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A DSC STUDY OF γ-AMINOBUTYRIC ACID GAMMA IRRADIATED

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abstract: The behaviour on heating of irradiated with gamma rays and non-irradiated γ-amino-butyric acid (γ-AMB) was investigated by means of differential scanning calorimetry. γ-AMB decomposes before melting.

key words: ionizing radiation; thermal decomposition; γ-amino-butyric acid; DSC.

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Introduction

The most commonly used physical property in order to characterize an organic compound is the melting point. However its applicability to amino acids still remains a problem, since different studies have yielded divergent values of this property, due to their decomposition. Two features of the thermal behavior of amino acids make such quantitative measurement difficult. Firstly a variety of products are generally formed, even in the initial stage, making their identification and quantitative measurement difficult. Secondly, the formation of products is often preceded by an induction period extending over a wide temperature span. Organic crystalline solids undergo decomposition when heated at relatively high temperatures. The decomposition temperatures are of little value and cannot be used to assign the identity or to confirm the structure of an amino acid.

It should be noted too that decomposition of all amino acid is subjected to change with the heating rate and the decomposition temperature is uncertain unless the heating conditions are strictly controlled.

It is difficult for an investigator to determine exactly when decomposition starts, in the case of many amino acids. Consequently it is more reliable to utilize the temperature (t_m) associated with the higher maximum inflection point of the decomposition curve DSC at which the decomposition reaction is occurring most rapidly [1].

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Consequently, the melting points usually found for amino acids are without fundamental significance since they vary according to the technique used by the observer. This is illustrated by t_m values of the γ-amino butyric acid (γ-AMB) found by different authors ranging over a wide interval: 203ºC [2], 193 ºC [3], 196 ºC [4], 200 ºC in the present study.

γ-AMB is not a constituent of proteins but it is found in vegetables (lettuce, potatoes), in fruits (apples) and in cereals (rice, barley, corn). The role played by it in plants is unclear but it seems that it operates as a pH regulator and it stores nitrogen contributing as a protector in the development of plants [5].

The γ-amino-butyric acid's function when delivered in the central nervous system of humans is that of a main neurotransmitter inhibitor [6]. The low level of this acid in brain is associated with many psychic and neurological disturbances such as: anxiety, depression, insomnia and epilepsy [7].

All these states of agitation can be improved by γ-AMB, which produces the relaxation of the organism, acting as an inhibitor. Many efficient anti-anxiety medicines containing this amino acid were synthesized, due to the relationship between the low level of acid and these states [8,9].

Most amino acids change their structure in function of state parameter values and environment. In solid state or in a polar environment they form zwitterions H_3N^+-R-COO^- while in gas phase or in matrix isolated molecules in the neutral form H_2N-R-COOH are more stable. Whereas the solid-state structure is easily accessible by X-ray methods, the investigation of isolated molecules is experimentally extremely difficult, since most amino acids decompose before melting. γ-AMB acid is no exception: crystals of zwitterions H_3N^+-$(CH_2)_3$-COO^- form the solid state.

Ramek and Flock [10] stated by following the crystalline structure of γ-AMB by means of quantum computation that the non-neighbour atoms of the molecules of this amino acid are involved in multiple interactions. Intramolecular hydrogen bonds of the types: N-H·····O=C, N-H·····OH, C-H····O=C, C-H····OH, N-H····H-C are present in this molecule, according to the above-mentioned authors.

No DSC study of both non-irradiated and irradiated amino butyric acid was reported in literature.

Experimental

The γ-AMB used in this research was a Fluka reagent of purity ≥ 99.5%.

Amino acid samples were irradiated with gamma rays from a ^{137}Cs source (Gammator type). The irradiation dose rate was $1.05 \cdot 10^2$ Gy/h while the exposure dose ranged between $7.2 \cdot 10^2$ Gy and $1.3 \cdot 10^4$ Gy. DSC measurements were performed using a DSC Perkin Elmer 1B calorimeter and working in static air. The γ-AMB samples of 3-7 mg were sealed in standard aluminium pans with non-pierced lid. The measurements were performed between 42ºC and 300ºC at the scanning rate of 4ºC min^{-1} and with a sensitivity of 4 mcal/s.

