Available online at www.sciencedirect.com

International Journal of Mass Spectrometry 227 (2003) 281–288

www.elsevier.com/locate/ijms

Temperature dependence of dissociative electron attachment to molecules of gentisic acid, hydroquinone and *p*-benzoquinone

S.A. Pshenichnyuk*, N.L. Asfandiarov, V.S. Fal'ko, V.G. Lukin

Institute of Physics of Molecules and Crystals, Russian Academy of Sciences, October Pr. 151, Ufa 450075, Russia

Received 28 February 2003; accepted 31 March 2003

Abstract

The temperature dependence of dissociative electron attachment to molecules of gentisic acid (2,5-dihydroxybenzoic acid) as well as to the products of its decomposition, namely molecules of hydroquinone and *p*-benzoquinone, has been investigated by means of electron capture negative ion mass spectrometry (ECNI-MS). We have obtained the negative ion yield as a function of incident electron energy for decay channels corresponding to m/z 108 which are $[M - CO_2 - H_2]^-$ anion in the spectrum of gentisic acid, $[M - H_2]^-$ anion in the spectrum of hydroquinone and $[M]^-$ parent long-lived anion in the spectrum of *p*-benzoquinone. It was shown that molecules of carbon dioxide may be formed at high temperature not only by decarboxylation of gentisic acid but also by capture of low-energy electrons (about 1 eV). Such a fact has a great importance for understanding of the problem of analyte desorption in matrix assisted laser desorption/ionization (MALDI) mass spectrometry in according to CO_2-MALDI model where the desorption of large biomolecules is explained in terms of gas-bubbles formation by thermal decomposition of molecules of MALDI matrix.

Keywords: Dissociative attachment; Negative ions; MALDI; 2,5-DHB

1. Introduction

Matrix assisted laser desorption/ionization (MALDI) is a widely used mass spectrometric technique for analysis of large biomolecules [1,2]. However, there is no clear statement of the processes of desorption/ionization of analyte and matrix molecules to date [3,4]. In the recent works [5,6] it was shown that free low-energy electrons exist in MALDI and energy distribution of free electrons in MALDI plume has a maximum at approximately 1 eV which position on electron energy scale depends on the nature of substrate for MALDI sample. Thus a process of dissociative attachment of above-mentioned electrons takes place necessarily in MALDI and may play an important role especially for negative ions formation. As it was shown in our latest work [7] in the negative ion mass spectrums of MALDI matrices (most of which is organic acids having carboxylic group) there is a decay channel $[M - CO_2 - H_2]^-$ (here and below M represents the intact molecule). Anion yield of that channel increases dramatically as the temperature of target molecule increases. Such an effect may be attributed in part by the well-known process of decarboxylation of carboxylic acids [8]. Since the existence of CO_2 molecules in MALDI sample may lead to

---

[*] Corresponding author. Tel.: +7-3472-313538; fax: +7-3472-313538.
E-mail address: sapsh@anrb.ru (S.A. Pshenichnyuk).

1387-3806/03/$ – see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S1387-3806(03)00168-4

formation of gas bubbles and subsequent desorption of analyte molecules according to CO_2-MALDI model [9], we decided to investigate that process in more detail by means of electron capture negative ion mass spectrometry (ECNI-MS) with purpose to specify the possible role of low-energy electron attachment in formation of gas molecules in MALDI.

2. Experimental/materials and methods

Electron capture reactions were studied in the gas-phase by passing an electron beam through ionization chamber of the mass spectrometer where an equilibrium flow of molecules under investigation was created. The yield of negative ions formed was recorded mass spectrometrically as a function of incident electron energy. Such a dependence, namely yield of defined negative ion versus electron energy, was recorded for each peak in the negative ion mass spectrum. If the lifetime of transient parent molecular anion is as long as 10^{-6} s and more then it is observable mass spectrometrically and corresponding peak is seen on the negative ion mass spectrum as well as the fragment anions.

Process of low-energy electron attachment was investigated by means of ECNI-MS using the mass spectrometer MI-1201 under the following conditions: full-width at half-maximum of electron energy distribution 0.4 eV, electron trap current 1 μA, electron energy range 0–12 eV, accelerating voltage 4 kV. The electron energy scale was calibrated by yield of SF_6^- ion from SF_6 molecule which gives the well-known resonance peak at approximately 0 eV. Briefly the mass spectrometer used consists of negative ion source, magnetic analyzer and electron multiplier. Deploying of electron energy and mass number were carried out by IBM-comparable computer using CAMAC interface.

