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Selective Background Suppression in MALDI-TOF Mass Spectrometry

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Rapid bipolar pulsing of an electrostatic particle guide (EPG) provides a method of both ion deflection and enhanced ion transmission for TOF mass spectrometry. This mode of operation effectively eliminates the intense low-mass component of the spectrum which normally saturates microchannel plate (MCP) detectors, while at the same time transporting the higher mass component with high efficiency. The ability to selectively reduce the effects of unwanted background peaks is achieved using a tailored pulse sequence for specific elimination of ions of a given m/z ratio. The mass selectivity of this technique and improvements in signal-to-noise ratio are examined.

Time of flight mass spectrometry (TOF-MS) has come to the forefront in high-mass analysis due to the high sensitivity and extended mass range that characterize the instruments.¹⁻³ Superb sensitivity has been shown for sample sizes in the range of a few hundred attomoles,^{3,4} and theoretically, the mass range for TOF-MS is unlimited.⁵ More recently, TOF-MS has become a valuable technique for the study of high-mass biomolecules, particularly since the introduction of ²⁵²Cf plasma desorption techniques⁶ and matrix-assisted laser desorption ionization (MALDI).⁷ Although the sensitivity and mass range are strong arguments for the use of MALDI-TOF, the spectra are complicated by the high-intensity background peaks resulting from the matrix. This high-intensity background results in limitations in both resolution and detector response, reducing the effectiveness of the technique for high molecular weight biomolecule analysis.

Microchannel plate (MCP) detectors are ideally suited for high-sensitivity applications due to the rapid response coupled with a very high gain.^{2,8} However, these same characteristics make the MCP detectors susceptible to saturation resulting from the low mass ion component in the MALDI spectrum. An individual channel of the microchannel plate array becomes saturated following generation of the electrode cascade, and a recovery of up to several milliseconds is required before the channel is restored to an active state. Because a large component of the ion flux in MALDI is composed of low-mass ions ($m/z < 300$), early saturation of the detector occurs frequently, resulting in a reduced capacity to detect high-mass ions.²

Efforts to increase the dynamic range of the MCP detector have focused on the use of pulsed deflector plates that are situated at the early stages of ion entrance to the flight region.⁹ 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.¹⁰

An alternate approach to background reduction has been based on an electrostatic particle guide (EPG). 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.¹¹ Ions that are accelerated slightly perpendicular to the ion optical axis are captured in the potential field and transported to the detector, resulting in a dramatic improvement in sensitivity.^{12,13} Although pulsed plates permit reduction of unwanted peaks by ion deflection, such methods have been proven to be ineffective when used in conjunction with an EPG. A novel system designed to reduce the background neutral component of the TOF spectrum utilizes pulsed, positive voltages on an EPG in the early stages of the ion flight region.¹⁴ This system achieves removal of background neutrals by the deflection of ions past a particle barrier located downstream of the pulsed EPG.

More recently, selective elimination of low molecular weight matrix ions was demonstrated by use of a bipolar pulsed EPG.¹⁵ 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 capability of an EPG that traverses the full length of the flight region. 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 toward the detector. This mode of operation permits the selective elimination of low-mass ions by operating the bipolar pulsed EPG as a high-pass filter.

The mechanism and selectivity of the ion elimination can be described on the basis of ion motion about the EPG derived by Oakey and MacFarlane.¹¹ A particle injected into the potential field of a centrally located EPG will have a velocity with vector components parallel and perpendicular to the ion axis. The perpendicular component of the ion's velocity, v_p , results in an eccentric spiral motion around the EPG. Ions trapped by the radial electric field are transported to the

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detector by the velocity component parallel to the ion axis. It is important to note that the parallel component of the velocity is unchanged by the radial electric fields and therefore does not alter the time of flight of the ion. When the polarity of the EPG is made positive to create a repulsive electric field, the ions are accelerated toward the flight tube walls. Ions are therefore eliminated by collision with the walls of the flight tube. If the polarity of the applied voltage is changed to create an attractive electric field (i.e., negative) prior to collision with the flight tube, the ion will be recaptured into a stable orbit. This ability to recapture and detect deflected ions results in a highly selective ion elimination technique.

The work presented here demonstrates the selective reduction of unwanted background peaks using mass-specific elimination by bipolar pulsing of an EPG. Because the ions eliminated using the bipolar pulsed EPG are pulse width dependent, specific ion elimination can be accomplished by use of a tailored pulse sequence. The increases in the dynamic range and sensitivity created by this selective deflection technique permits simultaneously detection of trace levels of both low and high molecular weight compounds using a matrix-assisted ionization technique. The mass selectivity of this technique and improvements in signal-to-noise ratio are examined.

