A High Resolving Power Multiple Reflection Matrix-assisted Laser Desorption/Ionization Time-of-flight Mass Spectrometer

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Two electrostatic mirrors, mounted symmetrically on the same optical axis facing each other, are used to increase the time-of-flight of molecular ions produced in matrix-assisted laser desorption/ionization (MALDI). The mirrors, which are used in the non-compensating mode, are located between a MALDI ion source and a stop detector. The source is operated at 10.5 kV acceleration voltage using the delayed extraction technique. The high voltage for the mirror arrangement is switched on after the desorption event when the molecular ions have drifted into the region between the mirrors. The ions are trapped by successive reflections of the opposite electrostatic fields in the mirrors until the electric fields are switched off. The number of reflections depends on the speed of the ions when they enter the mirror trap and the on-time of the mirrors. When the electric fields are removed during the motion of the ions towards the stop detector, the ions penetrate the grids of the mirror and reach that detector. The extension of the flight path due to the number of reflections is used to increase the resolving power in time-of-flight spectra. Values of 55 000 for substance-P (MW 1346.7) and 31 000 for bovine insulin (MW 5734) were obtained for single laser shot spectra. Copyright © 1999 John Wiley & Sons, Ltd.

Although the time-of-flight (TOF) technique in mass spectrometry was introduced in the late 1940s by Stephens,[1] as well as by Cameron and Eggers,[2] it is only in recent years that it has become well established. A drawback with the TOF technique has been the poor mass resolving power due to the spread of the initial velocities and the space distribution of the ions generated in the ion source. Two important steps to improve the resolving power were taken in 1955 when Wiley and McLaren[3] invented time-lag energy focusing for compensation of the space charge distribution and in 1973 when Mamyrin[4] invented the electrostatic reflector for compensation of the initial kinetic energy.

MALDI[5,6] TOF is today one of the most powerful organic mass spectrometry techniques but it has often suffered from too broad peaks in the spectra. However, by using the combined techniques of time-lag focusing for the spatial distribution and an electrostatic mirror for the velocity focusing, high performance MALDI spectra have been obtained with e.g. 23 000 resolving power for the isotopes of bovine insulin.[7]

In a linear TOF instrument of length *l* the flight time *T* of an ion with mass/charge *m/z* that has been accelerated with the potential *U* is

$$T \propto l\sqrt{m/(zU)} \qquad (1)$$

The mass resolving power of a peak is

$$\frac{m}{\Delta m} = \frac{T}{2\Delta T} \qquad (2)$$

where ΔT is the full-width at half maximum (FWHM) of an ion peak at flight time *T*. The resolving power can either be increased by increasing the flight time *T* or decreasing the time dispersion ΔT of the ion packet arriving at the stop detector. An increase in the flight time can be obtained by increasing the flight path length, *l*, or decreasing the acceleration voltage *U*, according to Eqn. 1.

An increase in the flight path, without increasing the physical dimensions of the spectrometer by much, can be achieved by using electrostatic reflectors placed in different geometries. Spectrometers using more than one reflector have been used by several groups for different purposes. For example, two reflectors have been used to obtain structural information of biologically interesting molecules by fragmenting them in a collision cell located between the mirrors.[8,9] Several different geometries for multiple reflections were described by Wollnik in 1990.[10] He pointed out that switchable ion mirrors without grids can be used to achieve high resolving power. A similar gridless reflector arrangement was used by Benner in his electrostatic ion trap[11] where ions are detected by the voltage induced when the ions move back and fourth through a detector tube.

Recently, Scherer *et al.*[12] have demonstrated a multiple TOF mass spectrometer which has been developed for the Rosetta comet rendezvous mission. In this multiple reflection TOF instrument, a resolving power of up to 5700 has been achieved for the [132]Xe isotopes.