Among all of the MALDI matrices gentisic acid (GA) was chosen for investigation in this work because this is an organic acid which undergoes the thermal decomposition and this is one of the best matrices in MALDI at the end. Besides $[GA - CO_2]^-$ is a molecule of hydroquinone (HQ) and $[GA - CO_2 - H_2]^-$ is a molecule of *p*-benzoquinone (*p*-BQ) and these decay channels are also the subject of this work. Thus compounds under investigation are GA, HQ and *p*-BQ. All of compounds under investigation are solid under standard conditions. They were introduced into ionization chamber of the mass spectrometer using a rod which may be heated up to 110 °C with purpose to evaporate of substances under investigation. The rod was made from stainless-steel and was introduced in the ion source of the mass spectrometer through the sluice chamber. The ion source of the spectrometer was equipped with an oven to heat molecules under investigation up to 300 °C with purpose to obtain temperature dependence of electron attachment process.

3. Results

The relative dissociative attachment cross-sections for molecules investigated are shown in Table 1. A method for determining the relative dissociative attachment cross-section was described previously [10, 11]. Briefly determination of the relative dissociative

Table 1
Melting point[a], the relative dissociative attachment cross-section and temperature of the rod used for evaporation of molecules under investigation into reaction chamber of the mass spectrometer

	Compound		
	Gentisic acid	Hydroquinone	*p*-Benzoquinone
Melting point (°C)	205 (decomposition)	172–175	113–115
σ_{DA} ($\times 10^{-16}$ cm^2)	0.14	10	0.3
T_{rod} (°C)	105	80	Ambient

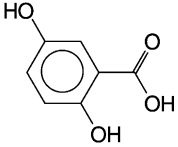
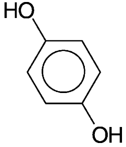
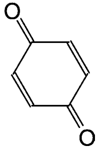
[a] Taken from Catalog Handbook of Fine Chemicals by Aldrich Chemical Company Inc.

attachment cross-section was carried out by comparison of the negative ion currents for substance investigated and SF_6 which has a well-known cross-section for the negative ion formation [12]. The relative vapor pressure of the different substances in the ion source was determined by means of switching the mass spectrometer in positive ion mode.

The temperature of the rod used in this study for evaporation of compounds under investigation into reaction chamber of the mass spectrometer and melting point for the compounds taken from the Catalog Handbook of Fine Chemicals by Aldrich Chemical Company Inc. are listed in Table 1. The temperature of the rod was chosen so to obtain satisfactory intensity of the main dissociative or associative channel for each substance under investigation.

The negative ion mass spectrum of gentisic acid was reported in detail previously [7]. In table form the negative ion mass spectrums of gentisic acid, hydroquinone and *p*-benzoquinone at 80 °C are shown in Table 2. The negative ion mass spectrum of *p*-benzoquinone agrees well with earlier experimental results [13]. In this work we present anion yields corresponding to *m*/*z* 108 only, namely $[GA - CO_2 - H_2]^-$, $[HQ - H_2]^-$ and *p*-BQ^- ions for compounds under investigation. The temperature dependencies of the negative ion yield for these decay channels are shown in Figs. 1–3 (solid line) for gentisic acid, hydroquinone and *p*-benzoquinone, respectively. Intensity shown on the figures is in arbitrary units but the same for each spectrum. In addition anion yield of one more intense decay channel in negative ion mass spectrums of compounds under investigation is shown in Figs. 1–3 (open circles), namely anion yield for $[M - H]^-$ decay channel in the negative ion mass spectrums of gentisic acid (Fig. 1) and hydroquinone (Fig. 2) and for $[M - COH]^-$ channel in the spectrum of *p*-benzoquinone (Fig. 3).

