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Characterization of bis-carboxyethyl germanium sesquioxide and its complexes with amino acids using electrospray QqTOF mass spectrometry

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ABSTRACT

This work deals with the application of electrospray ionization mass spectrometry (ESI-MS) with QqTOF analyzer for the characterization of Ge-132 complexes with different amino acids in aqueous solution with the emphasis on the determination of elemental composition. ESI mass spectra provide complementary structural information in both polarity modes. Some reaction products were suggested based on the interpretation of high resolution mass spectra. Moreover, the experimental isotopic distributions of ions were compared with theoretical calculated isotopic clusters. The superposition of many ion overlays was observed due to the wide isotopic distributions of studied polyisotopic complexes. The high resolution QqTOF analyzer enabled the discrimination of these ion signals differing at least by 0.12 mass units. The occurrence of overlaid signals from ions with smaller mass difference was successfully recognized based on the shift of isotopic distribution, and their elemental composition was verified using mass accuracies of non-overlaid isotopes at the borders of the isotopic cluster. Mass spectra obtained with ion trap and single quadrupole analyzers support QqTOF data.

1. Introduction

Organometallic compounds are molecules consisting of an organic molecule with one or more metal elements incorporated into their structures. As the properties of these compounds become apparent, their applications continue to grow to include reagents, catalysts, the production of dyes, pharmaceuticals, agrochemicals and chemotherapy drugs [1,2]. Early studies even reveal an anticancer activity of some organometallics. However, many of them are usually perceived as being too toxic and their applications in medicine are accepted only in fields, where there is no viable therapy, such as organotin and organoantimony compounds [3–6]. In contrast to these, organogermanium compounds exhibit a relatively low toxicity. Many works report their medical applications [7–10]. The main nutritional supplement form of germanium $O((Ge)CH_2CH_2COOH)_2$ is known as germanium-132 (Figure S1, see Supplementary Information section), Ge-132 or bis-carboxyethyl germanium sesquioxide [11]. Clinical experiments have suggested its antitumor activity and suitability for other medical applications [11,12]. Therefore, germanium sesquioxide is used as a food supplement in various countries, sometimes even without the official registration. The claimed beneficial effects are, however, offset by the toxicity of inorganic germanium oxide which may be present as trace contamination. This has been reported to have lead to the death of some humans who consumed high amounts of organic germanium supplements contaminated with the nephrotoxic germanium dioxide, and died from renal failure [11,13]. Due to this fact, chromatographic separation methods were reported for the confirmation of the purity of Ge-132 [14,15]. It is noteworthy that Ge-132 is soluble in water but not in organic solvents like acetonitrile and alcoholic solvents. Considering per os consumption of Ge-132, the structural characterization for the explanation of its behavior in the biological system is necessary. Various instrumental techniques can be used for this purpose. One of the most suitable ones is mass spectrometry (MS) which can be coupled to liquid-phase separation techniques for speciation analysis [16]. Another possibility, only applicable for pure compounds, is the use of direct infusion MS, which we have successfully applied for the characterization of organotin and organonickel compounds [17–19]. Only a few papers dealing with MS analysis of Ge-132 have been published so far [20–23].

This work is devoted to the detailed investigation of Ge-132 complexation with six different amino acids (1:1) in aqueous solution using electrospray ionization (ESI) MS. A QqTOF analyzer was used for the study of reaction products with the emphasis on the determination of elemental composition and detailed interpreta-

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tion of mass spectra. Moreover, ion trap and single quadrupole analyzers were used to support these conclusions.