The acquisition of experimental data was performed by means of a multimeter HP 34812A serving as an interface with the computer provided with the Benchlink data acquisition software. The acquired data were transferred to the Origin 5.0 software for graphical processing and calculation of thermal effects.

Results and discussion

The DSC thermogram of non-irradiated γ-AMB recorded with sealed crucibles is shown in Fig. 1.

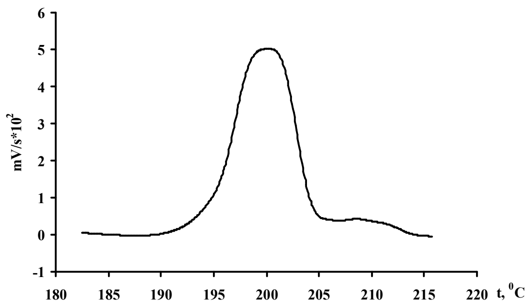


Fig. 1 *DSC thermogram of non-irradiated γ-AMB recorded with sealed pan.*

It is noticed in Fig.1 that the DSC curve of the non-irradiated γ-AMB, contains a single wide peak extending in the temperature range between 187ºC and 214ºC, i.e. on 27ºC.

The DSC curves of three irradiated samples, with doses between $7{,}5\cdot10^2$ and $2{,}5\cdot10^3$ Gy are shown in Fig. 2.

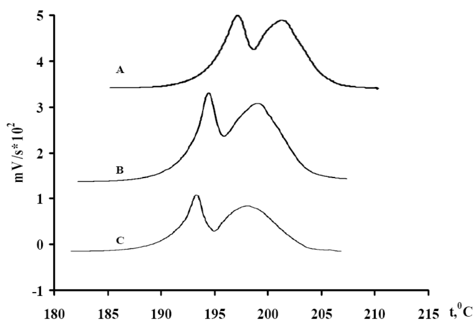


Fig. 2 *The effect of irradiation on the shape of the DSC curves of γ-AMB recorded with sealed pans: $7.5\cdot10^2$ Gy (A), $1.6\cdot10^3$ Gy (B), $2.5\cdot10^3$ Gy (C).*

Unlike the non-irradiated sample (Fig. 1) the DSC curves of irradiates samples of γ-AMB show two peaks, i.e. two endothermic effects in a narrower temperature range (21ºC). It is noticed in Fig. 2 that the maximum of the first peak tends to move toward lower temperatures as the irradiation dose increases.

The DSC thermograms of two samples of γ-AMB irradiated with high doses – $7.6\cdot10^3$ Gy (3B) and $1,3\cdot10^4$ Gy (3C) are shown in Fig. 3. In the case of the sample irradiated with $7.6\cdot10^3$ Gy (3B) the two peaks tend to unite.

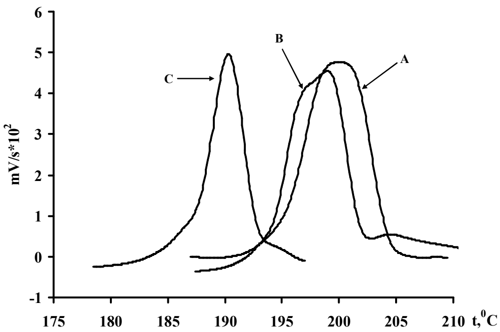


Fig. 3 *DSC thermograms of γ-AMB recorded with sealed pans: non-irradiated (A), irradiated $7.6\cdot10^3$ Gy (B), irradiated $1,3\cdot10^4$ Gy (C).*

On the DSC curve of the sample irradiated with a dose of $1,3\cdot10^4$ Gy (3C) a single symmetrical peak is observed, as in the case of the non-irradiated sample (3A). But the peak of curve 3C is much displaced toward lower temperatures and is narrower (about 15ºC). The onset (t_i), completion (t_f) and peak maximum (t_m) temperatures and the temperature range of the endothermic peaks (Δt) of samples of γ-AMB non-irradiated and irradiated with different doses are gathered in Table 1.

