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(44) I(Leu) gives either $H_2N-CH=CH_2 + H_3C-CH^+-CH_3$ by heterolytic simple bond cleavage (A) or $[H_2N-CH=CH_2]^{\cdot+} + H_3C-^{\cdot}CH-CH_3$ by homolytic cleavage (B).

(A) $\Delta H°_f(H_2C=CH-NH_2) = 29 \text{ kJ·mol}^{-1}$

         $\Delta H°_f(H_3C-CH^+-CH_3) = 799 \text{ kJ·mol}^{-1}$

         $\rightarrow E(A) = 826 \text{ kJ·mol}^{-1}$

(B) $\Delta H°_f(H_2C=CH-NH_2)^{\cdot+} = 820 \text{ kJ·mol}^{-1}$

         $\Delta H°_f(H_3C-^{\cdot}CH-CH_3) = 93 \text{ kJ·mol}^{-1}$

         $\rightarrow E(B) = 913 \text{ kJ·mol}^{-1}$

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Neutral-Ion Correlation Measurements: A Novel Tandem Mass Spectrometry Data Acquisition Mode for Tandem Magnetic Sector/Reflectron Time-of-Flight Instruments

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A new instrument concept for tandem mass spectrometry is described. The instrument consists of a high-performance magnetic sector (Kratos MS-50) as MS-I and a reflectron time-of-flight as MS-II. The instrument concept is compatible with pulsed and continuous ionization sources. This paper describes the basic instrument concept and presents preliminary data for tandem mass spectrometry experiments. This paper emphasizes the use of neutral-ion correlation measurements for obtaining metastable ion and collision-induced dissociation spectra by using a continuous ion source.

INTRODUCTION

Ionization methods for nonvolatile, thermally labile molecules are now sufficiently developed that virtually any class of organic molecule can be produced as an intact ion,[1] and considerable efforts are now focused on developing structural mass spectrometry to a comparable level. Ionization methods such as field-desorption,[2] plasma desorption,[3] fast-atom bombardment (FAB) ionization,[4] electrospray[5] ionization (ESI), and matrix-assisted laser desorption[6] (MALD) ionization have spawned explosive growth in biomolecule mass spectrometry. The advantage of each of these ionization methods is that the dominant ion in the mass spectrum corresponds to the protonated molecule $[M + H]^+$ (in the case of ESI multiple-charged ions are produced), metal ion adducts of the molecule, $[M + Na]^+$, or the corresponding anion, $[M - H]^-$ or $[M + Na - 2H]^-$. A disadvantage of these ionization methods is that very little structural information is obtained from the normal mass spectral data,[7] and with the exception

of FAB, there is considerable difficulty associated with adapting these methods to tandem mass spectrometry experiments. In the case of ESI, identification of the charge state of collision-induced dissociation product ions is difficult, especially for unknowns,[8] and MALD ionization is pulsed and not directly compatible with most tandem mass spectrometers. Because ionization methods such as fast-atom bombardment (FAB) or liquid-SIMS are easily adapted to tandem instruments, FAB/tandem mass spectrometry experiments have developed rapidly and are now firmly established as routine structural methods.[9]

The tandem mass spectrometry experiment adds new dimensions to structural characterization studies. First, the $[M + H]^+$ ion of the analyte can be separated from impurity ions, and the dissociation reactions of the $[M + H]^+$ ion can be examined free of interference ions, assuming the interference ions and analyte ions have different m/z values that can be separated at the working resolution of MS-I. Secondly, changes in the nature of the parent ion, e.g., selecting the $[M + Na]^+$ ion rather than the $[M + H]^+$ ion, can provide detailed/specific structural information.[10] Thirdly, tandem mass spectrometry facilitates probing of the ions by a range of methods, such as collision-induced dissociation,[11] photodissociation,[12] surface-induced dissociation,[13] and endothermic ion–molecule reactions.[14] Although the kinds of structural information obtained by each of these methods are quite similar, often times there are significant differences in the spectra.

In terms of increasing the sensitivity of mass spectrometry, an important feature of tandem mass spectrometry is the elimination of interference signals, thereby enhancing the

signal-to-noise ratio. For example, background ions that arise from sample impurities or ionization of matrix materials complicate the mass spectrum (due to "chemical noise").[15] The chemical noise in the mass spectrum can be greatly attenuated, if not eliminated entirely, by performing tandem mass spectrometry experiments.[16] In addition, background ions (chemical noise) from the matrix can be reduced further by using pulsed ionization,[17] and pulsed ionization methods are less sensitive to ion yield variation due to the presence of impurities. Although pulsed ionization methods are not normally used with scanning (e.g., magnetic sector or quadrupole) tandem instruments, we recently demonstrated that matrix-assisted laser desorption ionization combined with tandem mass spectrometry can (under ideal conditions) be used for the detection of subfemtomole levels of peptides.[18]

On the basis of current or planned work in several laboratories it is quite clear that the next major frontier in structural mass spectrometry involves the maturing of tandem mass spectrometry and the development of greater sensitivity, specifically the improvement in the signal-to-noise ratio, especially in terms of background noise. Several new instrument designs are in the developmental stage, e.g., multisector instruments with spatial array detectors[19] and time-array detectors,[20] Fourier transform ion cyclotron resonance (FTICR),[21] quadrupole ion traps,[22] and sophisticated time-of-flight instruments.[23] Each instrument design possesses specific advantages, in terms of operating parameters and range of experiments, but it is too early to evaluate in detail the specific advantages/disadvantages of a particular instrument concept.

