A homemade high-resolution orthogonal-injection time-of-flight mass spectrometer with a heated capillary inlet

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We describe a homemade high-resolution orthogonal-injection time-of-flight (O-TOF) mass spectrometer combing a heated capillary inlet. The O-TOF uses a heated capillary tube combined with a radio-frequency only quadrupole (rf-only quadrupole) as an interface to help the ion transmission from the atmospheric pressure to the low-pressure regions. The principle, configuration of the O-TOF, and the performance of the instrument are introduced in this paper. With electrospray ion source, the performances of the mass resolution, the sensitivity, the mass range, and the mass accuracy are described. We also include our results obtained by coupling atmospheric pressure matrix-assisted laser deporption ionization with this instrument. © *2008 American Institute of Physics.* [DOI: 10.1063/1.2832334]

I. INTRODUCTION

Mass spectrometry (MS) has become one of the most important tools in the biochemical sciences with capabilities ranging from small molecule analysis to protein characterization. Among all types of mass analyzers, time-of-flight mass spectrometers (TOF-MSs) are the most advantageous for the analysis of biomolecules. At the beginning of the 1990s, two new ionization methods, electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI), coupled to TOF analyzers[1] that avoided the difficult to detect with good sensitivity and analyze with good resolution, and revolutionized the role of mass spectrometry in biological research. These methods allow the high-precision analysis of biomolecules of very high molecular weight. Now, ESI-TOF-MS and MALDI-TOF-MS have been the basis of the new field of biological mass spectrometry.

The orthogonal-injection time-of-flight (O-TOF) mass spectrometry is relatively new and has been designed to be coupled to ESI and recently to atmospheric pressure matrix-assisted laser desorption ionization (AP-MALDI). The O-TOF mass spectrometers began to gain popularity in the 1980s. By using reflectron scheme,[2] the time-of-flight reflectron mass analyzer has advantages of the high efficiency, sensitivity, and accuracy. Also, the time-of-flight reflectron offers high resolution over a simple time-of-flight instrument by increasing the path length and kinetic energy focusing through the reflectron. The TOF reflectron analyzer combined with AP-MALDI can separate postionization fragment ions from the same precursor ions. The reflectron takes advantages of the fact that the fragment ions have different kinetic energies and separates them based on this property, thus producing a fragment ion spectrum. It should be noted that electrospray has also been adapted to TOF reflectron analyzer, where the ions from the continuous ESI source are pulsed into the analyzer. Thus, the necessary electrostatic pulsing creates a time zero from which the TOF measurements can begin. A TOF reflectron mass analyzer with an ESI ion source has gained wider use due to the fast scanning capabilities, good mass range (up to $\sim 10\,000 m/z$), and the high accuracy that the TOF reflectron can offer ($\sim$10 part per million, 10 ppm).[3]

However, the analyzer is in the high vacuum and the ESI, AP-MALDIs are atmospheric pressure ionization ion sources, how the ions transfer from atmospheric pressure to the low vacuum mass analyzer? For all these atmospheric pressure ion sources, the gas-phase ions are transferred to the lower pressure regions through conductance limits such as a metal capillary or an orifice.[4,5] Since ions must enter the MS through a small aperture or capillary with limited cross section, the ion cloud expansion and dispersion can significantly decrease the ion transmission to the lower pressure region. So there are some people research the interface to improve the efficiency of ion transmission from atmospheric pressure to the first differentially pumped region.[6–12] In these researches, we learned that the heated capillary inlet can provide more efficient introduction of ions, resulting in a significant enhancement in mass spectrometer sensitivity and detection limits. The use of a heated capillary tube is a convenient and effective desolvation method.

