International Journal of Applied and Natural Sciences (IJANS)
ISSN(P): 2319-4014; ISSN(E): 2319-4022
Vol. 3, Issue 4, July 2014, 29-36

International Academy of Science, Engineering and Technology
Connecting Researchers; Nurturing Innovations

STUDY ON THE EPR/DOSIMETRIC PROPERTIES ON DL-TRYPTOPHAN

S. EID & A. MANSOUR

National Center for Radiation Research and Technology, Atomic Energy Authority, Cairo, Egypt

ABSTRACT

Polycrystalline DL-tryptophan is promising material for EPR dosimetry, because it has a large yield of stable radicals due to gamma radiation. The free radical concentrations in DL-tryptophan proportional to the absorbed dose. The EPR spectra of DL-tryptophan have a spectroscopic splitting factor of g = 2.00922 ± 0.02107 and hyperfine constant *A*= 3.875 ± 0.787 mT. Dl-tryptophan have specified EPR signal developed under irradiation in the dose range from 0.5-200 kGy. The obtained number of free radicals per 100 eV (G value) was found to be 0.063 ± 0.01. The pre and post- irradiation stability was found to be satisfactory.

KEYWORDS: EPR – Radiation Dosimetry – DL-Tryptophan

INTRODUCTION

The electron paramagnetic resonance (EPR) is a technique commonly used for low and high doses dosimetry of the gamma, X, neutron and electron radiations, accident dosimetry, studies of defects, analysis of radicals, etc. The EPR technique detects unpaired electrons trapped in the crystalline lattice. The intensity of the EPR signal is proportional to the absorbed dose. The non-destructive nature of the detection also allows the study of species trapped in biological samples such as bone, tissue, drug, teeth, hair, fingernails and dry skin [1, 2, and 3].

The use of the alanine as a dosimeter happened after the discovery of EPR technique. The alanine spectrum presents multiply resonance lines and a high quantity of free radicals formed by absorbed dose unit [4], it is an amino acid with effective atomic number very close of the human tissue, it presents a stable and simple signal, with low background, low coast, easy handling and available universally [5-8]. Photochemistry and photolysis of aqueous tryptophan has been widely studied over last decades [9]. Contrary, there have been very few studies on the radiation chemistry of solid state tryptophan [10]. Sagstuen et al., have reported that EPR spectrum measured of a single crystal of L-tryptophan·HCl irradiated with 4MeV electrons indicates the formation of at least two different radical species [11]. The objective of the present study is preparation of a new EPR dosimeter. Tryptophan has been selected because it's stable towards environmental conditions, easily handling. The possibility of using the new dosimeter as EPR dosimeter material is examined.

MATERIALS AND EXPERIMENTAL

Materials

DL-tryptophan (Merck code is T61613) has the molecular formula of $C_{11}H_{12}N_2O_2$, molecular weight 204.23 g, and the melting point is 289 ^{o}C. Both hot melt stick adhesive based on ethylene vinyl acetate copolymer (Tec-Bond 232/12, Power Adhesives Limited, England) and paraffin wax (congealing point 65-71 ^{0}C, BHD) were used in rods preparation of DL-tryptophan.

Preparation of DL-Tryptophan Rods

An equal weight mixture of paraffin wax and ethylene vinyl acetate copolymer (EVA) was melt at 85-95^{o}C in a water bath. 5, 10, 20 and 40% fine powdered DL-tryptophan material was added to the hot mixture solution and mechanically stirred for about 15 minutes at the same temperature to obtain a homogeneous mixture. The hot solution is sucked into polypropylene tubes (inner diameter 3 mm) and was left to solidify by cooling. Tryptophan mixture rod was obtained by removing the polypropylene tube then cut into rods (3 x10 mm dimensions). The average mass of the prepared rods was found to be 0.0714 ± 0.0035 g.

