Recycling and Reuse Technology Transfer Center

http://www.rrttc.uni.edu

Selective Elimination of Low Molecular Weight Ions in Maldi-TOF Mass Spectrometry Using a Pulsed Electrostatic Particle Guide

Publication: 1993 – 010

Curtiss D. Hanson, Craig L. Just

RAPID COMMUNICATIONS IN MASS SPECTROMETRY, VOL. 7, 502–506 (1993)

93-010

Selective Elimination of Low-molecular-weight Ions in MALDI-TOF Mass Spectrometry Using a Bipolar Pulsed Electrostatic Particle Guide

Craig L. Just and Curtiss D. Hanson*
Department of Chemistry, University of Northern Iowa, Cedar Falls, IA 50614-0423, USA

SPONSOR REFEREE: Professor R. D. Macfarlane, Texas A&M University, TX 77843-3255, USA

This study addresses a problem inherent in the use of microchannel plates as detectors for matrix-assisted laser desorption/ionization. We have developed a unique new method of ion transport using fast bipolar pulsing of an electrostatic particle guide to provide ion deflection and enhanced ion transmission. This mode of operation effectively eliminates the intense low-mass component of the spectrum which normally saturates microchannel-plate detectors, while at the same time transporting the higher-mass component with high efficiency. Enhancement of the resulting signal-to-noise ratio has been achieved.

Time-of-flight mass spectrometry (TOF-MS) has become a valuable technique for the study of high-mass biomolecules particularly since the introduction of matrix-assisted laser desorption ionization (MALDI) methods pioneered by Karas and Hillenkamp.[1] They have reported the detection of molecular masses up to 274 800 Da[2] while other groups, including Beavis and Chait,[3,4] have demonstrated the matrix-assisted laser desorption of biomolecules up to 148 000 Da using TOF-MS with a linear geometry.

TOF-MS has come to the forefront in high-mass analysis due to the high sensitivity and extended mass range that characterize the instruments. Superb sensitivity has been shown for sample sizes in the range of a few hundred fmol[4] and, theoretically, the mass range for TOF-MS is unlimited.[5] Although unparalleled sensitivity and mass range are strong arguments for the use of time-of-flight instruments, limitations in resolution and the dynamic range of certain detectors must be addressed for the successful study of large biomolecules.

Many successful TOF-MS instrument designs utilize an ion extraction surface that is perpendicular to the ion-optical axis. This geometry prevents the loss of mass resolution due to spatial distribution effects related to sample position and the laser focal size.[5,6] Using this geometry, ions can acquire velocity components perpendicular to the ion-optical axis, making it difficult to focus ions onto the detector. Large acceleration potentials have been used to limit the effects of the initial kinetic energy distribution of extracted ions, thus enhancing mass resolution.[5,6]

Another technique used to improve resolution is lengthening of the flight region, but again, the extra length makes it more difficult to transport ions to the detector. Therefore, a significant loss of sensitivity can be experienced when going to the longer drift region. This loss in sensitivity was addressed by Oakey and Macfarlane with the introduction of an electrostatic particle guide (EPG).[7] The EPG is an isolated wire electrode that spans the length of the flight tube, creating a potential field in the center which effectively 'guides' ions to the detector. Ions that are accelerated slightly in a direction perpendicular to the ion-optical axis are captured in the potential field and transported to the detector, resulting in a dramatic improvement in sensitivity.[8,9]

Although the ion-transport ability of the EPG can improve sensitivity because of enhanced ion transmission, detector saturation can result, due to the increase in the incident ion flux. Microchannel plate (MCP) detectors are ideally suited for high-sensitivity applications due to the very high gain produced from a low-current source.[3,10] However, this high gain coupled with high ion flux makes the MCP detectors susceptible to saturation, owing to the abundant low-mass ion component in the MALDI spectrum. An individual channel of the microchannel plate array becomes saturated following generation of the electron cascade and a recovery time of up to several milliseconds is required before the channel is restored to an active state. This slow recovery period means that if many channels are saturated by early arriving, low-mass ions, the detector would be essentially paralyzed for milliseconds. Because a large component of the ion flux in MALDI is comprised of low-mass ions (m/z <300), saturation of the detector results in a reduced capacity to detect high-mass ions.[3]

