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Thermochemical Study of Some e-beam Irradiated Guanidine Derivatives Compounds

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Abstract: *The aim of this study was the investigation of the electron beam (e-beam) irradiated compounds derived from guanidines, implicated in the urea cycle using thermodynamic methods. The irradiation was performed in the range (0- 100) kGy using accelerated electron beam in order to establish if irradiation can alter significantly the characteristics of the compounds. The studied compounds present biochemical importance (creatine monohydrated and anhydrous, creatinine and arginine) and their transformations: dehydration, fusion and decomposition processes arising from heating were revealed from the recorded thermal profiles in the temperature range (25- 500) °C, using a coupled thermogravimetry (TG) and differential scanning calorimetry (DSC). The thermodynamic parameters (dehydration/ fusion/ decomposition temperatures, enthalpies, mass loss and purity) of the investigated compounds were slightly affected after e-beam irradiation with absorbed dose between (0- 100) kGy. In order to identify the characteristic groups, the corresponding chains and their conversion after irradiation, the Fourier transformed infrared (FTIR) method was used. The Mass Spectrometer (MS-MS) technique was employed to evidence the yields of the molecular products of the irradiation.*

Keywords*: irradiation, guanidine compounds, DSC, TG, MS-MS, FTIR*

1. Introduction

The amino acids irradiation is a method of interest due to their importance in dosimetry [1-4] and the performance that amino acids play in biological processes [5-8].

Radiosterilization is a suitable technology applied for a wide spectrum of bioproducts and pharmaceuticals.

The research of radicals resulted after irradiation and chemical amounts in drugs is needed in order to determine the utility of the radiation therapy and to manage it. Numerous studies have been performed in radiobiological research to highlight the relationship between absorbed dose and the corresponding biological effect.

At radiation action, amino acids show a complex chemical behavior, the radiolysis products being highlighted through numerous spectroscopic techniques, at different temperatures (including room temperature and negative ones) [9-10].

The guanidine category designates chemical and physicochemical characteristics of numerous substances of medical concern and guanidine derivatives establish a large important group of therapeutic factors useful for the curing of a broad spectrum of affections [11]. Electron beam (e-beam) irradiation could be an adequate and fast technique to enhance microbiological quality of some food supplements including guanidine derivatives without serious modification in their effective and nutritive fundamentals depending on dose of irradiation [12-13].

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Among the studied guanidino compounds, arginine (Arg) is a polar aliphatic amino acid (an essential amino acid, being part of 20 essential amino acids), involved in the removal of ammonia from the body, hormone secretion and immune modulation [14-15] and known as a growth hormone releaser and as a dietary supplement [16]. Another important function of arginine is that it can be used in the treatment for congestive heart failure, hypertension, coronary heart disease, preeclampsia etc [15]. The other two studied amino acids are biologically considerable constituents also, creatinine and creatine being utilised as substantial indicators for renal function. Creatine is not an essential nutrient and in human body it is produced from L-arginine, glycine and L-methionine in the kidney and liver. It is carried throught blood and taken by muscles. Relatively 95% of the human body's creatine amount is located in skeletal muscle.In humans and animals, approximately half of retained creatine come from food. Creatine monohydrate is a healthful supplement, being a support for athletes, it helps to enhance lean body mass and strenght in healthful humans [17-18].

The dehydration process of monohydrated creatine occurs at about 100°C to form anhydrous creatine. At a temperature over 230°C, the anhydrous creatine eliminates a water molecule and simultaneously performs an intramolecular ciclisation turning in to creatinine [17].

Creatine is a biological relevant substance, its phosphate acting a crucial role in the physiological cycling of life. In solid state, both anhydrous and hydrated forms of creatine present a zwiterionic structure [19-20]. The crystalline structure of creatine monohydrate presents a molecule linked through hydrogen bonds between the guanidine group with the carboxyl group, resulting a supramolecular network through bridges with water molecules [21]. It seems that water molecules play an important role in creating the supramolecular structure.

Creatinine, a substance containing nitrogen, is present in muscle tissue and blood. It is commonly excreted in the urine as a physiological wasting. Creatinine is the decomposition yield of creatine phosphate in muscle and is mostly generated at a quite regular proportion by the body (rely on muscle structure). Creatine is involved in providing the energy needed for our muscles. Creatinine also, serves as a reliable indicator of renal function [22]. Creatinine is the dehydrated form of creatine, its biochemical significance is that it helps to remove creatine from the body through the urine.

Typically, researches on irradiated substances were conducted with the goal to study if radiation can alter one or more characteristics of the substance in an significant manner. For example, irradiation may develop network deficiencies or captured charges which can alter any afterward quantified processing, such as thermic constancy [23].

To the best of our knowledge, in the literature, the effects of e-beam irradiation on the guanidine derivates investigated in this paper have been not studied yet. The goal of the present work was to analyse the e-beam irradiation impact of the mentioned guanidines derivatives on their thermal stability, applying two investigation techniques, particularly DSC and TG. Thus, their thermodynamic parameters such as enthalpies of melting-decomposition, temperature ranges in which transformations occurs and weight losses were obtained. Structural characterisation of the irradiated and non-irradiated studied guanidines was performed by FT-IR technique. The irradiation yields of the investigated compounds were analyzed by the MS-MS technique.

Highlighting the chemical alteration induced by radiations in these guanidines derivatives is important due of their contribution in biological complexes, being used as therapeutic compounds with a broad spectrum of medicinal activity, their irradiation having functional role in areas such as food conservation and pharmacological sector.