This paper introduces a new instrument under development in our laboratory. The instrument incorporates a high-resolution magnetic sector as MS-I (Kratos MS-50)[24] and a reflectron time-of-flight (TOF) as MS-II. The instrument design is mechanically simple, operator friendly, and compatible with pulsed ionization methods. In this paper we present the first detailed description of the instrument, but the major focus of the paper is on the use of neutral-ion correlation measurements, a specialized time-of-flight data acquisition mode for obtaining tandem mass spectra. Time-of-flight methods have been used previously for tandem mass spectrometry experiments. For example, Enke and co-workers have described a spectrometer that simultaneously measures momentum (magnetic analyzer) and velocity (time-of-flight) of ions. They demonstrated that this method can be used to acquire normal mass spectra as well as collision-induced dissociation spectra.[25] Glish and co-workers developed a tandem quadrupole/time-of-flight instrument and demonstrated the utility of the instrument for tandem mass spectrometry experiments.[26] Although the resolution of the tandem quadrupole/TOF instrument was not optimized (the TOF spectra were obtained by using a linear TOF) the reported data are comparable (in terms of resolution) to that obtained with mass-analyzed ion kinetic energy (MIKE) spectrometers. More recently Standing's group used neutral-ion correlation measurements[27] to detect ions formed by unimolecular dissociation reactions in the field-free region of a reflectron time-of-flight analyzer. The utility of this method for peptide sequence analysis was demonstrated on an unknown sample of dynorphin.[28]

The instrument under development in our laboratory differs from the earlier tandem mass spectrometry involving time-of-flight methods in several ways. First, MS-I is a high-performance/high-resolution magnetic sector instrument with an upper mass range limit of 3500 Da at full accelerating potential, and the upper mass limit can be extended by approximately a factor of 2 by reducing the accelerating voltage to 4 kV. Thus, the instrument is well suited for the analysis of modest size biomolecules. Secondly, the reflectron time-

of-flight instrument is designed for high sensitivity and modest mass resolution ($m/\Delta m$ = 500–1500 and 1000–5000 for analyte m/z values of 1000 and 5000, respectively). The reflectron differs from those used on most time-of-flight instruments, e.g., two-stage reflectrons used for high resolution of ions formed by direct ionization in the ion source,[29] in that it is designed to focus product ions which are formed by dissociation in a field-free region and therefore have a broad range of kinetic energies. The kinetic energy of the fragment ion is dependent on the ratio of the daughter ion mass to the parent ion mass, e.g., $E_k(m_i) = E_k(m_1)(m_i^+/m_1^+)$.[30] Other factors contributing to the kinetic energies for a particular fragment ion mass include kinetic energy release,[31] collisional scattering, and the initial kinetic energy distribution of the parent ion. Finally, the instrument is designed for operation in the tandem mass spectrometry mode with either continuous or pulsed ionization sources. In this regard the instrument is comparable to the new generation four-sector tandem instruments equipped with spatial array detectors; however, it should be noted that the cost of the four-sector tandem instruments with spatial array detectors far exceeds the cost of the two-sector/R-TOF tandem instruments.

EXPERIMENTAL SECTION

A schematic of the tandem magnetic sector (EB)/reflectron time-of-flight (TOF) instrument is shown in Figure 1. The instrument consists of a Kratos MS-50 and a home-built reflectron time-of-flight instrument. The instrument is equipped with a 30-keV Cs^+ ion gun (Phasor Scientific) that can be operated in a continuous or pulsed mode, an excimer laser (Questek Model 2440) for performing pulsed laser desorption and/or photodissociation, and a Laser Science, Inc., cartridge type N_2 laser (Model VSL 337 and VSL 337ND). Time-of-flight spectra can be obtained by using a time-to-digital converter (TDC) (LeCroy Model 4208 TDC), a 350-MHz digital oscilloscope (LeCroy Model 9450), or a transient recorder (LeCroy Model TR8828D 200-MHz digitizer). All of the TOF data acquisition modules are interfaced to PC/AT computers by using a National Instruments GPIB mainframe card. Event sequences for the various experiments are controlled by an EG&G Princeton Applied Research digital delay generator Model 9650. A bias collision cell and deflector lens are available but are not used in the present work.

The reflectron TOF instrument currently in use has a total flight path of 2 m and the reflection angle is approximately 0°.[32] The advantages of a collinear reflectron are high ion transmission and high mass resolution in comparison to that obtained with larger (5–20°) reflecting angles. The collinear arrangement is also convenient for positioning detectors to detect both neutrals and ions. For example, neutrals formed by metastable ion dissociation reactions, collision-induced dissociation (CID), or photodissociation in the region between MS-I and MS-II are detected by a microchannel plate detector (detector no. 1) positioned behind the reflectron. Ions are detected by the microchannel plate detector (detector no. 2) (25 mm diameter, 32 μm c–c, 25 μm diameter pore Chevron detector with 6 mm diameter center hole from Gallileo) positioned at the entrance to the reflectron TOF instrument. The reflector is 40 cm in length consisting of 33 electrodes with each electrode having a 7 cm inner diameter. The front plate of the reflectron is a grid with 98% transmission (20 lines/in., copper grid). A linear electric field gradient is produced by a resistor bridge placed in the vacuum system using 1 MΩ ± 1% glass-encapsulated resistors from Victoreen. The vacuum in the reflectron housing is maintained with a 60 L/s turbo pump (Balzers Model No. TPU 060).

