Polymer track membranes for extraction of ions from aqueous solutions at atmospheric pressure

Alexander. A. Balakin,* Alexander F. Dodonov, Mikhail I. Markin, Lyudmila I. Novikova, Ilia V. Soulimenkov and Victor L. Talroze
Institute for Energy Problems of Chemical Physics, Russian Academy of Sciences, 142432 Chernogolovka, Moscow region, Russia
E-mail: balakin@binep.ac.ru

Bogdan A. Budnik, Kim F. Haselmann and Roman A. Zubarev
University of Southern Denmark, DK-5230 Odense M, Denmark

The possibility of the application of the electromembrane technique for production of ions of biological molecules at atmospheric pressure is demonstrated. This technique has previously only been used for extraction of ions from liquids directly into vacuum. The membrane technique for ion extraction at atmospheric pressure was tested with both time-of-flight and Fourier transform ion cyclotron resonance mass spectrometers. The mass spectra of intact molecular ions obtained from aqueous solutions of peptides and proteins are presented. The possible mechanisms of non-destructive ion extraction are discussed. The new technique is promising for achieving absolute sensitivity (charging every analyte molecule) and for performing spatially-resolved analysis of liquid biological samples.

Keywords: ion extraction from solutions, track membranes, mass spectrometric analysis of polypeptides

Introduction

There were several motivations for undertaking an attempt to apply a track membrane technique for the field extraction of ions from liquid solutions at atmosphere pressure conditions. Currently, there is only one technique, electrospray ionization (ESI), [1] that is used widely in mass spectrometry for analysis of liquid samples. The ESI technique is now a well-recognized tool for the characterization and structural analysis of large molecules.[2] In its classical realization, ESI involves spraying the solution under investigation from a 100 μm-diameter capillary tip placed in a strong electrostatic field. It is also well known that smaller (1–10 μm) ESI capillary tips possess a much higher efficiency for the transformation of dissolved analyte molecules into gas-phase ions suitable for mass spectrometric analysis.[3] This efficiency is directly linked to the sensitivity of ESI-MS analysis, which is a key issue in, for example, proteomics research. Recently, it has been shown that a "brush-shaped" capillary, working in so-called multi-spray mode, increases the sensitivity of the mass spectrometric analysis as well.[4] Extrapolating this trend, one can expect that absolute sensitivity (at which every dissolved molecule is converted into a gas-phase ion) will be reached in a massively parallel electrospray device with many spraying tips each of sub-micrometer diameter.

Besides the desire to improve the sensitivity of the analysis, another motivation was to address the issue of spatially-resolved analysis of liquid samples. This latter is connected with molecular imaging of biological objects at a cellular level. The existing techniques, including matrix-assisted laser desorption/ionization (MALDI) mass spectrometry, [5] are oriented towards the use of solid samples,[6] although many biological objects can be viewed as solutions, albeit rather concentrated. With MALDI, the spatial resolution achieved for the mapping of surrogate markers of cellular components is close to 25 μm.[7] The track membrane can be considered as a structured matrix consisting of multiple channels, with the average distance between them of 1–3 μm. The possibility of selective extraction of ions from each individual channel could open up the prospect of mass spectrometric mapping of biological objects with a high spatial resolution.

The aim of this work was to show the concept of the application of etched-track membranes on a mass spectrometer with introduction of ions at atmospheric pressure.

Etched-track membranes with sub-micrometer size channels have previously been used in electromembrane ionization (EMI) that extracts ions directly into a vacuum from glycerol-based solutions.[8,9] The mechanism of ion production in EMI is believed to be different from that of ESI. Thus, whereas the instability of the liquid–air interface is essential for ESI, it has been shown, theoretically, that in certain conditions in EMI, a stable curvature of the liquid–vacuum interface can exist which is sufficient for direct field evaporation of ions from the liquid.[10] The ion population near the surface can be constantly replenished due to the presence of a high local electric field across the membrane, which enables the effective transfer of ions through the membrane channels.[11] EMI is a soft ionization method, as it produces intact complex bioorganic ions. In fact, the method may be too soft, since a solvent cluster shell usually surrounds the extracted ions. Cluster ions consisting of a central ion and several solvent molecules have already been observed in early investigations of this ion extraction method.[12,13] The solvent shells of ions have recently been investigated with different substances dissolved in glycerol–water mixtures.[14]