2. Materials and methods

2.1. Materials

The compounds studied in the present work, namely: anhydrous creatine [CAS 57-00-1] and creatine monohydrate [CAS 6020-87-7] were provided in powder forms from Fluka and the other two, arginine [CAS 74-79-3] and creatinine [CAS 60-27-5] from Merck. The mass fraction purities are:

>99% (anhydrous creatine), >99% (creatine monohydrate), >99% (arginine) and ≥99% (creatinine). All compounds were characterised without further purification.

2.2. Methods

2.2.1. Electron beam irradiation

A linear electron accelerator (ALID-7) was used to perform irradiations in solid state at room temperature and in the absence of oxygen. The average energy was 6 MeV. The device is installed in the Accelerators Laboratory from INFLPR (National Institute for Lasers, Plasma and Radiation Physics, Măgurele, Romania). Irradiations were carried out at an average beam current of 10 μA, pulse length of 4 μs and pulse repetition rate of 53 Hz at an operating filament voltage of 12 V [24]. The absorbed doses were 15, 25, 50 and 100 kGy and the dose rate of 4 kGy/min [25]. The investigated substances were white fine crystallite powders anterior to irradiation. No alteration of the occurrence of samples was noted after irradiation.

2.2.2. Fourier transforms infrared (FTIR) spectroscopic analysis

The identification and confirmation of the existing functional groups or spectral alterations of compounds in solid state was performed, using the ATR-FTIR spectrophotometer from Thermo Scientific Nicolet IS 10. The FT-IR spectra were recorded in the (3500-525) cm^{-1} wave-lenght range, having a spectral resolution of 4 cm^{-1}at room temperature. For determine the intensities and peak locations it was used the Omnic software (Nicolet Instrumentation Inc., Madison, WI, USA).

2.2.3. Mass Spectrometer method (MS-MS)

Varian 310 – MS LC/MS/MS triple quadrupole mass spectrometer fitted with an electrospray ionization interface (ESI) was used. Air was used as drying gas at a pressure of 19 psi and temperature according to experiment. The nebulizing gas was nitrogen to 40 psi for positive ionization and air to 55 psi for negative ionization. The needle voltage had been established to the potential 5000V for positive ionization and -4500 V for negative ionization. The compound was dissolved in water and was introduced into the interface using a syringe pump Harvard 11PLUS, with a 0.020mL/min flow. Thus, protonated or deprotonated molecular ion acquired was elected by the first quadrupole. Into the second quadrupole, the protonated or deprotonated molecular ion was fragmented by collision with an inert gas (argon) to 1.5 mTorr pressure. Fragments were examined by the third quadrupole. Prior to these determinations it was conducted the tuning of mass spectrometer using PPG (polipropilenglicol) both for positive and negative.

2.2.4. Differential Scanning Calorimetry-Thermogravimetry

A high temperature thermogravimetry (TG) coupled with a differential scanning calorimetry (DSC) were used, in order to determine the thermodynamic parameters of the guanidines compounds, before and after irradiation. The measurements were performed using a TGA/DSC SETSYS Evolution 17 device, between (20- 500)°C, with a heating rate of 10°C/min, in alumina crucibles, using Ar flow. The mass used was about 1.3 mg. All thermal and thermogravimetrical results (enthalpies and weight losses correlated with the fusion and/or decomposition processes, temperature of transformations) were analyzed using Calisto software.

In order to determine the purities of the studied samples, a Perkin Elmer DSC 8500 was utilized. The calibration of the device was performed with indium ($\Delta_{fus}H$ = 28.46 J g^{-1}). The DSC measurements were performed at a scan rate of 10 °C/min. The recorded scans and purity data were processed with Pyris Software.

3. Results and discussions

3.1. Detection of the constituents by FTIR

Infrared spectroscopy is an additional method to clarify the nature and configuration of radiation yields and FT-IR spectra of the compounds before and after irradiation were registered at ambient temperature. Significant discrepancies between the recorded spectra, due to any intermediary generated after irradiation, were not observed. This fact was expressed to be feasible considering the generated intermediary irradiation yields were very negligible. The observed IR comparative spectra of non-irradiated and 100 kGy irradiated sample, at room temperature, are presented in Figure 1.