Table 1 *DSC parameters for γ-AMB non-irradiated and irradiated with different doses.*

Dose, 10^3Gy	t_{onset},° C	t_{end}, °C	Δt (t_{onset}-t_{end}), °C	t_{max},°C peak 1	t_{max},°C peak 2	Number of peaks
0	187.3	213.5	26.3	199.7	–	1
0.74	185.3	205.5	20.2	196.0	202.0	
1.6	183.9	206.0	22.1	194.5	200.1	2
2.5	183.1	206.4	23.3	194.0	196.0	
5.0	185.2	207.1	21.9	193.8	198.1	
7.6	186	206.0	20.0	198.3	–	1
13.0	187.5	202.5	14.9	193.7	–	

The data of Table 1 indicate that the decomposition process starts at lower temperatures for samples irradiated with low and moderated doses. The onset temperatures tends to get back to the value for the non-irradiated sample, as the irradiation dose increases.

The mean final temperature (t_{end}) of the DSC peaks of irradiated samples is 206.2 ± 2.4ºC much lower than that of the non-irradiated amino acid sample – 213.5ºC. The temperatures corresponding to the maxima of the two peaks are close; they are separated by 2 - 6ºC. The decomposition enthalpies of γ-AMB both non-irradiated and irradiated with different doses are shown in Table 2.

Table 2 *Total decomposition enthalpies, enthalpies of the first peak process and mass losses of non-irradiated and irradiated γ-AMB.*

Dose, 10^3Gy	ΔH, kJ/mol	ΔH_1, kJ/mol	$100\Delta H_1/\Delta H$ (%)	Mass loss Δm, %	Number of peaks
0	119.4	–	–	98.6	1
0.74	105.7	11.64	11.04	97.8	
1.6	89.5	10.12	11.30	97.4	2
2.5	84.8	13.88	16.36	97.3	
5.0	78.4	9.85	12.56	97.2	
7.6	74.0	–	–	96.5	1
13.0	72.8	–	–	95.8	

The total decomposition enthalpy - ΔH decreases continuously from 119.4 kJ mol^{-1} for the non-irradiated sample to 72.8 kJ mol^{-1} for the sample irradiated with the highest dose ($1{,}3\cdot10^4$ Gy). The profile of decrease of the decomposition enthalpy of γ-AMB with the irradiation dose is shown in Fig. 4.

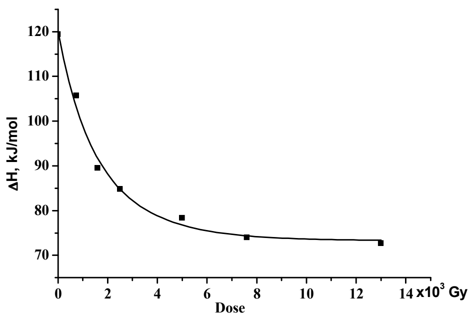


Fig. 4 *Decrease of the decomposition enthalpy of γ-AMB with the irradiation dose*

It may be noticed that the decrease of the enthalpy is important for small doses (lower than $5\cdot10^3$ Gy) while for high doses the enthalpy tends to a constant value.

The decrease of the enthalpy of thermal decomposition of γ-AMB with the increase of the irradiation dose is a behaviour consistent with DSC data obtained on irradiation of other organic compounds used in medicine [11,12].

The values of the enthalpies corresponding to the first peak (ΔH_1) for the four irradiated samples which undergo two endothermic processes (see Table 2) amount 11-14 kJ mol^{-1}, or 11-16 % from the total decomposition enthalpy. These values increase with the dose up to $2.5\text{-}13\cdot10^3$ Gy then they decrease as the two peaks tend to unify.

The following statements about the thermal decomposition of γ-AMB can be made starting from the experimental data presented above:

1. The almost total mass loss (98.6 %, see Table 2) of the non-irradiated sample and the shape of the DSC curve (Fig. 1) show that decomposition proceeds in a single step in this case.