It should be stressed that, in beginning this work, we did not presume an equivalence between massively parallel electrospray and atmospheric-pressure EMI (AP-EMI). In general, one should separate the empirical observation of mass spectra (which was the subject of this study) from the elucidation of the specific mechanism of gas-phase ion formation (which requires separate study).

Experimental

The AP-EMI technique was tested on mass spectrometers with two different analyzer types, both originally built for ESI. The ESI interface of the home-built time-of-flight mass spectrometer (ToF-MS) with orthogonal acceleration of ions[15] has a small entrance orifice of about 0.1 mm in diameter made in a thin foil. Two quadrupole stages were employed between the orifice and the ToF analyzer to provide declustering and collisional focusing of the ions. The nominal resolving power at *m/z* 100 was > 10,000. The other mass spectrometer used in this work was a 4.7 T Ultima (IonSpec, Irvine, CA, USA) Fourier transform ion cyclotron resonance (FT-ICR) instrument. A capillary of about 20 cm in length was used in the FT-ICR instrument to introduce ions from the atmosphere. The inner capillary diameter was close to 0.5 mm and the outer diameter was about 1.4 mm. This instrument is capable of providing a resolving power in excess of 10^5 for *m/z* 1000, provided the number of trapped ions is below the limit imposed by the space-charge effect.

The schematic diagrams of the experimental set-ups are shown in Figure 1. A drop of 10 μL of the liquid sample was placed into a round (3 mm diameter) depression made in a stainless steel electrode. The sample was covered by a thin poly(ethylene terephthalate) membrane with etched channels so that the liquid filled all the volume under the membrane. The channels were made using a track-membrane technology[16] by chemical treatment of a polymer film irradiated by high-energy xenon ions. The diameter of the channels was estimated from the airflow through the dry membrane. These membranes with channel diameters in the range of 350–920 Å were used. The thickness of the membranes was about 10 μm and the surface density of the channels was about 10^7 cm^{-2}.

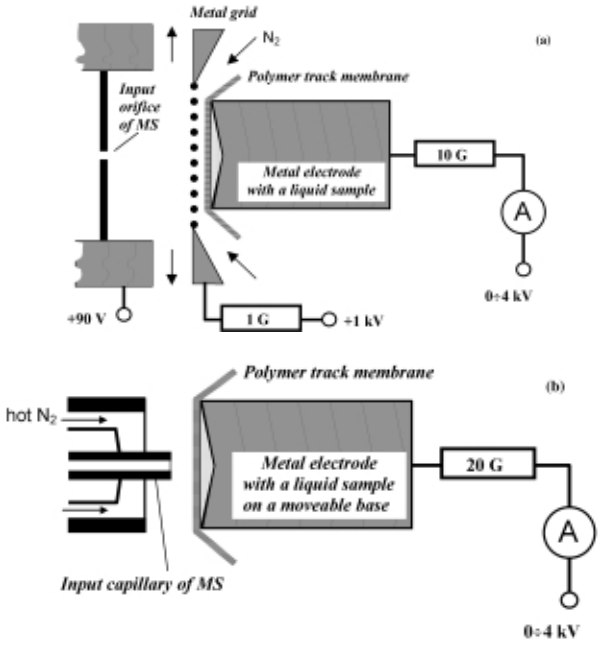

Figure 1. Set-up diagrams of the atmospheric-pressure membrane-based liquid-sample ion source employed with: (a) ToF mass spectrometer; (b) FT ICR mass spectrometer.