2. Experimental

Amino acids alanine (Ala), leucine (Leu), histidine (His), glutamine (Gln), threonine (Thr) or aspartic acid (Asp) were purchased from Serva (New York, USA). Amino acids were added to the Ge-132 (Sigma–Aldrich, St. Louis, USA) aqueous solution in the 1:1 molar ratio to study the formation of reaction products which were characterized mainly using the hybrid QqTOF analyzer (micrOTOF-Q, Bruker Daltonics, Germany). The concentration of Ge-132 was 0.1 mg/ml for QqTOF measurements and 0.3 mg/ml for ion trap and single quadrupole experiments. QqTOF mass spectra were measured in the mass range m/z 50–700. The instrument was externally calibrated using sodium formate clusters before individual measurements. Individual mixtures were analyzed by direct infusion at a flow rate of 3 μl/min. Interface parameters were set as follows: capillary voltage = −4.5 kV, drying temperature = 200 °C, the flow rate and pressure of nitrogen were 4 l/min and 0.4 bar, respectively. Data were acquired by summation of 50,000 scans with 10 rolling averages to obtain the accurate masses. Experimental conditions of other used mass spectrometers are described below. Ion trap analyzer (Esquire 3000, Bruker Daltonics, Germany) was used for the measurement in the mass range m/z 50–700. Samples were analyzed by direct infusion at the flow rate of 5 μl/min. The ion source temperature was 300 °C, the flow rate and the pressure of nitrogen 4 l/min and 10 psi, respectively. Selected precursor ions were further analyzed by MS/MS analyses under the following conditions: the isolation width m/z = 8, the collision amplitude in the range of 0.8–1.0 V depending on the precursor ion stability. Single quadrupole HP 1100 Series LC/MSD (Agilent Technologies, Waldbronn, Germany) was used for the measurement in the mass range m/z 150–550. Samples were analyzed by the flow injection analysis of 5 μl of sample solution at the flow rate of 0.5 ml/min. The ion source temperature was 315 °C, the flow rate and the pressure of nitrogen 9 l/min and 35 psi, respectively. Three values of fragmentor voltage (60, 80, and 100 V) were used for all measurements.

3. Results and discussion

The full scan mass spectra of pure Ge-132 were previously measured using ion trap analyzer and particular ions having the polymeric character were described [20]. Similar ionic structures were identified in the full scan mass spectra in our measurements, as shown in Fig. 1A and B. When individual amino acids were added to the aqueous solution of Ge-132 in 1:1 molar ratio, the formation of new ions was observed (Fig. 1C–F). This fact was ascribed to the adduct reaction of Ge-132 with amino acids resulting in the ions described in Table 1. The simpler spectrum was observed in the negative-ion mode and all reaction products were explained, as illustrated in Fig. 1E for the Ge-132 mixture with alanine (Table 1). Mass accuracies (1) and relative mean mass accuracies [19] of these ions were calculated and their elemental composition was determined (Table 2).

$$\text{mass accuracy} = 10^6 \frac{m/z_{\text{exp}} - m/z_{\text{theor}}}{m/z_{\text{theor}}} \quad (1)$$