Irradiation

^{60}Co irradiation facility was used for irradiation of the prepared rods. The absorbed dose rate was about 3.078 kGy/h overall the time of the experimental part. Three rods at each dose were irradiated together at the central position of the sample chamber using a specially designed holder made from polystyrene to ensure electronic equilibrium.

EPR Measurements

EPR signals were recorded at room temperature by using an X-band EPR spectrometer (Bruker EMX, Germany). The operating conditions are, microwave power 5.053 mW, modulation amplitude 0.5 mT, modulation frequency100 kHz, sweep width 20 mT, microwave frequency 9.721 GHz, time constant 81.92 ms and conversion time 20.48 ms. The bottom of the EPR tube was adjusted at fixed position to ensure reproducible and accurate positioning of the rods in the sensing zone of the cavity. EPR spectra were recorded at two orientations of each rod in the EPR cavity (0^0 and 90^0) to reduce the orientation effects. The readings were corrected by using reference standard material DPPH (α- α-diphenyl β picrylhydrazyl).

RESULTS AND DISCUSSIONS

Radical Species of Irradiated DL-Tryptophan

The EPR spectra of non-irradiated and irradiated tryptophan rods to a dose of 35 kGy measured at the same parameters and at room temperature were shown in Figure 1a (Inset Figure 1a is molecular structure of DL-tryptophan). Figure 1a shown that, the EPR signal developed as a result of radiation-induced radials, also no signal has detected for the non-irradiated tryptophan. A typical EPR signal of irradiated DL-tryptophan is shown in Figure 1b. The signal consists of four major peaks: a broad one observed at g = 2.00922 with line-width ***dH*** = 2.29 mT (named Trp^1 stable radical) and the other peaks were observed at g = 2.03216, 1.99248 and 1.98713 (named Trp^2 transient radicals). Also, EPR spectra of the tryptophan radicals are dominated by the large hyperfine splitting constant (A = 3.875 ± 0.787 mT) [12].

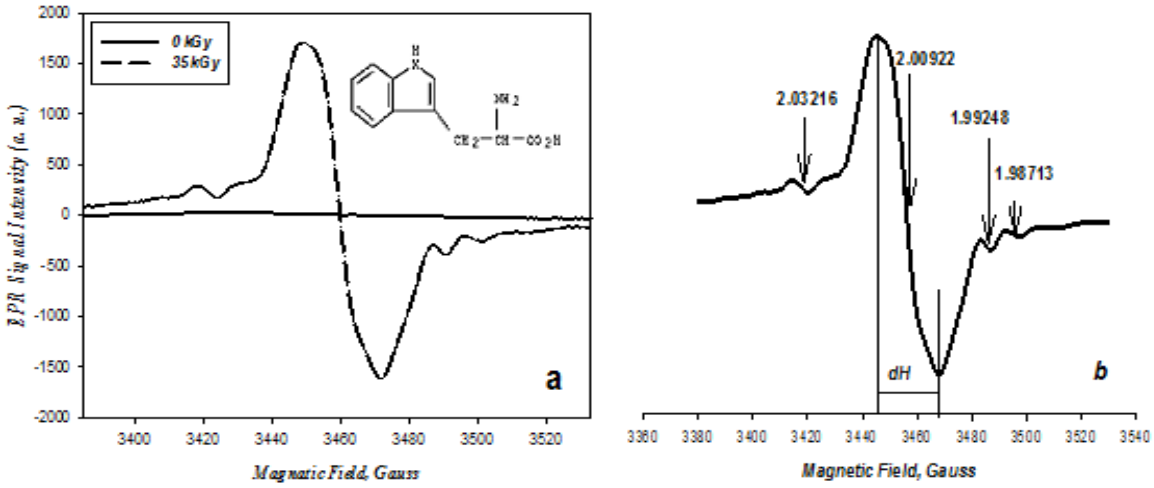

Figure 1: (a) EPR Spectra of Non-Irradiated and Irradiated DL-Tryptophan Rods (40 %) at a Dose of 35 kGy, (b) g Values at Different Peaks

The calculated g-values corresponding to Trp^1 and Trp^2 radicals are in good agreement with the data were obtained by F. G. Wertz [18].