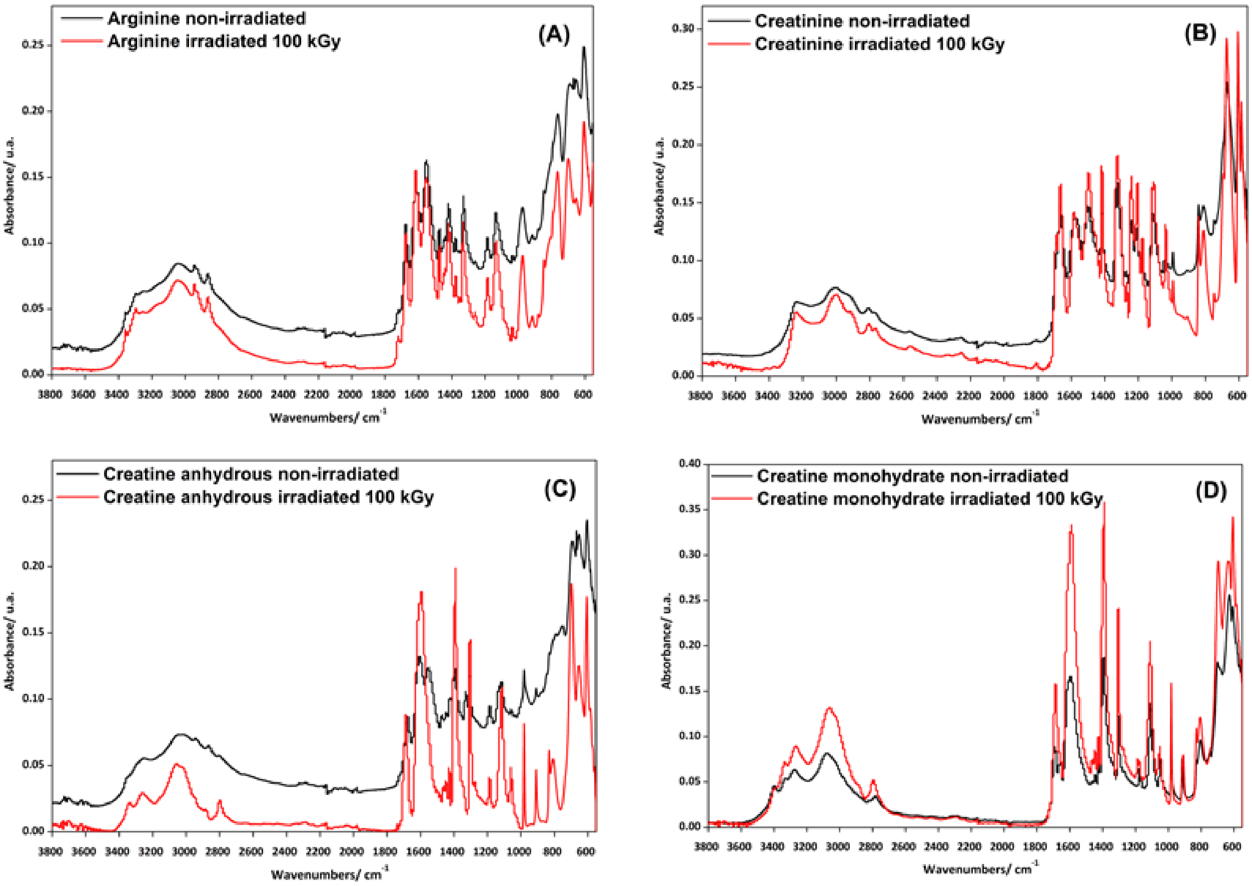

Figure 1. FTIR spectra before and after 100 kGy irradiation of arginine (A), creatinine (B), creatine anhydrous (C) and creatine monohydrated (D)

Insignificant alterations in the FT-IR spectra of samples after irradiation with 100 kGy, by comparation with the spectrum recorded before irradiation of the substance were observed (Figure 1). The modifications were noticed in the magnitude of the registered peaks. The characteristic groups of the studied guanidines were detected by FT-IR spectroscopy study (Table S1-4 in Supplementary Material); according with the FT-IR spectra, the e-beam irradiation with doses between 15-100 kGy does not produce changes in the physico- chemical properties of guanidine derivatives. The analogy between the sampling before and after 100 kGy irradiation demonstrated that their vibrational spectra express perfectly the distinct features of zwiterion.

The spectra were segmented into two areas: (3600-2600) cm^{-1} and (1800-600) cm^{-1} for all the compounds. In the literature [26] it was stated that amino acids present analogies in their infrared spectra in the area of (1587-1333) cm^{-1}. From the FTIR registered spectra, some common characteristics for the compounds before and after irradiation are revealed. For anhydrous and monohydrated creatine, a common characteristic was the presence in the range (3076-3057) cm^{-1} of the bands assigned to the (N-H…O) symmetrical stretching vibration. The consequence of hydrogen chains is really essential in a crystal. It will generated a down shifting of stretching mode of vibrations and up shifting of distorsion modes. The presence of intramolecularly hydrogen chain (N-H…O) stabilizes the crystal configuration, reflected in the shift of bands in the spectra. Another common characteristic is the presence of -NH stretching vibration in the area (3400-3200) cm^{-1}. The bands attributable to ν(C=O) stretching vibration were identified in the area (1719-1660) cm^{-1} for all the studied compounds. The C-N stretching vibration approach occurs highly in the area (1600-1500) cm^{-1} [27]. For the studied compounds, the C-N stretching occurence is complicated to assign since there are difficulties in determining these occurrences from other vibration [28] due to C-N stretching vibrations in the area 1386-1266 cm^{-1} for aromatic amines. Another characteristic for the studied guanidines is the presence of a high absorption band in the area (1600-1500) cm^{-1} attributable to C=N ring stretching vibration [27]. The asymmetric C-N stretching vibration was identified at 1480 cm^{-1}. The NH_2 rocking vibration appears at 1110 cm^{-1}.The symmetric C-N stretching vibration is appeared at 1000 cm^{-1}. The C-C stretching vibrational approach generally arise in the area (1650-1200) cm^{-1}, for the studied compounds, the asymmetric C-C stretching vibration or rocking is at 850 cm^{-1}. The band at 700 cm^{-1} is due to the CH_2 rocking vibration.

The C-H scissoring vibration appears at 1400 cm^{-1}. The band at about 1050 cm^{-1} is due to the C-N stretching vibration in IR spectra. In the lower wavenumber region (700-600) cm^{-1} was identified deformation or wagging for the carboxyl group.

The impact of irradiation is emphasized only by the spectral magnitude of the recorded peaks. The assigned frequencies characteristic of zwiterion groups come out in both before and after irradiation sample spectrum.

3.2 Irradiation products

The MS-MS spectra of the studied compounds (before and after irradiation with doses between (0-100) kGy) were recorded in order to explore the feasibility of radiation sterilization. For exemplification, in Figure 2 is shown the MS-MS spectra of the investigated compounds.

The development of radiation yields in the crystal configuration of amino acids is a result of the irradiation process [29-30]. Those having low molecular weight leave the crystal by diffusion, while other products stay captured in the affected crystal configuration together with the remaining quantity of not damaged amino acid. Applying mass spectrometry method, we have detected some of the irradiation yields and the potential of studied guanidines to preserve their original signature.