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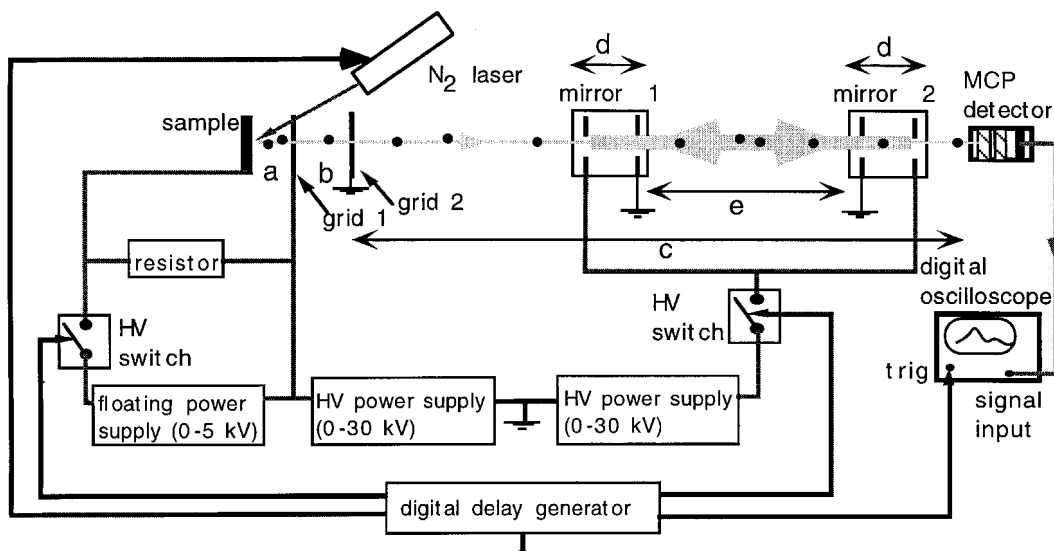
Figure 1. Schematic diagram of the multiple reflection MALDI TOF mass spectrometer. A laser pulse desorbs and ionizes sample molecules that are extracted from the ion source using a pulsed two grid system. The ions are injected into two switched electrostatic mirrors where they are trapped until the mirror voltages are switched off. The ions travelling towards mirror 2 in that moment will continue into the stop detector. Depending on the on-time of the mirrors different time-of-flights can be obtained for a certain mass. The distance **a** between the target and first grid is 1.18 cm and the distance **b** between the acceleration grids is 1.10 cm. The mirrors have a length **d** of 3.5 cm and are separated 81.6 cm, **e**. The flight path in direct mode, **c**, is .36 m.

In our construction the time-of-flight is increased and thereby the resolving power, in a MALDI TOF spectrometer by employing multiple reflections in two mirrors facing each other and time-lag focusing to extract the ions from the source. The opposite electric fields created by the two mirrors act as a trap for the ions. The number of reflections determine the total flight path for the ions. A suitable delay time for the delayed extraction is calculated according to the number of reflections (i.e. the increased distance) and the molecular mass of interest for highest possible resolving power.

EXPERIMENTAL

Sample preparation

Substance-P (Sigma) was dissolved in distilled water/0.1% TFA to a concentration of about 10^{-5} M. Small amounts of NaCl and KCl were added to the substance-P solution to generate $[M + Na]^+$ and $[M + K]^+$ ions. Bovine insulin (Sigma) was dissolved in distilled water/0.1% TFA to a concentration of about 10^{-5} M.

Solutions of 20 g/L of α-cyano-4-hydroxycinnamic acid (CHCA, Across Organics) in acetone and of 20 g/L of CHCA in 70% acetonitrile were used as MALDI matrices. The analyte was applied to the probe by first depositing 3–4 μL of the acetic acid matrix solution, allowing it to dry, and then depositing 4 μL of a 1:1 (v/v) mixture of the analyte and the acetonitrile matrix solution. The sample was then left to dry under a gentle stream of air.