In the case of the ToF instrument, a thin metal grid with 100 × 100 μm cells was mounted between the membrane and the input orifice of the mass spectrometer. This extraction grid was located at a distance of about 0.2 mm from the membrane; the distance between the grid and the input orifice was about 2 mm. An extraction potential difference was created between the metal electrode with the sample and the grid. The grid had a fixed potential of about 1 kV whereas the foil with the input orifice had a fixed potential of about 100 V. A high voltage of approximately 4 kV was applied to the electrode with the sample through a 10^{10} Ω resistor. To decrease the loss of extracted ions, a flow of dry nitrogen was applied towards the input orifice of the mass spectrometer.

In the case of FT-ICR-MS, the metal electrode with the sample covered by the membrane was located directly at a distance of about 0.2 mm from the entrance capillary and was mounted on an adjustable table positioned by microscrews. To initiate the ion extraction, the voltage between the electrode and capillary was applied through a 2×10^{10} Ω current-limiting resistor. In both cases, the total current through the resistor was measured.

Solutions of the following substances were under investigation. Gramicidin S, bradykinin des-[Arg1] and des-

[Arg9] fragments, human insulin and bovine ubiquitin, all purchased from Sigma. The amide of the peptide PHDTHESIS was synthesized in-house using the standard solid-phase Fmoc technique. The sample molecules were dissolved in the distilled water or in a water + alcohol (70 : 30 v/v) mixture.

All measurements were carried out in the positive-ion mode.

Results

Average total current through the membrane

The average total current through the membrane under atmospheric pressure conditions was measured in the circuit shown in Figure 1. At a distance of about 0.2 mm between the membrane and an extraction electrode, no current was observed at an extraction voltage below 1.2 kV. A voltage in the range of 1.2–1.4 kV resulted in an appearance of unstable current (less than 10 nA), which then became more stable at a higher voltage. This rather stable regime with the total current > 10 nA was employed for recording mass spectra. The stable current regime was obtained only for sufficiently high values of the resistor that prevented discharges by limiting the current in the gap between the membrane and the extracting electrode. The total current through the membrane was independent of the analyte concentration in the range 10^{-7}–10^{-4} M.

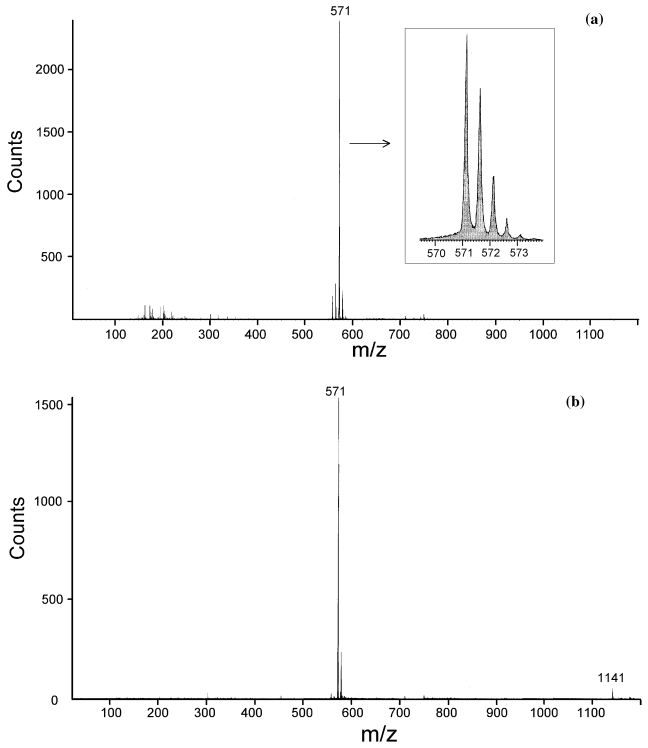

Figure 2. Mass spectrum of gramicidin S obtained with ToF-MS with: (a) AP-EMI, (b) ESI.