The radiolysis products identified in the MS spectra are the result of radicals assembling processes. The radicals formed in the radiolysis process participate at different reactions: interact reciprocal, interact with neutral molecules or re-organize. The products having higher molecular mass than the initial molecule are the results of the interplay with different radical or neutral molecule [31-32]. After arginine irradiation it was detected the ion m/z 115 due to allyl glycine formation and the ion m/z 160. The ion m/z 160 identified in the MS spectrum presents two structures, first one resulted after the depletion of α- amino group of the amino acid and the second one, obtained after eliminating the amine group from the guanidine position. In the case of irradiated and non-irradiated creatinine, the formation of the ion m/z 101 was highlighted because of depletion of a guanidine molecule. The ions m/z 349, m/z 523, m/z 698 are proton bound dimer and were identified in the MS spectra of arginine. The ion having m/z 226 is formed as result of creatinine dimerization.

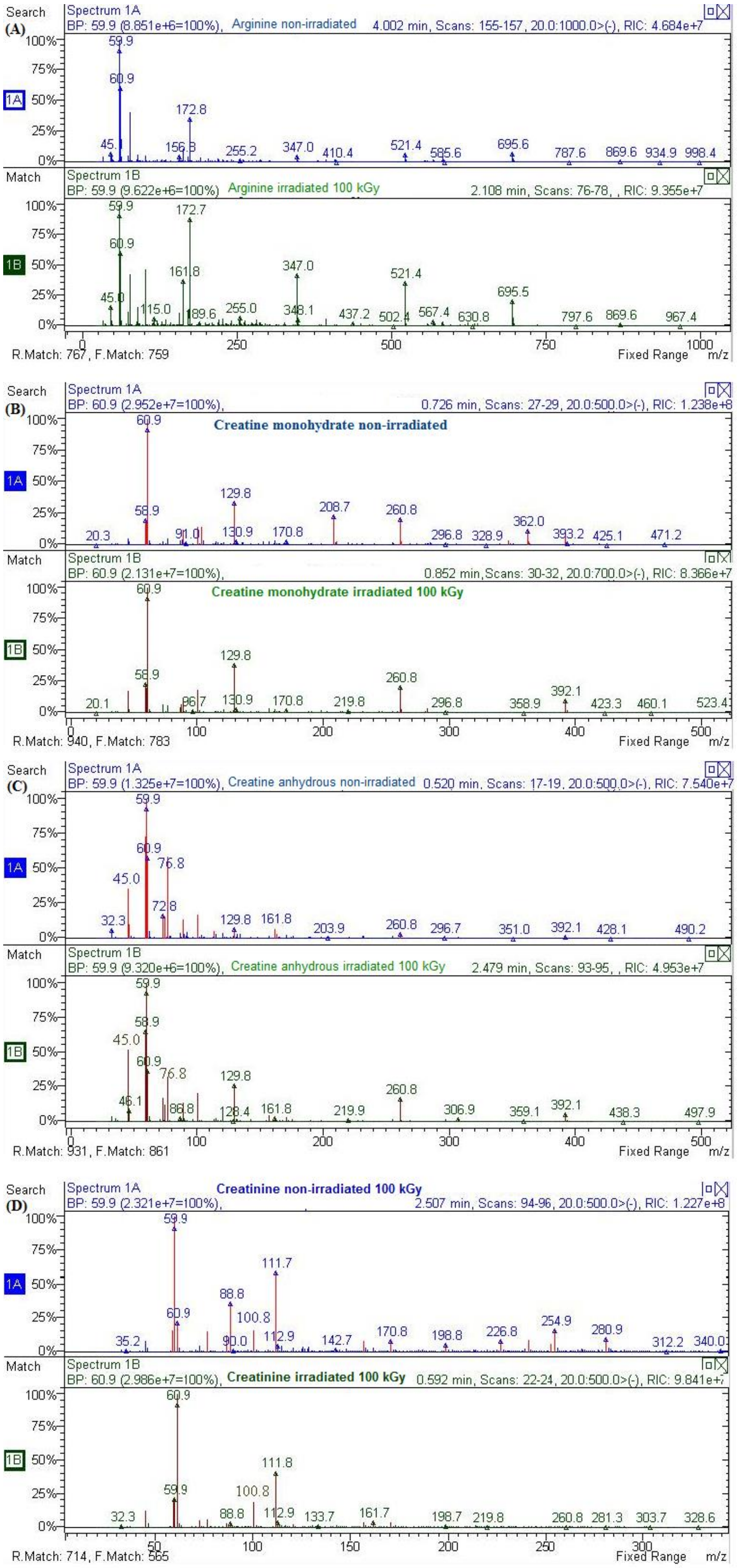

Figure 2. MS-MS full scan spectra of guanidine derivatives non irradiated and 100 kGy irradiated, arginine (A), creatine monohydrated (B), creatine anhydrous (C) and creatinine (D)

The recorded mass spectra of the studied guanidines are very close one to another and from Figure 2 it can be ascertained that there are no significant modification due to irradiation. This indicates that radiation degradation products are not formed in significant concentration to alter the physico-chemical properties of the studied compounds.

Grounding on the mentioned results, it was ended that MS spectroscopy could be employed as a prospective technique in controlling the radiosterilization of guanidines compounds and/or pharmaceutical delivering systems containing guanidine as working ingredient.

3.3. Thermochemical approach of the compounds before and after irradiation

The studied compounds were examined and their DSC curves were recorded before and after irradiation. The TG-DSC plots of the samples before irradiation and after 100 kGy irradiation are presented in Figure 3.

