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THERMAL ANNEALING KINETICS OF RADICALS FORMED BY THE RADIOLYSIS IN POLYCRYSTALLINE SOLID STATE OF ARGININE

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abstract: The radiolysis with gamma radiation of arginine, in solid polycrystalline state, was performed at room temperature. The EPR spectra, recorded after radiolysis allowed the identification of three paramagnetic centers. A possible mechanism of formation of the three radical species is suggested, based on literature data. It was established a third order kinetics for the thermal annealing process.

key words: arginine; EPR study; thermal annealing; radiolysis mechanism, unpaired electron.

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1. Introduction

Knowing the behaviour of amino acids to the action of ionizing radiation is of great importance because it helps indirectly to the obtaining information on the effects of radiation on proteins in which amino acids are the basic structure. The radicals formed in simple amino acids (glycine, alanine) irradiated with X and γ rays, in solid polycrystalline state have been extensively investigated using the EPR spectroscopy [1-4]. In order to obtain information on the mechanism of radiolytic processes, amino acids were irradiated with X and γ rays at low temperature, where the primary products formed by radiation are sufficiently stable.

Generally, amino acids even those having simple molecules show a complex chemical behaviour to the radiation action, due to the simultaneous trapping of a more free radicals Radiation damage manifests in the disturbance of a long range crystalline or supramolecular order [5] and in chemical modification of studied systems through free radical formation [6] or mass loss [7].

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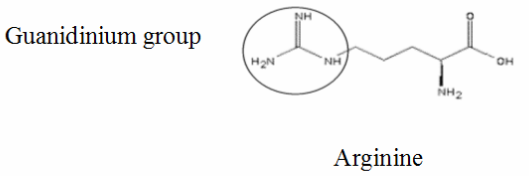
Arginine is an important biochemical nitrogen-containing compound. It plays an important role in nitrogen metabolism. Arginine is involved in the division of cells, in the removal of ammonia from the body (the urea cycle, involving ornithine, as well) in immunity mechanism and in hormone secretion [8].

The amino acid side chain of arginine consists of a 3-carbon aliphatic straight chain, the distal end of which is capped by a complex guanidinium group. The guanidinium group is positively charged in neutral, acidic and even most basic environments, and thus imparts basic chemical properties to arginine. Because of the conjugation between the double bond and the nitrogen lone pairs, the positive charge is delocalized, enabling the formation of multiple H-bonds [9].



Arginine

The analysis of EPR spectra of arginine irradiated in solid state indicated the certain existence of three radicals, stable at room temperature, two radicals which overlap resulted from decarboxylation and of one radical resulted from deamination process. The hyperfine structure of the spectrum results from the interaction of the unpaired electron with a hydrogen atom and with a nitrogen atom, both attached to α carbon and two with β non-equivalent protons [10].

2. Experimental

The polycrystalline samples of arginine provided by Loba-Chemie have been irradiated with gamma rays at room temperature by using a ^{137}Cs source having $23{,}54\cdot10^{12}$ Bq activity and a dose rate of $1{,}05\cdot10^{2}$ Gy/h. The EPR spectra were recorded with an EPR spectrometer ART 5 (IFIN-Magurele) operating in the X band having 100 kHz modulation. Mn^{2+} ion in CaO matrix was used as a standard for determining g factor.

3. Results and discussion

Through irradiation of polycrystalline solid state arginine (Arg) at room temperature, an EPR spectrum in the form of a singlet with a tendency to splitting into several components, is recorded (Fig. 1).

The spectrum presented in Fig. 1 is characterized by a factor g=2,003 and a total width of 5,58 mT. In order to establish the accumulation of radiolytically formed radicals in arginine with integral dose, equal weights of arginine samples were irradiated at different times. In Fig. 2 is presented the variation curve of EPR signal intensity (arbitrary units) versus irradiation time ($1{,}05\cdot10^{2}$ Gy/h, dose rate).