Instrumentation

The high resolving power MALDI TOF mass spectrometer was designed and constructed in Uppsala. The sample molecules are desorbed and ionized using a nanosecond UV-pulse (337 nm) from a standard nitrogen laser. The angle of incidence of the laser light is 60° with respect to the normal of the target surface. The secondary ions are accelerated and extracted from the ion source using the delayed extraction technique. Two switchable ion mirrors with grids are located between the ion source and the detector (Fig. 1). A dual micro-channel plate detector is used for ion detection at one end of the mass spectrometer. The distance between the two ion extraction regions of the source and the suitable delay time are selected in order to obtain space focus at the stop detector after a required number of reflections. The extraction voltage and the high voltages for the ion mirrors are switched by high voltage switches (Behlke Electronic, Germany) and timed by a digital delay generator (Stanford DG 535, USA). The on-time of the mirrors is defined by the number of reflections needed and the speed of the ions when they enter the mirror trap. The two mirrors are switched on simultaneously when the molecular ions have drifted into the region between the mirrors. When the electric fields are removed during the motion of the ions towards the stop detector, the ions penetrate the grids of the second mirror and reach the detector. Only the ions travelling from mirror 1, see Fig. 1, towards the stop detector will be registered in the spectrum when the mirrors are switched off. The switching rise time of the electronics is ≈15 ns, but the actual time to establish a stable and uniform field inside the mirror is longer, probably in the μs range. Usually the mirrors contain 25 rings with a resistor chain that distributes the high voltage uniformly from the grounded entrance grid to the high voltage grid at the rear of the mirror. However, in this application where the high voltage is switched fast, the response was too slow due to the resistor chain which had to be removed as well as the rings. This means that the mirrors act as plain mirrors and they are not compensating for the spread in the initial kinetic energy of the ions. A total voltage of 10.5 kV was
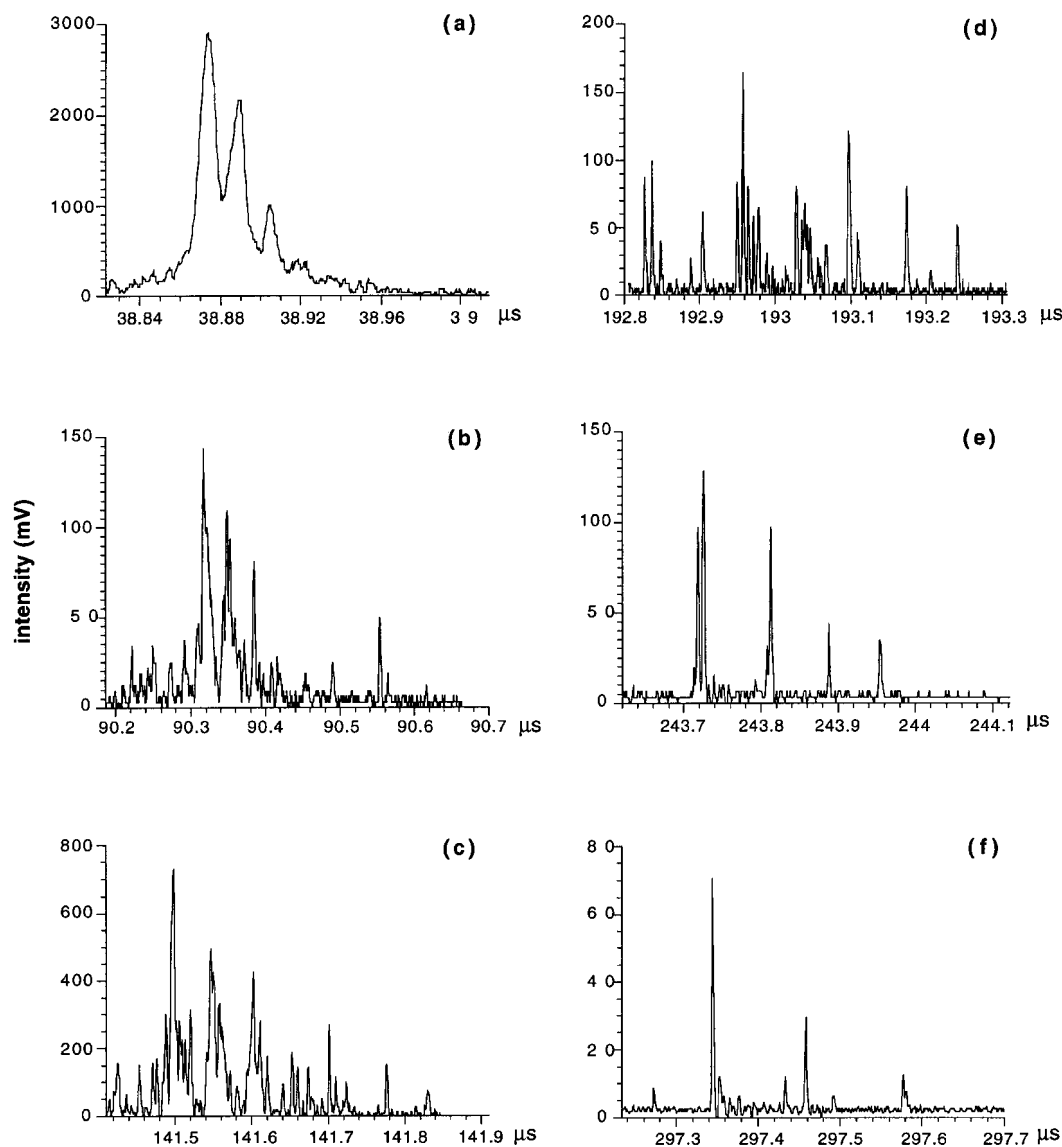
Figure 2. The figures show the molecular ion region in substance-P (MW 1347) spectra for (a) no reflections, i.e. a straight spectrometer, (b) 2 reflections, (c) 4 reflections, (d) 6 reflections, (e) 8 reflections, and (f) 10 reflections. In the last case the flight time of the monoisotopic ion is 297 µs and the half width 2.7 ns, corresponding to a mass resolving power of 55 000. All spectra are single laser shot acquisition.