EXPERIMENTAL SECTION

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 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 9310 digital sampling oscilloscope (100 megasamples/s). Background pressure for the system was maintained at 5×10^{-9} Torr by a Pfeiffer/Balzers TPU 220 turbomolecular pump.

Ions were generated by laser desorption using a Laser Science 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 dissolved in absolute ethanol was used for these experiments. A sample solution of $\sim 10^{-3}$ M gramicidin S was prepared, and 10 μ L portions of the matrix and sample solutions were mixed directly on the probe tip. The solvent of the sample/matrix mixture was allowed to evaporate at room temperature before the mixture was 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 (Figure 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. The

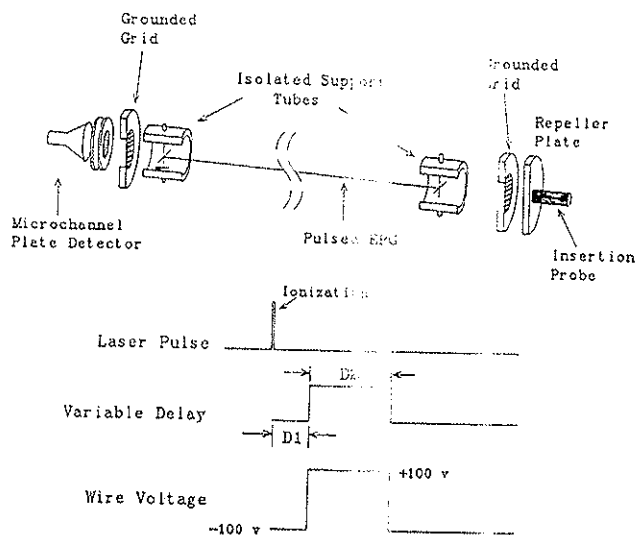


Figure 1. Isometric cutaway view of the TOF-MS used in the experiments. The EPG is supported by two isolated tubes and transverses the length of the flight tube. The voltages applied to the EPG are controlled by a variable delay circuit which permits both variable pulse delay (D1) and pulse width (D2).

cross-hair support for the EPG near the ion source was moved 5 mm off axis to introduce a small component of angular velocity into the ion trajectories to reduce ion collision with the wire. 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 an R. M. Jordan 1040 remote pulsing circuit that permitted voltage swings between +100 and -100 V. The pulse circuit provided rise times of <10 ns, resulting in a dramatic improvement in the S/N ratio for the transported ions. Both the duration of a variable delay (D1) and the pulse width (D2) were accomplished using a simple timing circuit triggered coincident with the laser pulse.

Electrostatic trajectories were calculated and displayed by use of the trajectory calculation program SIMION (version 4.0)¹⁶ on an IBM-compatible computer equipped with a math coprocessor. SIMION allows placement of electrodes in a user-defined array, permitting equipotential electric field lines to be calculated for the 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 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

The ion trajectories for the selective deflection/elimination of low molecular weight ions were evaluated by use of SIMION. A dynamics program was 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. Figure 2 is a vector plot for the calculated trajectories for ions of mass-to-charge ratios

(16) 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, 1988.