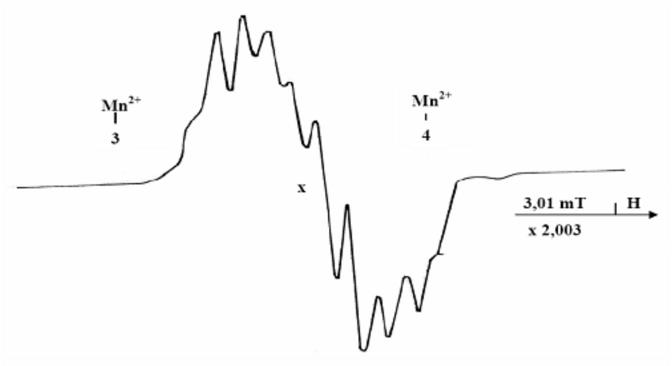


Fig. 1 *EPR spectrum of the arginine sample irradiated with 2·10*^{4} *Gy.*

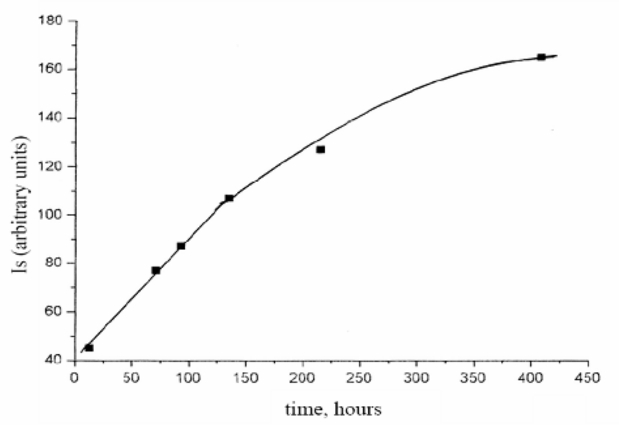


Fig. 2 *Variation of EPR signal intensity (arbitrary units) of a arginine sample, irradiated at room temperature versus irradiation time (dose rate 1,05·10*^{2} *Gy/h).*

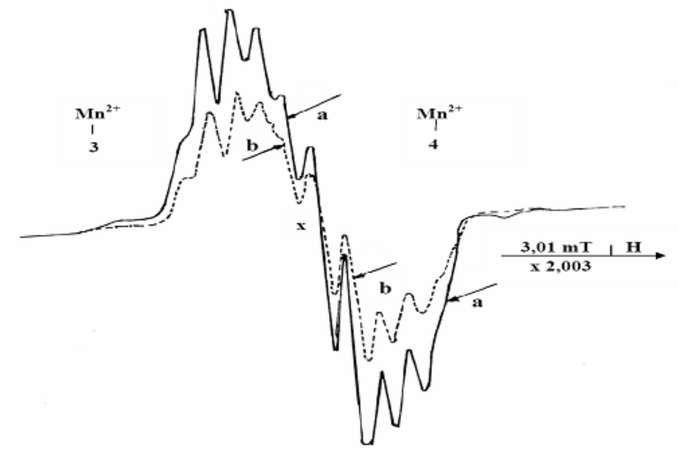


Fig. 3 *EPR spectrum of arginine sample gamma irradiated with a 4·10*^{3}*Gy: a) at room temperature b) same sample heated 5 minutes at 165*^{o}*C.*

From Fig. 2 it can be seen that the signal intensity, the radicals concentration respectively, increases at the beginning linearly with the irradiation dose, then slowly, tending to a plateau for high doses. This behaviour is similar for all amino acids [11] and is an evidence that in addition to the formation process the disappearance one occurs, also produced by radiation. When a constant concentration of radicals is reached, this means that the rates of the two processes, of formation and disappearance, become equal.

In Fig. 3 is presented the EPR spectrum of arginine sample irradiated with a $4\cdot10^3$ Gy dose to be compared with the same sample spectrum after heating for 5 minutes at 165^0C.