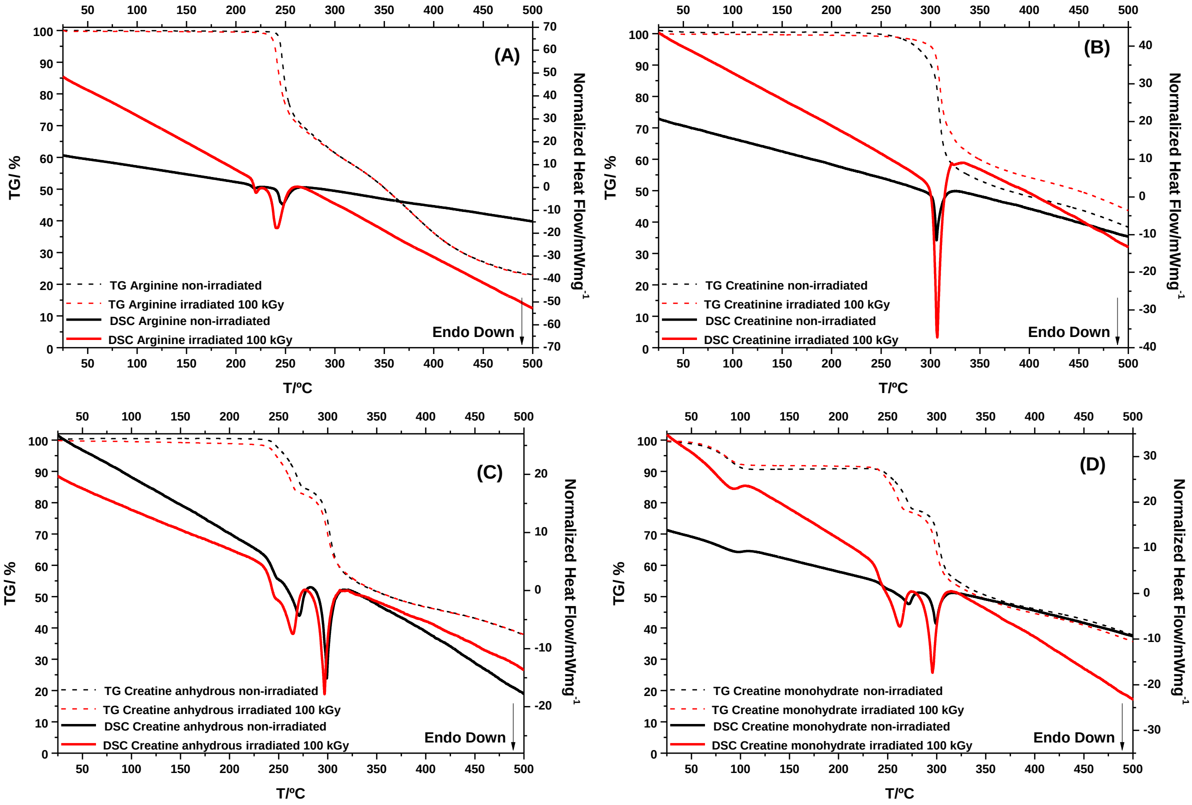

Figure 3. TG-DSC plots for non-irradiated and 100 kGy -irradiated arginine (A), creatinine (B), creatine anhydrous (C) and creatine monohydrated (D)

It is observed from DSC scans that the conformation of endothermic plots of the samples after 100 kGy irradiation is identical to that of the sample before irradiation and regarding of all irradiated samplings the registered peaks are slight moved to decreased temperatures.

Arginine melts and proceeds to decompose at about 215°C (Figure 3 A). The DSC plots reveal two peaks both for the samples before and after irradiation. It was noticed the absence of water molecules in the crystal, thus, no weight loss is seen before 215°C. The second endothermic effect in the DSC plot is seen at 243°C. This endothermic effect corresponds to a large mass loss in the TGA trace. An intense slope of the TGA plot in the field 220–250°C was mentioned by Malik [33] in good agreement with our results, in their work upon arginine dihydrate. This mass loss is comparable with the mass of two ammonia molecules (one originated from the α-carbon atom and the other from the guanidyl group). Decomposition of the primary carbon chain proceeds above 243°C with the formation of carbon dioxide. Kofler and Sitte [34] mentioned different values of the fusion temperature of arginine, achieved by various procedures. Arginine irradiated with a dose of 100 kGy revealed also two peaks, first due to melting at about 217°C and the second due to decomposition at 235°C.

DSC plots of creatine monohydrate (Figure 3D) revealed 3 endothermic peaks, first due to dehydration process starting at about 74°C and confirmed by the TG data. The second peak at 256°C is attributable to the loss of another water molecule of dehydrated creatine (anhydrous creatine) which undergoes an intramolecular cyclization to form creatinine (Figure 3D, 3C). This was illustrated by a challenging survey by Dash et al. [17]. The peaks registered about 300°C for creatine, as well as for creatinine (Figures 3 B, C, D) match to the fusion of the later, followed up by decomposition (a mass loss was observed in this temperature interval, too). The peak proceeds at a decreased temperature regarding creatine monohydrate, due to distinct structure of creatine derived from the dehydration step. An identical behavior was fulfill for other organic substances which creates hydrates [35].

The characteristic temperatures (T_{onset}/ºC and T_{max}/ºC) and enthalpies of each endothermic step were ascertained for both samples before and after irradiation with different doses and are presented in Tables 1-4.