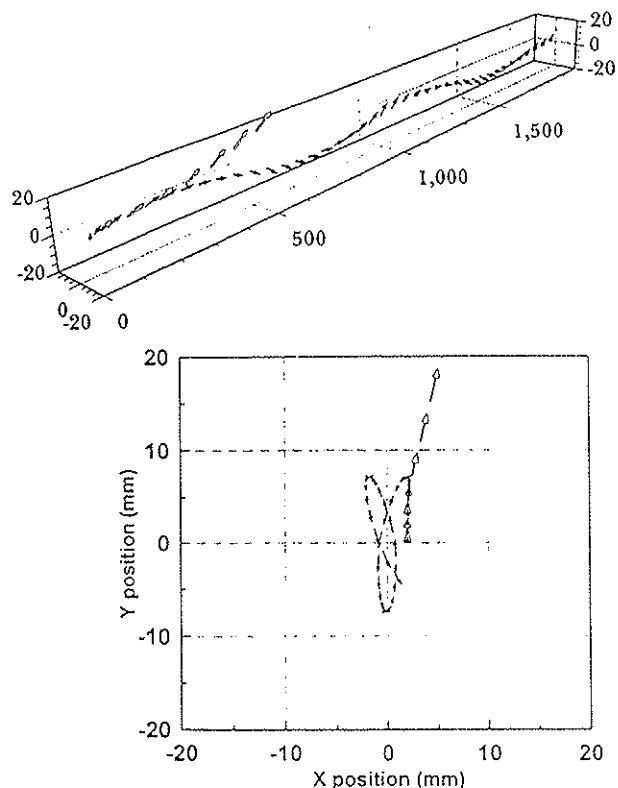


Figure 2. Trajectories of ions of m/z 100 (clear arrows) and 1000 (black arrows) represented in a vector plot. The initial potential bias of +100 V was switched to -100 V following the first 10 μs of the ion flight time. The cross section of the flight tube (X - Y plane) reveals a divergent trajectory for ions of m/z 100 compared to the stable elliptical orbit about the EPG ($x = 0$, $y = 0$) for ions of m/z 1000.

(m/z) 100 and 1000 accelerated through a 2 m flight region from an ion source held at 14 kV. The ions were accelerated into the flight region 5 mm off the central axis and given 2.5 eV of kinetic energy perpendicular to the ion optical axis to introduce a component of angular velocity into the trajectory. 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. It is clear from the resulting divergent trajectory that the resulting potential field was sufficient to repel the ions of m/z 100 into the walls of the flight tube. 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 toward the detector. It is important to note that the spiral orbit created by the initial angular momentum of the ions results in a low probability of ion loss due to collision with the EPG.¹¹ Therefore, by applying different potentials, the EPG can be used as both a highly efficient transport and deflection electrode.

The selective elimination of the low molecular weight component of the mass spectrum is demonstrated in Figure 3. Figure 3A is the MALDI-TOF mass spectrum of 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 a detector saturation due to the high abundance of low molecular weight ions. Although the molecular ion of gramicidin S (m/z 1214) is observed at 48 μs , its intensity was greatly reduced due to the saturation of the detector by the low molecular weight

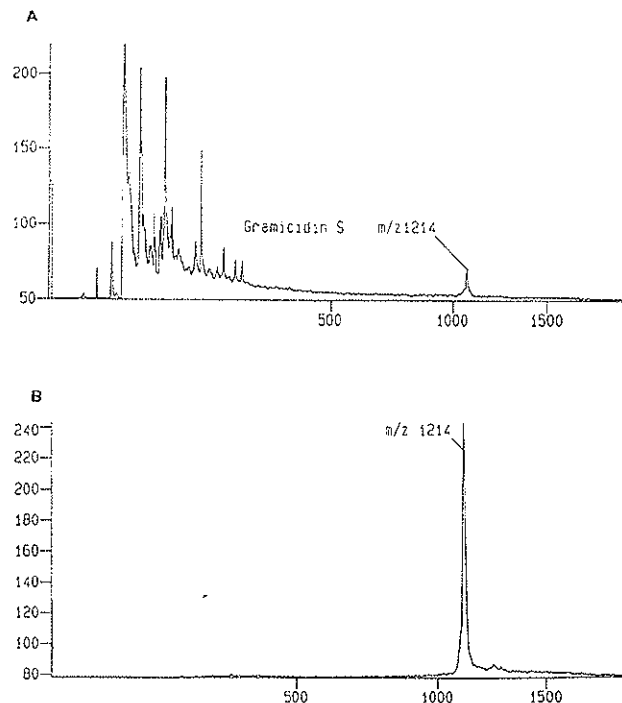


Figure 3. Selective elimination of the low molecular weight component of the MALDI-TOF mass spectrum of gramicidin S, resulting in a substantial improvement in the S/N ratio for the detection of the molecular ion (m/z 1214).

component of the mass spectrum. To enhance the detection of the molecular ion at m/z 1214, the EPG potential was switched from +100 to -100 V following a period of 12 μs , resulting in the selective elimination of the low molecular weight ions. The detection of the molecular ion peak was greatly enhanced, resulting in an increase in the S/N of ~ 20 using the bipolar pulsed EPG (Figure 3B).

