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Accurate Mass Measurements for Peptide and Protein Mixtures by Using Matrix-Assisted Laser Desorption/Ionization Fourier Transform Mass Spectrometry

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A new analytical scheme based on a combination of scanning FTMS, multiple-ion filling, and potential ramping methods has been developed for accurate molecular mass measurement of peptide and protein mixtures using broadband MALDI-FTMS. The scanning FTMS method alleviates the problems of time-of-flight effect for FTMS with an external MALDI ion source and provides a systematic means of sampling ions of different mass-to-charge ratios. The multiple-ion filling method is an effective way of trapping and retaining ions from successive ion generation/accumulation events. The potential ramping method allows the use of high trapping potentials for effective trapping of ions of high kinetic energies and the use of low trapping potentials for high-resolution detection of the trapped ions. With this analytical scheme, high-resolution broadband MALDI mass spectra covering a wide mass range of 1000−5700 Da were obtained. For peptide mixtures of mass range 1000−3500 Da, calibration errors of low part-per-millions were demonstrated using a parabolic calibration equation $f^2 = ML_1/m^2 + ML_2/m + ML_3$, where f is the measured cyclotron frequency and ML_1, ML_2, and ML_3 are calibration constants.

Mass spectrometry has made a profound impact on the current development of proteomic research[1−3] because of its high sensitivity and mass accuracy. Among various mass spectrometric techniques, matrix-assisted laser desorption/ionization (MALDI)[4] and electrospray ionization (ESI)[5] are the most commonly used methods to transform peptides/proteins into their characteristic gaseous ions for mass analysis. MALDI mass spectrometry is particularly suitable for analysis of tryptic digests because of the relatively homogeneous ionization efficiency for peptides of different masses and amino acid compositions and the simplicity of the mass spectrum generated.[6] An accuracy of 0.1 Da can normally be achieved for major digestion products in the mass range up to 3000 Da by using commercially available MALDI time-of-flight mass spectrometer equipped with delayed-ion extraction and reflectron mirror.[7] Further improvement in mass measurement accuracy is always desirable because of the increase in the confidence of identification of digestion products and hence facilitation of identification of target proteins.[8] One approach to improve the mass measurement accuracy (MMA) is to couple MALDI method to a Fourier transform ion cyclotron resonance mass spectrometer (FTMS). FTMS is an instrument that is capable of producing mass spectra with mass resolution in excess of hundreds of thousands.[9] High-mass resolution reduces the possibility of peak overlapping and improves mass measurement accuracy. Using a 7-T FTMS instrument, average MMA of 5.2 ± 7.4 and 0.7 ± 0.9 ppm have been observed by using external and internal calibration methods, respectively.[10] For FTMS instruments with external MALDI ion source, there exists a so-called time-of-flight effect in which ions of different mass-to-charge ratios arrive at the trapped-ion cell at different times.[11,12] Using typical experimental event sequences, lighter ions would enter and leave the trapped-ion cell before heavier ions would arrive. The effective mass range is therefore limited in MALDI-FTMS experiments. Li and co-workers[13] developed a master calibration equation for calibrating the FTMS instrument using spectra of peptide/protein standards acquired separately under different trapping conditions. An average accuracy of 3.3 parts-per-million (ppm) was observed for peptides/proteins with masses up to 5700 Da. However, it was noted that the exact masses of the melittin ions were miscalculated. For instance, the monoisotopic mass of melittin ions was calculated as 2845.738 Th rather than the expected 2845.761 Th.

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The calibration errors should therefore be larger than the reported values. In addition, the use of this advanced calibration equation does not alleviate the time-of-flight effect. Only samples with components falling in a narrow and preset mass range can thus be measured in a single analysis.

In an attempt to widen the effective mass range of MALDI-FTMS for unknown or mixture analysis, we previously described a "scanning" FTMS method.[12] This method was based on the spectral averaging method used in the gated deceleration experiments by Wilkins' group[14] for polymer analysis in a FTMS with internal MALDI ion source. With stepwise changes in the time interval between the ionization pulse and the gating of the trapping voltage (T_{gate}) while summing individual MALDI-FT mass spectra, a mixture of peptides with m/z range of 150−2000 Th had been obtained. However, there are several drawbacks associated with this "scanning" FTMS method. Since the initial desorption kinetic energy of MALDI desorbed ions are known to increase with their mass-to-charge ratios,[15] the trapping efficiency of high-mass ions is always lower in comparison with the low-mass ions. This is particularly true when low trapping potentials are used during the ion accumulation process. Although the use of a high trapping potential might reduce this mass discrimination effect, high trapping potentials would induce a substantial space-charge effect and lower the mass spectral resolution. A practical solution involves the use of a gas-assisted ion cooling/potential ramping method.[13,16] Ions of interest are first trapped using high trapping potentials and are subsequently cooled by a short pulse of inert gas through multiple low-energy collisions. The trapping potentials are then ramped down to low values before the ion excitation and detection processes. However, this gas-assisted ion cooling/potential ramping method is difficult to implement in conjunction with the "scanning" FTMS method because of the time-consuming gas-assisted ion cooling procedure. In addition, it is known that the buildup of noise is proportional to the square root of the number of spectral summation. Since the ions of a particular m/z would only be trapped and detected at a narrow range of T_{gate} values, the buildup of ion signal might even be less efficient than that of the noise. The "scanning" FTMS would intrinsically lead to poor signal-to-noise ratios. Furthermore, it is known that the precise cyclotron frequencies of particular ions are affected by the total abundance of the trapped ion, i.e., the space-charge effect. In the "scanning" FTMS method, ions of different mass-to-charge ratios are sampled in different acquisitions and the ion abundance in different acquisitions might differ from one another. The measured cyclotron frequencies for ions of different mass-to-charge ratios would deviate from the expected values using fixed calibration constants. This would prohibit the use of exact mass measurement for analysis of unknown mixtures.