Efforts to increase the dynamic range of the MCP detector have focused on the use of the pulsed deflector plates that are situated at the early stages of ion entrance to the flight region.[11] In a deflector-plate system described by Brown and Gilfrich, two deflector plates are placed opposite each other near the outer edges of the flight tube. By pulsing the positive and negative voltages on the plates, low-mass ions were shown to be deflected away from the ion-optical axis.[12]

It has been shown that use of an EPG in conjunction with deflector plates is an ineffective method for increasing sensitivity while maintaining the ability to deflect low-mass ions.[9] The ion-transport effects of the EPG offset the ion-repelling effects of the deflector plates by recapturing the deflected ions. A novel system described by Macfarlane utilizes pulsed, positive voltages on an EPG in the early stages of the ion flight region.[13] This system achieves removal of background

* Author to whom correspondence should be addressed.

0951–4198/93/060502–05 $07.50

Received 26 April 1993
Accepted 26 April 1993

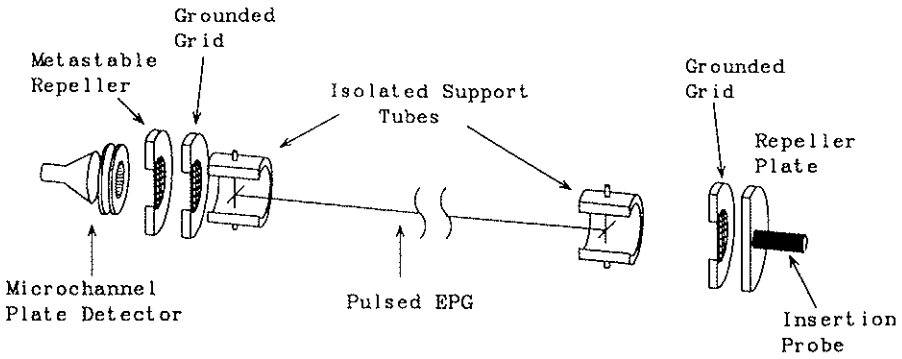

Figure 1. Isometric cutaway view of the TOF-MS system with an isolated electrostatic particle guide (EPG) used for pulsed elimination of low-molecular-weight ions.

neutrals by the deflection of ions past a particle barrier located downstream of the pulsed EPG.

The work presented here involves the use of a bipolar pulsed EPG that permits both enhanced transport and deflection of ions. The ability to apply pulsed positive and negative voltages on the EPG allows one to combine the deflection capability of pulsed plates with the transport ability of an EPG that traverses the full length of the flight region. The increased wire length permits an increase in ion-interaction time which allows for the use of lower wire voltages to achieve desired deflection/transport. The low voltage requirement allows for relatively simple pulsing electronics to control the system. The bipolar pulsed EPG not only deflects undesired ions away from the detector, but recaptures divergent high-molecular-weight ions into a spiral orbit back towards the detector. The increases in the dynamic range and sensitivity created by this selective deflection technique provide a means of detecting trace levels of high-molecular-weight compounds using a matrix-assisted ionization technique.

EXPERIMENTAL

Mass spectra were obtained on a prototype MALDI-TOF mass spectrometer built at the University of Northern Iowa. In order to obtain homogeneous field lines for the efficient extraction of ions produced in the source, a rear-entry stainless-steel probe with a single extraction grid was selected. The acceleration voltage was maintained at 14 kV. The laser optics were mounted in the vacuum chamber at a 45° angle with respect to the probe. Detection of the extracted ions was accomplished using a Comstock (Oak Ridge, TN, USA) CP625/M microchannel-plate detector with chevron geometry at the end of a 2 m flight tube. The 50 Ω output of the detector was shunted to ground by a terminator and the image current was digitized using a LeCroy (Chestnut Ridge, NY, USA) 9310 digital sampling oscilloscope (100 megasamples/s). Background pressure for the system was maintained at 5×10^{-8} Torr by an oil diffusion pump.

Ions were generated by laser desorption using a Laser Science (Cambridge, MA, USA) VSL-337ND-T nitrogen laser producing a 3 ns pulse at 337 nm. The matrix selected for the initial experiments was 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid) because of its strong absorbance at 337 nm. A 5–10 g/L solution of sinapinic acid was used for these experiments. A sample solution of approximately 10^{-3} M gramicidin S was prepared and 10 µL portions of the matrix and sample solutions were mixed directly on the probe tip. The sample/matrix solution was allowed to evaporate at room temperature before being inserted into the vacuum system.

