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Hadamard Transform Time-of-Flight Mass Spectrometry: A High-Speed Detector for Capillary-Format Separations

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This work demonstrates that with an intrinsic duty cycle of 50% and spectral storage speeds up to 277 spectra s^{-1} Hadamard transform time-of-flight mass spectrometry (HT-TOFMS) is a promising detector for any capillary-format separation that can be coupled to MS by electrospray ionization. Complete resolution of the components of a nine-peptide standard was achieved by coupling pressurized-capillary electrophoresis (pCE) to HT-TOFMS. The addition of pressure to the separation capillary decreased analysis times and stabilized the electrospray ionization source. Pulsed-pressurized injection of reserpine was used to experimentally simulate narrower peaks than those obtained in the pCE. HT-TOFMS was able to sample peaks having widths in the millisecond range.

The combination of capillary-format separations with mass spectrometric (MS) detection has proven to be a powerful technique for solving a diversity of problems.[1] Electrospray ionization (ESI) has become a preferred method for converting eluting solutes to gas-phase ions, thus making it possible to couple a multitude of separation techniques to MS. Depending on the efficiency of the separation, analyte peaks can have widths as narrow as hundreds of milliseconds. Consequently, MS detectors coupled to the separations must offer high speed. This requirement has proven to be a challenge.[1] This work focuses on the coupling of capillary electrophoresis (CE) to a newly developed high-speed MS detector that uses an on-line multiplexing technique. The discussion is valid, however, for any capillary-format technique.

While no type of mass analyzer is superior to others in all its characteristics, time-of-flight MS (TOFMS) detectors are the type most often coupled to separations, in large part because of their lack of expense. The most commonly used TOFMS setup is orthogonal-acceleration (OA) TOFMS,[2] which provides several pertinent advantages over conventional on-axis TOF instruments. The first is the improved spectral resolution that results when the ion packets are pulsed and accelerated in a direction perpendicular to their initial flight paths.[3] Another advantage of OA-TOFMS is the improved duty cycle. Ions are accumulated in an ion storage region during the analysis time of the preceding ion packet. Such storage leads to duty cycles between 5 and 15%, which is much higher than the few percent obtained in conventional TOFMS operated in beam raster mode.[4] OA-TOFMS also offers the ability to obtain spectra at a relatively high speed. Average reported speeds[5,6] range from 5 to 8 spectra s^{-1}, and the highest reported speeds[7] are near 80 spectra s^{-1}. Further improvements in the spectral acquisition rate of an OA-TOFMS are achieved by increasing the repetition rate of the repelling electrode, but a constant duty cycle cannot be sustained across the mass range.[4]

Hyperbolic ion trap TOF (IT-TOF) mass spectrometry is an alternative to OA-TOFMS,[8] but its moderate speeds[9] (1−4 spectra s^{-1}) limit its use to separations with peak widths greater than 5 s.[7] Linear ion traps are finding increased use as well and seem to be a promising alternative to hyperbolic traps owing to simple instrumentation and the ability to operate in several different modes.[10]

Recently, Brock et al.[11,12] developed a different approach that makes use of on-axis encoding of the ion beam followed by mathematical decoding of the acquired signal. This system achieves a 50% duty cycle over the entire mass range without sacrificing the high speed of TOFMS. As the technique makes use of a Hadamard convolution/deconvolution scheme, the name Hadamard transform time-of-flight mass spectrometry (HT-TOFMS) has been coined to describe it.

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In previous work,[13] theoretical background and experimental results were presented to demonstrate the capabilities and analytical advantages of HT-TOFMS using corona discharge ionization of N_2 and electrospray ionization of continuously infused solutions. In this work, HT-TOFMS has been coupled to a pressure-assisted capillary electrophoresis system to demonstrate its use in hyphenated separations and to explore the potential of HT-TOFMS to monitor transient signals.

EXPERIMENTAL SECTION

Standards, Reagents, and Sample Preparation. All reagents were refrigerated at 4 °C and only warmed to room temperature prior to use. Samples and standards were prepared in a CE buffer[14] consisting of a 50:50 v/v mixture of methyl alcohol (Merck, Darmstadt, Germany) and high-purity water (18 MΩ cm^{-1}) with 0.001 M acetic acid added (Sigma, St. Louis, MO). Both samples and buffer were filtered through a 0.45-μm Puradisc AS disposable cartridge (Whatman, Maidstone, U.K.). Bradykinin (Sigma), reserpine (11,17-dimethoxy-18-[(3,4,5-trimethoxybenzoyl)oxy]-, methyl ester, (3β,16β,17α,18β,20α)- (9Cl) (Aldrich, St. Louis, MO), and arginine (Sigma) were diluted in CE run buffer without further purification. A nine-peptide calibration standard was purchased from Sigma (Catalog No. P2693). The lyophilized peptide mixture was dissolved in 500 μL of the water−methanol buffer to give the following concentrations: bombesin, 31 μM; bradykinin fragment 1−5, 87 μM; leucine enkephalin, 90 μM; oxytocin, 50 μM; methionine enkephalin, 87 μM; substance P, 31 μM; human luteinizing release hormone, 42 μM, Arg8-vasopressin, 46 μM; and bradykinin, 47 μM. This solution was divided into 10 50-μL portions that were refrigerated until used.