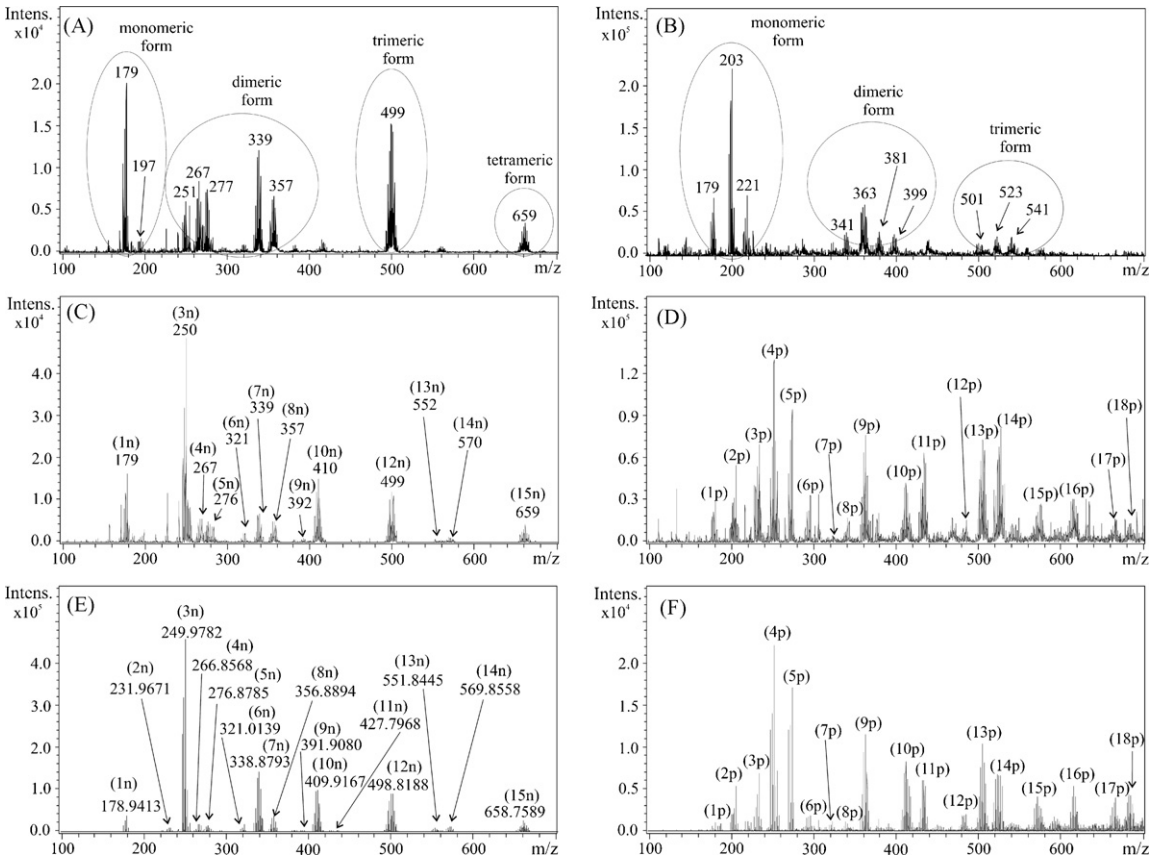

Fig. 1. Ion trap positive-ion (A) and negative-ion (B) full scan mass spectra of Ge-132. Ion trap positive-ion (C) and negative-ion (D) full scan mass spectra of 1:1 aqueous mixture of Ge-132 with alanine. QqTOF positive-ion (E) and negative-ion (F) full scan mass spectra of aqueous mixture of Ge-132 with alanine. The interpretation of individual ions is explained in Tables 1 and 2.

Table 1
Assignment of ions observed in the full scan negative-ion (n) and positive-ion (p) mass spectra of Ge-132 mixture with alanine (1:1) in Fig. 1E and F

Annotation	m/z_{exp}	Ion	Summary formula
(1n)	178.9413	$[M_{mono}-H]^-$	$C_3H_5O_4Ge$
(2n)	231.9671	$[A_1-H]^-$	$C_6H_8O_4NGe$
(3n)	249.9782	$[A_2-H]^-$	$C_6H_{10}NO_5Ge$
(4n)	268.8568	$[M_{di}-H-C_3H_4O_2]^-$	$C_3H_5O_5Ge_2$
(5n)	278.8757	$[M_{di}-H-CH_2O_3]^-$	$C_5H_7O_4Ge_2$
(6n)	321.0139	$[A_3-H]^-$	$C_9H_{15}O_6N_2Ge$
(7n)	340.8778	$[M_{di}-H]^-$	$C_6H_9O_7Ge_2$
(8n)	358.8893	$[M_{di}-H+H_2O]^-$	$C_6H_{11}O_8Ge_2$
(9n)	393.9043	$[A_4-H]^-$	$C_9H_{12}O_7NGe_2$
(10n)	411.9159	$[A_5-H]^-$	$C_9H_{14}O_8NGe_2$
(11n)	430.7968	$[M_{tri}-H-C_3H_4O_2]^-$	$C_6H_9O_8Ge_3$
(12n)	502.8175	$[M_{tri}-H]^-$	$C_9H_{13}O_{10}Ge_3$
(13n)	555.8406	$[A_7-H]^-$	$C_{12}H_{16}O_{10}NGe_3$
(14n)	573.8535	$[A_8-H]^-$	$C_{12}H_{18}O_{11}NGe_3$
(15n)	664.7565	$[M_{tetra}-H]^-$	$C_{12}H_{17}O_{13}Ge_4$
(1p)	180.9548	$[M_{mono}+H]^+$	$C_3H_7O_4Ge$
(2p)	202.9355	$[M_{mono}+Na]^+$	$C_3H_6O_4GeNa$
	205.9870	$[A_2-H_2CO_2+H]^+$	$C_5H_{10}O_3NGe$
(3p)	233.9813	$[A_1+H]^+$	$C_6H_{10}O_4NGe$
(4p)	251.9908	$[A_2+H]^+$	$C_6H_{12}O_5NGe$
	255.9631	$[A_1+Na]^+$	$C_6H_9O_4NGeNa$
(5p)	273.9743	$[A_2+Na]^+$	$C_6H_{11}O_5NGeNa$
(6p)	295.9573	$[A_2+2Na-H]^+$	$C_6H_{10}O_5NGeNa_2$
(7p)	323.0266	$[A_3+H]^+$	$C_9H_{17}O_6N_2Ge$
(8p)	342.8746	$[M_{di}+H]^+$	$C_6H_{11}O_7Ge_2$
(9p)	364.8744	$[M_{di}+Na]^+$	$C_6H_{10}O_7Ge_2Na$
(10p)	413.9186	$[A_5+H]^+$	$C_9H_{16}O_8NGe_2$
	417.9019	$[A_4+Na]^+$	$C_9H_{13}O_7NGe_2Na$
(11p)	435.9105	$[A_5+Na]^+$	$C_9H_{15}O_8NGe_2Na$
(12p)	484.9656	$[A_6+H]^+$	$C_{12}H_{21}O_9N_2Ge_2$
(13p)	508.8005	$[M_{tri}-H_2O+Na]^+$	$C_9H_{12}O_9Ge_3Na$
(14p)	526.9340	$[M_{tri}+Na]^+$	$C_9H_{14}O_{10}Ge_3Na$
	528.9311	$[A_6+2Na-H]^+$	$C_{12}H_{19}O_9N_2Ge_2Na_2$
(15p)	575.8551	$[A_8+H]^+$	$C_{12}H_{20}O_{11}NGe_3$
	579.8416	$[A_7+Na]^+$	$C_{12}H_{17}O_{10}NGe_3Na$
(16p)	619.8286	$[A_8+2Na-H]^+$	$C_{12}H_{18}O_{11}NGe_3Na_2$
(17p)	670.7403	$[M_{tetra}+Na-H_2O]^+$	$C_{12}H_{16}O_{12}Ge_4Na$
(18p)	688.7478	$[M_{tetra}+Na]^+$	$C_{12}H_{18}O_{13}Ge_4Na$