With the aim of extending the accessible mass range of the boardband MALDI-FTMS for analysis of unknown mixtures, we have derived a new analytical scheme based on a combination of a multiple-ion filling technique and potential ramping method. With this analytical scheme, we have been able to obtain high-resolution broadband MALDI mass spectra covering a wider mass range of 1000−6000 Th. Using a protein mixture covering this mass range, a mass measurement accuracy of a few parts-per-million was demonstrated.

EXPERIMENTAL SECTION

Materials. All materials were obtained commercially and were used without further purification. Luteinizing hormone releasing hormone (LHRH), gramicidin D, adrenocorticotropic hormone fragment 18−39 (ACTH), melittin, insulin chain B, and bovine insulin were obtained from Sigma Chemical Co. (St. Louis, MO). α-Cyano-4-hydroxycinnamic acid (α-CCA), 3-aminoquinoline (3-AQ), and glycerol were purchased from Aldrich Chemical Co. (Dorset, England). Methanol (HPLC grade) was obtained from Labscan Ltd. (Bangkok, Thailand).

Instrumentation. All experiments described in this paper were performed by using a 4.7-T FTMS system (APEX I, Bruker Instrument Inc., Boston, MA) equipped with a homemade external MALDI ion source. A nitrogen laser (λ = 337 nm) (VSL-337ND, Laser Science Inc., Newton, MA) was used to produce short pulses (3 ns) of photons. Details of the instrumental arrangement have previously been reported.[17] To perform potential ramping experiments, the cell voltage control board inside the internal electronic unit (IEU) was modified to include an add-on voltage control board, as shown in Scheme 1. Basically, a pair of fast switches was installed to toggle the voltages for the front (PV1) and rear (PV2) trapping plates between the computer-preset values (i.e., SET PV1 and SET PV2) and the hardware-preset values (PP PV1 and PP PV2). The computer-preset values were software controlled by the Xmass 4.0, whereas the hardware-preset voltages were manipulated by two precision potentiometers. A TTL pulse was used to toggle the trapping potentials between these sets of values. All MALDI-FT mass spectra were acquired in broadband mode using a 128-kbyte data set. The time-domain signals were zero-filled twice and multiplied with a trapezoid function prior to Fourier transformation.

Sample Preparation. All samples were prepared using a matrix solution composed of α-CCA, 3-AQ, and glycerol. Details of the performance of this matrix solution were previously described.[17] Briefly, α-CCA, 3-AQ, and glycerol were mixed with a weight ratio of 1:4:6 and the resulting slurry was treated in an ultrasonic bath until a transparent viscous matrix solution was obtained. All analyte solutions were prepared at concentration of 500 pmol/μL. LHRH, gramicidin D, and melittin solutions were prepared in methanol; whereas insulin chain B and insulin solution were dissolved in 1:1 methanol/water (v/v), with the addition of 1% acetic acid. Then 3.0 μL of each analyte solution was mixed with 3.0 μL of the matrix solution in an Eppendorf vial. The mixture was purged under a stream of dry nitrogen to remove volatile components. A 1.0-μL aliquot of the resulting sample was placed onto the sample plate and was inserted into the mass spectrometer for analysis.

RESULTS AND DISCUSSION

Multiple-Ion Filling (MIF). The technique of multiple-ion filling constitutes the key for this analytical scheme. This

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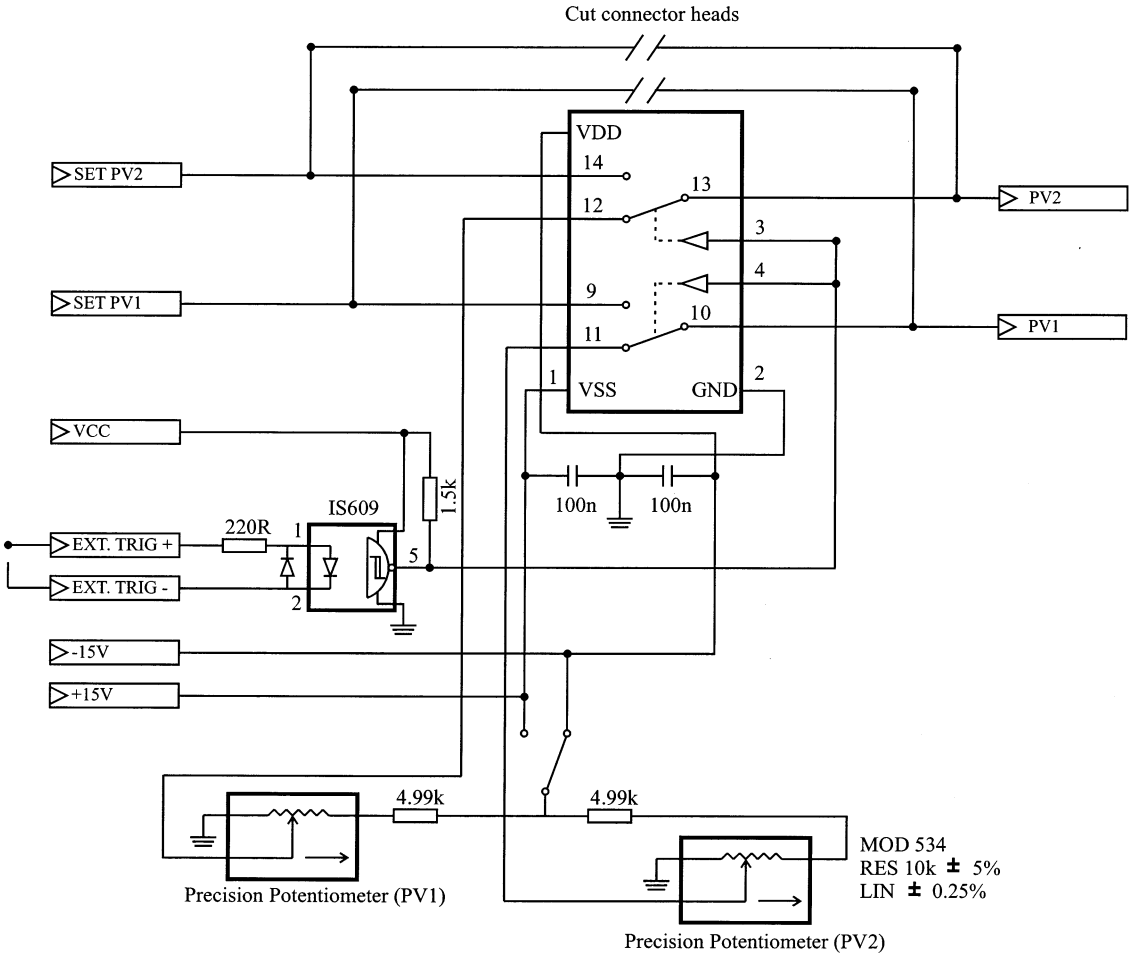
Scheme 1. Circuit Diagram of the Add-On Voltage Control Board for Potential Ramping Experiments