The relationship between mass-to-charge ratio and time of flight was calibrated using the mass peaks obtained by spraying 100 μM solutions of a poly(propylene glycol) standard (Scientific Polymer Products Inc., Ontario, NY) with an average molecular weight of 450 amu, tetraethylammonium *p*-toluenesulfonate (Eastman Fine Chemicals, Rochester, NY), and tetrabutylammonium perchlorate (Aldrich). These last two reagents were also used for ultrafast continuous infusion experiments.

The reagents used for capillary surface derivatization were the following: sodium hydroxide (Sigma), trimethoxy(7-octen-1-yl)-silane (Fluka, St. Louis, MO), acetic acid (Sigma), ammonium peroxodisulfate (Fluka), *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED, Fluka), and [3-(methacryloylamino)propyl]trimethylammonium chloride; (MAPTAC, Aldrich). A solution containing 2% (w/v) MAPTAC, 0.15% (w/v) ammonium peroxodisulfate, and 0.2% (v/v) TEMED was freshly prepared prior to the final capillary-coating step.

Inner Surface Capillary Modification. To eliminate solute−wall interactions, which cause band broadening, 65 cm long, 100 μm i.d. × 360 μm o.d. fused-silica capillaries (Polymicro Technologies, Tucson, AZ) were coated according to the procedure using MAPTAC described by Kelly et al.[15] This procedure was chosen instead of the more traditional (aminopropyl)trimethoxysilane derivatization because of the extended lifetime of the coating and reduced "bleeding" of the modifying reagent.

Electrospray Emitters and Electrospray Interface. Several types of CE-ESI interfaces have been described in the literature.[16−20] We chose to use metallized sheathless electrospray emitters[21,22] to minimize unwanted dilution and distortion of the sample plug. These emitters exhibit exceptional stability and have a lifetime of several months if discharges within the ESI source are carefully avoided.

The sheathless electrospray emitters were constructed following the procedure described by Barnidge et al.[21,22] Briefly, the tip of a MAPTAC-coated fused-silica capillary was mechanically sharpened. Electrical contact between the tip and the flowing liquid was provided by Au particles (2-μm diameter, Aldrich) glued to the tip of the capillary with Pyrolin polyimide sealing resin (Supelco, St. Louis, MO). During the coating process, the capillary was constantly purged with N_2 to avoid clogging. The polyimide resin was cured overnight at 200 °C.

Electrospray ionization experiments were carried out in a previously described system[13] under two different modes: (1) continuous infusion experiments for signal-to-noise optimization (lens tuning and emitter positioning) and (2) CE−MS experiments. In the continuous-infusion mode, solutions were nebulized by applying 35 hPa (0.5 psi) to a sealed sample vial connected to the ESI emitter (Figure 1). The solutions were driven at a nominal flow rate of 795 nL min^{-1}. Then 2500 V was applied to the emitter tip that was mounted in an xyz micropositioning stage facing the grounded counter plate. This counter electrode was mounted on the first differentially pumped region of the mass spectrometer (operating at 2.7 Torr and 125 °C). After optimizing the position of the ESI tip, the intensity of the continuous signal varied by no more than 2% over a 20-min period. Improved electrospray stability was obtained by spraying off-axis from the grounded inlet. The gap between the emitter and the ground plate inlet was ∼3 mm, offset by 4 mm with respect to the ground plate orifice inlet. A skimmer (3-mm i.d.) selected the core of the supersonic jet produced by the ground plate orifice. Ions passing through were collected and transported to the focusing cylindrical lenses by an octopole ion guide (238 mm long, 3.175-mm rod diameter, 14 mm clearance between rods). Prior to CE−HT-TOFMS analysis, the HT-TOFMS was tuned up using a continuous infusion of a 100 μM bradykinin solution prepared in separation buffer. The signal was acquired, deconvoluted, saved, and plotted in real time.

Pressure-Assisted Capillary Electrophoresis. For CE−HT-TOFMS operation, a MAPTAC-coated capillary with a built-in ESI emitter was installed in a P/ACE model 2000 CE system (Beckman-Coulter, Fullerton, CA), fitted with an external detector adapter (Figure 1). The P/ACE instrument was modified to allow the application of external pressure to the sample vial during pressure-assisted CE (pCE).

