 (1972).

[27] S. Osmanoğlu, M.H. Bakan, and T. Gner, Z. Naturforsch., **57a,** 909 (2002).

[28] P. Neta and R. W. Fessenden, J. Phys.Chem., **75,** 738 (1971).

[29] D. E. Wood and R. V. Lloyd, J. Chem. Phys., **53,** 3932 (1970).

[30] M.H. Baskan, M. Aydğn and S. Osmanoğlu, Radiat. Eff. Defects Solids, **163,** 65 (2008).

[31] S.I. Weissman, J. Chem. Phys., **22,** 1378 (1954).

[32] S.M. Blinder, J. Chem. Phys., **33,** 748 (1960).

[33] R. Köseoğlu, E. Köseoölu and F. Kksal, App. Radiat. Isot., **58,** 63 (2003).