ToF-MS experiments

The first experiments with the atmospheric pressure electromembrane technique were curried out by using the ToF device. Figure 2(a) presents the mass spectrum obtained for a 10^{-4} M solution of gramicidin S (a common mass spectrometry standard) in a water + alcohol mixture recorded at a total current through the membrane of 35 nA. The diameter of the channels in the membrane was about 750 Å. The most intense peak at *m/z* 571 corresponds to the doubly-protonated gramicidin S molecule (note the 0.5 unit spacing between the isotopic peaks in the inset). Peaks in the range $150 < m/z < 250$ are not connected with the presence of the analyte in the solution since they were also observed in the mass spectrum from pure solvent. In spite of the rather high stability of the current through the membrane, a long-term instability in the intensity of the mass spectra was observed.

For comparison, the same substance in a 7×10^{-5} M solution in acetonitrile with a small admixture of formic acid gave a similar spectrum in ESI [Figure 2(b)]. It was recorded at a sample flow rate of about 0.5 □L min^{-1} and a total ESI current of 10 nA. The molecular ion peak intensities in both ionization techniques were close (2×10^3 counts after 10^5 scans in both cases).

FT-ICR-MS experiments

Again, instability in ion peak intensities was observed despite the apparent stability of the total current through the membrane. The maximum ion intensity in the mass spectra was observed during the first several minutes after application of the extracting voltage. Because the area of the membrane exceeded that of the capillary entrance, it was possible to change the "working zone" of the membrane by moving the electrode with the sample to a new position. Such a fast change of the working zone restored the analyte peaks in the mass spectrum after depletion of the signal from the previous working zone.

The synthetic peptide PHDTHESIS-NH_2 was the first substance used for testing the AP-EMI source on the FT-ICR-MS instrument. The mass spectrum obtained from a

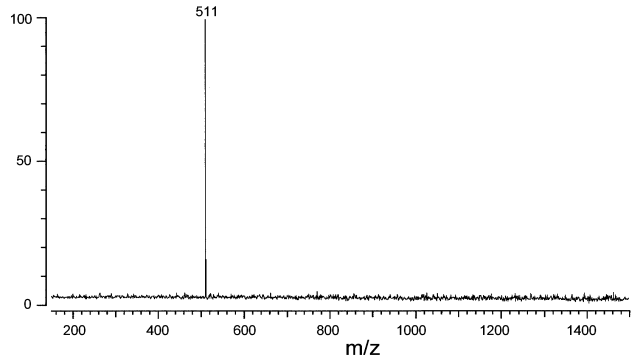

Figure 3. AP-EMI mass spectrum of the amide of the peptide PHDTHESIS obtained with FTICR-MS.

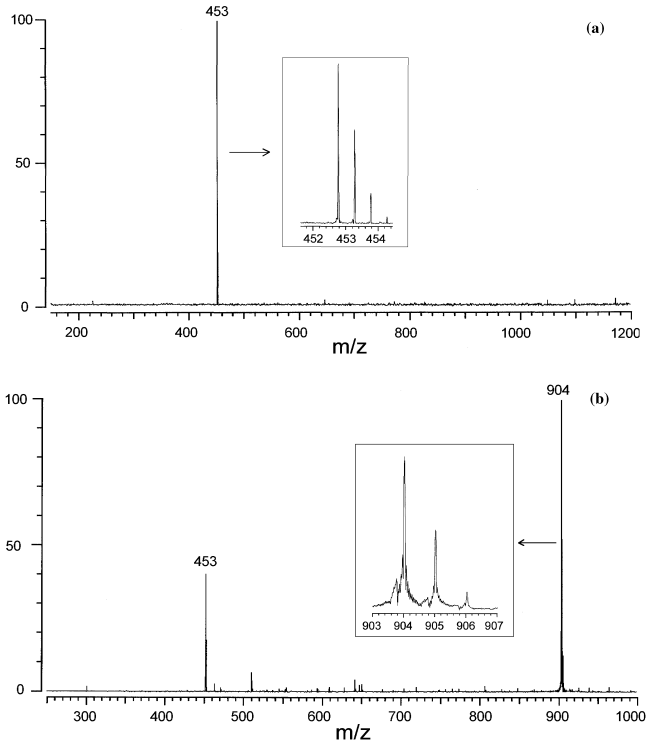

Figure 4. AP-EMI FTICR mass spectra obtained for: (a) des[Arg1]-bradykinin and (b) des[Arg9]-bradykinin.