Table 1. DSC results of arginine before and after e-beam irradiation

Dose/kGy	Peak	T_{onset}/ºC	T_{max}/ºC	ΔH/J g^{-1}
0	1	214	219	58.42
	2	242	247	433.11
15	1	217	221	57.21
	2	244	246	432.6
25	1	219	222	56.95
	2	244	246	433.3
50	1	220	223	57.86
	2	234	247	432.7
100	1	217	220	58.01
	2	235	246.5	433.20

Table 2. DSC results of creatine monohydrate before and after e-beam irradiation

Dose/kGy	Peak	T_{onset}/ºC	T_{max}/ºC	ΔH/J g^{-1}
0	1	74	93	157.19
	2	256	271	318.09
	3	295	300	323.13
15	1	75	93.8	156.9
	2	256.5	270.5	319.1
	3	295.2	299.5	321.5
25	1	74	94.1	157.3
	2	257.1	269.8	318.6
	3	294.8	298.9	320.86
50	1	73.8	94.8	157.8
	2	265.9	270.8	317.92
	3	296	298	323.4
100	1	64	89	164.55
	2	245	262	357.34
	3	290	296	315.63

Table 3. DSC results of anhydrous creatine before and after e-beam irradiation

Dose/kGy	**Peak**	***T*** T_{onset}/°C	T_{max}/°C	Δ*H*/J g^{-1}
0	1	256	248 271	357.37
	2	295	299	322.09
15	1	255.6	248.7	255.6
	2	297	299.3	321.57
25	1	256.3	249.1	357.82
	2	294	298.7	322.09
50	1	255.1	248.1	358.2
	2	293.8	297.3	321.9
100	1	238	248 264	346.33
	2	291	297	316.96

Table 4. DSC results of creatinine before and after e-beam irradiation

Dose/kGy	**Peak**	T_{onset}/°C	T_{max}/°C	Δ*H*/J g^{-1}
0	1	303	306	424.06
15	1	304	307.3	334.5
25	1	302	306.8	424.8
50	1	304.17	307.5	348.6
100	1	303	307	439.1

From DSC results (Tables 1-4) that irradiation does not significantly affect melting points and heat of fusion of studied guanidines and indicates its radiation stability in the (15-100) kGy range.To achieve additionaly details about the impact of ionising radiations on thermal comportment of studied guanidines, the weight losses of the samples before and after irradiation were evaluated using thermogravimetry technique. The obtained data are shown in Table 5. In the range (0-100) kGy, the weight loss is not affected by radiation and it is almost the same. For example, the non-irradiated arginine has a weight loss of about 77.25% and the 100 kGy irradiated sample 77.39%. The same tendency was obtained for anhydrous creatine, 62.92% (non-irradiated) and 62.33% (100 kGy irradiated) and monohydrated creatine 62.16% (non-irradiated) and 64.49% (100 kGy irradiated sample).

Table 5. The total weight loss (500 °C) of guanidine derivatives before and after irradiation acquired by TG technique and the calculated purity (%) from Pyris software

Compound /Doses , kGy	**Arginine**		**Creatine monohydrate**		**Creatine anhydrous**		**Creatinine**	
	Δm/%	purity/%	Δm/%	purity/%	Δm/%	purity/%	Δm/%	purity/%
0	-77.25	99.98	-62.16	99.88	-62.93	99.83	-63.06	98.54
15	-76.8	99.79	-62.1	99.04	-62.55	99.97	-62.81	98.54
25	-77.06	99.26	-61.9	98.74	-62.76	99.92	-63.2	99.53
50	-76.71	98.99	-62.7	98.95	-61.95	99.89	-63.7	98.60
100	-77.39	99.34	-64.49	98.87	-62.33	99.95	-56.76	98.79

The purity measured by DSC method is coupled to the fusion enthalpy data which is linked to the consistent energy of the molecular crystal. This method is suitable for calculate the total quantity of impurities including the unknown types [36]. From the fusion process one can estimate the fusion point of a pure compound and confront with that of a substance to get the extent of depression of the fusion point.

The principle of purity calculation by DSC is premised on the circumstance that impurities reduces the fusion point of a complex in an expected manner as defined using the van’t Hoff equation (1) [37].

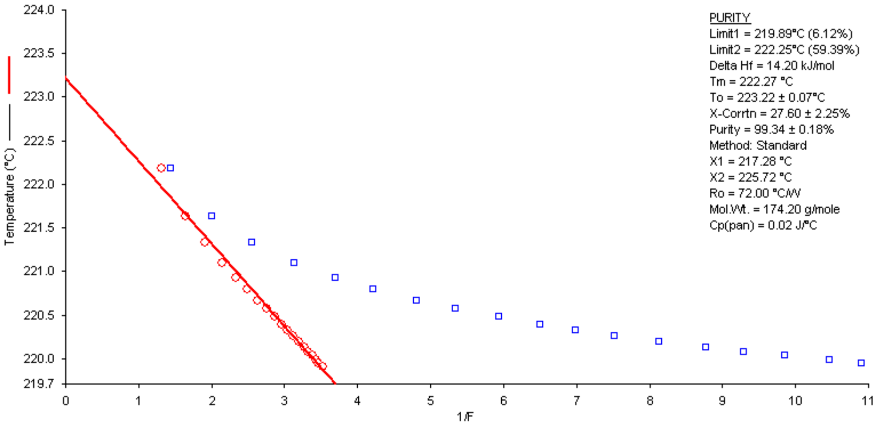

Figure 4. Estimation of purity using Van't Hoff equation for arginine after 100 kGy irradiation

In Figure 4 is illustrated the purity applying Pyris soft for arginine after100 kGy irradiation. The plot T vs $1/F_S$, shows how the results fit the Van't Hoff equation (1). The black circles represent the corrected points and the line drawn, how the results fit the Van't Hoff equation (1). The uncorrected values are shown as black boxes, for $1/F_S$ (fraction fusioned) at recorded temperatures.

$$1/F_S = [\Delta H/R] \cdot [T_0 - T_S]/T_0^2 \cdot [1/X_2] \qquad (1)$$

T_S- sample temperature and the fusion temperature, K; T_0- the fusion temperature of the pure substance (156.59°C, indium, purity> 99.999%); ΔH- heat of fusion for indium, (28.57 J/g), [38]; X_2- mole ratio of impurity in the substance; R- constant (8.314 $Jmol^{-1}K^{-1}$); F_S- ratio of the substance fusion at temperature T_S.

$$F_S = A_S / A_t \qquad (2)$$

A_S- area of the fusion endotherm up to T_S; A_t- total area of fusion endotherm.