A particular advantage of the neutral-ion correlation experiment is that either continuous or pulsed ionization/excitation can be used for the time-of-flight measurements. For example, dissociation reactions (unimolecular dissociation, collision-induced dissociation, or photodissociation) of the mass-selected M_1^+ ion that occur in the field-free region between MS-I and the reflectron produce a fragment ion (M_2^+) and a neutral (M_3^0). The velocity of M_2^+ and M_3^0 are the same as that of M_1^+. The neutral fragment will not experience any force due to the reflectron and will strike detector no. 1 and produce a signal. M_2^+ will be decelerated upon
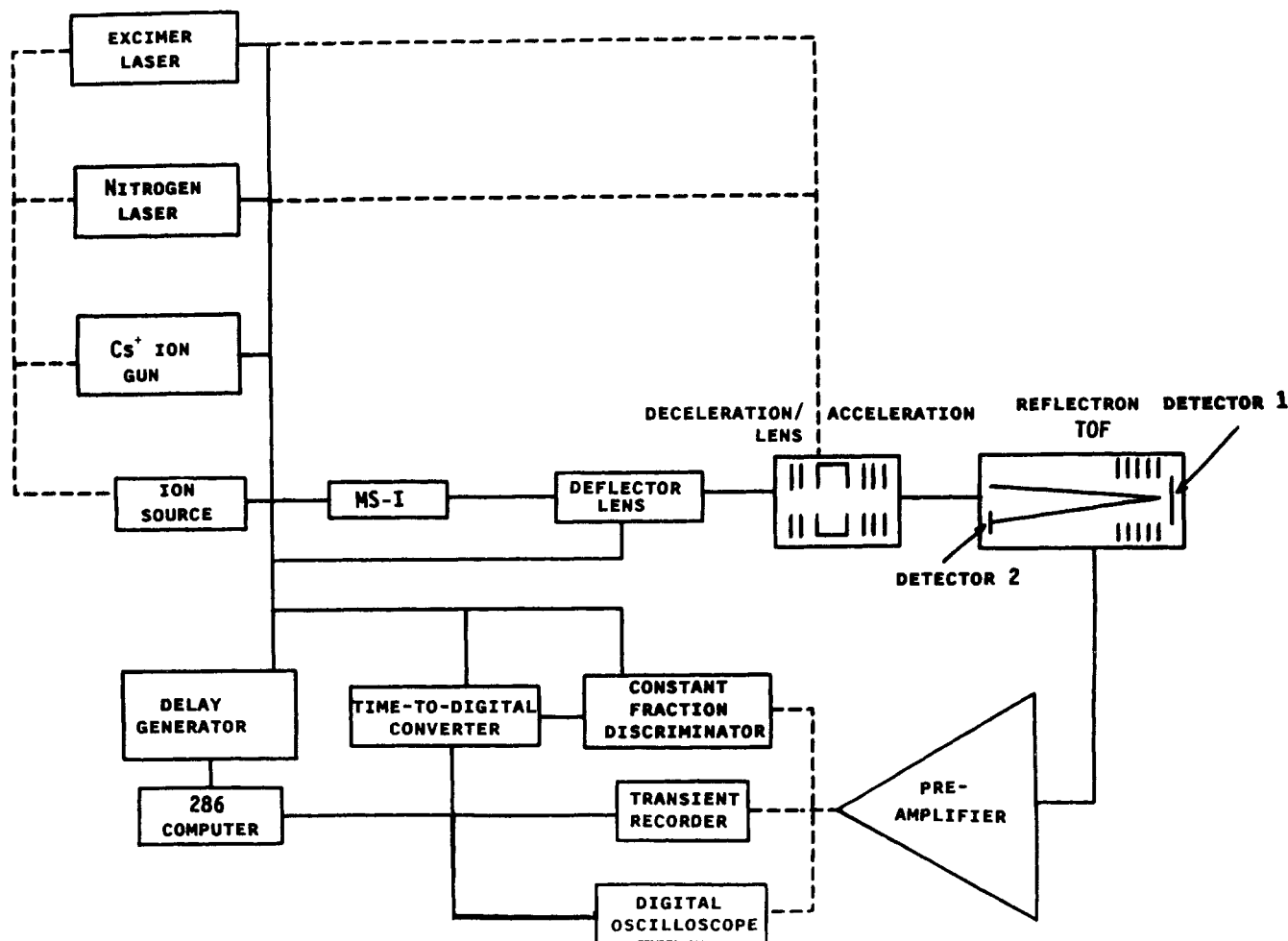
Figure 1. Schematic diagram of the magnetic sector/reflectron time-of-flight mass spectrometer.

entering the reflectron, eventually reaching zero velocity, at which point its direction is reversed. M_2^+ is then reaccelerated back to its initial velocity. Because the initial velocities of the fragment ions are independent of the m/z value, the time that an ion spends in the reflectron depends upon the rate of deceleration and reacceleration. The rate of deceleration/reacceleration is proportional to the m/z of M_2^+. Therefore, the difference in the arrival time of the neutral and the arrival time of M_2^+ is dependent upon the m/z of the ion.

The neutral-ion correlation experiment can be performed in two ways. The first is to use the neutral signal as the start for the time-of-flight measurement and measure the arrival time for the ion. The second method uses the ion as the start event for the time-of-flight experiment while delaying the signal from the neutral detector. In both modes the time delay between arrival of a neutral at detector no. 1 and the arrival time of the ion at detector no. 2 is used to assign the m/z of the fragment ion. The two methods should give identical spectra; however it has been our experience that the second method yields a better signal-to-noise ratio.

In order to understand the signal-to-noise ratio associated with this experiment, it is necessary to examine the signal and noise components separately. The signal observed in the neutral-ion correlation measurement is the result of a neutral and an ion striking the appropriate detector within a specified time interval. For example, if M_1^+ dissociates to M_2^+ and M_3^0, M_3^0 striking the neutral detector starts the TOF clock and M_2^+ must arrive at the ion detector within a time interval Δt. Similarly, if the TOF clock is started by M_2^+ striking the ion detector, the delayed signal for M_3^0 must fall within a time interval Δt. Δt is defined by the initial velocity of M_1^+, the value of M_2^+, and the dimensions of the reflectron (i.e., the deceleration/reacceleration for M_2^+). Because only one Δt is measured for each start signal, noise and signal are mutually exclusive.

The correlation between the number of false coincidences and the primary ion count rate for the neutral-ion correlation experiment is the same as that for photoelectron–photoion coincidence spectroscopy.[33] A special requirement of this experiment is the need for a low beam current density (ca. 10^3–10^4 ions/s for the M_1^+ ion) in order to eliminate (or minimize) the number of false correlations. There are two major sources of noise associated with neutral-ion correlation measurements: (i) "dark current noise" associated with the detector and (ii) noise associated with the ion beam (M_1^+ ions that penetrate the potential applied to the reflectron and/or neutrals formed by charge-transfer ion-molecule reactions between M_1^+ and the CID target gas, which cause false coincidences). We have found that the signal-to-noise ratio for the EB/R-TOF neutral-ion correlation experiment can be improved by adjusting the potential (E_R) of the reflectron to a value less then the kinetic energy of M_1^+, e.g., E_R = 7500 V for a nominal beam energy of 8 keV. This reduces the beam noise associated with the ion detector but does not overcome beam-related noise associated with the neutral detector. In the Results and Discussion section, we will demonstrate that CID spectra are currently limited by beam-related noise (M_1^+ and charge-transfer neutrals) striking the neutral detector. An instrument modification is currently underway that will improve the signal-to-noise ratio associated with the neutral detector. Another source of beam-related noise is due to sputtering of particles upon impact of M_1^+, M_2^+, and M_3^0 with one detector since the sputtered ions are then detected at the opposing detector.[34] This source of noise can be minimized if the fronts of the detectors are biased at the same voltage.