From Fig. 3 it can be noticed that after heating the irradiated sample, the spectrum structure remains unchanged. This behaviour was found to all irradiated and heated samples, during the radicals thermal annealing study. The fact that after heating the irradiated samples, no modification of the spectrum structure is found, could be an argument that through irradiation of arginine at room temperature, a single radical species is formed. The amino acid sample being irradiated as a powder, the identification of radicals is difficult because the paramagnetic centers have all possible orientations towards the external magnetic field and that is the EPR spectrum is in the form of a broad singlet. For this reason, the isotropic components of the hyperfine coupling will contribute to the increasing of lines broadening and the anisotropic components can not be distinguished.

The thermal stability of the radicals formed on Arg irradiation required the plotting of reaction isochronous. To this purpose, a gamma irradiated sample ($5\cdot10^4$ Gy, dose) was gradually heated for 5 minutes in stepwise (each step=10^0C), from room temperature up to the temperature of the complete disappearance of radicals. Thus, the irradiated sample has been maintained for the same period at each temperature. After each isothermal heating time, was recorded the EPR spectrum at room temperature. In Fig. 4 is presented the signal intensity versus heating temperature.

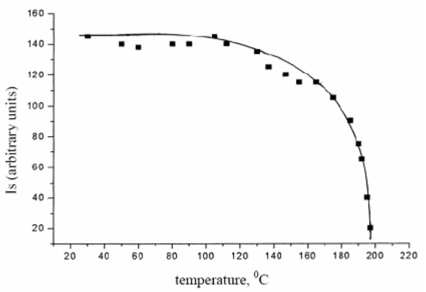


Fig. 4 *Isochronous variation of EPR signal intensity (arbitrary units) versus heating temperature of a $5\cdot10^4$ Gy irradiated Arg sample.*

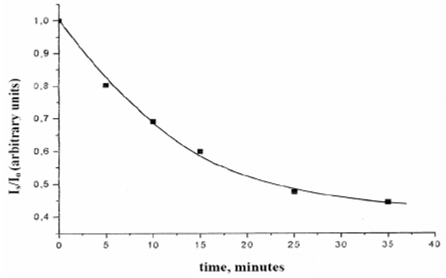


Fig. 5 *Isothermal variation of the EPR signal intensity versus heating time at 185^0C for an irradiated arginine sample.*

Fig. 4 shows that the decrease of the signal intensity until 150°C does not occur. From this temperature to 180°C it can be seen a slow decrease and then a steeper one starts. The complete disappearance of the radicals under the mentioned work conditions occurs at 200°C. The kinetic study of the paramagnetic centers thermal annealing required the plotting in isothermal conditions of the radicals concentration, respectively the peak-peak EPR signal intensity against heating time at different temperatures. In Fig. 5 is plotted the isotherm representing the relative intensity (I_s/I_o) versus heating time at 185^{o}C. I_s is the signal intensity at t moment and I_0 represents the initial intensity. From Fig. 5 it is found that at 185^{o}C the isotherm shows first a strong decrease of the radicals concentration and after that tends to a plateau for longer heating times. This behaviour must be correlated with the radicals stability by proving that at this temperature a certain concentration of radicals still remains and do not disappear because being more strongly trapped in the crystalline network they have high thermal resistance. The thermal annealing process of the radiolitically formed radicals in Arg samples is due to the increase of the radicals species mobilities produced by the influence of the temperature.

In order to determine the reaction order and calculate the rate constants, the integral kinetic equations for all fractional and integers orders were plotted using the graphic method. The reaction order was established from the graphical representation which gave a straight line, the other representations were curves. For calculating the rate constants it was used the dimensionless parameter (I_s/I_0) relative intensity instead concentration. For the temperatures between 155-195^{o}C, linear plots are obtained only when the ratio $1/(I_s/I_0)^2$ is expressed as a function of the time of isothermal heating. This proves that the radicals thermal annealing obeys the kinetics of 3 order. The plot $1/(I_s/I_0)^2$ as a function of the heating time at 185^{o}C is shown in Fig. 6, for exemplification. The values of the rate constants for each heating temperature, were calculated from the slopes of the straight lines (Table 1).