Coupling the CE instrument to the MS required lengthening the separation capillaries (from 30 to 65 cm). To compensate for

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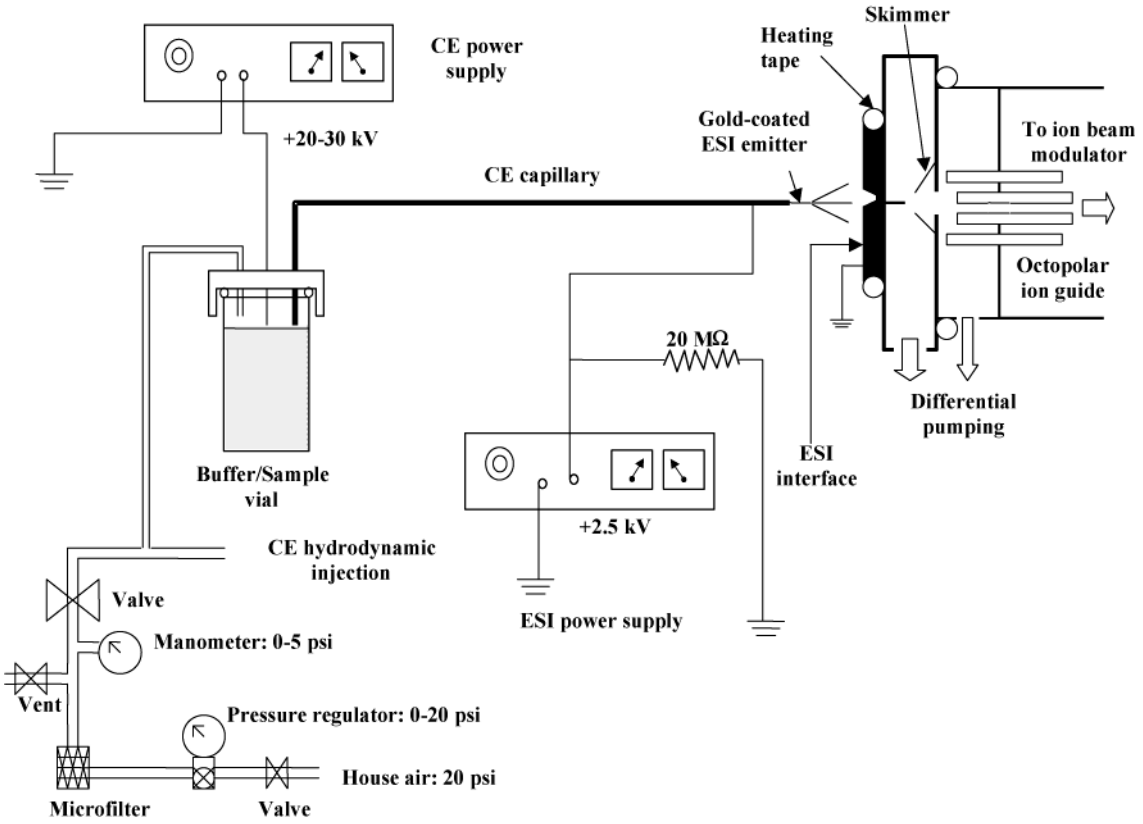

Figure 1. Schematic of the pressure-assisted CE-electrospray ionization interface.

this increase, compressed air was added to the internal nitrogen line used for sample injection. Several authors have shown that such pressure assistance can efficiently reduce CE analysis times.[23,24] Additionally, the increased flow rate stabilized the ESI.[25] The application of 14 hPa (0.2 psi) increased the flow rate by 300 nL min^{-1} and halved the analysis time. We believe that pressure-assisted operation is quite advantageous.

Figure 1 shows the electrical connections used for simultaneous CE and ESI operation. The CE separation voltage is applied to the sample vial by the P/ACE power supply (−28 kV against ground). Working under these conditions, the analytes are positively charged and migrate against the anodal electroosmotic flow.[15] During CE separations using UV or LIF detection, the outlet of the CE capillary is usually grounded. In CE−ESI-MS operation, the ESI spray voltage is applied to the capillary outlet by an external power supply (+2.2 kV against ground). The circuit is closed by the separation buffer wetting the gold-coated ESI emitter that is connected to the ESI power. The electrophoretic and electrospray currents share this common point, making it necessary to balance the currents in the system to avoid a voltage drop in the ESI source.[26] This balance was achieved by introducing a 20 MΩ resistor, as detailed in Figure 1.