The polyisotopic elements, such as Ge, Sn, Ni, have a characteristic distribution of isotopic peaks which helps in the interpretation of their mass spectra [18,19]. Therefore, the presence and exact number of germanium atoms in individual ions was verified by the comparison of experimental isotopic distributions with the theoretical ones. It is noteworthy that organogermanium ions exhibit a similar isotopic distribution as organotin ions, which can be used for the illustration of the applicability of our approach for differentiation of polyisotopic metal ions with the similar isotopic pattern. The comparison of two isotopic distributions for different organogermanium and organotin ions with comparable masses is shown in Figure S2 and theoretical and experimental relative abundances are compared in Table S1. The monoisotopic mass (M) corresponds to the most abundant peak. Isotopic patterns of both ions are rather similar. The main differences are in the relative abundances of isotopic peaks M−3, M−4 which are higher in case of tin-containing ions. Moreover, the peak M+4 is totally missing for organogermanium ion.

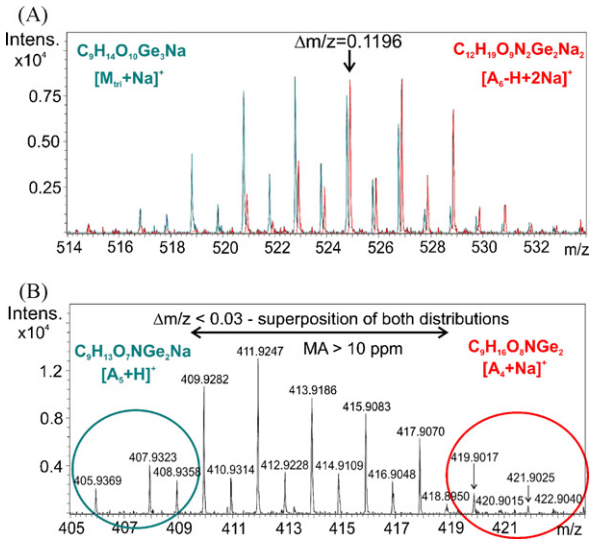

Fig. 2. Details of full scan positive-ion ESI mass spectra of (A) mixture of Ge-132 with alanine in the interval of m/z 514–534 including summary formulas of two sets of ions $[M_{tri}+Na]^+$ and $[A_6-H+2Na]^+$; (B) mixture of Ge-132 with alanine in the interval of m/z 405–424 containing two overlaid signals of $[A_5+H]^+$ and $[A_4+Na]^+$.