In our experiments, the fronts of the detectors are at ground and a positive potential is applied to the backsides of the microchannel plates and collection anodes (2000 and 2500 V, respectively). The collection anode is then capacitively coupled to a constant-fraction discriminator (Canberra Model Nos. 2128 and 2126). The output from the constant-fraction discriminators can then be used as inputs to the TDC or to the delay generator.
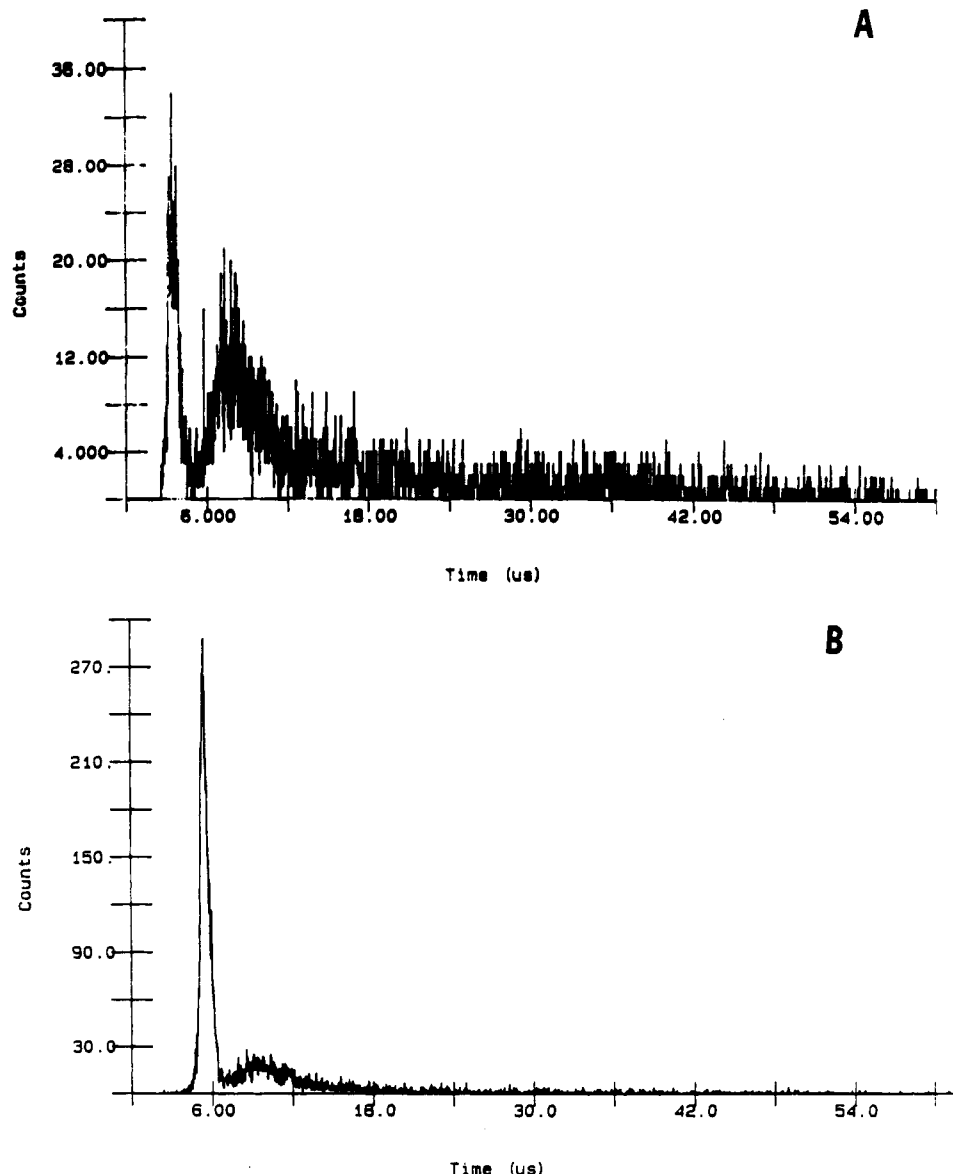
Figure 2. Arrival time distributions of the mass-selected $(M + H)^+$ ion of (A) sinapic acid $(m/z = 225)$ and (B) $[Aus^{1,6}]$ oxytocin $(m/z = 957)$ after a 3-ns laser pulse (337 nm).

The m/z value of the fragment ions are assigned by using eq 1 and 2, where C_1, C_2, and C_3 are calibration constants, m_1 is the

$$m/z = A + C_3(1 - A/m_1) \qquad (1)$$

$$A = (C_1(t_{ion} - t_{neutral})m_1^{1/2} + C_2m_1^{1/2}) \qquad (2)$$

parent ion mass, and $t_{ion} - t_{neutral}$ is the time difference that is measured by the neutral-ion correlation measurements. A is a theoretical calibration where the first term reflects the time in the reflectron and the second term is used to correct for the fact that the distance from the exit of the reflectron to the ion detector is different from the distance from the entrance of the reflectron to the neutral detector.[27] The second term in equation 1 was found empirically by measuring the fragment ions of known compounds, e.g., butylbenzene, metal carbonyls (e.g., $Mn_2(CO)_{10}$), and glycerol clusters, to fit previously reported mass-to-charge ratios of the fragment ions. Because C_1 reflects the penetration depths of the ions, C_1 will change with the reflectron voltages, therefore, C_1 is calibrated every day with a known compound.

RESULTS AND DISCUSSION

The standard mode of operation for time-of-flight instruments is to form ions by using a pulsed ionization source, e.g., a pulsed Cs^+ ion source or a pulsed laser (for desorption or multiphoton ionization). If the ions are formed with a small