The obtained data are presented in Table 5. It can be ascertained that the purity of the studied guanidines are not affected by the irradiation process in the range 15-100 kGy. The mass loss data are in accordance with the purity data of the samples. In the literature, it was reported that through irradiation, at higher doses >100 kGy, the content of the pure sample decreases due to the radiolytic products formation, thus, the mass loss and purity decrease [39]. At increased doses of 3.2 MGy, Cataldo et al. [39] evidenced a decrease in the fusion enthalpy of the irradiated substance attributable to the fragmentary decomposition of the amino acids into further yields and thus a reduction of purity. Regarding guanidines irradiation with doses between (15-100) kGy, the amount of the radiolytic products are not high enough to damage the crystalline structure and to produce the decrease of the determined thermochemical parameters.

4. Conclusions

The thermochemical investigations performed on arginine, creatine monohydrated, creatine anhydrous and creatinine revealed that the studied guanidines derivatives showed two endothermic effects for arginine, creatine monohydrated and creatine anhydrous and only one endothermic for creatinine, attributed to the dehydration (creatine monohydrated), intramolecular cyclization (anhydrous creatine), followed by melting/decomposition processes.

FTIR spectra revealed a higher intensities of the bands ascribed to the irradiated samples.

The nominated frequencies were attributed to the zwitterion grouping which occur in both the samples spectra before and after irradiation, the broader differentiation being established for COO^- and can be attributed to incomplete decarboxylation generated by irradiation. The e-beam irradiation induced an insignificantly modification of the thermic behavior of the investigated compounds. The negligible displacement of the peak location can be assigned to few potential formations of short-live radiolytic products.

The weight loss of studied guanidines is due to the CO_2 and NH_3 removal, except monohydrated and anhydrous creatine which adding loss water, an identical proceeding to the result of irradiation. However, in the case of irradiation, besides decarboxylation and deamination products, the radiolytic species are present in small quantities.

The studied guanidines can be considered a category of substances resistive to the action of ionising radiation. No changes of their thermal behavior were observed after irradiation with a dose up to 100 kGy which indicates that this guanidine derivates can be exposed to radiation sterilization in solid state. The thermal study indicate that the result of e-beam irradiation was conditioned by their complex chemical composition, these nitrogen – containing biochemical compounds presenting a common feature, namely the guanidine moiety, being one of the most hydrophilic functional groups known.

Supplementary material

Table S1. IR band absorbtion frequencies: our experimental data for arginine samples before and after 100 kGy irradiation, over a range of 600-3400 cm^{-1} compared with frequencies from literature

Band position (cm^{-1})			
Arginine non-irradiated	**Arginine irradiated 100 kGy**	**Assignments***	**References**
3357	3358	$\nu_s(NH_2)$	[40]
	3296	$\nu(NH)$	[41-42]
3045	3042	$\nu_s(NH_3^+)$	[30,43]
2943	2943	$\nu_a(CH_2)$	[41-42]
2862	2862	$\nu_a(CH_2)$	[41]
1719	1721	$\nu(C=O)$	[43-44]
1674	1673	$\nu_{as}(CN_3H_5^+)$	[44-45]
1633		$\delta_a(NH_3^+)$ $\nu_s(CN_3H_5^+)$	[43-44] [44]
1612	1614	$\nu_a(COO^-)$	[46-47] [44]
1551	1552	$\delta(NH_2)$ $\delta(NH_3^+)$	[41] [40]
1473	1473	$\delta_a(CH_2)$	[41,48] [42]
1419	1418	$\delta_s(CH_2)$	[44,48]
1376	1375	$\omega(CH_2)$ $\nu_s(COO^-)$	[41] [40]
1331	1331	$\omega(CH_2)$ $\delta(CH)$	[41] [40]
1184	1184	$\rho(NH_3^+)$	[41] [40]
1134	1130	ν (CN)	[40]
974	974	$\delta(NH_3)$	[43,49]
915	915	$\rho(CH_2)$	[41,47]
845	845	$\rho(COO^-)$	[40]
764	764	$\gamma(NH_2)$	[41]
690	**700**	$\gamma(NH_2)$	[40-41]
668	667	$\delta(COO^-)$	[41-42]
657		$\delta(COO^-)$	[41-42]
607	606	$\omega(COO^-)$	[41]

* ν_s- symmetric stretching, ν_a- asymmetric stretching, δ- distortion, δ_a- asymmetric distortion, ρ- rocking, ω- waving, τ- twisting, γ- off surface folding.