technique was originally developed[18] for boosting the analyte signal intensity by trapping and retaining analyte ions from successive laser shots. Scheme 2 shows a diagram of the pulse sequence for the multiple-ion filling experiment. At the beginning of each acquisition, a quench control pulse was first sent to the cell voltage control board to change the potential of the ring electrode located at the rear trapping plate to −10 V for removal of residual positive ions in the trapped-ion cell. After this "quenching" pulse, a short delay D1 was applied for the restoration of the ring electrode potential back to the preset voltage of PV2. An "ion accumulation" pulse was executed, and the voltages at the deflector electrodes and the entrance electrodes of the Infinity Cell, were adjusted to some preset values to direct the ion beam toward the analyzer cell. A delay of 1 ms (D2) was given for the implementation of these voltages. The nitrogen laser was then triggered. Ions generated were transmitted to the Infinity Cell by using a series of electrostatic ion lens. The deflector voltages and the cell entrance potentials were restored to ground voltage and the preset PV1, respectively, after the ion accumulation pulse. The length of the ion accumulation pulse (which is subsequently called "gate time" or "T_{gate}") governs the m/z's of the trapped ions. An empirical relationship between the m/z and T_{gate} had previously been quantified and reported.[12] After the ion accumulation pulse, a pulse of carbon dioxide gas (∼1300−1500 μs) was introduced into the trapped-ion cell to remove excess translational energy of the MALDI-derived ions through presumably multiple low-energy collisions. During the gas pulsing, the pressure inside the trapped-ion cell was raised temporally to about 10^{-6}−10^{-7} Torr. After a short pumping delay of 0.1−1.0 s (D4), the pulse program was looped back to the ion accumulation event. The number of looping cycles determines the signal amplification factor. When the multiple-ion filling method is used for signal amplification, the ΔT is set to zero. After a fixed number of cycles, a long pumping delay of 5−20 s (D5) was applied to restore the cell pressure to less than 10^{-8} Torr prior to ion excitation and detection. Panels a and b of Figure 1 show the MALDI-FTICR mass spectra of LHRH with and without the use of MIF, respectively. Figure 1a was obtained by using five successive ion accumulations and gas-pulsing (i.e., five cycles of MIF). Figure 1b was obtained with a single laser shot. Both spectra were obtained at the same sampling spot using the same laser irradiance. The trapping potentials were set at 1.0 V, and the T_{gate} was 1250 μs. From the relative intensities of LHRH molecule ion signals, it is demonstrated that the high-pressure pulse in the trapped-ion cell can effectively remove excess z-axial translational energy of the MALDI-derived ions and prevent substantial loss of the trapped ions during the admission of another pulse of ions. After showing the capability of MIF for increasing the sensitivity of the detection, attempts have been made to analyze higher molecular weight compounds. Panels a

(18) Private communications with Dr. Christian Berg, Bruker Daltonic Inc.