The selectivity of ion elimination is related to the time required for an ion to collide with the flight tube when the EPG is held at a repulsive polarity. Because the fields produced within the flight region are perpendicular to the flight axis, the parallel and perpendicular components of the ion's velocities are decoupled and can be treated separately. Therefore, the time required to eliminate an ion by collision with the flight tube can be calculated on the basis of the flight time for an ion released from rest in an electric field between two concentric conducting cylinders of radii a and b , where $b > a$ (eq 1).

$$T = r_0 \left(\frac{2m}{qV_0} \ln \left(\frac{b}{a} \right) \right)^{1/2} \int e^{u^2} du \quad (1)$$

In eq 1, m , q , and r_0 are the mass, charge, and initial radial location of the ion, V_0 is the potential applied to the center electrode, and the integral $\int e^{u^2} du$ is the changing field potential integrated from zero to $(\ln(b/r_0))^{1/2}$. This type of integral is in the form of Dawson's integral for which numerical values have been determined.¹⁷ From this equation, elimination of all ions having $m/z \leq 300$ from an initial radius of 5 mm in a 5 cm flight tube using a voltage of +100 V on a 0.25 mm EPG would require a repulsive field for a duration of

(17) *Handbook of Mathematical Functions with Formulas, Graphs, and Mathematical Tables*; Abramowitz, M., Stegun, I. A., Eds.; Applied Mathematics Series 55; National Bureau of Standards, U.S. Government Printing Office: Washington, DC, 1972.

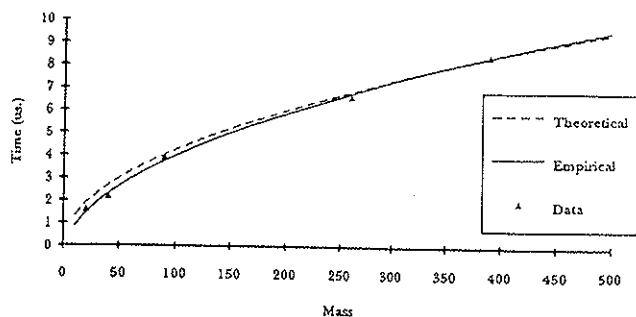


Figure 4. Flight times required for ion elimination by collision with the flight tube, calculated by eq 1 and compared with experimental data. Experimental data were collected by recording the pulse width required to eject ions of a given m/z ratio.

$\sim 7.3 \mu\text{s}$. During that time, all ions having $m/z \leq 300$ would be accelerated into the cylinder wall and lost. If the repulsive field of the EPG is switched to an attractive polarity after $8 \mu\text{s}$, ions having a m/z of >300 would be recaptured by the EPG field and transported to the detector.

To test the theoretical times predicted by eq 1, the pulse width of the repulsive potential applied to the EPG was incrementally increased and the time at which observed masses were eliminated was recorded. Because the pulse width required for ion elimination is directly proportional to the square root of the mass of the ion, a proportionality constant and therefore an empirical formula can be derived. Figure 4 is a comparison of experimentally observed times for the ion elimination to the theoretical times calculated using eq 1. The good correlation between the observed data and the theoretical times required for ion elimination is consistent with the ions being quenched by impact with the walls of the flight tube. Furthermore, the detection of ions that have been accelerated out to radii approaching that of the flight tube indicates that ions are successfully transported by the attractive EPG even though they have a large translational displacement perpendicular to the flight axis. This ability to recapture and transport deflected ions gives rise to high selectivity.

The limitation to the selectivity of the ion elimination is the time range required to completely eliminate ions of a given m/z . Based on eq 1, the elimination flight time is proportional to the square root of the mass of the ion in a constant system. Therefore, an empirical proportionality constant, k , for mass and time can be derived

$$m/z = kt^2 \quad (2)$$

where m/z is the mass of the ion being eliminated and t the time required for elimination by collision with the flight tube. The selectivity of the elimination, $\Delta m/z$, can be derived as the range of masses ($m_1 - m_2$) affected during Δt (eq 3)

$$\Delta \frac{m}{z} = 2k(\Delta t) \left(\frac{m}{zk} \right)^{1/2} + k(\Delta t)^2 \quad (3)$$

For the system used with $k = 2.1 \text{ amu}/\mu^2$ and a $\Delta t = 170 \text{ ns}$, elimination of ions of $m/z 300$ would result in a $\Delta m/z$ of ~ 15 amu. Therefore, during the elimination of ions having a m/z of 300 amu, partial elimination of ions from $m/z 300$ to 315 will also occur. For example, shown in Figure 5 is the TOF