10^{-4} M solution in a water + alcohol mixture with a total current through the membrane of 70 nA is shown in Figure 3. The diameter of the membrane channels was about 950 Å. The main peak at *m/z* 511 corresponds to the doubly-protonated molecule.

The mass spectra for the bradykinin des[Arg1] and des[Arg9] fragments are shown in Figure 4. The concentration of both peptide solutions was 10^{-4} M. In the case of des[Arg1]-bradykinin, the membrane channel diameter was approximately 500 Å and the total current through the membrane was 20 nA. In the case of des[Arg9]-bradykinin, the membrane channel diameter was 750 Å and the total current 30 nA. Both samples produced abundant doubly-charged molecular ions at *m/z* 453. In the second case, the spectrum was dominated by the singly-charged ion at *m/z* 904

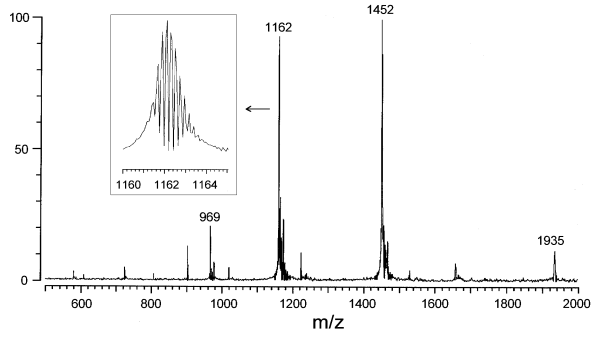

Figure 5. Human insulin AP-EMI FTICR mass spectrum. Note that the space-charge effect reduced the resolving power.

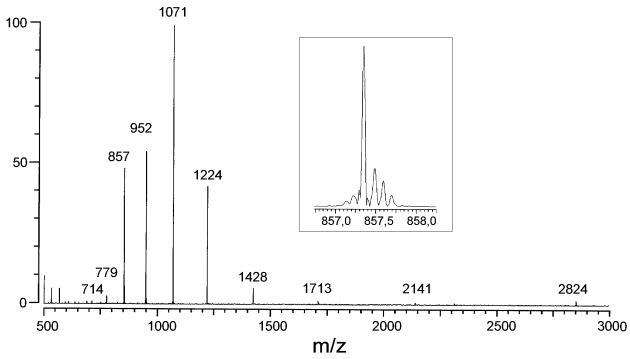

Figure 6. Bovine ubiquitin AP-EMI FTICR mass spectrum. Note that the space-charge effect reduced the resolving power.

(protonated molecule). The signal was so intense that the space-charge effect reduced the FT-ICR resolution [Figure 4(b), inset].

Figures 5 and 6 show the mass spectra for 10^{-3} M solutions of two proteins: both spectra show multiply-charged states. Human insulin (5.8 kDa, Figure 5) gave dominant 4+ (*m/z* 1453) and 5+ (*m/z* 1162) ions at a current of about 10 nA (920 Å membrane channels). Bovine ubiquitin (8.6 kDa, Figure 6) gave charge stages from 4+ up to 11+ at a current of 30 nA (720 Å membrane channels). In both cases, the space-charge effect was quite severe.

Discussion

We note that the ions detected in these experiments are not necessarily the same species that were ejected from the membrane. This is due to the collision-induced declustering process in the nozzle-skimmer region of the air/vacuum interface of both instruments, and to the presence of a low-mass cut-off (about *m/z* 100) in the quadrupole region of the ToF instrument[13] and in the hexapole of the FT-ICR instrument. Despite these circumstances, the fact that the same molecular ions were observed with both ESI and EMI is significant.