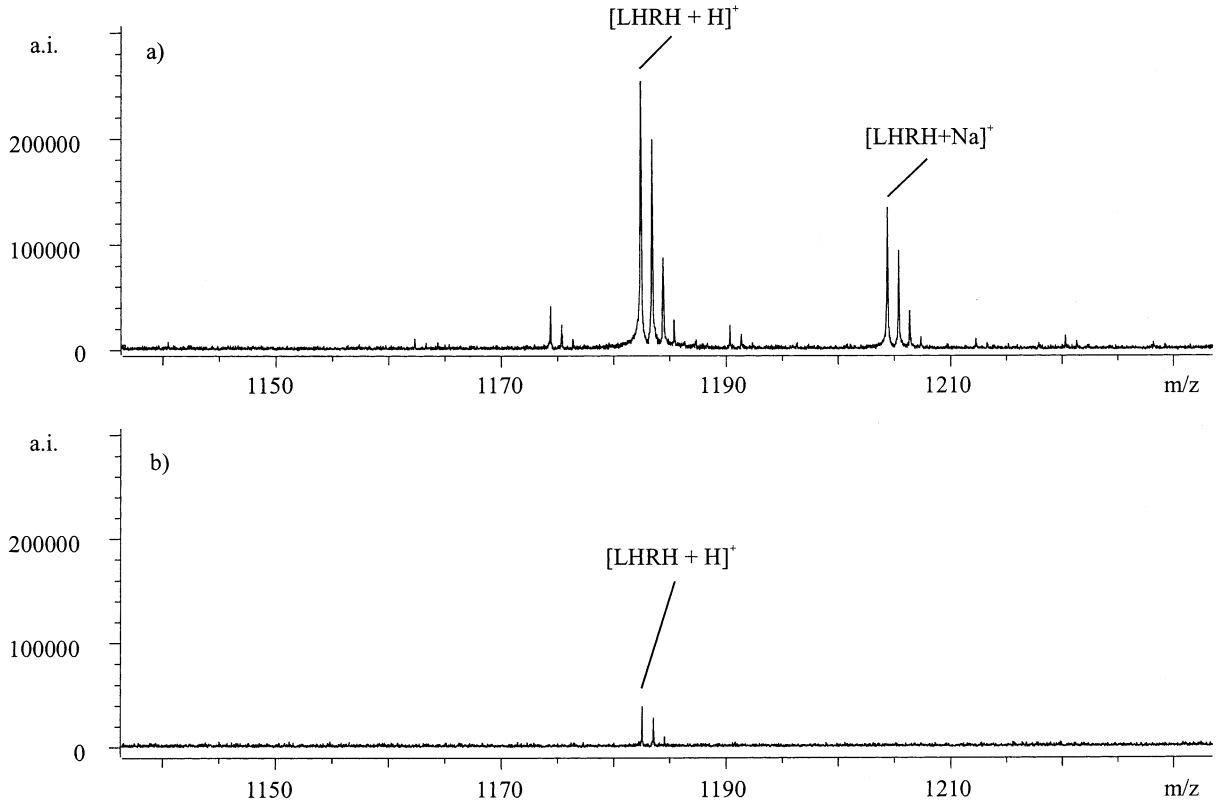
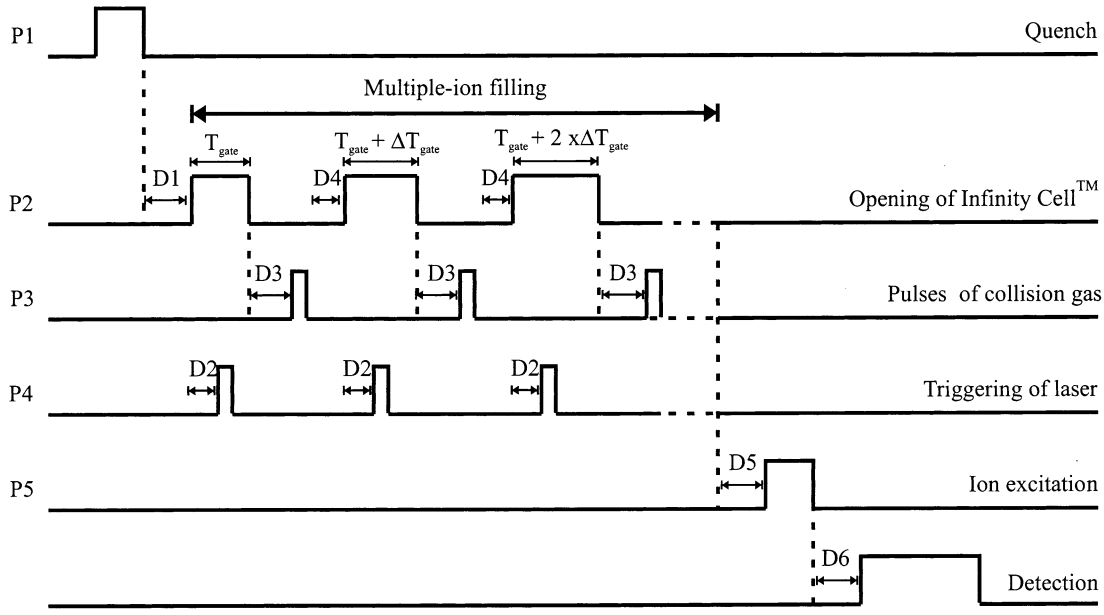


Figure 1. Single-scan MALDI-FT mass spectra of luteinizing hormone releasing hormone (a) with five cycles of multiple-ion filling and (b) without the use of multiple-ion filling. α-CCA/3-AQ/glycerol was used as matrix solution.

Scheme 2. Diagram of the Pulse Sequence for the Multiple-Ion Filling Experiment Using MALDI-FTMS.



D1 : Quenching Delay
D2 : Laser Triggering Delay
D3 : Trapping Delay
D4 : Pumping Delay
D5 : Pumping Delay
D6 : Excitation Delay

and b of Figure 2 show the MALDI-FTICR spectra of bovine insulin with and without the use of MIF, respectively. The trapping potentials were set at 3.0 V, and the T_{gate} was 1850 μs as determined from the equation derived previously.[12] Figure 2a was obtained by using four cycles of MIF, and Figure 2b was obtained by summing four single-shot spectra. With the use of MIF, a series of board peaks was observed. These peaks were tentatively attributed to the multiple adduction of alkali metal ions, such as sodium and potassium ions, onto the insulin molecules. By comparing Figure 2a and b, it is clearly demonstrated that MIF