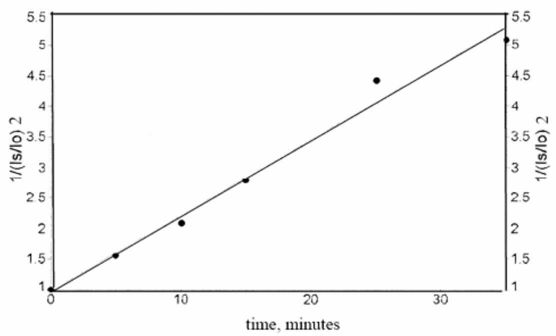


Fig. 6 *The plot of the kinetic equation corresponding to a reaction of third order versus the time for isothermal heating at 185^{0}C for a γ irradiated arginine sample.*

The third order kinetics experimentally found signifies the implication in the thermal annealing process of a three radicalic species whose structure is presented in the radiolysis mechanism.

Table 1 The kinetic parameters values for the thermal annealing of the radicals formed by gamma radiations in arginine.

t^{o}C	T,K	1/T·10^3, K^{-1}	k·10^2, s^{-1}	lgk+2
155	428,15	2,336	1,13	0,053
165	438,15	2,283	2,89	0,460
175	448,15	2,232	6,45	0,809
185	458,15	2,183	12,33	1,090
195	468,15	2,136	22,93	1,360

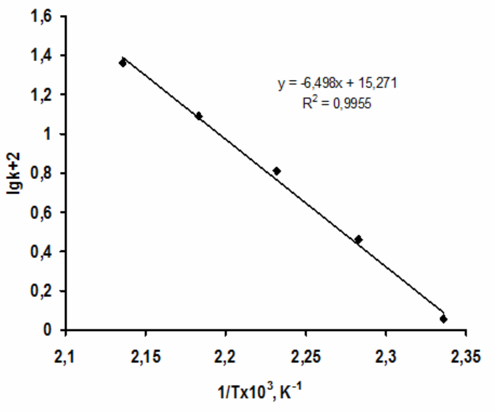


Fig. 7 *The plot of lgk as a function of 1/T.*

An activation energy Ea=124,35 kJ/mol and a pre-exponential factor A=$1{,}8894\cdot10^{13}$ s^{-1} were calculated from the Arrhenius graphic plot (Fig.7)

We mention that these values do not have the same meaning as those from the theory of the gas state collisions. In this case, the reactant entities, the radicals respectively, do not move freely as gas molecules and the recombination process takes place only after the increase of the mobility of these species due to the temperature. Knowing the pre-exponential A factor, the activation entropy $\Delta S_0^* = -19{,}07$ J/mol·K, for the third order, using the formula:

$$\Delta S_0^* = R\left[\ln\frac{Ah}{k_B T} - m\right] \tag{1}$$

where: $h=6{,}625.10^{-34}$J·s, $k_B=1{,}38.10^{-23}$J/K, R=8,31 J/mol·K and m=3 (the reaction order), was calculated.

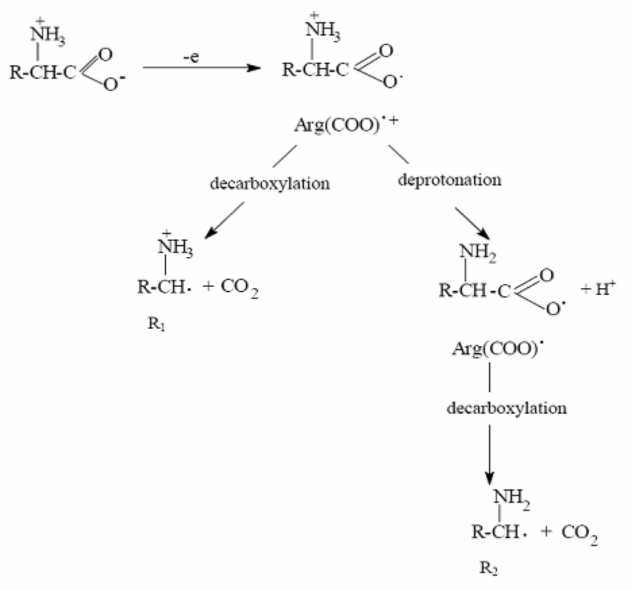
The negative value of the activation entropy means that the activated complex has a more ordered structure than the reactants. The involvement of the three radicals in the reaction is accompanied by loss of the rotational and translational degrees of freedom.