spatial distribution and a narrow kinetic energy distribution, the mass resolution of the time-of-flight measurement is limited by the temporal characteristics of the ionization pulse.[35] Thus, ionization pulse durations of 1-3 ns are required for high mass resolution measurements. Although pulse widths of 1-10 ns can be obtained by rastering a conventional Cs^+ ion gun across a small slit,[36] this mode of operation requires summing a large number of events (10^4 or greater) to obtain a complete mass spectrum. The requirement of a large number of ionization events presents a major obstacle to performing tandem mass spectrometry by using a pulsed ion source. For example, the efficiency of collision-induced dissociation is quite low ranging from a few percent to 0.1-0.5% for large molecules.[37] If we assume a collision-induced dissociation efficiency of 0.1%, then 10^4 primary ions yield 10 fragment ions. Because the number of reaction channels available to the collisionally activated ion is quite large (say 10-15 structurally significant fragment ions are typical for relatively small peptides), it may be necessary to accumulate data from 10^5-10^8 ionization events in order to collect a statistically significant data set. If the repetition rate of the ionization source is 10 kHz, a data set of 10^8 events can be obtained in 100 s. However, as the ion yield or collision-induced dissociation efficiency decreases, as is observed for
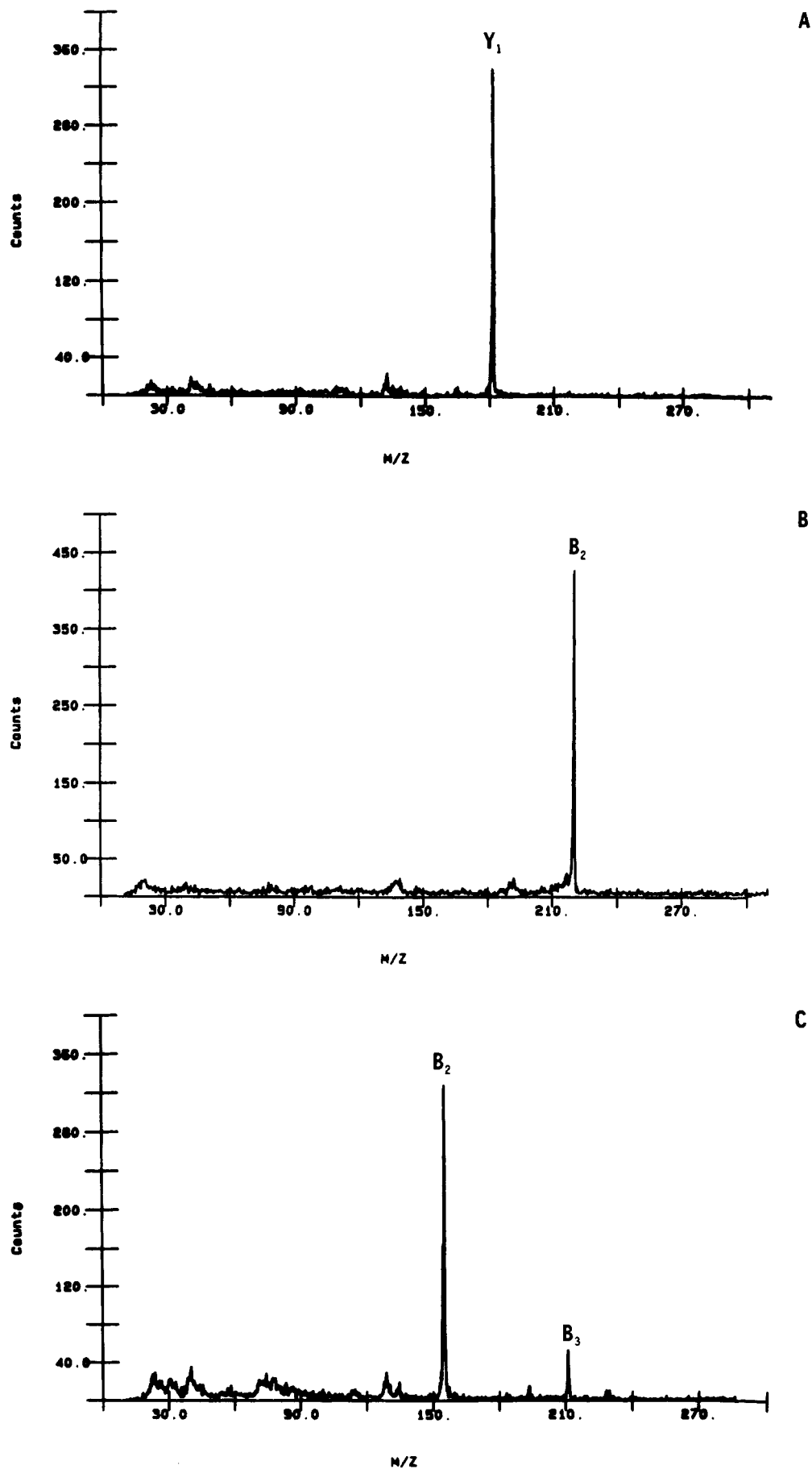
Figure 3. Metastable ion spectra of the $(M + H)^+$ ion of (A) glycine-alanine-tyrosine $(m/z = 310)$, (B) glycine-tyrosine-alanine $(m/z = 310)$, and (C) glycine-proline-glycine-glycine $(m/z = 287)$ obtained by neutral-ion correlation measurements on the EB/R-TOF instrument.
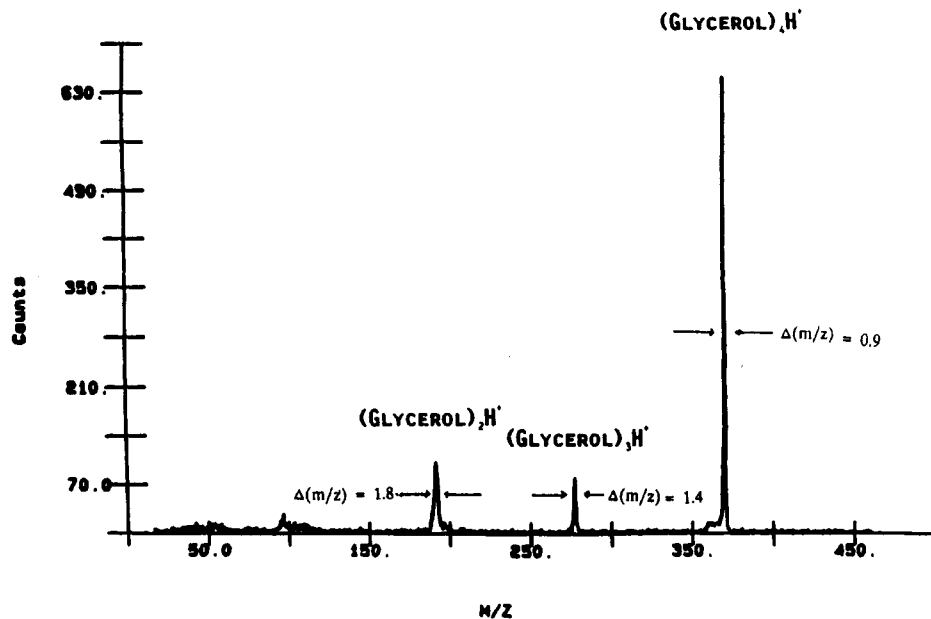
Figure 4. Metastable ion spectrum of $((C_3H_6O_3)_5H^+)$ ($m/z = 461$) obtained by neutral-ion correlation measurements on the EB/R-TOF spectrometer.

increasing molecular weight, the number of events required to collect a statistically significant data set also increases.