The positive-ion full scan mass spectra are rather complex which is caused mainly due to the presence of both sodium ion and proton adducts. Consequently, the ion superposition of polygermanium ions containing wide isotopic distributions is observed in the

Table 2
Mass accuracies (MA) and relative mean mass accuracies (MMA) for ions observed in the negative-ion full scan mass spectra of Ge-132 mixture with different amino acids (values are in ppm)

Ion	m/z_{theor}	Ge-132 + Ala		Ge-132 + Thr		Ge-132 + Leu		Ge-132 + Asp		Ge-132 + His		Ge-132 + Gln	
		MA	MMA	MA	MMA	MA	MMA	MA	MMA	MA	MMA	MA	MMA
$[A_1-H]^-$	217+R	−0.4	−2.7	−0.4	0.8	−4.7	−3.7	2.9	0.4	−3.7	−3.2	−0.3	−1.1
$[A_1-H-72]^-$	145+R	–	–	–	–	–	–	8.8	3.2	−1.8	−2.4	–	–
$[A_2-H]^-$	235+R	1.6	−1.0	0.7	0.2	−0.7	−1.0	−2.2	−0.3	−4.4	−6.9	−0.3	1.2
$[A_3-H]^-$	291+2R	−3.1	−2.7	–	–	2.7	−1.7	–	–	−5.7	−5.3	−0.9	−0.1
$[A_4-H]^-$	379+R	−1.5	−0.4	1.4	1.1	–	–	2.0	−0.7	−3.0	−1.8	−1.1	−1.3
$[A_5-H]^-$	397+R	1.0	0.8	−0.2	−0.2	−1.5	−2.4	−0.9	0.2	−9.4	−5.0	0.2	−0.9
$[A_6-H-H_2O]^-$	435+2R	–	–	0.4	1.4	–	–	−3.4	−1.5	–	–	–	–
$[A_7-H]^-$	541+R	−3.9	−1.4	−0.5	−0.9	–	–	−2.3	−0.4	1.6	0.4	−5.2	1.3
$[A_8-H]^-$	559+R	0.2	0.4	–	–	−7.0	−5.3	–	–	–	–	−5.7	−1.5
$[M_{mono}-H]^-$	179	3.9	8.0	8.9	6.8	1.7	1.8	6.1	8.0	1.7	2.6	3.9	4.8
$[M_{di}-H-GeO]^-$	251	–	–	−5.6	−3.7	−7.6	−4.4	−5.2	−4.5	–	–	2.8	3.3
$[M_{di}-H-CH_2O_3]^-$	269	−7.5	−3.9	7.8	−1.1	−3.9	−6.0	−7.9	1.9	1.1	−2.6	−1.4	−4.7
$[M_{di}-H-C_3H_4O_2]^-$	279	−0.7	−1.3	−1.5	1.3	0.0	−1.1	5.9	−3.5	1.1	0.9	−1.1	4.2
$[M_{di}-H]^-$	341	−1.2	0.1	1.8	1.2	−1.2	−2.5	−1.2	−1.0	−1.5	−2.1	0.9	−0.2
$[M_{di}-H+H_2O]^-$	359	1.4	−1.8	0.6	−0.4	−3.9	−3.5	−1.9	−3.6	–	–	0.0	−1.0
$[M_{tri}-H-C_3H_4O_2]^-$	431	4.4	−0.5	3.7	1.9	−2.1	−2.2	1.9	0.5	–	–	−0.5	2.7
$[M_{tri}-H]^-$	503	2.9	2.3	1.6	1.4	−2.0	−2.1	2.2	0.5	0.8	−1.2	3.8	−1.2
$[M_{tetra}-H]^-$	665	3.7	3.0	3.7	3.3	−1.5	−0.7	1.5	1.5	−3.3	1.6	−1.4	−0.4

R corresponds to the amino acid side chain (15 for Ala, 45 for Thr, 57 for Leu, 59 for Asp, 81 for His, and 72 for Gln).