used to accelerate the ions and 13 kV was used as the mirror voltages.

Data is collected by a 500 MHz LeCroy 9354 digital oscilloscope (LeCroy, Chestnut Ridge, NY, USA) with suitable settings for maximum resolving power.

RESULTS AND DISCUSSION

Figure. 2(b)–(f) show single laser shot spectra of molecular ions from substance-P (MW 1347) obtained for a series of even-number reflections. The substance-P molecular ion needs ≈38.9 µs to reach the stop detector in direct mode (Fig. 2(a)) and ≈51.5 µs (t_{cycle}) to complete two reflections or one cycle between the mirrors. Therefore, t_{cycle} is used as the time increment factor for the mirrors for each cycle. After five cycles, the TOF of the substance-P molecular ions has increased from 39 to 297 µs.

In the direct mode (Fig. 2(a)), the separation between two consecutive isotopes of substance-P is 15 ns. For each cycle,

the separation increases by 19 ns. After five cycles, the separation is 110 ns (Fig. 2(f)). At the same time, the half-widths of the isotopes reduce with increasing TOF from ≈8.5 ns in direct mode (Fig. 2(a)) to ≈2.7 ns after five cycles (Fig. 2(f)). The monoisotopic peak in the substance-P spectrum (Fig. 2(f)) has thus a flight time T of 297 µs and a width ΔT of 2.7 ns. This corresponds to a resolving power of 55 000 according to Eqn. 2.

The half-widths of the isotope peaks in Fig. 2, as a function of their flight times, are plotted in Fig. 3(a). For short flight times the half-width decreases rapidly but for longer flight times it is nearly constant which is supposed to be determined primarily by the length of the laser pulse which is 3 ns at FWHM. The mirrors are run in non-compensating mode. The reduction of the half-widths comes only from the time-lag focusing technique. Figure 3(b) shows the almost linear variation of the resolving power as a function of the flight times for substance-P molecular ions.
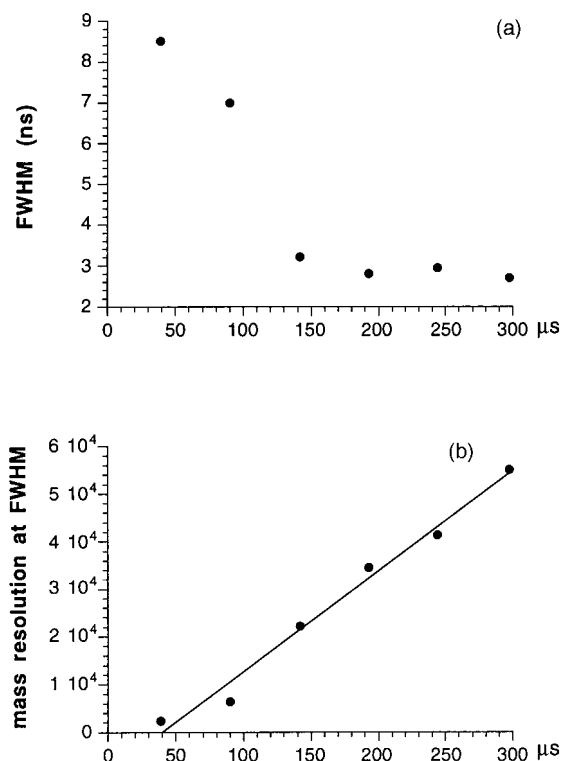
Figure 3. In the upper part (a) of the figure, the full-width at half maximum as a function of the flight time (i.e. number of reflections) for molecular ions of substance-P is plotted. In the lower part (b), the corresponding mass resolving power as a function of flight time is plotted.
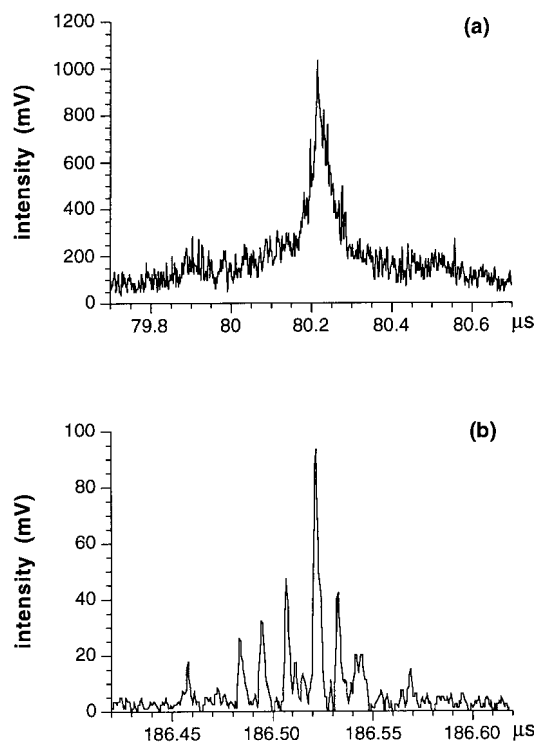