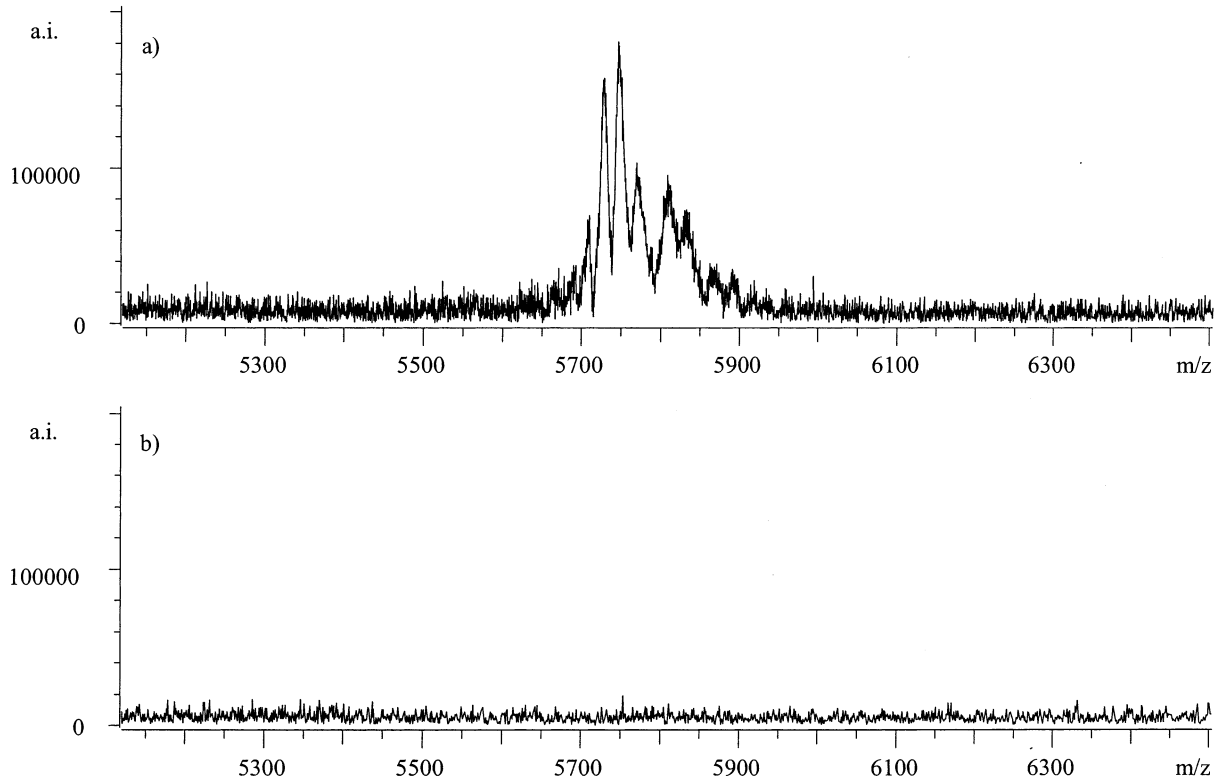


Figure 2. MALDI-FT mass spectra of bovine insulin (a) with four cycles of multiple-ion filling and (b) without the use of multiple-ion filling. Trapping voltages were at 3.0 V. α-CCA/3-AQ/glycerol was used as matrix solution.

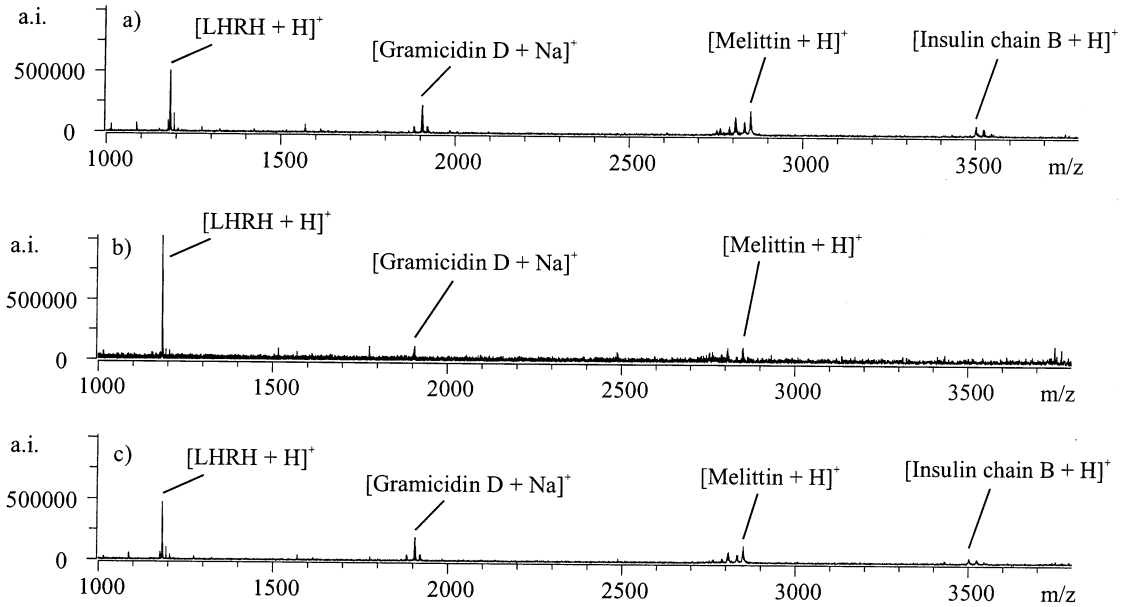


Figure 3. MALDI-FT mass spectra of a peptide mixture of LHRH, gramicidin *D*, melittin, and insulin chain B using (a) scanning MIF-FTMS method with T_{gate} incremented from 800 to 2600 μs, (b) "scanning" FTMS method, and (c) scanning MIF-FTMS method with T_{gate} decremented from 2600 to 800 μs. α-CCA/3-AQ/glycerol was used as matrix solution.

can effectively enhance the molecule ion signals for high-mass ions.