Table 3
Comparison of theoretical and experimental isotopic distributions of ions observed in Fig. 2A including mass accuracies (MA) in ppm and relative abundances (RA) in %

Isotope	Ion_1-exp. (m/z)	Ion_1-theor. (m/z)	MA (ppm)	RA (%)	Ion_2-exp. (m/z)	Ion_2-theor. (m/z)	MA (ppm)	RA (%)
M−12	514.8106	514.8207	−19.6	6	–	–		
M−11	515.8660	515.8241	81.2	3	–	–		
M−10	516.8095	516.8185	−17.4	15	–	–		
M−9	517.8419	517.8204	41.5	12	–	–		
M−8	518.8128	518.8171	−8.3	50	520.9440	520.9365	14.4	25
M−7	519.8155	519.8185	−5.8	18	521.9318	521.9396	−14.9	8
M−6	520.8136	520.8157	−4.0	91	522.9395	522.9344	9.8	46
M−5	521.8174	521.8171	0.6	38	523.9413	523.9364	9.4	30
M−4	522.8122	522.8145	−4.4	100	524.9313	524.9332	−3.6	99
M−3	523.8128	523.8158	−5.7	44	525.9353	525.9348	1.0	35
M−2	524.8117	524.8134	−3.2	88	526.9340	526.9320	3.8	100
M−1	525.8088	525.8149	−11.6	34	527.9352	527.9336	3.0	37
M	526.8122	526.8126	−0.8	70	528.9311	528.9311	0.0	80
M+1	527.8143	527.815	−1.3	15	529.9356	529.9338	3.4	17
M+2	528.8127	528.8124	0.6	17	530.9281	530.9312	−5.8	18
M+3	529.8158	529.8154	0.8	10	531.9533	531.9342	35.9	6
M+4	530.8247	530.8126	22.8	6	532.9266	532.9319	−9.9	5
M+5	531.8621	531.8160	86.7	6	–	–		
M+6	532.8165	532.8135	5.6	1	–	–		

M corresponds the monoisotopic mass.

spectra which complicates their interpretation. Two examples are shown here for the full scan positive-ion mass spectra of the Ge-132 mixture with alanine. Fig. 2A represents more favorable situation, when the resolution was sufficient for the separation of two different sets of ions observed in the interval of m/z 514–533. The calculation of individual mass accuracy parameters was performed after determining the tentative elemental composition of each recorded ion (Table 3). The mass difference of $\Delta m/z$ 0.12 enables almost the baseline separation of all twin ions with the elemental composition $C_9H_{14}O_{10}Ge_3Na$ and $C_{12}H_{19}O_9Ge_2Na_2$ (Fig. 2A).

However, the second example (Fig. 2B) illustrates the situation, where two ions with the elemental composition $C_9H_{13}Ge_2NO_7Na$ and $C_9H_{16}Ge_2NO_8$ are too close to each other and the resolving power of about 10^4 or slightly more does not enable their separation. Therefore, only one distribution is formed by the superposition of individual isotopic peaks of both ions. A useful indicator of overlaid ion distributions is the non-constant difference of m/z values within one isotopic envelope. In principle, this values should be similar (±5 mDa) for all peaks with unaffected isotopic distribution in contrast to the distribution formed by the superposition of two ions, which is indicated by variable differences between individual isotopic signals. This is the case of our example and therefore the mass accuracies and mean mass accuracies of individual overlaid peaks are higher than 10 ppm in the superposition region. The only possibility of verifying the elemental composition of these ions is the calculation of mass accuracies for non-overlaid isotopes at the borders of isotopic distributions, i.e., at m/z ratios lower than 410 and higher than 419 in this example (Table 4).

Finally, the complementary information obtained from both polarity modes ($[M-H]^-$ vs. $[M+H]^+$ and $[M+Na]^+$) was used for the molecular weight assignment of reaction products and their elemental composition was determined on the basis of their accurate masses and relative isotopic abundances. Subsequently, the proposed reactions leading to the adduct formation were suggested based on the knowledge of elemental composition of starting compounds (Ge-132, amino acid) and the products. This approach is illustrated on the example of Ge-132 mixture with alanine. Summary formulas of products are summarized in Table 5. Considering the isotopic patterns of polygermanium compounds and mainly by accurate masses, the number of germanium atoms was verified in reaction products and the polymeric form of Ge-132 reacting with alanine was established based on this information. The detail