Table S2. IR band absorbtion frequencies: our experimental data for creatine anhydrous samples before and after 100 kGy irradiation, over a range of 600-3400 cm^{-1} compared with frequencies from literature

Band position (cm^{-1})			
Creatine anhydrous non-irradiated	**Creatine anhydrous irradiated 100 kGy**	**Assignments***	**References**
	3337	ν(NH)	[50]
3252	3261	ν(N-H...O)	[51]
	3057	ν_s(N-H…O) and (O-H…O)	[52]
2945		$\nu_a(CH_2)$	[27]
	2883	ν_a(CH)	[27]
2863		ν_a(CH)	[27]
	2798	ν(CH)	[27]
	1685	$\nu_{as}(C=N_3)$	[52]
1677		$\nu_{as}(C=N_3)$ ν(C=O)	[52] [27]
1604		$\delta_{as}(NH_3^+)$ $\delta_{as}(NH_2)$	[27] [51]
	1595	$\nu_{as}(COO^-)$	[52,27]
1552		δ(NH)	[27]
	1483 **1461**	ν_{as}(C-N)+$\rho(CH_2)$	[22]
1473		δ(CH)	[27]
1448	1447	$\delta(CH_2)$ scissoring	[53]
1420	1426	ν(CN)	[22,53]
1392	1391	$\nu_s(COO^-)$	[50-52]
1329		$\nu_s(COO^-)$	[27]
1304	1304	$\delta(CH_2)$	[53]
	1274	$\delta(CH_3)$	[53]
1185 1129	1186	$\rho(NH_2)$	[22]
1114	1113	ν(CO)	[22]
1059	1059	ν_{as}(CN)	[51-52]
981	980	ν_s(CN)	[52]
909	909	ν_s(CCN)	[51]
	831	$\nu_s(CN^+)$	[53]
	808	$\rho(NH_2^+)$	[27]
792		$\rho(NH_2^+)$	[27]
750		γ(CH)	[27] [54]
692	697	δ(NH)	[27]
668		$\delta(COO^-)$	[45]
651	653	$\gamma(COO^-)$	[27]
604	606	$\delta(CCO^-)$	[51]

*The symbols have the same meaning as in Table S1.

Table S3. IR band absorbtion frequencies: our experimental data for creatine monohydrated samples before and after 100 kGy irradiation, over a range of 600-3400 cm^{-1} compared with frequencies from literature

Band position (cm^{-1})			
Creatine monohydrate non-irradiated	**Creatine monohydrate irradiated 100 kGy**	**Assignments***	**References**
3400		ν(OH)	[55]
3331	3334	ν(NH)	[27]
	3266	ν(OH)	[27]
3076	**3062**	ν_s(N-H…O) and (O-H…O)	[52]
	2797	ν(COOH)	[27]
2786		$\nu(CH_3)$	[56]

2285	2288	combination bands $\delta(NH_3^+)+\tau(NH_3^+)$	[27]
1688	1685	$\nu_{as}(C=N_3)$	[52]
1660		$\nu(CO)$	[57]
1593	1590	$\nu_{as}(COO^-)$	[52]
1463	1463	$\delta(CH_2)$	[51,27]
1447	1447	$\delta(OH)$	[27]
1422	1424	$\nu_{as}(C\text{-}N)+\delta(CH_2)$	[22]
1394	1392	$\nu_s(COO^-)$	[52]
1306	1304	$\delta(NH)$	[27]
	1186	$\gamma(CH)$	[27]
1173	1174	$\rho(NH_2)$	[22]
1109	1110	$\gamma(CO)$	[57]
1049	1050	$\nu_{as}(CN_3)$	[52]
982	981	$\nu_s(CN_3)$	[52]
915	**910**	γ (CH)	[27]
829	830	$\nu_{as}(CC)$	[22]
804	806	$\tau(CN)$	[22]
747	746	$\gamma(CH)$	[27]
700	697	$\rho(NH_2^+)$	[27]
629	633	ω (COO^-)	[22]
606	603	$\gamma(C=O)$	[22,55]

*The symbols have the same meaning as in Table S1.

Table S4. IR band absorbtion frequencies: our experimental data for creatinine samples before and after 100 kGy irradiation, over a range of 600-3400 cm^{-1} compared with frequencies from literature

Band position (cm^{-1})			
Creatinine non-irradiated	**Creatinine irradiated 100 kGy**	**Assignments***	**References**
	3374	$\nu(NH)$ aromatic	[58-59]
3238	3237	$\nu_s(NH)$	[60]
3003	2999	$\nu_{as}(CH)$	[59-60]
	1689	$\nu_{as}(C=N_3)$ $\delta(HCH)$ $\delta(NH_2)$ $\nu(C=O)$	[57] [60] [22] [21]
1664	1664	$\nu(CO)$ $\delta(HCH)$	[57] [60]
1580	1581	$\delta(HNH)$	[21]
1497	1496	$\nu_{as}(C\text{-}N)+\delta(CH_2)$	[22]
	1434	$\nu(C\text{-}N)$	[59-60]
1417	1416	$\nu_{as}(C\text{-}N)+\delta(CH_2)$ $\delta(CH_2)$	[22] [60]
1326	1324	$\delta(NCH)$	[60]
1269	1268	$\delta(CNC)$	[60]
1205	1205	$\delta(NCH)$	[60]
1176	1175	$\nu(C\text{-}N)$ $\delta(CO)$	[60]
1116	1116	$\omega(CN)$ $\delta(CN)$	[60] [57]
1037	1036	$\nu_s(C\text{-}N)+\delta(CH_3)$	[22]
993	992	$\rho(CH_2)$	[27]
840	841	$\rho(CC)$ $\rho(CH_2)$	[22] [60]
812	812	$\nu_{as}(C\text{-}C)$ $\omega(NH_3^+)$	[22] [60] [58]
747	747	$\gamma(CH)$	[59]
675	674	$\delta(COO^-)$	[45]

		δ(NCO), δ(CNC), δ(CCN)	[60]
607	606	ω(N-C), δ(HCH)	[60]

*The symbols have the same meaning as in Table S1.

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