In CE−HT-TOFMS mode, samples were hydrodynamically injected for 10 s (160 nL), followed by a 3-s buffer injection (50 nL). This buffer injection was performed to reduce analysis time[27] by pushing the sample plug closer to the capillary outlet. The MS data acquisition, the auxiliary pressure, and the separation voltage were turned on simultaneously. This procedure is simpler than the approach used by Schramel et al. in which the pressure was applied after the separation was complete.[28] The window for the UV detector (operated at 214 nm) was situated 25 cm downstream of the capillary inlet.

Hadamard Transform Time-of-Flight Mass Spectrometer. A detailed description of the operation of the HT-TOFMS spectrometer can be found in previous works.[11−13] Briefly, ions transported by the rf ion guide are collimated by a set of cylindrical lenses onto a finely spaced Bradbury−Nielson gate.[29] A pseudo-random voltage sequence[30] is applied to the two wire sets of this gate. This sequence is binary and periodic, repeating after 2047 elements (1's or 0's) have been applied to the wires. When a 0 is output to the gate, the voltage of one set of wires is held at −1235 V and the voltage of the other wire set is held at −1265 V; this setting causes the ions to be deflected. When a 1 is output to the gate, both wire sets are brought to −1250 V, the acceleration voltage of the ions, and the ions pass undeflected through the gate. Beyond the gate, ion packets are steered into a reflectron by a set of deflection plates and subsequently strike a 40-mm-diameter multichannel plate (MCP) detector. This detector is masked by a slit that prevents the deflected ions from being

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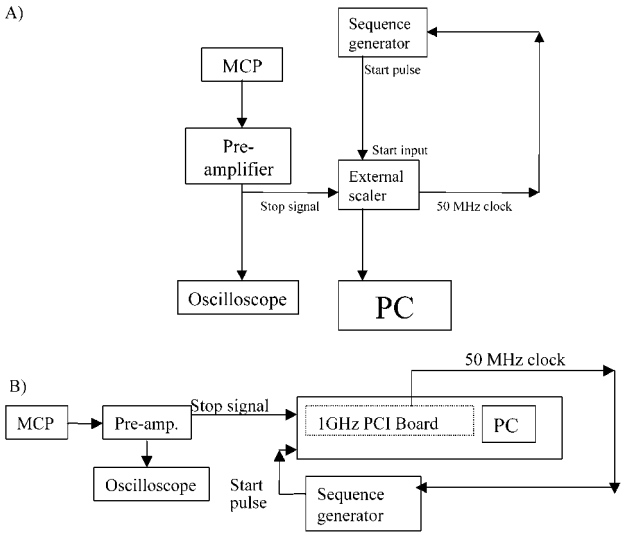

Figure 2. Different data acquisition setups for Hadamard transform time-of-flight mass spectrometry: (A) single external multichannel scaler with a custom-built external clock output; (B) single PCI multichannel scaler.

detected. By reconfiguring the modulation electronics, it is possible to use pseudorandom sequences with a larger (8191, 4095) or smaller (1023) number of elements. The time duration of each of the elements in the modulation sequence (also called "modulation bin width") is normally 100 ns (10 MHz), although 40 ns (25 MHz) can be used if improved resolution is desired.[13] A conventional TOF spectrum is recovered after deconvoluting the acquired signal with the inverse Hadamard transform.[31]

Data Acquisition System and Data Processing. Pulses produced at the MCP detector were amplified by a preamplifier (EG&G Ortec model VT120C, Oak Ridge, TN) and fed to the discriminator input of a multichannel scaler (EG&G Ortec model Turbo-MCS), which was customized by the manufacturer to provide a 50-MHz clock output for synchronization purposes (Figure 2A). A four-bit synchronous binary counter (MC74F161) divides this clock output down to the desired modulation frequency (10 or 25 MHz). This timing signal is used to clock the modulation electronics that drive the pseudorandom sequence generator. The multichannel scaler has an inherent end-of-sweep dead time that is a function of the number of bins in each sweep and the length of each bin. Operating with 2047 100-ns bins, the dead time is roughly 300 μs per sweep. When high spectral storage rates were required (more than 15 spectra min^{-1}), the Ortec multichannel scaler was replaced by a 1-GHz PCI-board time-to-digital converter (model P7886S, FastComtec GmbH, Oberhaching, Germany) operated in direct memory access (DMA) mode with a 4-ns bin width (Figure 2B). The dead time of this board is less than 200 ns per sweep, which can be accommodated in the last two elements of a 10-MHz Hadamard sequence. The net effect is zero dead time.

The nine-peptide separation data were smoothed using a sequential paired covariance algorithm.[32] An 11-point, third-degree Savitzky−Golay smoothing routine was applied to the ultrafast acquisition data.