Table 4
Comparison of experimental isotopic distribution shown in Fig. 2B with theoretical distributions of (1) m/z 418 ($C_9H_{13}O_7NGe_2Na$), and (2) m/z 414 ($C_9H_{16}O_8NGe_2$) including the resolving power (RP) for particular isotopic peaks and mass accuracies (MA) in ppm for both ions

Ion_1-theor. (m/z)	Ion_2-theor. (m/z)	Exp. (m/z)	MA (ppm)		Relative abundance (%)			RP
			Ion_1	Ion_2	Theor. 1	Theor. 2	Exp.	
405.9355	–	405.9369	3.4	–	18	–	16	13,848
407.9334	–	407.9323	−2.7	–	47	–	31	11,655
408.9353	–	408.9358	1.2	–	18	–	21	12,792
409.9322	409.9069	409.9282	−9.8	52.0	94	18	82	11,958
410.9336	410.9101	410.9314	−5.4	51.8	27	2	23	11,806
411.9309	411.9048	411.9247	−15.1	48.3	100	47	100	13,012
412.9325	412.9066	412.9228	−23.5	39.2	33	18	27	11,277
413.9300	413.9036	413.9186	−27.5	36.2	75	94	75	8,293
414.9327	414.9050	414.9109	−52.5	14.2	13	27	19	6,744
415.9301	415.9023	415.9083	−52.4	14.4	24	100	64	9,667
416.9332	416.9038	416.9048	−68.1	2.4	3	33	21	14,824
417.9307	417.9014	417.9019	−68.9	1.2	3	75	48	12,107
418.9337	418.9041	418.895	−92.4	−21.7	0.3	13	6	5,899
–	419.9014	419.9017	–	0.7	–	24	13	10,202
–	420.9046	420.9015	–	−7.4	–	3	5	18,523
–	421.902	421.9025	–	1.2	–	3	2	32,274

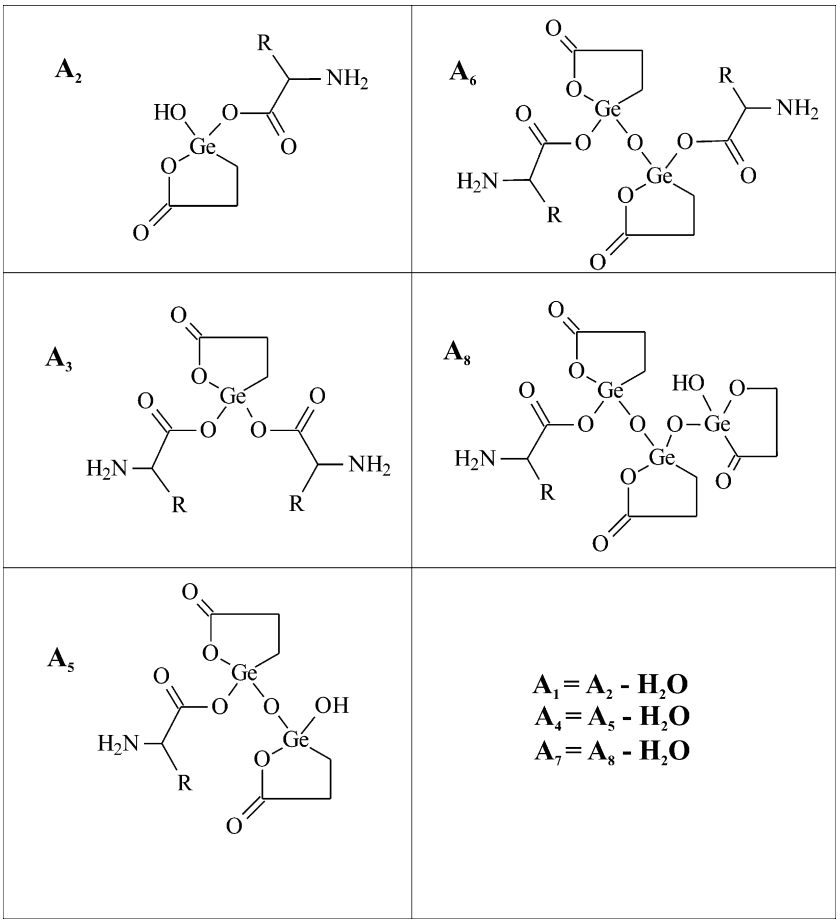

Fig. 3. Proposed structures of product ions formed in aqueous mixtures of Ge-132 with amino acids, where R is CH_3 (Ala), CH_2COOH (Asp), $CH_2CH(CH_3)_2$ (Leu), $CHOHCH_3$ (Thr), $(CH_2)_2CONH_2$ (Gln) or $CH_2C_3H_3N_2$ (His).