Figure 4. The molecular ion region of bovine insulin (MW 5734) for single laser shot acquisition. (a) In the direct mode the isotopes cannot be resolved. (b) After two reflections the isotopes can be resolved. The mass resolving power is about 31 000.

The TOF of a certain mass varies slightly from one acquisition to another. This could be due to path instability which is a result of several possible paths for different molecules with the same mass during the reflections between the mirrors. These different paths could be due to non-uniformity of the mirror fields and field penetration into the field free region. Introduction of a wire ion guide between the mirrors could be one way to reduce the number of different paths.

It is observed that low mass ions can travel longer distances (more reflections) than heavier ones without losing intensity. This is demonstrated for molecular ions of bovine insulin (MW 5734) shown in Fig. 4. The upper spectrum (Fig. 4(a)) is in direct mode where the isotopes cannot be resolved. However, after two reflections the isotopes can be resolved with a resolving power of about 31 000 in single laser shot acquisition (Fig. 4(b)). Although the signal is strong after the second reflection, it is weak compared to the substance-P case. Dissociation or scattering due to collisions[13] with residual gas molecules inside the spectrometer are phenomena that could cause this weak detection as well as a more divergent beam for high masses compared to low masses. The mass spectrometer has a base pressure around 10^{-6} mbar. We expect that an improved vacuum and introduction of an ion guide could give better residence time for larger molecular ions.

The spectrometer contains two grids in the ion source, two in each reflector and one in the stop detector. The transmission T of the grids employed was 88.6 %. For every cycle in the spectrometer, i.e. two reflections, the intensity of a peak should theoretically be reduced by a factor T^2 due to losses at the grids. Consider the intensity drop of the

$[M + H]^+$ molecular ion of substance-P as a function of the number of reflections as shown in Figs 5(a)–(f). The intensity ratio between the direct spectrum, I_{direct}, and after n reflections, I_n, due to losses in the grids is given by

$$I_{direct}/I_n = T^{-n} \qquad (3)$$

After Six reflections the intensity should be reduced by a factor of 2 according to Equ 3 but in the spectra it is a factor of 16. This discrepancy is attributed to non-optimized ion optics and unimolecular decay of the molecular ions. Dissociation and scattering due to collisions[13] with residual gas molecules can also contribute.

When working with substance-P it has also been noticed that the intensity of the protonated molecular ions $[M + H]^+$ is reduced much faster, relative to the molecular ions produced by alkali ion attachment, $[M + Na]^+$ and $[M + K]^+$, as a function of flight time (see Fig. 5). Since the flight path is common for all three types of molecular ions (i.e. the collision probability is the same for all molecular ions), this difference in the beam attenuation may be due to different stabilities of the molecular ions.