RESULTS AND DISCUSSION

pCE−HT-TOFMS Analysis of a Nine-Peptide Mixture. A standard nine-peptide mixture was selected as a test sample to investigate the ability of HT-TOFMS to detect transient signals produced by the separation of a complex mixture. Figure 3 shows a contour plot (migration time vs mass-to-charge ratio) of the separation using pCE−HT-TOFMS with an auxiliary pressure of

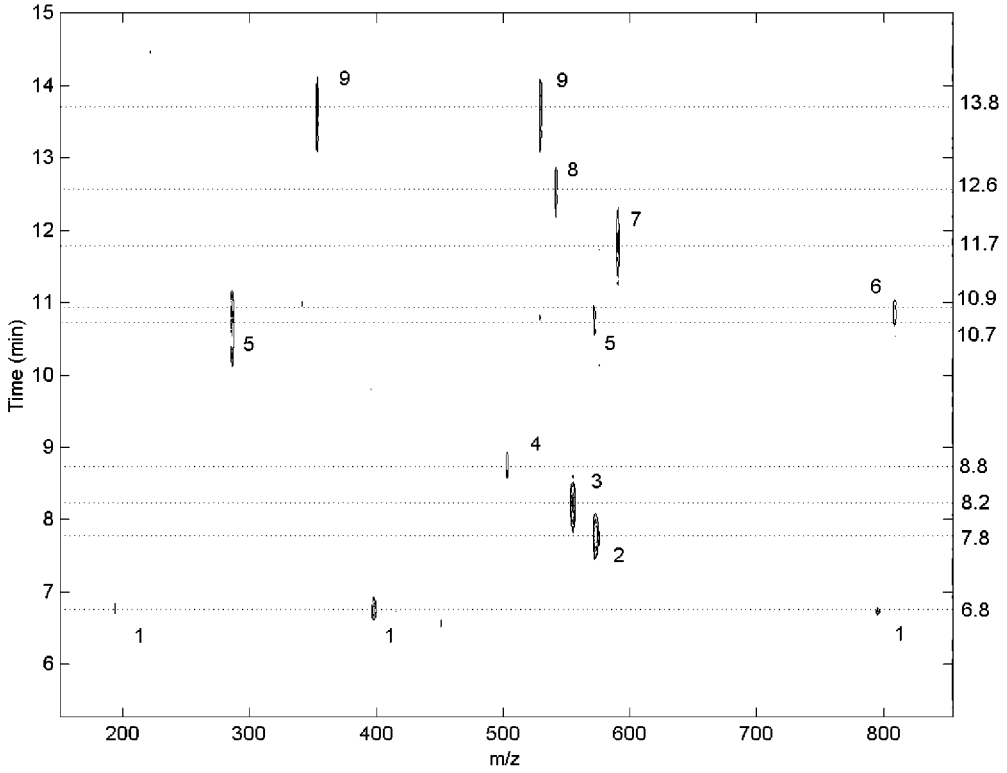

Figure 3. Separation of a nine-peptide mixture by pressure-assisted CE and electrospray-ionization Hadamard transform time-of-flight detection. Peak assignments: (1) bombesin, (2) bradykinin fragment 1−5, (3) leucine enkephalin, (4) oxytocin, (5) methionine enkephalin, (6) substance P, (7) human luteinizing release hormone, (8) Arg8-vasopressin, and (9) bradykinin. Auxiliary pressure, 7 hPa; separation voltage, −28 kV.

Table 1. Detection Limits of the Peptides Separation Shown in Figure 3

compound	detection limit (μM)	absolute detection limit (pmol)
bombesin	1	0.1
bradykinin fragment (1−5)	7	1.0
leucine enkephalin	4	0.6
oxytocin	26	4.2
methionine enkephalin	3	0.5
substance p	10	1.6
human luteinizing release hormone	7	1.1
Arg8-vasopressin	12	1.9
bradykinin	8	1.3

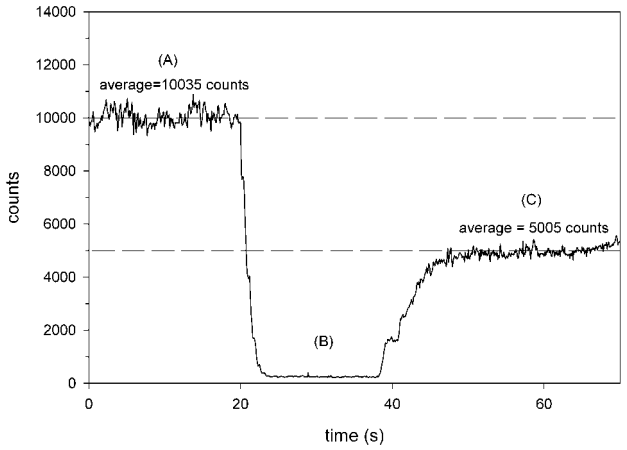

Figure 4. Changes in ion signal intensity when (A) no sequence is applied to the Bradbury−Nielsen gate, (B) a constant bias voltage is applied, and (C) a 10-MHz Hadamard sequence is applied to the gate. The signal corresponds to the total ion counts for poly(propylene-glycol), $\langle Mw \rangle = 450$, infused at a flow rate of 250 nL min^{-1}.