Effect of Concentration on the EPR Signal Intensity

Four different concentrations of DL-tryptophan namely 5, 10, 20, 40% were irradiated to a dose of 15 kGy and the obtained spectra were shown in Figure 2, from which it could be concluded that the peak intensity increases with the increase of DL-tryptophan concentration.

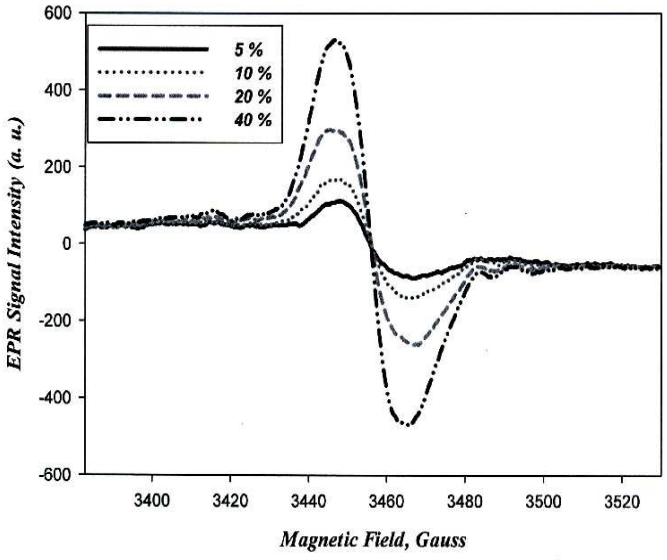

Figure 2: EPR Spectra Recorded for Tryptophan Rods of Different Concentrations Irradiated to a Dose of 15 kGy

Dosimetric Properties

Micro-Wave Power Dependence

A general rule for quantitative EPR measurements is to keep the microwave power in the unsaturated region.

The parameter optimization was based on the dependences of the signal amplitude on microwave power. This dependence was measured using DL-tryptophan rod (40%) irradiated to 35 kGy. Figure 3 shows the peak-to-peak signal amplitude as a function of the square root of the microwave power. The optimum value for carrying out the measurements is 5.053 mW (corresponding to $P^{1/2}$ = 2.248 mW) was selected because the signal intensity increases as a function of microwave power without reaching saturation. The spectrum obtained at this value has the best line shape and accurate line-width.

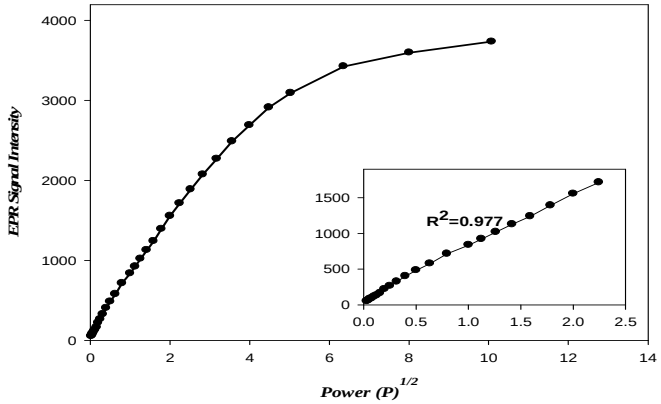

Figure 3: Relationship between the Square Root of Microwave Power ($mW^{1/2}$) and EPR Signal Intensity of γ–Irradiated Tryptophan Rod (35 kGy)

Dose Response

Figure 4 represents the EPR spectra of DL-tryptophan rods (20%) were recorded after irradiation to doses 0.5, 5, 10, 35, 75, and 100 kGy as well as non-irradiated rod. From this figure it can be seen that the EPR signal begins to develop upon irradiation and its amplitude increases gradually with increasing the absorbed dose of γ-rays. The kinetics of the small peaks could not be accurately established due to spectral overlap with the main peak, this means the rapid radical Trp^2 migrate to main peak Trp^1 [14].