Table 2
The negative ion mass spectrums of molecules under investigation at 80 °C

Compound	*m*/*z* ratio	Ion structure	Energy of maximum (eV)	Intensity (%)
Gentisic acid	154	$[M]^-$	0.0	0.36
	153	$[M - H]^-$	0.83	100
			3.4	15
	152	$[M - H_2]^-$	6.6	36
	135	$[M - OH - H_2]^-$	5.0	2.2
	109	$[M - COOH]^-$	4.9	15
	108	$[M - CO_2 - H_2]^-$	7.6	15
	107	$[M - COOH - H_2]^-$	6.0	1.1
Hydroquinone	109	$[M - H]^-$	1.6	100
			4.2	8.8
	108	$[M - H_2]^-$	5.2	28
			8.7	7.0
	92	$[M - H_2O]^-$	1.3	0.57
	25	$[C_2H]^-$	10.3	1.5
	17	$[OH]^-$	10.2	0.65
p-Benzoquinone	108	$[M]^-$	1.4	100
	82	$[M - C_2H_2]^-$	4.7	0.15
	80	$[M - CO]^-$	2.0	0.12
	79	$[M - COH]^-$	4.8	0.65
			6.8	0.44
	41	$[C_2OH]^-$	5.1	0.14
			6.3	0.34
	25	$[C_2H]^-$	6.3	0.35
			7.8	0.38

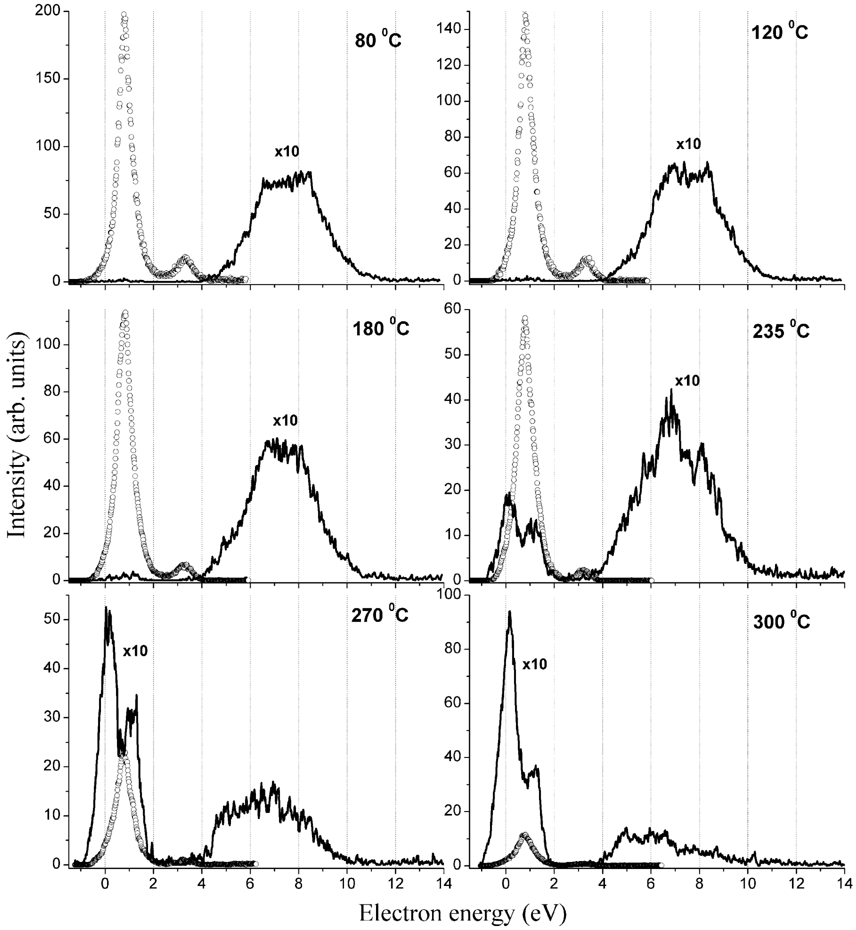


Fig. 1. Negative ion yield for decay channels $[GA - H]^-$, *m*/*z* 153 (open circles) and $[GA - CO_2 - H_2]^-$, *m*/*z* 108 (solid line) in electron capture negative ion mass spectrum of gentisic acid (GA) at different temperatures.

4. Discussion

The temperature of molecules in MALDI sample is varied from ambient temperature (before applying the laser pulse) to approximately 500–700 K (after applying the laser pulse) as it was shown elsewhere [14]. Thus the matrix molecules (molecules of gentisic acid for example) which may capture free electrons which exist in MALDI [5] are rather "hot". In addition molecules of carboxylic acid undergo the decomposition at high temperature with escape of carbon dioxide molecule (decarboxylation) and fragments formed may in turn capture electrons. So on the basis of our experimental results we can define what is happened when molecule of gentisic acid as well as the products of its decomposition capture low-energy electron at different temperatures. In other words we suppose that processes taking place in the ion source of the mass spectrometer may be a model of MALDI processes concerning at least dissociative electron attachment to matrix molecules in conventional MALDI experiments.