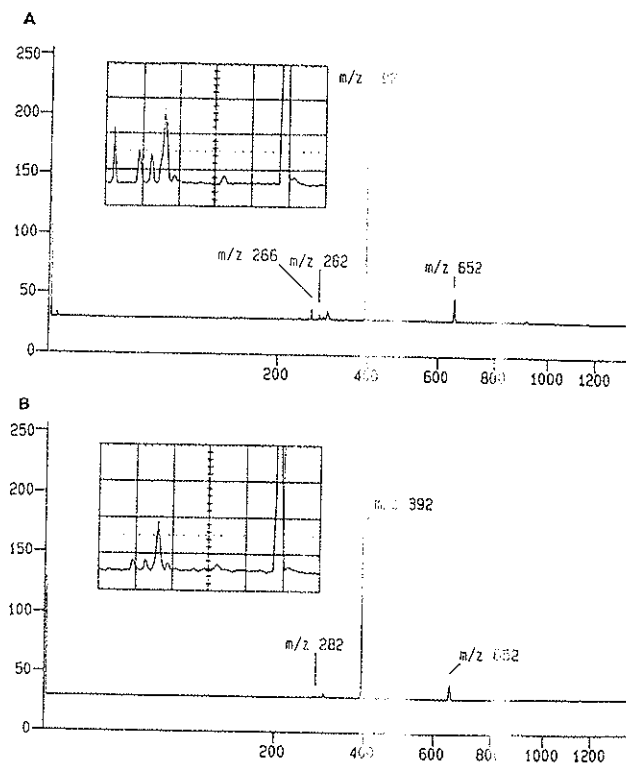


Figure 5. Selectivity of the bipolar pulsed EPG as illustrated by the selective elimination of ions of $m/z 266$ (inset A) while $m/z 282$ is still detected (insets A and B). Although the reduction of intensity of the peak corresponding to $m/z 282$ indicates an effect of the pulsed voltage, it is important to note that the flight time was not affected. This illustrates the decoupling of the acceleration potentials in the X-Y plane.

mass spectrum following laser desorption/ionization of CsI following the selective elimination of all ions having $m/z < 260$ amu. The intensity of the peak corresponding to the arrival of CsI ions ($m/z 266$) at $21.68 \mu\text{s}$ shows no decrease following a pulse width of $6.45 \mu\text{s}$ (inset of Figure 5A). The intensity of the peak drops to zero if the pulse width is increased by 170 ns to a pulse width of $6.62 \mu\text{s}$ (inset of Figure 5B). It is the range in the pulse width, Δt ($\sim 170 \text{ ns}$), required for the elimination of ions of a given m/z that limits the selectivity of the method. The effective selectivity using the bipolar pulsed EPG is limited by the range of m/z affected during that time (eq 3). This effect is illustrated in Figure 5B, where the peak corresponding to the arrival of ions of $m/z 282$ ($22.35 \mu\text{s}$) is reduced in intensity during the elimination of ions having a m/z of 266. Although the amplitude is diminished by $\sim 50\%$, it is important to note that the arrival time is unaffected. This again illustrates that the radial or perpendicular components of the velocities are decoupled from the axial or parallel components of the velocities.

In addition to the selective elimination of the low molecular weight component, a pulsed EPG can also be used for the mass-selective elimination of any ions within a spectrum. Figure 6A contains a laser desorption TOF mass spectrum of CsI cluster ions. Using the pulse sequence shown in Figure 2, it is possible to achieve elimination of only the Cs^+ ions arriving at $16.7 \mu\text{s}$. By holding the EPG at a negative potential initially, it is possible to transport low molecular weight ions to the detector (Figure 6B). This potential ensured that all ions would be transported to the detector. Following the arrival of the K^+ ions ($D1 = 8.4 \mu\text{s}$), the potential on the EPG was