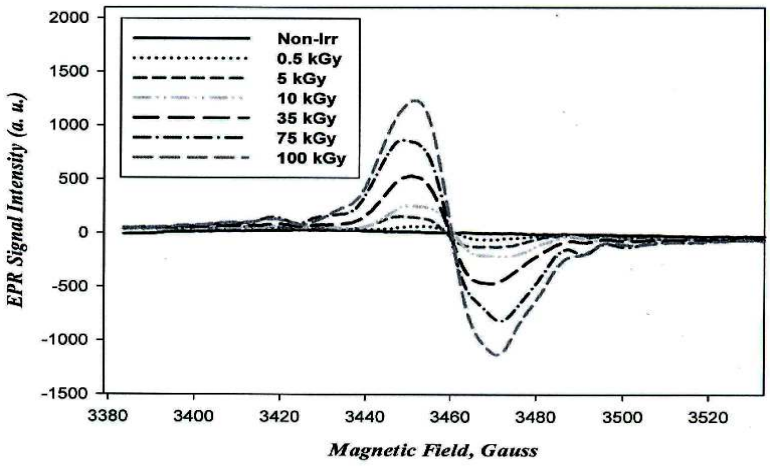

Figure 4: The EPR Spectra of Tryptophan Rods Irradiated to Different Doses (20%)

Figure 5 represents the response curves for the four sets of tryptophan rods concentrations (5, 10, 20 and 40%) to different absorbed dose (0.5 - 200 kGy) to reach saturation. EPR spectra were recorded to establish the response curves (each dose point is represented by the average value of 3 rods measurements). The response curves obtained for the irradiated samples in terms of average peak-to-peak amplitude normalized to rod mass (peak height/mass) versus the

absorbed dose. It can be seen that the EPR signal intensity of tryptophan rods increases with the increase of absorbed dose (inset Figure 5) and tryptophan concentration in the rods.

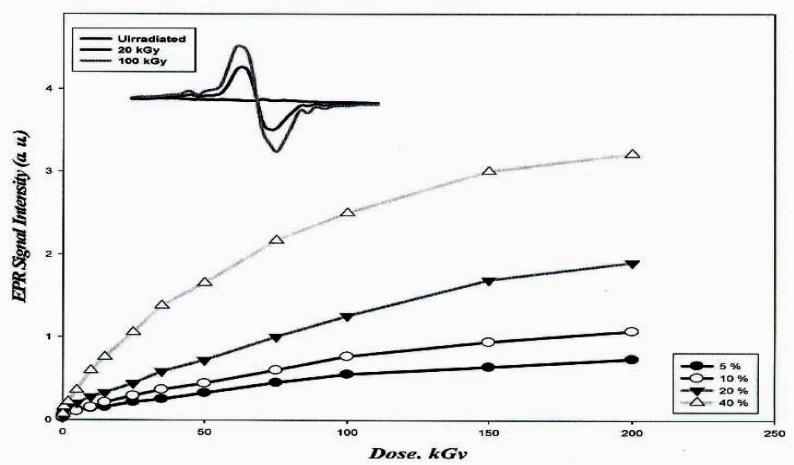

Figure 5: Dose Response of Irradiated Tryptophan Rods at Different Doses for Different Concentrations

Radiation Sensitivity G-Value

The efficiency of a dosimeter is expressed by a G-value, i. e. the number of radicals or ions produced by the absorbed radiation per 100eV. The radical formation efficiency was determined by double integration of the first derivative spectra of strong pitch (DPPH) and compared with those of irradiated rods. The absolute spin concentration was estimated by using the following equation [15].

$$n = \frac{A_{sample} \times n_{strong\ pitch}}{A_{strong\ pitch} \times \text{Dose (Gy)} \times m\ (g)}$$

$= 6.25 \times 10^{13}.G\ (Gy^{-1}g^{-1})$

Where, A_{sample}, $A_{strong\text{-}pitch}$, $n_{strong\text{-}pitch}$ and m are the areas of integrated signals of sample and strong pitch, number of spin in strong pitch and the mass of the sample, respectively. The G value of the whole area was found 0.063 $\pm$ 0.01.