In the case of gentisic acid (Fig. 1) decreasing of $[GA - H]^-$ ion yield as the temperature increases follows decreasing of amount of gentisic acid in the reaction area of the ion source because some molecules

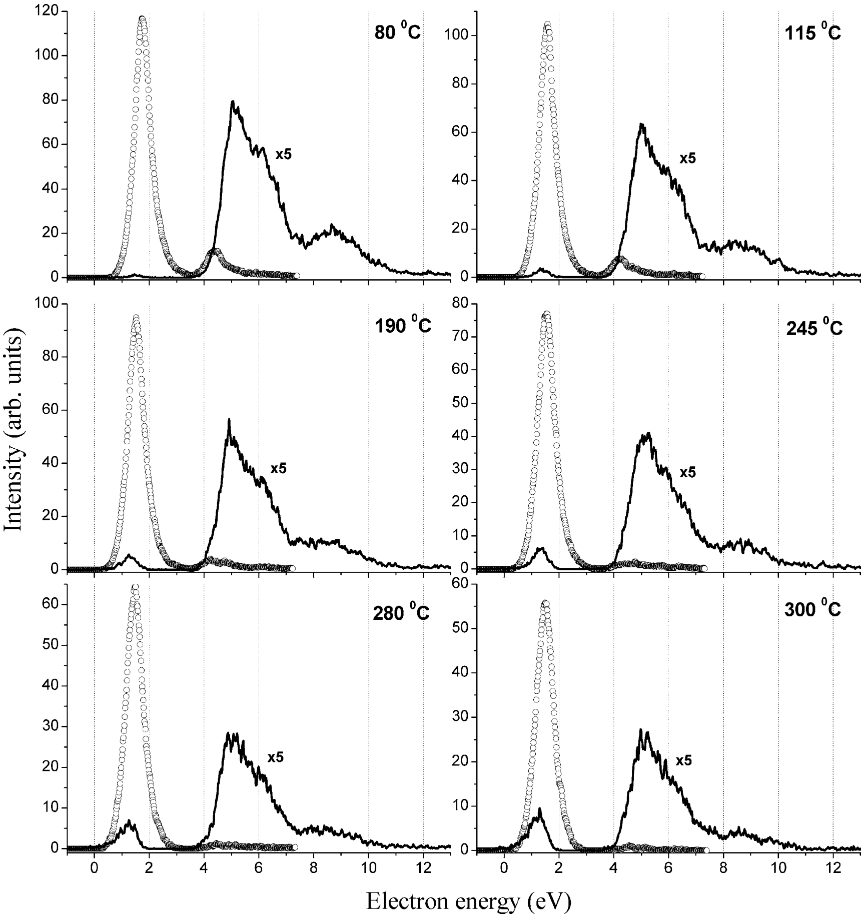


Fig. 2. Negative ion yield for decay channels $[HQ - H]^-$, *m*/*z* 109 (open circles) and $[HQ - H_2]^-$, *m*/*z* 108 (solid line) in electron capture negative ion mass spectrum of hydroquinone (HQ) at different temperatures.

undergo decarboxylation before they capture electron. Indeed some amount of free CO_2 molecules appears in the ion source that was controlled by measuring of O^- anion yield from CO_2 molecule (corresponding results are not shown here). Such a decreasing of $[GA - H]^-$ ion yield may be a temperature effect as well. According to CO_2-MALDI model [9] it is very appropriate when carbon dioxide molecules are formed in MALDI sample because they may assist the desorption of analyte molecules. Below we are going to show that an origin of gas molecules in MALDI may be attributed to electron attachment processes as well as to decarboxylation. Indeed, in the negative ion mass spectrum of gentisic acid there is decay channel $[GA - CO_2 - H_2]^-$ (*m*/*z* 108) that gives two gas molecules at once. Moreover, as the temperature of the target molecule rises the strong features appear on the anion yield corresponding to that decay channel (see Fig. 1) at low-energy electrons. These are the resonant states at approximately 0.15 and 1.25 eV. However, some molecules of gentisic acid have undergone the thermal decomposition inevitably before they capture electron. Thus some amount of hydroquinone appears in the reaction area of the mass spectrometer.