The electrostatic particle guide consisted of a 0.25 mm diameter stainless steel wire suspended down the center of the flight tube (Fig. 1). The wire (180 cm long) was supported by cross-hair wires anchored in a small section of an isolated 76 cm diameter tube located 10 cm downstream from the ion source and terminated 5 cm prior to a grounded grid. A second metastable-repeller grid was maintained at a ground potential (relative to the flight tube) so both parent and metastable ions were detected. The wire voltages were controlled by a specifically designed pulsing circuit that was triggered by the laser pulse and allowed voltage swings between +100 V and −100 V. Initially, the wire was +100 V and was switched to −100 V after a variable delay of from 3 to 50 µs (Fig. 2). The duration of the variable delay was adjusted to provide an acceptable low-mass cutoff.

Electrostatic contours were calculated and displayed using the trajectory calculation program SIMION (version 4.0)[14] on an IBM-compatible computer equipped with a mathematical co-processor. SIMION allows placement of electrodes in a user-defined array, permitting equipotential electric field lines to be calculated for this user-defined electrode array. Voltage gradients are calculated for the points which surround a specific ion's location in the potential array. The potential at each point is calculated via linear interpolation

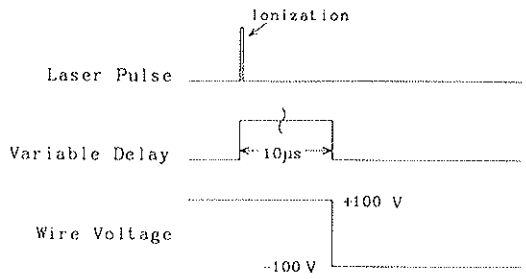

Figure 2. Pulse sequence used to control electrostatic particle guide voltages to achieve selective deflection of low-mass ions.

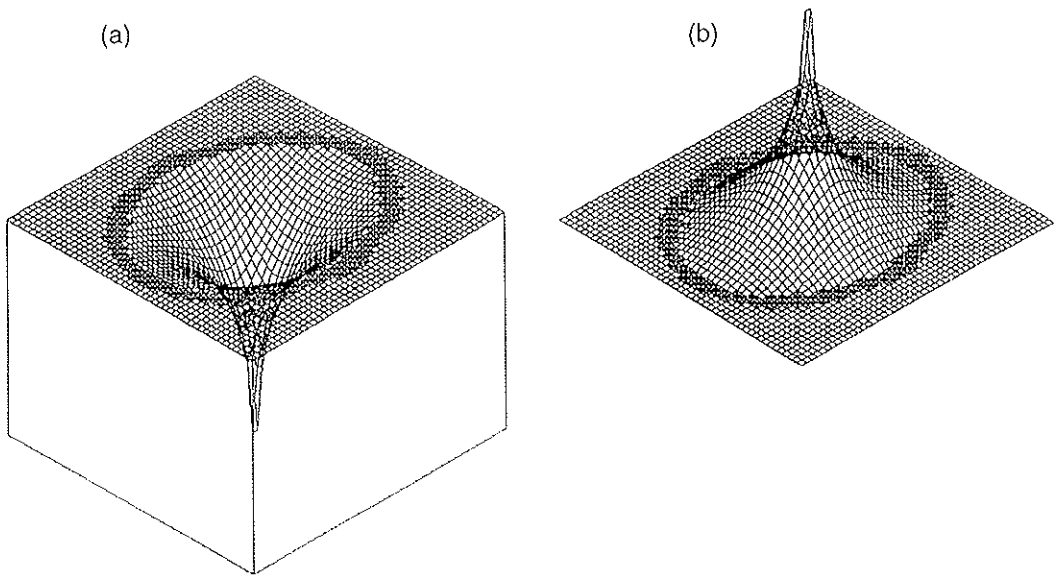

Figure 3. Potential surfaces for the cross-sections of a circular tube with a centrally located electrostatic particle guide having (a) negative bias relative to flight chamber (transport mode) and (b) positive bias (deflection mode).

using the surrounding grid points. A standard fourth-order Runge–Kutta method is used for the numerical integration of the ion's trajectory. This approach has good accuracy and the ability to use continuously adjustable time increments.