An alternative approach to performing measurements with a pulsed ion beam is to form large numbers of ions per ionization pulse and collect the data by using a transient recorder. This is a common mode of operation for matrix-assisted laser desorption where the yield of ions per laser pulse is high; e.g., Standing has reported that approximately 10^4 ions are formed for each laser pulse;[38] however, the numbers of ions per laser pulse varies with the laser power density, the type of matrix used, and the matrix-to-analyte ratio. The limitation of this experiment is the high rate of sample consumption and the inherent loss in sensitivity over single ion-counting experiments. The sample consumption rate does not affect acquisition of normal mass spectral data, but this does pose a problem for signal-averaging. In addition, because the efficiency of the activation/dissociation process is low, the transient recorder mode is less effective for acquisition of collision-induced dissociation and/or photodissociation data.

A complicating factor associated with laser desorption ionization, and especially matrix-assisted laser desorption, is the temporal evolution of ions following laser excitation. Figure 2 contains a plot of the ion abundance for the [M + H]$^+$ ion of sinapic acid and oxytocin as a function of time following the laser pulse. The laser pulse width is approximately 3 ns but the arrival time distribution for the [M + H]$^+$ ions is bimodal. One portion of the arrival distribution has a width at half-height of approximately 500 ns, but the total arrival time distribution extends to 50–70 us. Although data such as that shown in Figure 2 are important in terms of the fundamentals of MALD and further studies of this observation are underway, in this paper we want to focus on those factors that directly impact the performance characteristics of the EB/R-TOF instrument. That is, the extended arrival time distribution complicates tandem mass spectrometry experiments with an instrument such as shown in Figure 1 in two ways: (i) the start signal for the time-of-flight experiment is not sufficiently defined for high mass resolution, and (ii) to achieve reasonable mass resolution, it is necessary to pulse the ion beam entering MS-II such that a small slice of the arrival distribution is selected. Although such an experiment achieves the desired result, the sensitivity is reduced because a substantial portion of the ions produced are not analyzed. In order to achieve the desired sensitivity and resolution with

a reflectron TOF instrument, the ions must be formed with an infinitely short pulse (1–10 ns) with at least one ion in each pulse. A low current density ion beam (e.g. 100–10 000 ions/s) can be considered a series of infinitely short pulses that contain one ion of interest. Because the timing of the pulse is not known, two particles (e.g. an ion and a neutral) that strike different detectors can be used to synchronize the time-of-flight clock.[39]

Figure 3 contains the metastable ion spectra for selected peptides (Gly-Ala-Tyr, Gly-Tyr-Ala, Gly-Pro-Gly-Gly) obtained by using the neutral-ion correlation experiment. In these experiments the ion is used to synchronize the TOF clock and the neutral signal was delayed (see Experimental Section). The metastable ion spectra for the (M + H)$^+$ ions of small peptides are relatively simple, with a single fragment ion typically dominating the spectrum. The major product ions are assigned according to the notation introduced by Roepstorff.[40]

Figure 4 shows the metastable ion spectrum for the glycerol cluster $((C_3H_6O_3)_5 + H)^+$. Peak widths at half-height for each of the daughter ion signals are indicated. Ions were produced by Cs$^+$ ion bombardment of neat glycerol. The glycerol clusters may be considered to be simple solvated species.[41] The peak widths of the fragment ions increase as the mass of the daughter ion decreases, e.g., as the ratio m_2^+/m_1^+ decreases. The temporal focusing of the reflectron depends on the penetration depth of the ions into the reflectron. The daughter ions with lower m/z values have lower energy; therefore, the lower m/z ions will not penetrate the reflector as far as the higher mass ions.

Neutral-ion correlation measurements can also be used for acquisition of collision-induced dissociation spectra. The collision-induced dissociation spectrum of the glycerol cluster $((C_3H_6O_3)_5H^+)$ is contained in Figure 5.