In negative ion mass spectrum of hydroquinone there is a decay channel $[HQ - H_2]^-$ with the same

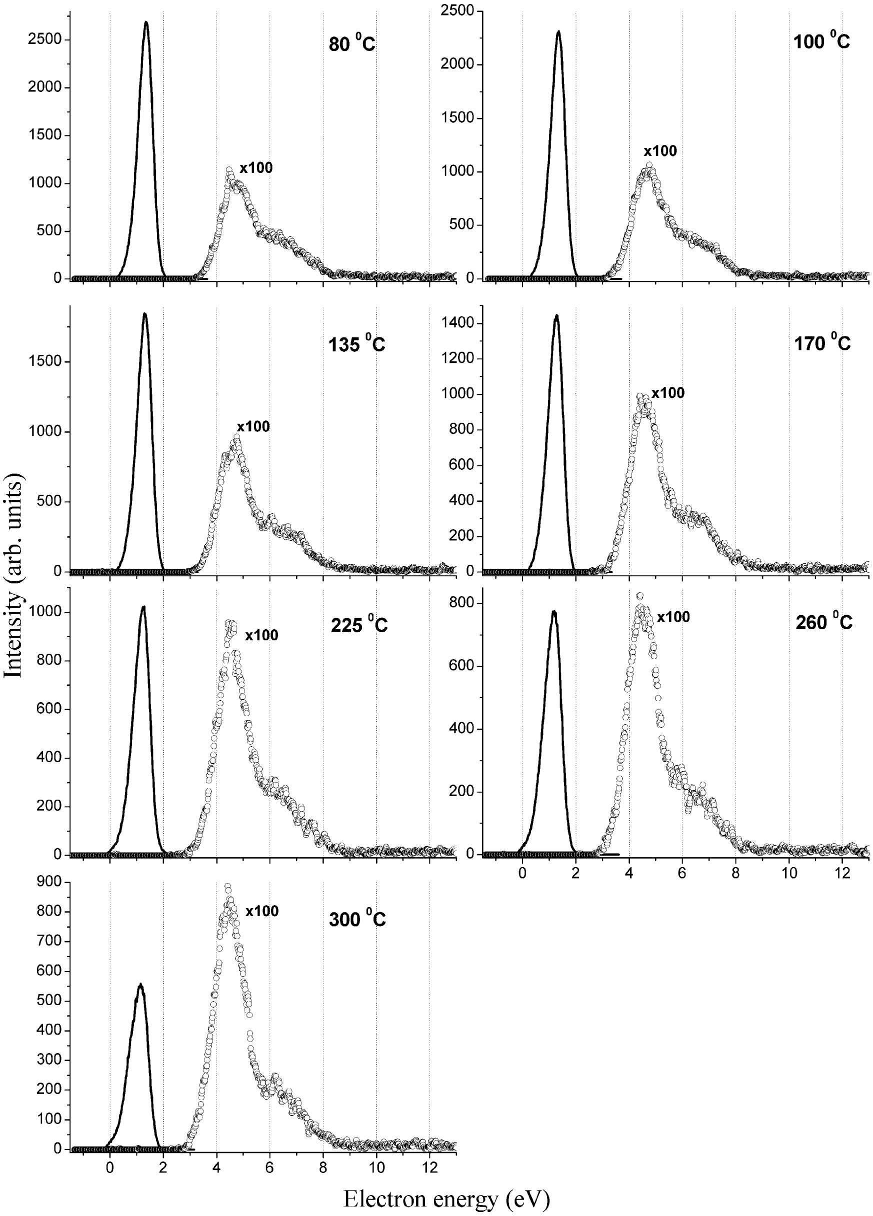


Fig. 3. Negative ion yield for decay channel $[p\text{-BQ} - \text{COH}]^-$, m/z 79 (open circles) and for formation of parent long-lived anion $p\text{-BQ}^-$, m/z 108 (solid line) in electron capture negative ion mass spectrum of *p*-benzoquinone (*p*-BQ) at different temperatures.

m/*z* ratio as for $[\text{GA} - \text{CO}_2 - \text{H}_2]^-$ decay channel in the spectrum of gentisic acid. One can see in Fig. 2 that the resonant state at approximately 1.25 eV appears on the anion yield curve for $[\text{HQ} - \text{H}_2]^-$ decay channel in the spectrum of hydroquinone as the temperature rises. The yield of $[\text{HQ} - \text{H}]^-$ ion decreases along with increasing the temperature of target molecule just as it is in the spectrum of gentisic acid. This may be attributed to the thermal decomposition of hydroquinone in the gas phase which is the ejection of two hydrogen atoms from molecule of hydroquinone. Such a process leads to formation of *p*-benzoquinone in the reaction area of the mass spectrometer.