Scanning MIF. For analysis of unknown mixtures, the multiple-ion filling experiment was modified to include a variable term in the gate time (i.e., $\Delta T_{gate} \neq 0$). The initial T_{gate} was initially set at a low value and was increased by a stepwise increment at each cycle of the MIF. The number of cycles would therefore determine the range of the T_{gate} and was calculated in accordance with the acquisition mass range using a predetermined time-of-flight equation. Figure 3a shows the "multiple-ion filling" MALDI-FTICR mass spectrum of a peptide mixture comprising LHRH (m/z = 1183 Th), gramicidin *D* (m/z = 1880 Th), melittin (m/z = 2846 Th), and insulin chain B (m/z = 3465 Th). The sample was prepared using α-CCA/3-AQ/glyceol as matrix solution. The T_{gate} was initially set at 800 μs. In each cycle, the value of T_{gate} was increased incrementally by 100 μs, and the T_{gate} was scanned

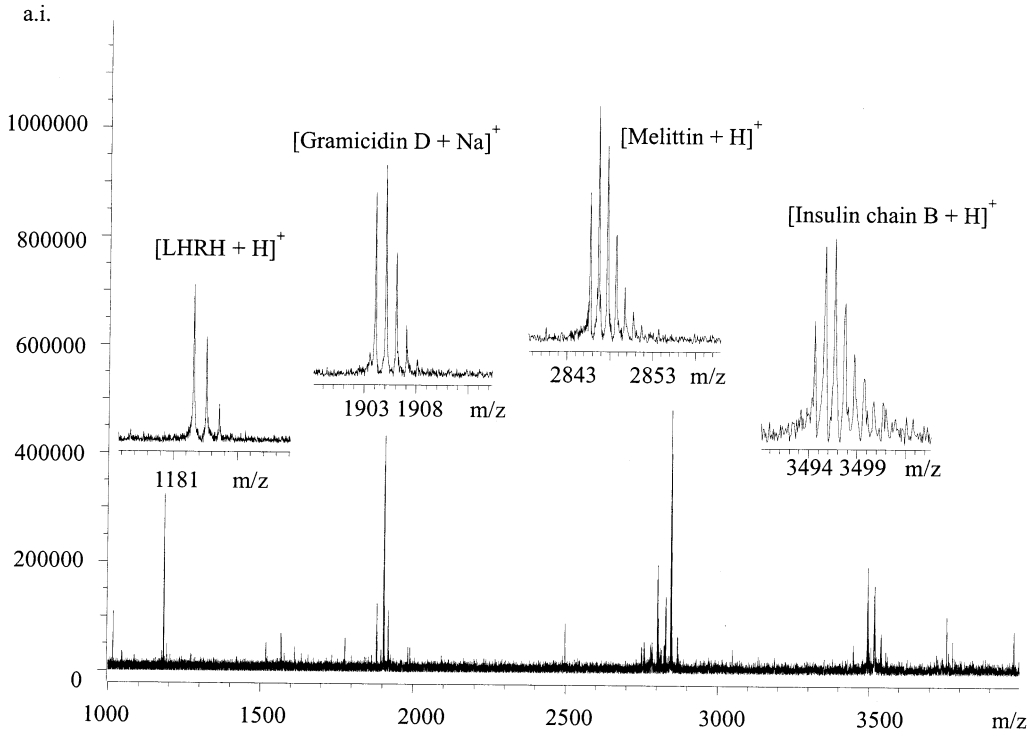


Figure 4. MALDI-FT mass spectrum of a peptide mixture of LHRH, gramicidin D, melittin, and insulin chain B using scanning MIF-PR-FTMS method. T_{gate} was incremented from 800 to 2600 μs, and the trapping potentials were ramped from 3.0 to 1.0 V (front trapping electrode) and 0.13 V (rear trapping electrode). α-CCA/3-AQ/glycerol was used as matrix solution.

from 800 to 2600 μs. Twenty scans were summed to improve signal-to-noise ratios. A static trapping voltage of 3.0 V was used. To illustrate the effectiveness of this MIF method, the same mixture was analyzed using the previously described "scanning" FTMS pulse program.[12] For consistency, a total of 360 single-shot FID spectra were accumulated. The resulting spectrum was shown in Figure 3b. Using the scanning FTMS method, the signal intensities of gramicidin D, melittin, and insulin chain B were very low. The trapping efficiency for ions of m/z above 2000 is relatively low even at trapping potentials of 3 V. Using the scanning MIF FTMS method, the ion signals for gramicidin D, melittin, and insulin chain B were significantly enhanced. Substantial reduction of the noise level has also been observed for spectra obtained using the MIF FTMS method. One of the main concerns for the use of MIF method is the possibility of leakage of trapped ions during the subsequent ion-filling processes. To evaluate the possibility of ion leakage, the MIF experiments were repeated by reversing the sequence of T_{gate} increment, i.e., from 2600 to 800 μs. If ion leakage exists, the relative signal intensities for these peptide species would be changed and the signals at the high-mass end would be suppressed. The resulting MIF scanning mass spectrum is shown in Figure 3c. There was no obvious change in the relative abundance of different components. It is therefore concluded that there is no evidence for significant ion leakage during the MIF procedures.

Scanning with Potential Ramping (PR). One of the main objectives for obtaining the broadband MALDI mass spectrum is to improve the mass measurement accuracy. Using the MIF method with a static trapping potential of 3 V, the mass resolution was limited due presumably to the substantial space-charge effect. The isotopic species of insulin chain B could only be partial resolved. A logical solution was to include the potential ramping method with the scanning MIF experiment. By reducing the trapping potential prior to ion excitation and detection, the space-charge effect could be minimized. Figure 4 shows the scanning MIF mass spectrum of the peptide mixture comprising LHRH, gramicidin D, melittin, and insulin chain B. In this experiment, the trapping voltages were set at 3 V on both trapping electrodes and were ramped to 1.0 (PP PV1) and 0.13 V (PP PV2). As expected, the mass resolution was much improved. All components were isotopically resolved. The mass resolution of the insulin chain B ions was ~20 000 (fwhh). From the time-domain representation (data not shown), it was found that the FID signal was truncated because of insufficient memory. In the present instrumentation, 128 kbytes was the largest data size for broadband acquisition. The spectral resolution could be higher if a larger data size is available. To evaluate the mass accuracy, the same MALDI-FTMS measurement was repeated three times. The cyclotron frequencies for the two most abundant isotopic peaks of each molecule ion were extracted and were calibrated using two different calibration equations:

$$f = \mathrm{ML}_1/m + \mathrm{ML}_2 \quad (1)$$

$$f^2 = \mathrm{ML}_1/m^2 + \mathrm{ML}_2/m + \mathrm{ML}_3 \quad (2)$$

in which f is the measured cyclotron frequency in hertz of ion species i; m_i is the theoretical mass of the same ion; and ML_1, ML_2, and ML_3 are calibration constants. Equation 1 is a linear calibration equation requiring the measurement of the cyclotron frequencies of two or more ions of known masses for experimental evaluation of the calibration constants, ML_1 and ML_2. This

Table 1. Accuracy of Mass Measurements for a Peptide Mixture Composed of LHRH, Gramicidin D, Melittin, and Insulin Chain B Using the Scanning MIF-PR Method

peptide molecule ions	theoretical m/z (Th)	experiment 1 measured freq (Hz)	experiment 1 calibrated m/z (Th)	experiment 1 error (ppm)	experiment 2 measured freq (Hz)	experiment 2 calibrated m/z (Th)	experiment 2 error (ppm)	experiment 3 measured freq (Hz)	experiment 3 calibrated m/z (Th)	experiment 3 error (ppm)
(a) Calibration Equation: $f = ML_1/m + ML_2$										
$[LHRH + H]^+$	1182.5802	60 988.542	1182.5811	+0.77	60 988.389	1182.5815	+1.08	60 988.565	1182.5811	+0.74
	1183.5831	60 936.804	1183.5850	+1.60	60 936.681	1183.5848	+1.46	60 930.810	1183.5853	+1.89
$[gramicidin D + Na]^+$	1904.0597	37 875.748	1904.0538	−3.08	37 875.626	1904.0535	−3.29	37 875.752	1904.0537	−3.16
	1905.0627	37 855.814	1905.0562	−3.39	37 855.683	1905.0563	−3.36	37 855.834	1905.0553	−3.90
$[melittin + H]^+$	2846.7642	25 330.354	2846.7451	−6.71	25 330.262	2846.7408	−8.23	25 330.359	2846.7432	−7.38
	2847.7670	25 321.431	2847.7479	−6.70	25 321.328	2847.7448	−7.78	25 321.457	2847.7437	−8.10
$[insulin chain B + H]^+$	3495.6535	20 626.447	3495.6712	+5.06	20 626.181	3495.6943	+11.67	20 626.337	3495.6870	+9.57
	3496.6559	20 620.300	3496.7129	+16.29	20 620.244	3496.7003	+12.71	20 620.315	3496.7074	+14.74
av error				5.45			6.12			6.19
statistical error										5.9 ± 0.4
(b) Calibration Equation: $f^2 = ML_1/m^2 + ML_2/m + ML_3$										
$[LHRH + H]^+$	1182.5802	60 988.542	1182.5796	−0.49	60 988.389	1182.5799	−0.25	60 988.565	1182.5794	−0.64
	1183.5831	60 936.804	1183.5836	+0.38	60 936.681	1183.5832	+0.12	60 930.810	1183.5837	+0.51
$[gramicidin D + Na]^+$	1904.0597	37 875.748	1904.0617	+1.07	37 875.626	1904.0619	+1.17	37 875.752	1904.0625	+1.45
	1905.0627	37 855.814	1905.0642	+0.76	37 855.683	1905.0648	+1.09	37 855.834	1905.0641	+0.71
$[melittin + H]^+$	2846.7642	25 330.354	2846.7503	−4.89	25 330.262	2846.7465	−6.23	25 330.359	2846.7490	−5.34
	2847.7670	25 321.431	2847.7531	−4.89	25 321.328	2847.7505	−5.80	25 321.457	2847.7494	−6.17
$[insulin chain B + H]^+$	3495.6535	20 626.447	3495.6505	−0.86	20 626.181	3495.6721	+5.31	20 626.337	3495.6640	+3.01
	3496.6559	20 620.300	3496.6921	+10.34	20 620.244	3496.6781	+6.34	20 620.315	3496.6844	+8.15
av error				2.96			3.29			3.25
statistical error										3.2 ± 0.2