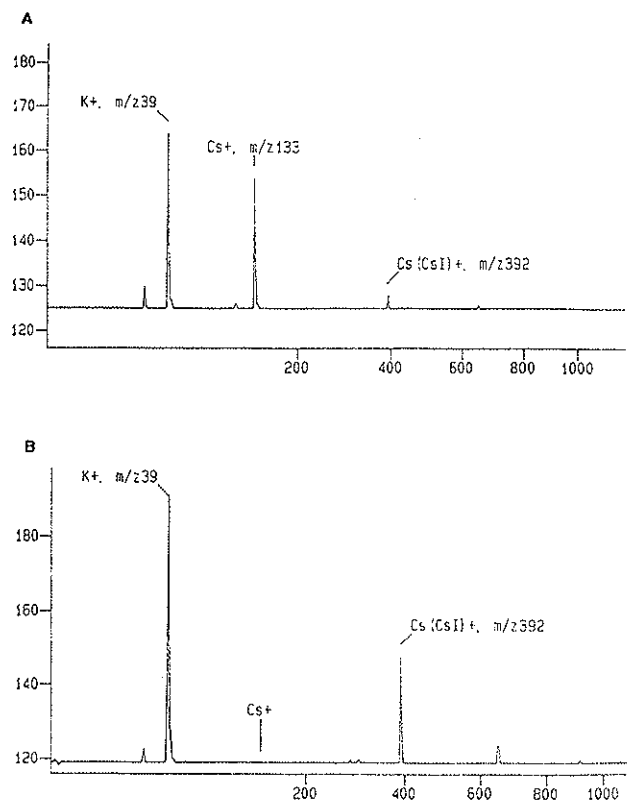


Figure 6. Mass-selective elimination of Cs^+ ions (m/z 133), achieved by holding the EPG at an initially negative potential, permitting the detection of low-mass ions. Following the detection of K^+ (m/z 39), the potential on the EPG is switched to a positive voltage for a duration of 4.5 μs . This pulse selectively eliminates the Cs^+ ions and then recaptures and detects the higher molecular weight cluster ions.

switched to +100 V, resulting in the elimination of the higher molecular weight Cs^+ ions. Once the Cs^+ were eliminated ($D_2 = 4.5 \mu\text{s}$) the potential on the EPG was returned to -100 V, recapturing and transporting the high component of the mass spectrum to the detector. The reduction in detector saturation again results in an enhancement in the S/N for the $n = 1$ and $n = 2$ clusters of $\text{Cs}(\text{CsI})_n$.

CONCLUSIONS

Time of flight mass spectrometry is quickly becoming the analytical technique of choice for biomolecule analysis. This is largely due to the recent improvements in electronics and timing circuitry which permit high-mass analysis with high sensitivity. With the development of faster electronics, the limiting factor for mass resolution has become the response of the detector associated with ion detection. The response time for microchannel plate detectors has been shown to be faster than other comparable detection equipment. Although these detectors permit rapid response to ion arrival, they are susceptible to saturation and therefore loss of effective mass range. Rapid bipolar pulsing of the EPG permits selective elimination of unwanted background peaks that would normally saturate the detector, thus enhancing the dynamic range of the detector.

The limiting factor for increasing the signal-to-noise ratio using the bipolar pulsed EPG has been determined to be the switching time of the pulsing circuitry. Initial data using a prototype pulsing circuit resulted in nonselective ion scattering and, therefore, considerable ion noise.¹⁵ This nonselective ion scatter reduced both the gains in signal-to-noise ratio and the selectivity of ion ejection. Data reported here highlight the impact of a fast pulsing circuit on the ability of bipolar pulsing to eliminate specific ions. The nanosecond switching times afforded by the 1040 pulsing circuit demonstrates a marked improvement in the selectivity and signal-to-noise ratio.

For selective ion ejection to be practical, it needs to have a predictable mathematical relationship between pulse duration and the masses eliminated. Because ions are lost by collision with the vacuum chamber, ion elimination times are based on the TOF of selected ions from the center of the flight tube to the cylinder wall. Because of this simple relationship, pulse durations for selective ion elimination can be determined as the inverse square root of the mass of the ion. The close correlation of calculated and actual pulsed durations required for ion elimination validates the relationship. Furthermore, this mathematical correlation coupled with the switching time of the bipolar pulsed EPG permits predictions of the mass selectivity of the technique. The demonstrated selectivity has been determined to be ~ 15 amu at m/z 300. This selectivity adequately eliminated competing background noise from sample ion detection.

The selectivity of the fast pulsing circuitry also permits a "notched" ion elimination technique capable of ejecting unwanted ions from any point in the mass spectrum. This allows versatility when samples are analyzed that produce a closely knit mass spectrum where ions of interest can be masked by other nearby background ions. By notching out a portion of the unwanted mass spectrum before a peak of interest, the signal-to-noise ratio for that peak can be improved by reducing the detector saturation that would have occurred without ejection.

These improvements afforded by rapid bipolar pulsing permit an enhancement of the dynamic range of MCP detection for TOF mass spectrometry. This will expand the utility of TOF mass spectrometry for the analysis of high molecular weight biomolecule analysis.

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