The most intense peak in the negative ion mass spectrum of *p*-benzoquinone corresponds to formation of parent long-lived anion having *m*/*z* 108. Yield of that channel is on two orders of magnitude more intense than others [13]. So we believe that thermal decomposition of hydroquinone takes place in the gas phase and a resonant state at 1.25 eV on the anion yield curve for $[HQ - H_2]^-$ at 300 °C corresponds to molecular anion of *p*-benzoquinone. Moreover, the relative autodetachment lifetime for that resonance in the negative ion mass spectrum of hydroquinone at 300 °C was estimated to approximately 26 μs. This value agrees reasonable with autodetachment lifetime of molecular anion of *p*-benzoquinone at the same temperature which was estimated to approximately 20 μs. A method for determination of the relative autodetachment lifetime was described previously [15]. Decreasing of parent anion yield in the case of *p*-benzoquinone (see Fig. 3) as the temperature of the target molecule increases is a general effect of autodetachment as it was discussed elsewhere [16,17].

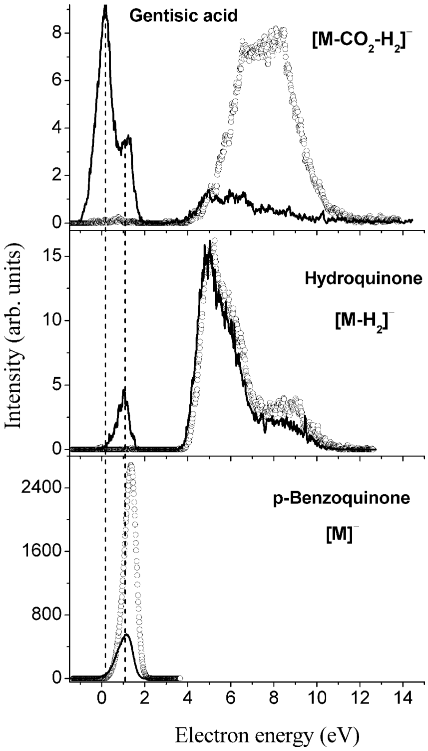


Fig. 4. Negative ion yields for all compounds under investigation for dissociative and associative electron capture channels corresponding to *m*/*z* 108 at 80 °C (open circles) and 300 °C (solid line).

The anion yields corresponding to *m*/*z* 108 for all compounds under investigation are shown in Fig. 4 at the minimal and maximal temperatures. We propose the following assignment of the anion yield observed for $[GA - CO_2 - H_2]^-$ decay channel in the negative ion mass spectrum of gentisic acid at 300 °C. The resonant states above 3.5 eV correspond mainly to the ion $[HQ - H_2]^-$ in the spectrum of hydroquinone which appears in the ion source owing to a process of decarboxylation $GA \rightarrow CO_2 + HQ$ at high temperature. These resonant states are unimportant for MALDI because there are no free electrons with such energies in MALDI plume. The peak at approximately 1.25 eV corresponds to the ion *p*-BQ^- in the spectrum of *p*-benzoquinone which appears in the ion source owing to processes both $GA \rightarrow CO_2 + H_2 + p\text{-BQ}$ and $HQ \rightarrow H_2 + p\text{-BQ}$ at high temperature. We were unable to measure the relative autodetachment lifetime for this resonance directly in the spectrum of gentisic acid because of intensity of the neutral component was too small to be detected. Position of this resonant state on electron energy scale corresponds to 1.25 eV both in spectrum of gentisic acid and hydroquinone at 300 °C that allows us to propose that *p*-benzoquinone molecules formed by thermal decomposition are heated to the same temperature as it is seen from the anion yield for *p*-BQ^- in Fig. 3 at 300 °C.