RESULTS AND DISCUSSION

SIMION was initially used to study numerically the use of an EPG as a pulsed deflection electrode. Shown in Fig. 3 is the calculated potential surface of a cross-section of a circular vacuum tube with a centrally located EPG. Under situations where the EPG is at a negative potential (Fig. 3(a)), positive ions are captured by the potential field. These ions achieve a spiral orbit and are continuously redirected toward the detector. If, however, a positive potential is applied to the EPG (Fig. 3(b)), the resulting potential field will accelerate positive ions into the vacuum chamber walls. It is important to note that a single deflection electrode located in the center of a circular vacuum tube results in a radially homogeneous deflection field. This type of repulsive field permits uniform acceleration of ions regardless of any radial divergence from the source.

The ability to deflect selectively low-molecular-weight ions was evaluated using a dynamics program written to change the electrostatic potential on the EPG from an initially positive potential (deflection mode) to a negative voltage (transport mode) following a variable delay (Fig. 2). Contained in Fig. 4 are the theoretical trajectories for ions of m/z 100 and 1000, accelerated through a 2 m flight region from an ion source held at 14 kV. The ions were given 5 eV of kinetic energy perpendicular to the ion-optical axis to illustrate the ability of the EPG in transport mode. The initial potential bias (relative to the flight tube) of the EPG was held at +100 V for the first 10 μs of the ion flight time. The resulting potential field was sufficient to repel the ions of m/z 100 into the walls of the flight tube. It is important to note that ions having an m/z of 1000 had not interacted with the field over a significant distance, due to their low velocities. Following the 10 μs delay, the potential of the EPG was changed to −100 V. This change in potential recaptured the divergent ions of m/z 1000 and resulted in a spiral trajectory towards the detector. Therefore, by applying different potentials, the EPG can be used as both a transport and deflection electrode.

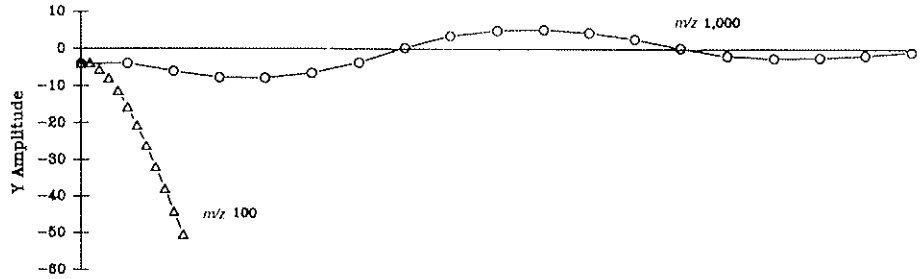

Figure 4. Theoretical trajectories of ions of mass-to-charge ratio 100 and 1000 as calculated by SIMION utilizing a user-defined electrodynamics program to simulate the pulse sequence shown in Fig. 2. It is important to note that ions of m/z 100 have divergent trajectories while ions of m/z 1000 are recaptured by the potential field of the EPG and redirected to the detector.

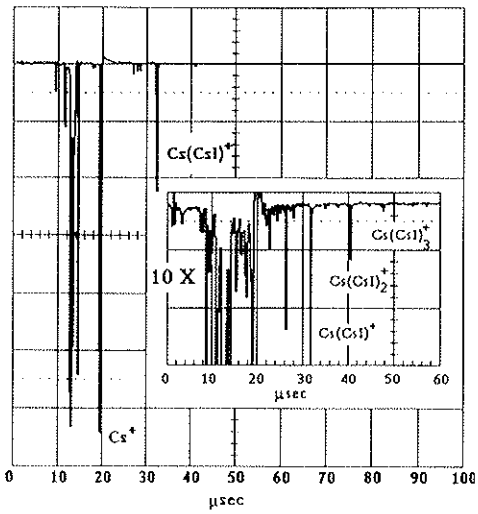

Figure 5. Oscilloscope trace of the time-of-flight mass spectrum of CsI, illustrating detector saturation caused by low-mass ions with the EPG held at a constant −100 V (transport only). Although there is an increase in the ion yield at the detector, detection of the $n = 3$ cluster (10× inset) barely achieves a signal-to-noise ratio of 2, due to detector saturation.