By comparison of Figures 4 and 5, it can be seen that the peak widths at half height in the collision-induced dissociation spectra are larger then the peak widths at half height in the metastable ion spectra. The increased peak widths in the collision-induced dissociation spectra can be attributed to the spread of kinetic energies of the M_i^+ ion due to translation energy loss accompanying collisional activation[42] and the scattering of ions that occur upon kiloelectronvolt collisions. The higher mass daughter ions appear to be less affected by the translational energy losses and scattering. The effect of
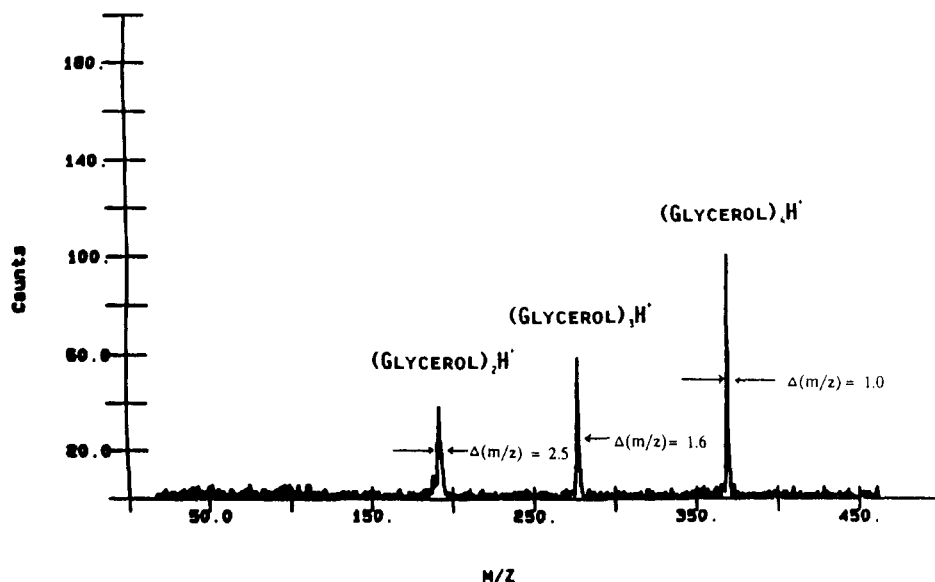
Figure 5. Collision-induced dissociation spectrum of $((C_3H_6O_3)_5H^+)$ $(m/z = 461)$ (8 keV, 25% attenuation, He collision gas) obtained by neutral-ion correlation measurements on the EB/R-TOF instrument.
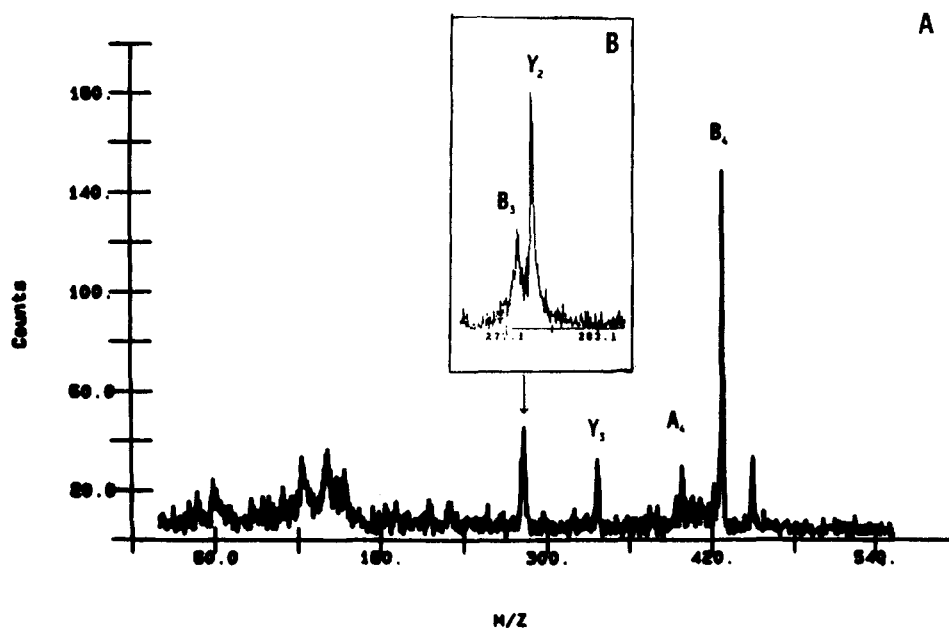

Figure 6. Metastable ion spectrum of leucine enkephalin $(M + H)^+$ $(m/z = 556)$ obtained by neutral-ion correlation measurements on the EB/R-TOF spectrometer. (A) Full spectrum with reflectron voltage at 8000 V. (B) Spectrum in the region of m/z 278, 279 with the reflector voltage at 5000 V.

the penetration can be seen by comparison of the peak widths of the $(glycerol)_4H^+$ and the $(glycerol)_2H^+$ daughter ions in Figures 4 and 5. While the peak widths of the $(glycerol)_4H^+$ daughter ions are approximately equal in Figures 4 and 5, the peak width of the $(glycerol)_2H^+$ peak is 40% larger for the collision-induced dissociation spectra than for the metastable spectra.

To obtain higher resolution on the lower mass daughter ions, the potential on the reflector can be lowered to change the penetration depth of the lower mass daughter ions. Figure 6 contains the metastable spectrum of leucine enkephalin. The largest signal corresponds to the B_4 fragment, but the Y_3 and the B_3 and/or Y_2 fragment ions also are observed.[43] The B_3 and Y_2 $(m/z = 278$ and $279)$ fragment ions are not resolved in this spectrum, but when the reflectron was lowered to 5000 V, the doublet could be resolved (see Figure 6B).[23b,c]

The spectra in Figures 3–6 illustrate the utility of the neutral-ion correlation experiment for acquisition of tandem mass spectra. However, the signal-to-noise ratio for colli-