It is important to understand the origin of the observed current through the membrane, which was by a factor of 3–10 larger than the typical ESI current. Judging from the molecular ion intensities, which were comparable to those in ESI, it appears unlikely that all the AP-EMI current was due to the flow of ions extracted from the solution. The suggestion that the major part of the observed current was not due to the analyte ions agrees with the fact that the current value remained almost the same when the membrane was replaced by a thin metal plate without channels. An average electrostatic field strength in the gas gap between the membrane and the extracting electrode was about 6×10^4 V cm^{-1}. This is not enough to provide field extraction of ions from the liquid. At the same time, 6×10^4 V cm^{-1} is higher than the discharge threshold for air at ambient conditions. It is, therefore, reasonable to assume that at least part of the cur-

rent through the membrane was due to other charged species, produced in, for example, a low-intensity gas discharge.

The origin of the molecular ions also requires an explanation. In conventional ESI sources, the typical electric field strength exceeds 10^5 V cm^{-1}. The assisting circumstance in ESI is the sharp curvature of the spraying tip, which concentrates the electrostatic field in its vicinity. This circumstance is absent in EMI, where the membrane surface is flat. To spray an aqueous solution from the track membrane channels with a diameter < 1000 Å, an electric field strength > 10^7 V cm^{-1} is necessary.[10,11,13] Another way to transfer the ions from the liquid into the gas phase is direct field evaporation without spraying the liquid. In accordance with evaluations based on the experimental results,[10,17] an electric field strength >10^6 V cm^{-1} is necessary in this case. Therefore, to explain the observed molecular ions in the frame of both the electrospray and the direct ion evaporation processes, we need to assume that there is some mechanism that creates, near to the liquid–gas interface, a local electric field with a strength that is significantly more than the average electrostatic field strength in the gas gap between the membrane and the extracting electrode (about 6×10^4 V cm^{-1}).

The bombardment of the liquid surface by the discharge ions could be considered as an alternative mechanism of ion extraction into the gas phase. This mechanism also requires a strong electric field near the liquid–gas interface. To evaporate one cluster ion of the relevant size from water, an energy of about 3 eV is required.[14] The kinetic energy of gas-phase ions near the liquid surface can reach this value at an electric field strength of about 3×10^5 V cm^{-1}, with the free-flight path of ions equal to 10^{-5} cm at atmospheric pressure. Taking into account the facts that ions dislodged from the liquid may have a larger size and that only part of the kinetic energy can be utilized in the cluster-ion evaporation process, the required electric field should be even stronger. Thus, the question about the formation of a local electric field on the liquid–gas interface is important for this mechanism as well.

One of the possible mechanisms for strong local electric field formation can be charging-up of the membrane surface by ions of the opposite sign to that of the extracted ions. This mechanism is believed to act in direct field evaporation of ions into a vacuum in EMI.[11,17] The ions from the gas discharge in the gap could provide this charging, with a sufficiently strong local field to evaporate polypeptide ions directly. In this case, the electrical conductivity of the polymer surface is important, as it determines the maximum achievable local field strength. The presence of humid air near the membrane or heating the membrane by the current passed can result in an increase of the surface conductivity and thus in the interruption of the ion extraction process. Bombardment of the polymer by ions from the gas discharge can also influence its surface conductivity.

Another possible initiation mechanism is the fluctuation of the electric potential on the membrane surface due to a special feature of the current in the gas gap at the high value of the current-limiting resistor. These fluctuations can induce formation of strong local electric fields, the strength of which exceeds the threshold value for an electrospray process (or direct ion evaporation) for one or several channels near such a fluctuation.

Perhaps other possible mechanisms of ion extraction from the membrane channels will also be proposed in the future. Whatever the nature of the ion production mechanism turns out to be, it is clearly rather soft, since no intense fragments of molecular ions were observed. Another feature is the abundance of multiply-charged ions of polypeptides even for rather concentrated (10^{-3} M) solutions. As far as we know, there are as yet no data about the observation of any multiply-charged ions generated by the EMI technique in the case of the direct extraction of ions into vacuum where the field evaporation mechanism for singly-charged ions was postulated. Finally, the molecular-ion yield was comparable to that in ESI, at least for gramicidin S.