A sample of CsI was analyzed experimentally to evaluate the theoretical increase in the dynamic range of a multichannel electron multiplier. The oscilloscope trace shown in Fig. 5 was acquired with the EPG held at a constant negative potential of −100 V. Under these conditions, most of the ions formed in the source were guided through the flight tube into the detector. Saturation of the detector due to the influx of low-molecular-weight ions is evident by the overshoot of the signal following the arrival of the high-abundance Cs^+ ions (m/z 132, 18.28 µs). Furthermore, the 10× inset illustrates that after signal averaging 1000 scans,

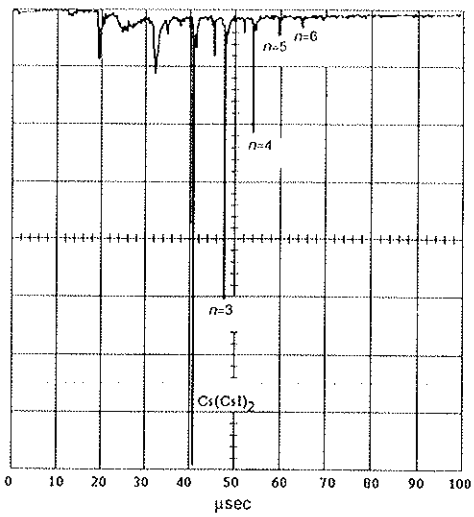

Figure 6. Oscilloscope trace of the time-of-flight mass spectrum of CsI, illustrating the improved sensitivity and dynamic range achieved using the bipolar-pulsed EPG. The dynamic range improvement is illustrated by the detection of the $n = 6$ cluster ($Cs(CsI)_6^+$, m/z 1692), while the sensitivity improvement is illustrated by the increase in signal-to-noise ratio by an order of magnitude for the $n = 3$ cluster.

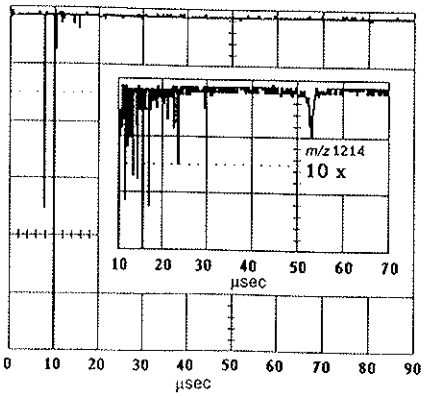

Figure 7. Oscilloscope trace of the time-of-flight mass spectrum of a 10 pmol sample of gramicidin S (m/z 1214) using sinapinic acid matrix with the EPG held at a constant −100 V (transport only). Detector saturation results from the high abundance of low-mass ions. the 10× inset shows the detection of the molecular ion, but sensitivity has been greatly reduced.

the $Cs(CsI)_3^+$ ions (m/z 912) barely exceeded the detection limit due to previous saturation of the multichannel plates.

The ability to eliminate selectively ions using the EPG in a pulsed mode is illustrated in Fig. 6. The oscilloscope trace shown is the result of the potential of the EPG being pulsed from +100 V to −100 V following a 10 µs delay (Fig. 2). Under these conditions, the low-molecular-weight ions were deflected into the vacuum chamber and the higher-molecular-weight ions were redirected to the center of the flight tube. Using this technique, cesium iodide cluster ions of $n = 6$ ($Cs(CsI)_6^+$, $m/z = 1692$) were detected. The gain in signal-to-noise ratio can be illustrated in the gain of the $n = 3$ cluster ions, which was increased by an order of magnitude.

The ability of the bipolar pulsed EPG to enhance sensitivity for the detection of biomolecules using a matrix-assisted ionization technique was tested using a 10 pmol sample of gramicidin S, loaded on the probe tip as described in the Experimental section. Maintaining a constant negative voltage on the EPG again resulted in detector saturation due to the high abundance of low-molecular-weight ions. The 10× inset in Fig. 7 shows that the signal-to-noise ratio for the molecular ion of gramicidin S ($m/z = 1214$) was greatly reduced due to the saturation of the detector. Using the pulse sequence shown in Fig. 2, the potential placed on the EPG was switched from +100 V to −100 V following a specified delay period of 10 µs, resulting in the selective elimination of the low-molecular-weight ions. Using the bipolar pulsed EPG, the detection of the molecular-ion peak was greatly enhanced, permitting a 40-fold increase in the detected signal (Fig. 8).