7 hPa. Complete separation was achieved for all of the peaks. Peaks five (methionine enkephalin) and six (substance P) are overlapped in the electrophoretic dimension, but the HT-TOFMS resolves these peaks satisfactorily. A partial overlap also occurs in the electrophoretic dimension between peak two (bradykinin fragment 1−5) and peak three (leucine enkephalin). The separation was completed in 15 min, a 45% reduction in analysis time compared to the nonpressurized CE separation.

The use of pressure distorts the plug profile typical of CE, superimposing a parabolic flow profile. This distortion leads to decreases in separation efficiency. As an example, the migration times of the first four species were each reduced by 50% when 7 hPa were applied. The effective plate number was reduced from 33 000 to 14 000 for bombesin, 3900 to 3000 for bradykinin fragment 1−5, 7900 to 2800 for leucine enkephalin, and 5900 to 3500 for oxytocin. The loss in efficiency is partially compensated for by the higher peak capacity obtained through the hyphenation of CE and MS.

Table 1 lists the detection limits for the nine peptides. These detection limits are in the low-micromolar range, which corresponds to absolute detection limits between 0.1 and 4.2 pmol. These detection limits are still far from the attomole level reported using CE and Fourier transform ion cyclotron resonance MS[33] but for many purposes will be quite adequate.

Duty Cycle, Mass Range, Data File Size, and Resolution of the HT-TOFMS Detector. Hadamard transform time-of-flight mass spectrometry offers unique possibilities when coupled to on-line separations. First and foremost, it offers a 50% duty cycle over an entire mass range. This efficiency may be observed in Figure 4 where the effect of applying a Hadamard sequence to a continuous ion beam obtained by electrospraying a 100 μM poly-(propylene glycol) solution is shown. The total signal, shown in Figure 4A, drops to values close to zero (∼2%), as shown in Figure 4B, if the ions are constantly deflected. When the ions are modulated following a Hadamard sequence (at 10 MHz in this particular example), the average total count number equals 50% of the original (nondeflected) signal, as shown in Figure 4C. This high duty cycle allows an increased spectral storage speed. For example, if a 2047-element 10-MHz sequence is used, 1024 packets (100 ns each) are detected per sweep. The multiplexing scheme used in HT-TOFMS improves the statistical efficiency, which is important in applications where the signal-to-noise ratio is low.[34] The increase in the injected number of ion packets produces a signal-to-noise ratio gain of $(N/2)^{1/2}$, where N is the number of elements in the sequence. The duty cycle could be increased to 100% (SNR gain of $(N)^{1/2}$) if a dual MCP configuration were used to detect all of the ion packets (deflected and undeflected).

A common bottleneck when coupling separation techniques with high-speed TOF detectors is the size of the data files