4. The radiolysis mechanism

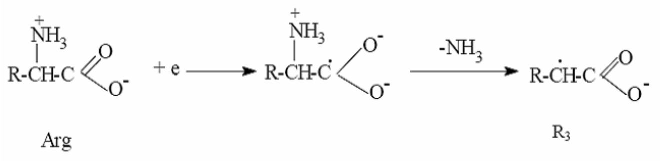
It should be noted that in literature there is no study on paramagnetic centers formed by ionizing radiation in arginine. In order to establish the radicalic species structure formed on Arg irradiation, correlations of the above presented results with those existing in literature, obtained from irradiation of other amino acids, will be made [12].

Arginine, like all amino acids, having an alkaline and acidic group, reciprocally neutralizes intramolecular, resulting a dipolar structure. Direct ionization of arginine molecule by gamma radiation, although one can not determine experimentally than 77 K, should lead to radical-cation $\mathrm{Arg(COO)}^{\bullet+}$ formation, with deprotonated carboxyl group, in which the spin density is located on carboxyl group. The $\mathrm{Arg}(\mathrm{COO})^{\bullet+}$ disappearance could follow two paths:

1. The CO_2 expulsion from $\mathrm{Arg}(\mathrm{COO})^{\bullet+}$ radical, followed by Arg decarboxylated radical formation.
2. The amino group deprotonation produced by the breaking of the shortest hydrogen bond of amino group with neighboring molecules. The result of deprotonation is the unstable radical $\mathrm{Arg}(\mathrm{COO})^{\cdot}$ which decarboxylates forming the radical $\mathrm{Arg}^{\bullet}$, stable at room temperature.



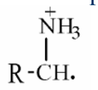
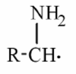
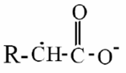
Scheme 1



Scheme 2

Decarboxylation is sustained by highlighting the formation of CO_2 using the gas chromatography method of the irradiated samples of alanine and asparagines [13].The radiolysis mechanism of Arg, in solid state must also include the formation of a radical-anion , reduced species, complementary to the oxidized species. The expulsed electron from the primary process of interaction after the thermalization process is captured by the carboxyl group of a parent molecule to form the radical anion $\mathrm{Arg}(\mathrm{COO})^{\bullet-}$. From the radiolysis products of glicine and alanine, formed by irradiation at 77 K, only the radical anion was identified by EPR [14,15]. Subsequent conversion of Arg radical anion

$\mathrm{Arg(COO)}^{\bullet -}$ occurs at room temperature and consists in its deamination and formation of the R_3 entity, stable at room temperature [16-18].

In conclusion, the EPR spectra recorded at room temperature comes from the superposition of two radicals  $\overset{\overset{+}{N}H_3}{\underset{|}{}}\mathrm{R\text{-}CH}^{\bullet}$ (R_1) and  $\overset{NH_2}{\underset{|}{}}\mathrm{R\text{-}CH}^{\bullet}$ (R_2) resulted from decarboxylation and of one radical  $\mathrm{R\text{-}\dot{C}H\text{-}}\overset{\overset{O}{\|}}{C}\mathrm{\text{-}O^-}$ (R_3) resulted from deamination.

5. Conclusions

The radiolysis at room temperature in polycrystalline solid state of arginine was performed. From the kinetic study was observed that the reaction order of thermal annealing process is 3. The activation energy and entropy were calculated. It was developed a mechanism of the radiolytic formation of the radicals in agreement with the kinetic study. The great reaction order (3) sustains the consideration about the complexity of the mechanism of the thermal disappearance of radicals. It must contain various processes, such as recombination, decomposition and reaction with neighbor molecules.

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