CONCLUSIONS

Development of a bipolar pulsed EPG to expand the dynamic range of the detector offers several advantages. Because the EPG described in this work spans the entire length of the flight tube, ion interaction with the deflection fields is enhanced to a greater extent than if small deflection plates located outside the source

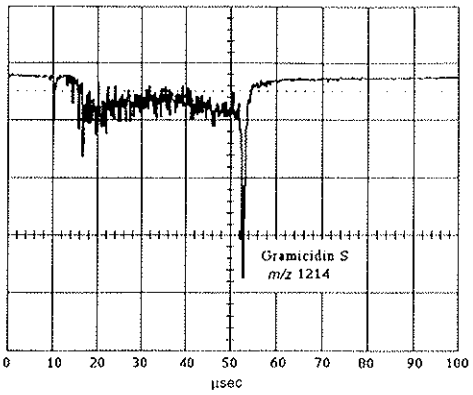

Figure 8. Oscilloscope trace of the time-of-flight mass spectrum of a 10 pmol sample of gramicidin S (*m/z* 1214) using sinapinic acid matrix with the EPG in pulsing mode. The sensitivity has been greatly improved for the molecular ion by selectively deflecting low-mass ions away from the detector while selectively transporting higher-mass ions.

were used. This increased interaction time permits the use of a smaller deflection potential for the removal of low-molecular-weight ions. A smaller deflecting potential makes it easier to pulse between deflection and transport modes (±100 V) permitting simple, fast electronic pulsing circuits to be used. Such circuits allow higher ion selectivity. In addition to the increased interaction time, the radial homogeneity of the repulsive field ensures that all ions traveling down the flight tube are uniformly deflected towards the chamber walls. Finally, a bipolar pulsed EPG not only deflects undesired ions away from the detector, but recaptures divergent high-molecular-weight ions. These inherent advantages result in enhanced detector response for the detection of low-abundance, high-molecular-weight ions.

Extending the dynamic range by detecting low-abundance ions selectively has been used extensively in other mass spectrometric techniques such as Fourier-transform ion cyclotron resonance.[15] Use of a bipolar pulsed EPG increases both sensitivity and the dynamic range by transporting low-abundance, high-molecular-weight ions selectively to the detector, improving the ability to detect trace levels of high-molecular-weight compounds. Such enhancements should permit routine use of microchannel-plate detectors with matrix-assisted laser desorption/ionization techniques.

Acknowledgement

We would like to express our gratitude for the funding provided by the Research Corporation (Cottrell Science Grant) and the Iowa Space Grant Consortium.

REFERENCES

1. M. Karas and F. Hillenkamp, *Anal. Chem.*, **60**, 2299 (1988).
2. M. Karas, U. Bahr, A. Ingendoh and F. Hillenkamp, *Angew. Chem., Int. Ed. Engl.*, **28**, 760 (1989).
3. R. Beavis and B. Chait, *Rapid Commun. Mass Spectrom.*, **3**, 233 (1989).
4. R. Beavis and B. Chait, *Anal. Chem.*, **62**, 1838 (1990).
5. R. Opsal, K. Owens and J. Reilly, *Anal. Chem.*, **57**, 1884 (1989).
6. R. Cotter, *Biomed. Environ. Mass Spectrom.*, **18**, 513 (1989).
7. N. Oakey and R. Macfarlane, *Nucl. Instrum. Methods*, **49**, 220 (1967).
8. P. Geno and R. Macfarlane, *Int. J. Mass Spectrom. Ion Processes*, **74**, 43 (1986).
9. R. Brown and N. Gilfrich, *Rapid Commun. Mass Spectrom.*, **6**, 697 (1992).
10. B. Wolf, P. Mudget and R. Macfarlane, *J. Am. Soc. Mass Spectrom.*, **1**, 28 (1990).
11. W. deHeer and P. Milani, *Rev. Sci. Instrum.*, **62**, 670 (1991).
12. R. Brown and N. Gilfrich, *Anal. Chim. Acta.*, **248**, 541 (1991).
13. B. Wolf and R. Macfarlane, *J. Am. Soc. Mass Spectrom.* **3**, 706 (1992).
14. SIMION PC/PS2 V4.0 was developed by D. C. McGilvery and modified by D. A. Dahl. The program is distributed by D. A. Dahl, Idaho National Laboratory, Idaho Falls, ID, USA (1988).
15. M. L. Gross and D. L. Rempel, *Science*, **226**, 261 (1984).