One can see in Fig. 4 that threshold peak at 0.15 eV corresponds only to the molecule of gentisic acid. This decay channel leads to formation of pseudomolecular anion with a structure of *p*-benzoquinone that seems more probable. Corresponding to this decay channel neutral fragment is a molecule of carbon dioxide. Such a fact is very useful for MALDI because in such a way there is an origin of gas molecules follows by electron attachment to matrix. Thus low-energy electron attachment by molecule of gentisic acid in solid MALDI sample just after laser pulse produces the free electrons may assist the desorption of analyte molecules into the gas phase in according to the model described elsewhere [9]. Arising of the threshold peak of pseudomolecular anion along with the temperature may probably be interpreted in terms of Illenberger's model [16] for the case when corresponding vibration level lies near the crossing point of molecular and dissociative terms. But this research requires additional precise calculations but it is not a subject of this work.

5. Conclusions

The temperature dependence of dissociative and associative electron attachment processes by molecule of gentisic acid as well as by the products of its thermal decomposition in the ion source of the mass spectrometer at high temperature has been investigated by means of ECNI-MS. On the basis of these results and measurements of the relative autodetachment lifetimes the yield of $[GA - CO_2 - H_2]^-$ anion in the negative ion mass spectrum of gentisic acid was interpreted. It was shown that dissociative attachment by molecule of gentisic acid may lead to formation of gas molecules. Thus that process may assist the desorption of MALDI sample in conventional MALDI experiments [9]. This finding is especially important for understanding MALDI desorption because there are thermally stable matrices (6-aza-2-thiothimine, for example). In such a case matrix never gives gas molecules by the thermal decomposition but only by the capture of low-energy electrons [7]. On the other hand, it will be interesting to investigate along the same line the others unstable matrices, like caffeic and sinapinic acids, for example. But this is a subject of subsequent investigations.

Acknowledgements

The authors thanks Prof. R.A. Zubarev (Uppsala University, Uppsala, Sweden) for providing with samples of gentisic acid and hydroquinone as well as Dr. A.N. Leksin (Institute of Energy Problems of Chemical Physics, Russian Academy of Sciences, Moscow, Russia) for providing with *p*-benzoquinone. This work was supported by INTAS grant #99-00647.

References

[1] M. Karas, D. Bachmann, F. Hillenkamp, Anal. Chem. 57 (1985) 2935.

[2] M. Karas, F. Hillenkamp, Anal. Chem. 60 (1988) 2299.

[3] K. Dreisewerd, Chem. Rev. 103 (2003) 395.

[4] R. Zenobi, R. Knochenmuss, Mass Spectrom. Rev. 17 (1998) 337.

[5] M.V. Gorshkov, V.E. Frankevich, R. Zenobi, Eur. J. Mass Spectrom. 8 (2002) 67.

[6] V.E. Frankevich, R. Knochenmuss, R. Zenobi, Int. J. Mass Spectrom. 220 (2002) 11.

[7] N.L. Asfandiarov, S.A. Pshenichnyuk, A.I. Fokin, V.G. Lukin, V.S. Fal'ko, Rapid Commun. Mass Spectrom. 16 (2002) 1760.

[8] I.O. Sutherland, Comprehensive Organic Chemistry, Volume 2. Carboxylic Acids, Phosphorous Compounds, Pergamon Press, Oxford, 1979.

[9] V.L. Talroze, R.J. Jacob, A.L. Burlingame, M.A. Baldwin, in: E. Gelpi (Ed.), Advanced in Mass Spectrometry, Wiley, 2001, p. 481.

[10] D. Rapp, D.D. Briglia, J. Chem. Phys. 43 (1965) 1480.

[11] M.V. Muftakhov, G.M. Tuymedov, R.F. Tuktarov, V.A. Mazunov, High Energy Chem. 30 (1996) 405. O.G. Khvostenko, private communication.

[12] L.G. Christophorou, J.K. Olthoff, Int. J. Mass Spectrom. 205 (2001) 27.

[13] C.D. Cooper, W.T. Naff, R.N. Compton, J. Chem. Phys. 63 (1975) 2752.

[14] C.D. Mowry, M.V. Johnson, J. Phys. Chem. 98 (1994) 1904.

[15] V.I. Khvostenko, Negative Ion Mass Spectrometry in Organic Chemistry, Nauka, Moscow, 1981.

[16] I. Hahndorf, E. Illenberger, Int. J. Mass Spectrom. Ion Process. 167/168 (1997) 87.

[17] L.G. Christophorou, Electron–Molecule Interactions and their Applications, Academic Press, Orlando, FL, 1984.