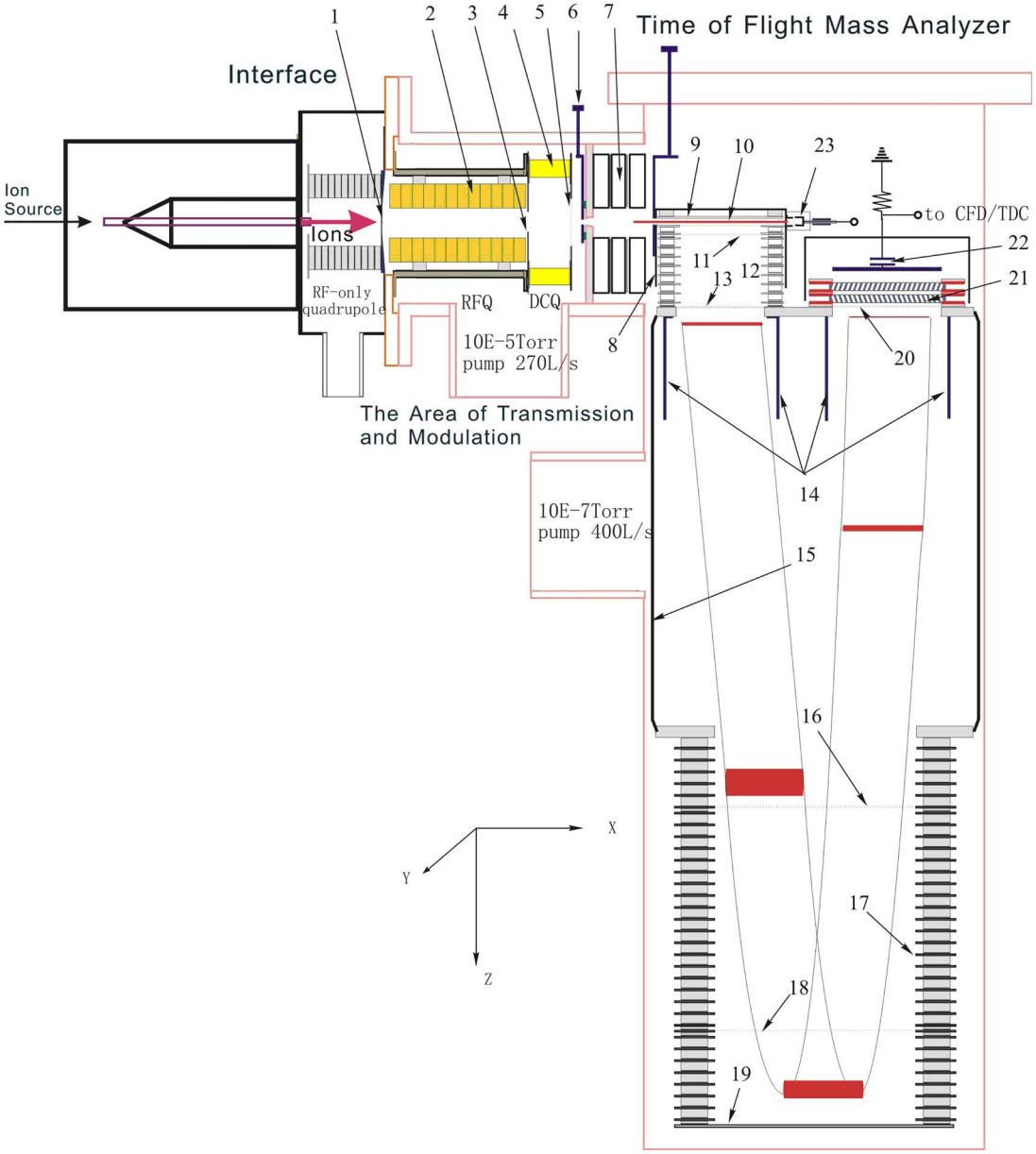

FIG. 1. (Color online) Schematic of the orthogonal-injection time-of-flight mass analyzer. (1) Skimmer 1, (2) rf quadrupole, (3) skimmer 2, (4) dc quadrupole, (5) output orifice, (6) slit, (7) einzel lens, (8) field screen plate, (9) repeller plate, (10) grid 1, (11) frid 2, (12) Acceleration region, (13) grid 3, (14) deflector, (15) field-free flight tube, (16) grid 4, (17) first reflector stage, (18) grid 5, (19) backplate, (20) grid 6, (21) MCP, (22) coupling capacity, and (23) faraday cup.

In this paper a home made high-resolution O-TOF mass spectrometer with a heated capillary inlet is presented. Combining a quadrupole analyzer, the O-TOF exploits the quadrupole's ability to select a particular ion and the ability of O-TOF MS to achieve simultaneous and accurate measurements of ions across the full mass range. Because of using the reflectron mass analyzer, higher mass resolving power could be achieved in our instrument. The heated capillary interface combined with a rf-only quadrupole is helpful of desolvation and focusing ions, then could increase the transmission efficiency of ions. The capillary inlet has provided an efficient means of coupling ESI and AP-MALDI to O-TOF instrument. The goal in this thesis was to perform comprehensive theoretical and experimental investigations of our instrument.

II. PRINCIPLE AND STRUCTURE OF THE INSTRUMENT

A. O-TOF MS

The design of the O-TOF MS is based on a system developed by Dodonov *et al.*[13–15] An overview of the O-TOF MS amended by gas-filled quadrupole is presented in Fig. 1. It consists mainly of an atmospheric pressure ion source (ESI or AP-MALDI), a capillary interface combined with rf-only quadrupole, and a reflecting time-of-flight mass analyzer. Ions produced in the ion source first entered to a capillary interface, then to the area of transmission and modulation. The area of transmission and modulation contains a rf quadrupole (RFQ) (2) and a dc quadrupole (DCQ) (4). RFQ can reduce space distribution and energy distribution of the ions, and DCQ matches this ion beam to the ion modulator region.

TABLE I. The main parameters of the O-TOF MS.