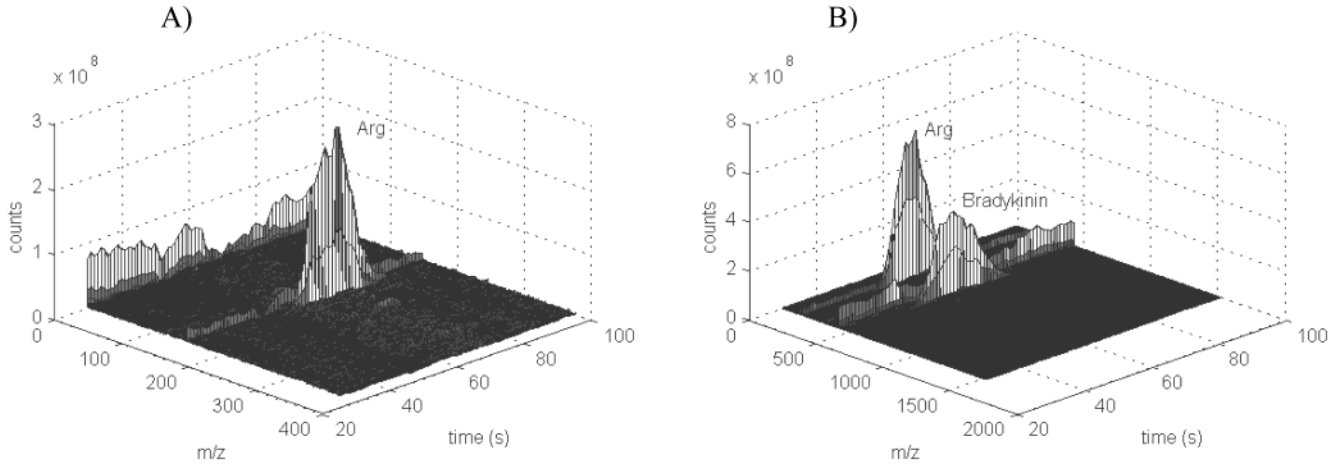

Figure 5. Effect of different Hadamard sequences on the mass range of the acquired mass spectra of eluting compounds: (A) arginine (*m*/*z* 175) scanned using a 1023-element Hadamard sequence; (B) arginine and bradykinin (*m*/*z* 531) scanned using a 2047-element sequence.

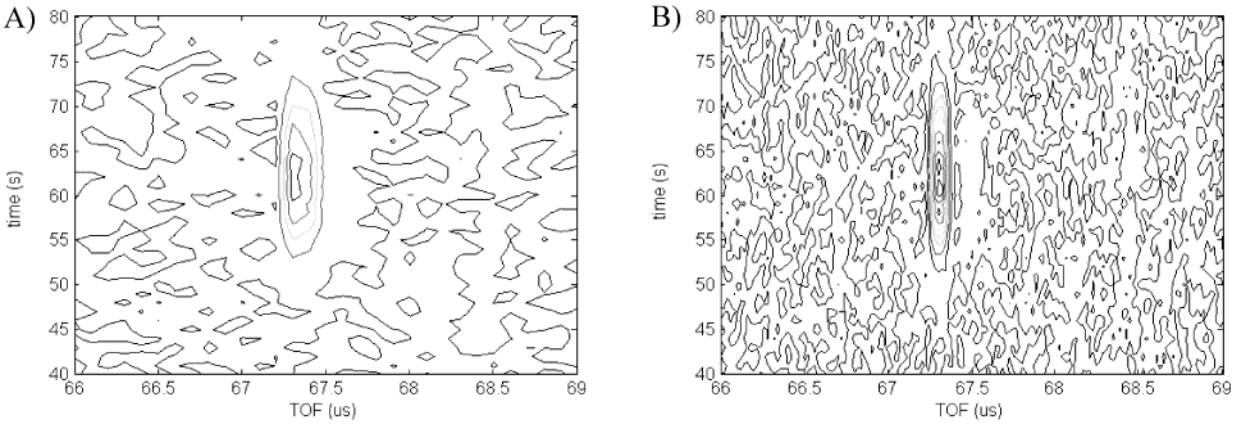

Figure 6. Effect of the acquisition frequency on spectral resolution: (A) contour plot of the arginine peak acquired at 10 MHz; (B) the same peak acquired at 250 MHz and deconvoluted in 20-ns intervals.

generated. An interesting characteristic of HT-TOFMS is that a decrease in the number of elements in the Hadamard sequence leads to a proportional reduction of the sweep length and the data file size. Short sequences are output at the same frequency as long ones, and thus, they do not require faster electronics. Figure 5A demonstrates this point where an arginine peak is acquired with a 1023-element Hadamard sequence. Applying this sequence at 10 MHz with a flight path of ~2.5 m and an acceleration voltage of −1250 V, results in a mass range of 380 Da, and a spectral acquisition rate of 10 000 sweeps s^{-1}.

If a higher mass compound is present, the mass range can be increased to 1.5 kDa by using a 2047-elements sequence. Figure 5B shows the real-time evolution of the mass spectra of two transient peaks of arginine and bradykinin acquired with a 2047-element sequence. In this case, the mass range was quadrupled while the file size was doubled. This mass range is sufficient for most peptides and multiply charged proteins. Higher mass ranges can be obtained by increasing the length of the sequence to values satisfying $2^n - 1$ (with $n = 12$, 13, etc.). The duty cycle is unaffected by changes in sequence length.

In our most common mode of operation for HT-TOFMS, the sequence is applied to the ion beam in 100-ns modulation bin widths and the data are collected by the multichannel scaler in 100-ns bins. This procedure yields the smallest file size. Alternatively, the convolution/deconvolution process can be carried out in a different way using the so-called oversampling procedure.[13] Instead of matching the modulation and acquisition bin widths, the data are collected in integer fractions of the modulation bin. For example, for a 100-ns modulation bin width, the multichannel scaler can sum counts in 5-, 10-, 20-, 25-, or 50-ns time bins. The inverse Hadamard transform is applied to each set of second, third, or whatever multiple was used in increasing the sampling density. Figure 6 compares the arginine peak contour under standard conditions (Figure 6A) and using 20-ns oversampling (Figure 6B). With no oversampling, the observed mass resolution is 180 ($m/\Delta m_{fwhm}$); if oversampling is used, resolution increases to 350. The resolution improvement obtained by oversampling comes at the expense of file size; a 60-s acquisition at a rate of 10 spectra s^{-1} with no oversampling produces a file of ~10 MB. If 20-ns oversampling is used, the file size increases to 45 MB. It must be emphasized that the laboratory-made software only provided the capability to perform the fast Hadamard transform algorithm and real-time visualization. Data compression has not been developed yet. Resolution can be further improved by shortening the modulation bin widths. If the ions are modulated each 40 ns, and a 20-ns oversampling is used, resolution for bradykinin can be increased to 1200. As explained in a previous paper,[13] by decreasing the modulation and acquisition bins to 20 and 5 ns, respectively, a significant improvement in both resolution and mass accuracy may be achieved (3300 and 70 ppm). The current electronics limit the ultimate performance of the system.