sion-induced dissociation spectra acquired in this way is less than that obtained for metastable ion spectra. This limitation can be readily seen by comparing the metastable ion spectrum for Leu enkephalin (Figure 6) with the collision-induced dissociation spectrum contained in Figure 7. The reduction in the signal-to-noise ratio for the collision-induced dissociation spectrum can be attributed to the background noise that is associated with neutralization of the incident ions upon collision with the target gas. For example, Figure 8 shows the arrival time distribution data for $[M + H]^+$ of sinapic acid and the arrival time distribution of the neutrals after the ion beam is attenuated by 25% by the addition of collision gas. Most of the attenuation of the beam can be accounted for by neutrals that are formed by a charge-transfer reaction; whereas, neutrals (and ions) formed by collision-induced dissociation account for less than 1% of the total neutrals. The large number of uncorrelated neutrals produces a significant background noise. Because noise and signal are mutually exclusive (see Experimental Section), the signal-
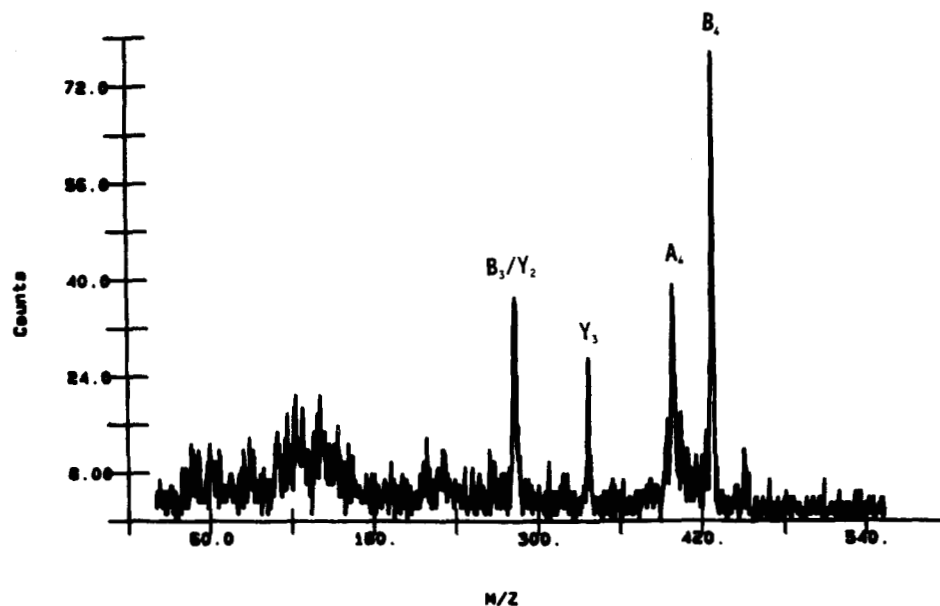
Figure 7. Collision-induced dissociation spectrum of leucine enkephalin (M + H)$^+$ (m/z = 556) (8 keV, 25% attenuation, He collision gas) obtained by neutral-ion correlation measurements on the EB/R-TOF instrument.
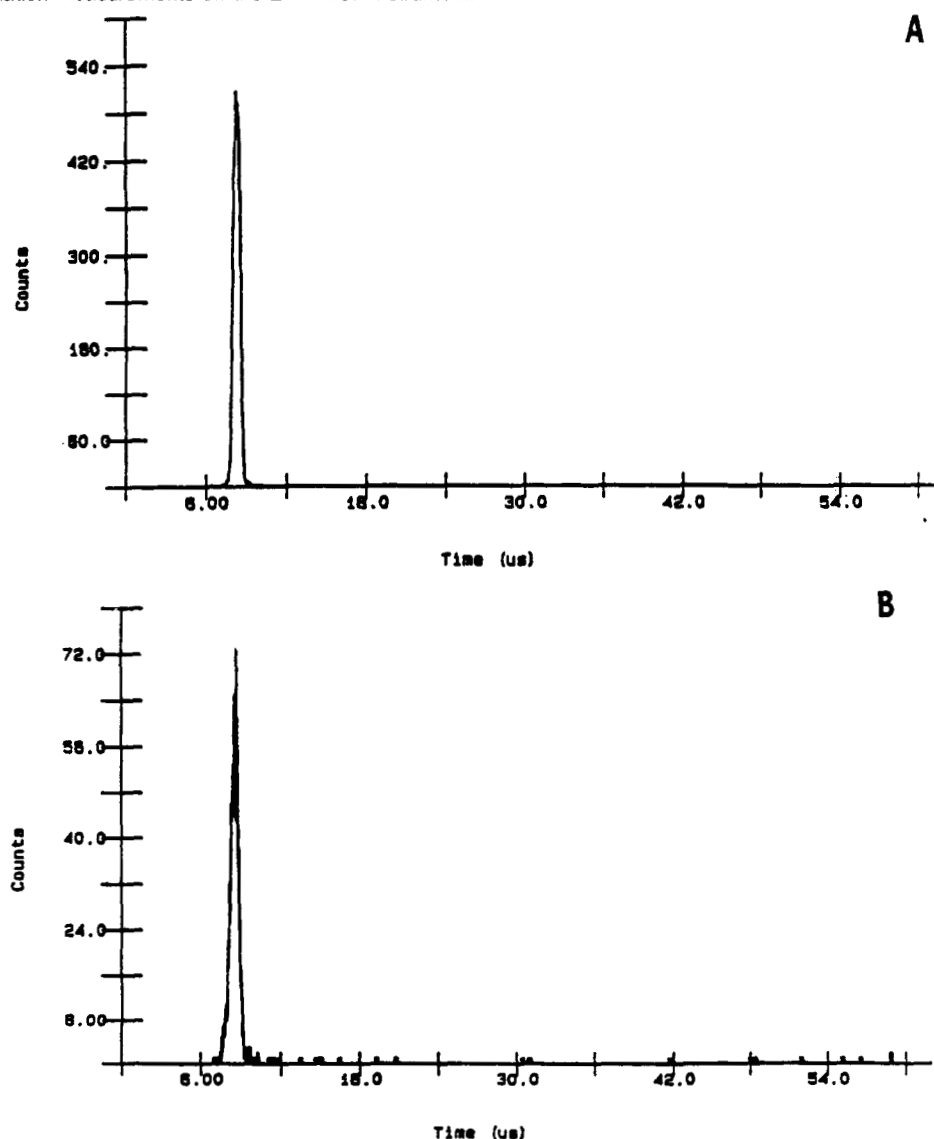

Figure 8. (A) Arrival time distribution without bias applied to the reflectron. Therefore both ions and neutrals of sinapic acid (M + H)$^+$ (m/z = 225) are detected using the line of sight detector (4-keV collision energy, 25% attenuation, He collision gas). (B) Arrival time distribution with 5-kV bias on the reflectron. Therefore neutral products of collisions of the sinapic acid (M + H)$^+$ (m/z = 225) ion with the He target gas are detected using the line of sight detector (4-keV collision energy, 25% attenuation, He collision gas).

to-noise ratio for the collision-induced dissociation product ions is reduced. The large numbers of neutrals detected in this experiment have direct bearing on the unresolved issue concerning charge-transfer reactions between kiloelectronvolt translational energy polyatomic ions and neutral target atoms, e.g., the competition between collisional activation and target gas excitation in the collision-induced dissociation experiment.[44-48] The neutral species measured in this experiment provides irrefutable evidence for the large cross-sections of endothermic charge-transfer reactions between the incident ion and rare gas target atoms.

The effects of a large background signal on the neutral-ion correlation measurement suggests that activation methods that do not produce significant amounts of neutrals will be best suited for this method. Thus, this measurement should be ideally suited for photodissociation,[12] electron-induced dissociation,[47] and possibly surface-induced dissociation.[14]

CONCLUSIONS

Ion-neutral correlation measurements provide a convenient method for acquiring metastable ion and collision-induced dissociation spectra for ions produced by a continuous ion source in a tandem magnetic sector/reflectron-time-of-flight instrument. Although the signal-to-noise ratio for the collision-induced dissociation data is reduced by random background from charge-transfer reactions, this may be overcome by signal-averaging a large number of dissociation events. Another approach to improving the signal-to-noise ratio is to reduce the background. Background reduction can be accomplished by using a pulse ionization source[17] or by using activation methods that do not contribute excess neutrals, e.g., photodissociation,[12] electron-induced dissociation,[47] and (possibly) surface-induced dissociation.[14]

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