CONCLUSIONS

Using multiple reflections employing two mirrors, together with the powerful technique of delayed extraction, it has been demonstrated that a time-of-flight spectrometer can reach a resolving power above 50 000 in MALDI applications. A drawback of the current design is that only a certain fraction of the whole spectrum can be analyzed in high resolving power mode at a time. This is also the case in the Fourier transform ion cyclotron resonance technique when
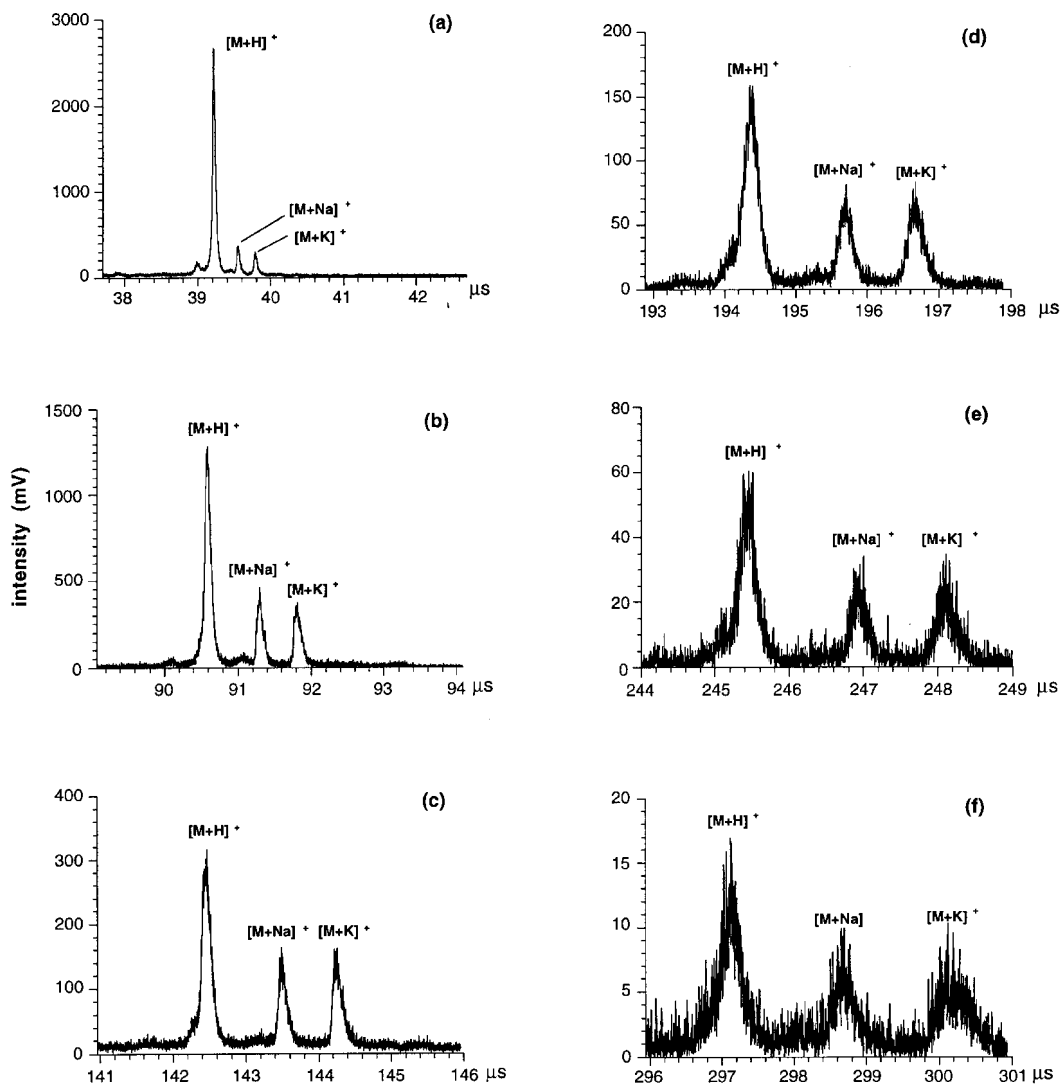
Figure 5. The molecular ion region in substance-P spectra in direct mode (a) and for 2,4,6,8 and 10 reflections in (b) to (f), respectively. A pronounced change in the relative intensities between the $[M + H]^+$ ion and the $[M + Na]^+$ and $[M + K]^+$ ions are observed for longer flight times. Each spectrum is the average of 40 laser shots.

switching from broadband detection to high resolution mode.

The mass spectrometer could be further optimized for high resolving power MALDI applications with an improved ion focusing technique that will reduce multiple paths and thereby make it possible to accumulate several spectra for improved statistics. A lower pressure in the spectrometer would also reduce the collision rate and thereby reduce dissociation and scattering of the analyte molecules. Another obvious improvement to the spectrometer is to run the mirrors in compensating mode.

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