Conclusions

The present work demonstrates the potential feasibility of using the membrane technique for mass spectrometric analysis of polypeptides, including small proteins. The membrane is easy to handle and it can be made less expensively than the high-quality pulled-glass nano-electrospray capillaries. The current limitations are the rather short period of stable production of molecular ions from a single working zone and the higher overall sample consumption compared with nano-ESI. On the other hand, the prospects of achieving spatially-resolved ion production from liquid samples and absolute sensitivity are sufficiently attractive to warrant future studies. These studies will be directed towards overcoming the current limitations as well as elucidating the mechanism of AP-EMI ion production.

Acknowledgment

This work was supported by INTAS grant 99-00647 and grant 00-15-97291 of the Council of grants of the President of the Russian Federation for Leading Scientific Schools, as well as grant N 0001242 of the Danish National Research Council for Technical Sciences.

References

1. R.D. Smith, J.A. Loo, R.R. Ogorzalek, M. Busman and H.R. Udseth, *Mass Spectrom. Rev.* **10,** 359 (1991).
2. J.B. Fenn, M. Mann, C.K. Meng and S.F. Wong, *Mass Spectrom. Rev.* **9,** 37 (1990).
3. (a) M. Wilm and M. Mann, *Anal. Chem*. **68,** 1 (1996); (b) M. Wilm and M. Mann, *Int. J. Mass Spectrom. Ion Processes* **136,** 167 (1994).

4. (a) H. Klesper, G. Klesper and G. Fußhöller, *Improved detection sensitivity in ES MS by a new capillary design: basic principles,* Proceedings of 48th ASMS Conference (CD ROM), Long Beach, California, USA, p. 1512, June 11–15 (2000); (b) G. Klesper, G. Fußhöller and H. Klesper, in *Proceedings of 48th ASMS Conference* (CD ROM), Long Beach, California, USA, p. 1514, June 11–15 (2000).
5. M. Karas and F. Hillenkamp, *Anal. Chem.* **60,** 2299 (1988).
6. R.M. Caprioli, T.B. Farmer and J. Geli, *Anal. Chem*. **69,** 4751 (1997).
7. J.M. Koomen, M. Stoeckli and R. Caprioli, *J. Mass Spectrom.* **35,** 258 (2000).
8. A.A. Balakin, B.V. Mchedlishwilly, L.I. Novikova, V.A. Alejnikov, A.V. Tolmachev, G.I. Florov, V.L. Talroze and B.S. Yakovlev, *Inventor's Certificate No 1542322*, USSR, February 24 (1988).
9. B.S. Yakovlev and V.L. Talroze, *Patent of Russia, No 4879085, Bull. No 3,* January 27 (1995).
10. B.S. Yakovlev, *High Energy Chem.* **29,** 389 (1995).
11. A.A. Balakin, A.F. Dodonov, L.I. Novikova and V.L. Talroze, *J. Electrostatics* **40/41,** 615. (1997).
12. B.S. Yakovlev, V.L. Talroze and C. Fenselau, *Anal. Chem.* **66,** 1704 (1994).
13. A.A. Balakin, A.F. Dodonov, L.I. Novikova and V.L. Talroze, *Rapid Commun. Mass Spectrom.* **10,** 512 (1996).
14. A.A. Balakin, A.F. Dodonov, L.I. Novikova and V.L. Talroze, *Rapid Commun. Mass Spectrom.* **15,** 485 (2001).
15. A.F. Dodonov, V.I. Kozlovski, I.V. Soulimenkov, V.V. Raznikov, A.V. Loboda, Z. Zhen, T. Horwath and H. Wollnik, *Eur. J. Mass Spectrom.* **6,** 481 (2000).
16. G.N. Flerow and V.S. Baraschenkov, *Usp. Phys. Nauk (USSR)* **114,** 351 (1971).
17. A.A. Balakin, V.V. Gridin and I. Schechter, *J. Phys. Chem.* **A 102**, 9470 (1998).

Received: 3 December 2001
Revised: 13 February 2002
Accepted: 19 February 2002
Web Publication: 14 March 2002