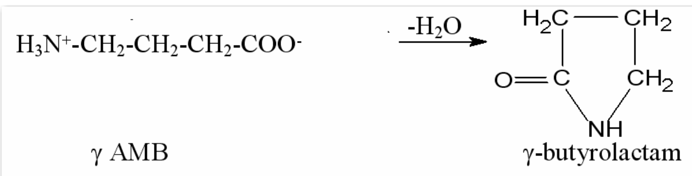
2. All irradiated samples show a mass loss slightly smaller than that of the non-irradiated one. This behaviour is due most probably to the elimination of gaseous inorganic molecules (NH_3, CO_2, CO, H_2O) produced by ionizing radiations. This supposition is sustained by the fact that no significant decrease of the mass loss with the irradiation dose is observed.

3. The endothermic chemical processes corresponding to the two peaks from the thermograms of the samples irradiated with doses between $(0.74\text{-}5)10^3$ Gy are overlapping. This is proved by the close position of the maxima of the two peaks. As the two endothermic processes are not completely separate, individual mass losses could not be determined for each process.

4. The almost total mass loss of both the non-irradiated and irradiated samples represent a strong evidence that no melting is proceeding during the non-isothermal heating of γ-AMB, but decomposition in a certain temperature range.

The decomposition mechanism

It is generally admitted [13] that a ring is closed when γ-amino acids are heated. In the case of γ-AMB a water molecule is eliminated between the two functional groups of the zwitterionic form of this molecule, resulting γ-butyrolactam.



The wide temperature range of the endothermic process includes for sure the reaction shown above.

The information obtained in the present study should be correlated with that obtained previously by means of electronic spin resonance (ESR) [14] in order to explain the different shape of the thermograms of non-irradiated and irradiated amino butyric acid and its dependence on the irradiation dose.

The first peak is due for the greatest part to the formation of γ-butyrolactam. This is sustained by the low values of the thermal effect ΔH_1. The elimination of a water molecule and consequently the ring closure proceeds more easily in the free radicals formed by irradiation than in amino acid molecules. The decrease in the values of the onset temperature in the dose range between $0.74 \cdot 10^3$ and $5 \cdot 10^3$ Gy pleads for that. The second peak registered on the DSC curves of samples irradiated only with doses in this range involves the radiolysis products i.e. it is associated with the disappearance process of free radicals.

The temperature rise to 184ºC produces the increase of the thermal energy of radicals that can be involved in many disappearance pathways compatible with the overall reaction order determined previously (reaction order three) [14]. One pathway is decomposition; another one is the reaction with adjacent parent molecules forming different molecular species, which decompose much more easily than the amino acid molecules.

The previous explanation is sustained by the shape of the DSC thermograms obtained with samples irradiated with large doses $(7.6\text{-}13)10^3$ Gy. It may be noticed in Fig. 3B that the two peaks become closer on the curve corresponding to $7.6\cdot10^3$ Gy while a single peak is observed on that of the sample irradiated with $13\cdot10^3$ (3C). This behaviour is explained by the accumulation of free radicals at high doses, which are in a majority involved in generating more unstable molecular species, which decompose at lower temperatures. The maximum temperature of the peak, when the highest rate of the decomposition process is registered is t_{max}= 193.7ºC for the highest dose, compared to t_{max}= 199.7ºC, for the non-irradiated sample. The DSC thermograms of γ-AMB show both similarities and differences if compared with those of the irradiated α-amino butyric acid investigated previously [15]. While the decrease of the decomposition enthalpy with the irradiation dose takes place in similar proportions for both amino acids, the mass loss is much smaller in the case of α-AMB than in that of γ-AMB, for the same irradiation dose.

Conclusions

The DSC curves of irradiated samples of γ-AMB differ from those of the non-irradiated acid. This behaviour proves that the radiolysis products greatly influence the mechanism of the thermal decomposition process. The shape of the DSC curves of the irradiated samples of γ-AMB depends on the irradiation dose: two peaks are registered at small doses, which unite at large ones in a single peak, situated at lower temperatures.

A significant decrease with the irradiation dose of the decomposition enthalpy of γ-AMB is noticed. The mass lose due to decomposition is almost total for non-irradiated samples as well as for irradiated.

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