Pre-Irradiation Stability

The pre-irradiation stability of DL-tryptophan rods was investigated by measuring the EPR signal of non-irradiated rods with concentration 20 % during storage period of 60 days. The rods were conditioned at 35 % RH and at room temperature (25 ± 2 ^{o}C) in dark and laboratory light, it was noticed that no detectable EPR signal was produced during the storage period at different storage conditions.

Post-Irradiation Stability

Short Term Decay

Figure 6 shows the obtained decay behavior of radiation induced-paramagnetic centers in DL-tryptophan rod (concentration 20%) at a dose of 35 kGy. The irradiated rod was fixed inside the EPR cavity center and measured for 5 hours. It can be seen the free radicals undergoes random orientation immediately in the first few minutes after irradiation. After that, EPR signal intensity tends to be slightly increased because the radicals attributed to Trp^2 migrates to Trp^1.

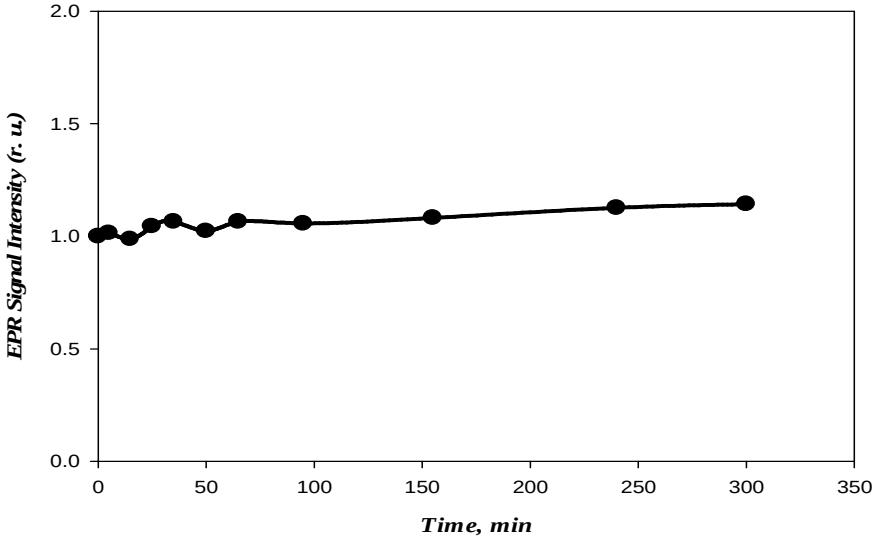

Figure 6: Decay of EPR Line of Irradiated Tryptophan (Conc. 20%) at a Dose of 35 kGy

Long Term Decay

DL-tryptophan rod was irradiated to a dose of 35 kGy and measure the signal intensity at different time intervals of 60 days as shown in Figure 7. The peak intensity values shows slight increase in the first two weeks, then tends to be stable to the end of storage period.

From inset Figure 7 it has been shown that transient species (Trp^2 represented by black line immediately after irradiation and red line represent the same radicals after 1 day from irradiation) generated by gamma ray are transported by diffusion (migrate) to Trp^1 and this leads to grow the peak height of EPR signal to reach maximum after 4 days (green line, notice that the small peaks have disappeared). After about two weeks from irradiation there is an absence of species attributed to Trp^2 and the still stable EPR signal due to Trp^1 (decay percent = 4.89 within the storage period).