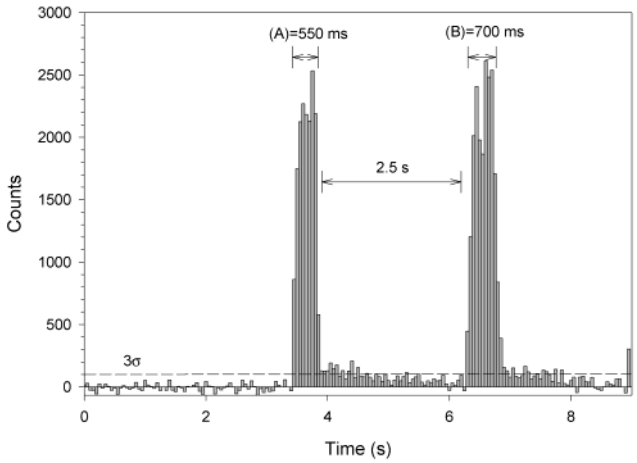

Figure 7. Scan of transient signals produced by pulsing the auxiliary pressure inlet. The sprayed solution is 100 μM reserpine at a flow rate of 795 nL min^{-1}. The spectral storage rate is 20 Hz, and the amount of reserpine detected in (A) is 729 fmol and in (B) is 861 fmol.

Spectral Storage Rate. The transient signals discussed in the previous sections have widths between 10 and 60 s. A spectral storage speed of 1 spectrum s^{-1} is more than sufficient to detect such peaks. Microchip and GC separations, which are also coupled to MS, have peak widths shorter than 1 s. To demonstrate the capabilities of HT-TOFMS at this time scale, we have created

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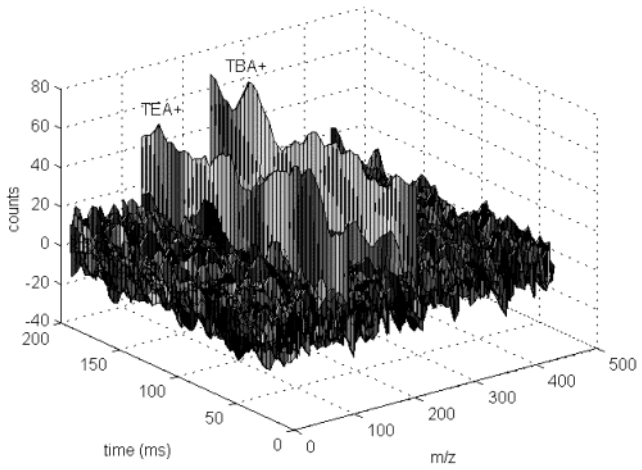

Figure 8. Ultrafast scanning of continuous infusion of a mixture of tetraethylammonium *p*-toluensulfonate (TEA, *m*/*z* 130) and tetrabutylammonium perchlorate (TBA, *m*/*z* 242). The spectral storage speed is 277 spectra s^{-1}.

millisecond peaks of a 100 μM solution of reserpine by pulsing the injection pressure of the continuous infusion setup at ~0.3 Hz. Figure 7 presents the time evolution of the peak at m/z 609 for two reserpine injections. With a spectral storage speed of 20 spectra s^{-1}, 11 spectra were recorded for peak A and 13 spectra for peak B.

The spectral storage speed can be made even faster. Figure 8 shows the results of a continuous infusion experiment in which spectra were collected at a rate of 277 spectra s^{-1}. This was accomplished by summing nine sweeps (2047 elements, 5 MHz) per spectrum. Each spectrum contains an average of 7 fmol of both tetraethylammonium and tetrabutylammonium ions. This spectral storage speed is 3.46 times higher than the fastest TOFMS instruments operating over a mass range of ~2 kDa.[7] It is worth noting that these results were obtained using an instrument whose ion optics can be further optimized. We expect that future improvements will push resolution and sensitivity even higher.

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