explanation can be illustrated on the example of adduct reaction product A_2 (M = 251, $C_6H_{11}GeNO_5$) containing one germanium and one nitrogen atom. The adduct corresponding to this ion could be probably formed by the reaction of sub-unit of Ge-132 with one molecule of alanine and the loss of one H_2O molecule due to the condensation reaction of sub-unit of Ge-132 with alanine, i.e., $C_3H_6GeO_4 + C_3H_7NO_2 - H_2O = C_6H_{11}NO_5Ge$. In general, this simple calculation can be used for the proposal of reaction mechanisms of other adducts, where the number of germanium atoms shows the information about the type of ions. Odd or even number of nitrogen atoms is determined according to the nitrogen rule and then correlated with the amino acid number participating in the reaction (Table S2). The loss of water is occurring for all reactions in agreement with the formation of polymeric structures of Ge-132, for which the occurrence of condensation products has been reported previously [20]. The most probable products of the condensation reaction are shown in Fig. 3. The alternative explanation, i.e., loss of water due to the formation of a peptidic bond, seems to be unlikely under ambient non-enzymatic conditions.The products of Ge-132 mixture with six amino acids (alanine, leucine, glutamine, threonine, histidine or aspartic acid) were also measured using ESI-MS with single quadrupole and ion trap analyzers. Concerning the ion trap analyzer, mass spectra in both polarities were similar to spectra obtained by QqTOF except of lower resolution and mass accuracy (Fig. 1C and D). On the other hand, the signal with single quadrupole analyzer was observed only in the negative-ion ESI mode for all studied complexes and the spectra provided only basic information with ions at *m*/*z* 197 and *m*/*z* 179 corresponding to Ge-132 sub-unit with the absence of any polymeric ions (Figure S3) unlike to QqTOF and ion trap measurements. Their relative abundances depend on the applied fragmentor voltage. The most abundant ion of quadrupole spectra is Ge-132 adduct A_2 with particular amino acids.

Table 5
Elemental composition of ions determined from spectra in Fig. 1E and F

Ion type	Summary formula
M_{mono}	$C_3H_6O_4Ge$
M_{di}	$C_6H_{10}O_7Ge_2$
M_{tri}	$C_9H_{14}O_{10}Ge_3$
M_{tetra}	$C_{12}H_{18}O_{13}Ge_4$
A_1	$C_6H_9O_4GeN$
A_2	$C_6H_{11}NO_5Ge$
A_3	$C_9H_{16}N_2O_6Ge$
A_4	$C_9H_{13}O_7NGe_2$
A_5	$C_9H_{15}O_8NGe_2$
A_6	$C_{12}H_{20}O_9N_2Ge_2$
A_7	$C_{12}H_{17}O_{10}NGe_3$
A_8	$C_{12}H_{19}O_{11}NGe_3$

4. Conclusions

This work shows the potential of ESI-MS with high resolution QqTOF analyzer for the characterization of Ge-132 mixtures with different amino acids based on the determination of the elemental composition and the theoretical isotopic pattern of observed product ions. Measured mass spectra have a very complex char-

acter because of wide isotopic distributions of organogermanium ions and also the formation of various adducts which makes the spectra interpretation complicated. A new approach of identifying potential overlap of ion signals with wide isotopic patterns is described here, based on the use of the accurate masses of overlaid and non-overlaid isotopes at the borders of isotopic envelopes. The combined information from both polarity modes was subsequently used for the elucidation of some structural aspects and the proposal of reaction mechanisms leading to the adduct formation. This paper contributes to a better understanding of the reactivity of germanium sesquioxide with amino acids which may be an important step in the explanation of possible action mechanisms of Ge-132 in the human body. The presented comprehensive approach is particularly useful in the structural analysis of molecules containing complex polyisotopic elements, and is more generally applicable for any organic, bioorganic and organometallic species.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijms.2008.09.003.

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