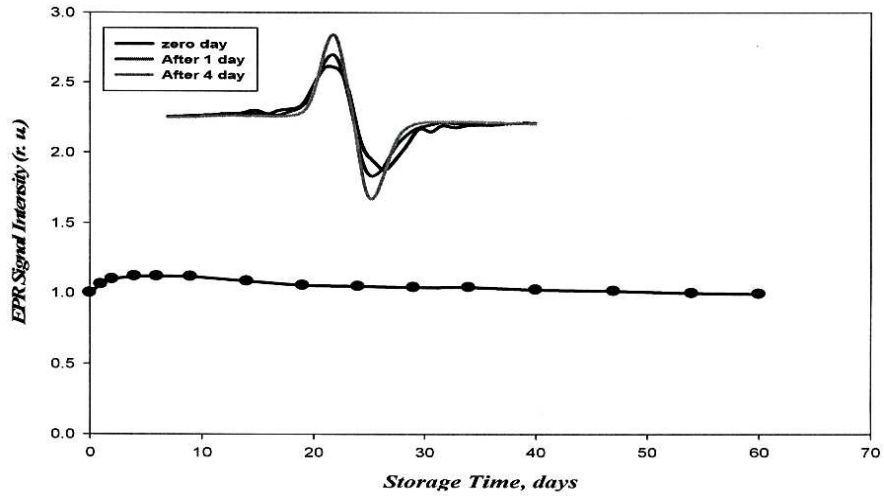

Figure 7: Post Irradiation Stability of Tryptophan Rods Irradiated to 35 kGy

Humidity during Irradiation

The effect of relative humidity during irradiation on the response of DL-tryptophan rods of concentration 20%

was investigated by the irradiation of the rods to a dose of 50 kGy at different humidity values. The rods were stored before irradiation for a 3 days period under the same relative humidity as when irradiated, so that equilibrium moisture content in the rods has been Established during irradiation. Figure 8 shows the variation in EPR signal intensity as a function of relative humidity during irradiation relative to the response value at 33% relative humidity. It can be conclude that DL-tryptophan rods has negligible humidity effect in the range of humidity from 0-100 % not exceeds 5%.

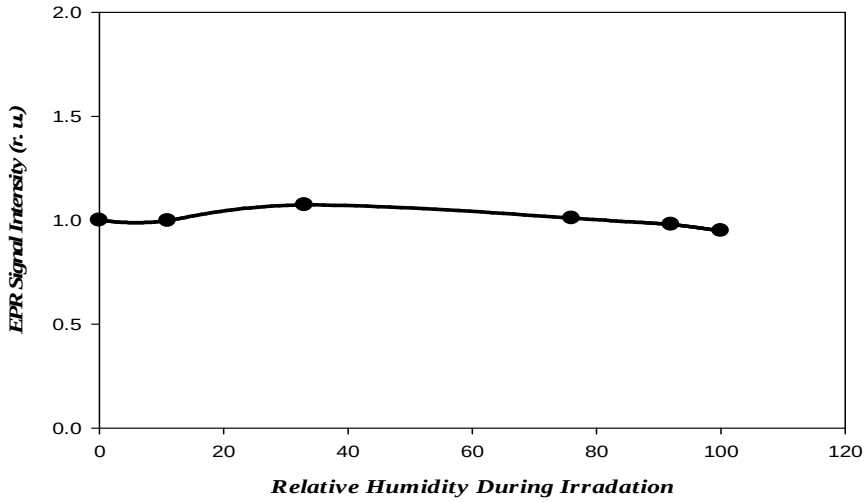

Figure 8: Variation of EPR Response of Tryptophan Rods as a Function of Relative Humidity during Irradiation (Conc. 20%)

CONCLUSIONS

From the data presented in this study, the following conclusions can be drawn

- A new Tryptophan rods have been prepared by a simple technique in the laboratory.
- The prepared rods have significant EPR signal developed upon irradiation and its intensity increases with the increase of absorbed dose and DL-tryptophan concentration. * The useful dose range was found to be 0.5-100 kGy (linear range is 0.5-35 kGy).
- The obtained number of free radicals per 100 eV (G-value) is 0.063 ± 0.01.
- About 5 % decay at room temperature was observed at storage period (60 day).
- The effect of humidity on response of this dosimeter can be neglected during irradiation (not exceeds 5 %)**.**
- The tryptophan rods may be a good candidate for dosimetric applications at the dose range of 0.5-100 kGy.