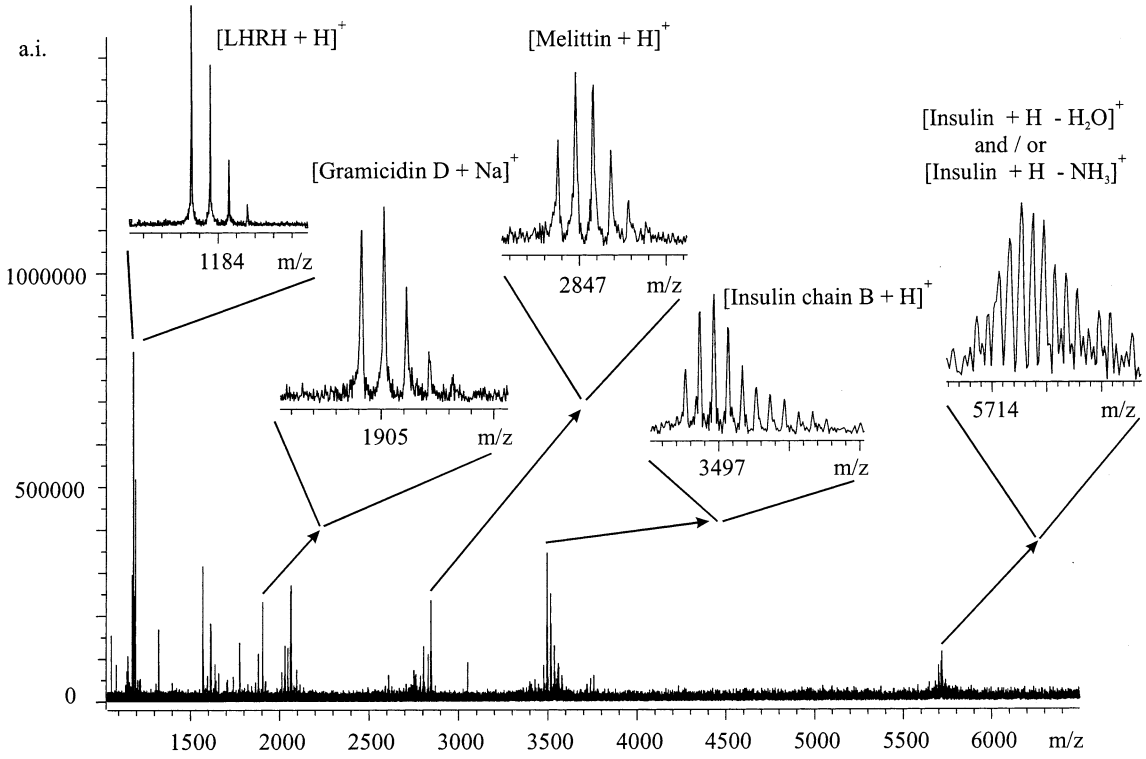


Figure 5. MALDI-FT mass spectrum of a peptide mixture of LHRH, gramicidin D, melittin, insulin chain B, and insulin using scanning MIF-PR-FTMS method. T_{gate} was incremented from 800 to 2600 μs, and the trapping potentials were ramped from 3.0 to 0.2 V (front trapping electrode) and 2.5 V (rear trapping electrode). α-CCA/3-AQ/glycerol was used as matrix solution.

equation is implemented in the operating system of our system, i.e., XMASS 4.0, for instrumental calibration. Equation 2 is a parabolic calibration equation requiring three or more calibration standard peaks for evaluation of the calibration constants, ML_1, ML_2, and ML_3. It has been suggested that eq 1 is a good approximation for narrow mass range calibration. Over a wider mass range, the parabolic calibration, i.e., eq 2, should give substantially better mass accuracy than the linear procedure, i.e., eq 1.[20]

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Table 1 summarizes the mass measurement accuracy of the scanning MIF-PR protocol for measurement of a peptide mixture using two different calibration equations. Table 1a utilizes eq 1 for calibration and gives an average mass measurement error of ~5.9 ± 0.4 ppm. Close inspection of the measurement errors across the mass range reveals a systematic variation of the errors. This implies that the higher-order correction term is no longer negligible in the presence case. By utilizing the parabolic equation for calibration, a substantial improvement in the mass measurement accuracy was obtained, as shown in Table 1b. An average mass measurement error of 3.2 ± 0.2 ppm was obtained by incorporating a higher-order correction terms.

To demonstrate the applicability of this method for wider mass range, bovine insulin (MW 5734 Da) was added into the peptide mixture. Figure 5 shows the scanning MIF-PR mass spectrum of a peptide mixture consisting of LHRH, gramicidin D, melittin, insulin chain B, and bovine insulin. The initial trapping potentials for both PP PV1 and PP PV2 were set to 3.0 V, and the final trapping potentials prior to excitation were 0.2 (PP PV1) and 2.5 V (PP PV2). Ion signals derived from different molecules can clearly be identified. Under the presence experimental conditions, the most predominant ion signals for bovine insulin correspond to the dehydrated (or deaminated) species. The intensity of intact protonated molecule ion of bovine insulin was weak. Without any knowledge on the relative contribution of dehydrated and deaminated species, theoretical values for the most abundant peaks of the isotopic cluster could not be obtained. No attempt was therefore made to assess the measurement accuracy at this mass range. Nevertheless, the dehydrated (or deaminated) insulin peaks were isotoptically resolved with a mass resolution of ~14 000 fwhh.

It is important to note that unknowns can be analyzed without the need to add internal calibration compounds into the sample during sample preparation. Calibration compounds and analyte sample can be prepared separately in adjacent positions on the sample holder. By toggling the sampling position while performing MIF experiments, ions of the unknown sample and calibration compounds can be sequentially injected into (and retained at) the trapped-ion cell prior to ion excitation and detection. Apart from analysis of unknowns and complicated mixtures, this scanning MIF-PR method should also be useful for the characterization of polymers. Having alleviated the time-of-fight effect and improved the trapping efficiency of high-mass ions, this MALDI-FTMS method should be able to analyze low-/medium-mass polymers (M_n < 10 000, where M_n is the number-averaged molecular weight) and provide important physical information, such as number-averaged molecular weight and molecular weight distribution. Through accurate molecular mass measurement and tandem mass spectrometry, end-group structures of the polymer could be obtained. Experimental evaluation of the utility of this scanning MIF-PR method for polymer analysis is currently under investigation.

CONCLUSIONS

We have developed an effective analytical scheme based on a combination of scanning FTMS, multiple-ion filling, and potential ramping methods for high-resolution detection of MALDI-derived ions of extended range of mass-to-charge ratios in a Fourier transform mass spectrometer. Baseline-resolved broadband MALDI mass spectra covering mass ranges as large as 1000−5700 Da were obtained. It has also been demonstrated that a parabolic equation is more appropriate than a linear equation for calibrating mass spectra covering such a wide mass range. Using a parabolic calibration equation, an average mass measurement error of 3.2 ± 0.2 ppm was achieved for peptide mixtures composed of LHRH, gramicidin D, melittin, and insulin chain B.

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