REFERENCES

1. M. Ikeya, J. Miyajima and S. Okajima. *ESR dosimetry for Atomic Bomb Survivors Using Shell Butons and Tooth Enamel*. J. Appl. Phys. Vol. **23**, p. 679-710, (1984).
2. Caracelli, M. C. Terrile and S. Mascarenhas. *Electron Spin Resonance Dosimetric Properties of Bone.* Health Phys., Vol. **50** (2), p. 259-263, (1986).
3. K. Sato. *Study of an Asymmetric ESR Signal in X-irradiated Human Tooth Enamel.* Calcif. Tissue Int.,

Vol. **29**, p. 95-99, (1979).

4. ISO/ASTM, Standard guide for selecting and calibration of dosimetry systems for radiation processing, ISO/ASTM 51261, *In Annual Book of ASTM standards*, Vol. **12.02**, ASTM International, USA, (2003).
5. W. Gordy, W. B. Ard and H. Shields. *Microwave Spectroscopy of Biological Substances*. *Paramagnetic Resonance in X-irradiated Amino Acids and Proteins*. Proc. Nat. Acad. Sci., Vol. **41**, p. 983-986, (1955).
6. M. S. Galante. *Caracterização de Compostos Quimicospara Dosimetria das Radiaçôesem Processos Industriais*. Dissertation, IPEN/CNEN, São Paulo, (1999).
7. P. N. Keizer, J. R. Noton and K. F. Preson. *Electron Paramagnetic Resonance Radiation Dosimetry: Possible Inorganic Alternatives to the EPR/Alanine Dosimeter*. J. Chem. Soc. Faraday Trans., 87 (19). p. 314-319, (1991).
8. T. Kojima, R. Tanaka and Y. Morita. *Alanine Dosimeters Using Polymer as Binders*. Appl. Radiat. Isot. Vol. **37**, p. 517-520, (1986).
9. R. V. Bensasson, E. j. Land and T. G. Truscott. *Flash Photolysis and Pulse Radiolysis*. Contributions to the Chemistry of Biology and Medicien, Pergamon Press, Oxford, (1983).
10. Z. P. Zagôrski. *Emission Spectra and Decay Kinetics of Pulse Irradiated Polycrystalline Tryptophan*. Radiat. Phys. Chem., Vol. **47**. No. 3, p. 385-388, (1996).
11. E. Sagstuen, H.G. Byrkjeland and T. Henriksen. *An ESR Study of Irradiated L-Tryptophan· HCl Single Crystal at 295 K*. Radiat. Res., **Vol**. 74, No. 1, p. 10-22, (1978).
12. F. Lendzian. *Structure and Interactions of Amino Acid Radicals in Class 1 Ribonucleotide reductase Studied by ENDOR and High-Field EPR Spectroscopy*. Biochimicaet Biophysica Acta. Vol. **170**, p. 67-90, (2005).
13. F. G. Wiertz. O. H. Richter, B. Ludwig and S. Vries. *Tryptophan Radical in Cytochrome Oxidase*. J. Biological Chem., Manuscript M705520200, (2007).
14. Z. P. Zagôrski. *Solid State Radiation Chemistry-Features Important in Basic Research and Applications*. Radiat. Phys. Chem., Vol. **56**, p. 559-565, (1999).
15. G. M. Hassan, U. Ulusoy and M. Ikeya. *Radical Formation in Lithium and magnesium oxalate*.J. Appl. Phys. Vol. **39**, Issue 11, p. 6236, (2000).