Parameter title	Parameter value
Length of the repelling region (mm)	12
Length of the acceleration region (mm)	61.6
Length of the field-free flight tube (mm)	833
Length of the first reflector stage (mm)	113
Length of the second reflector stage (mm)	42
Repelling voltage (V)	800
Accelerating voltage (V)	−4800
Grid 5 voltage (V)	−220
Back plate voltage (V)	1210
Repelling pulse frequency (Hz)	5000
Repelling pulse width (μs)	5

After extraction out of the area of transmission and modulation, the cooled ions passed an einzel lens (7) and entered to the time-of-flight mass analyzer. The einzel lens controls the overall focus action and adjusts the direction of the ion beam. In the analyzer, first the ions were pushed by a pulsed electric field in the direction perpendicular to the initial beam direction and were accelerated in the acceleration region (13). There is a Faraday cup (23) at the opposite end of the repelling plate (9) to monitor the ion current. Then the ions were reflected in two-stage ion reflector (17) after flying in the field-free drift tube (15) and were detected by microchannel plates (MCPs) (21). Finally, the electrons MCP produced were collected by a special plate anode, which is a back to back glued copper-covered kapton foil. A constant fraction discriminator (CFD) and a time-to-digital converter (TDC) served for spectra registration.

Such a construction allows a precise mechanical assembly as well as the removal of the complete mass analyzer in one piece from the vacuum chamber for modification. The main working parameters of the instrument are shown in Table I.

B. Heated capillary interface

The design of the interface is shown in Fig. 2. It consists of skimmer (1), mechanical pump bleeding point (2), first stage vacuum chamber (3), focusing electrode (4), heater (5), heater sheath (6), capillary (7), temperature sensor (8), capillary pedestal (9), rf-only quadrupole (10), and rf-only quadrupole pedestal (11). The capillary is a stainless steel tube, whose inner diameter is 0.35 mm and length is 100 mm. The inner diameter and length of the capillary have an effect on the first stage vacuum and ion transmission. Increasing the diameter and decreasing the length of the capillary allow introduction of more ions, but increase the gas load. Decreasing the diameter and increasing the length of the capillary can secure a better vacuum of the first stage vacuum chamber, but reduce signal intensity beyond the range acceptable for high-resolution study. The heating part of the capillary consists of heater, temperature sensor, and heater sheath, the temperature of the capillary can be adjusted continuously in the range of ambient temperature to 200 °C. Droplets that entered the heated capillary were strongly desolvated, but as

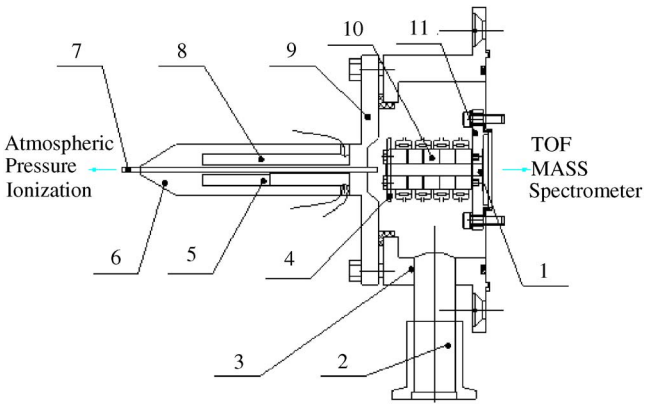

FIG. 2. (Color online) Schematic of the AP-interface. (1) Skimmer, (2) mechanical pump bleeding point, (3) first stage vacuum chamber, (4) focusing electrode, (5) heater, (6) heater sheath, (7) capillary, (8) temperature sensor, (9) capillary pedestal, (10) rf-only quadrupole, and (11) rf-only quadrupole pedestal.

they traveled toward the mass spectrometer, the temperature inside the tube facilitated evaporation of the solvent so that ions that exited the capillary tube were almost completely desolvated. A better first vacuum can also be acquired by heating the capillary. The rf-only quadrupole is located ~2 mm from the capillary exit along the axis. In rf-only quadrupole, the ions collided with buffer gas molecules under the radio-frequency electric field. So the ions can have further desolvation and can be focused to ion beams of which diameter less than 0.2 mm. The skimmer is made of 0.1 mm thick stainless steel plate, which has an orifice (diameter of 0.35 mm) in the center and which separates the first and second stages.

The heated capillary interface combined with rf-only quadrupole can provide more efficient introduction of ions. For electrospray ionization, the ion intensity detected on the time-of-flight mass spectrometer was seen to increase threefold compared with an orifice interface we used before (the diameter of the orifice is 0.12 mm, and aerating nitrogen to help desolvation). Taking the 10^{-9} mol/l gramicidin S as the sample, we discovered that in the spectrum 5300 ions can be accumulated per second with the heated capillary inlet, but 1700 ions were accumulated per second with the orifice inlet (the sample flow rates was 1 μl/min).

The use of a heated capillary tube is a convenient and effective desolvation method. The capillary tube can allow the introduction of more ions, and the effective rf potential keeps ions near the axis of the quadrupole. While the ions oscillate in velocities are reduced to near thermal values, this produces a beam with a small longitudinal velocity spread and a small lateral spatial spread. As a result both the sensitivity and the mass resolving power of the time-of-flight mass spectrometer are improved.

C. Ion source

1. ESI

ESI is a method routinely used with peptides, proteins, carbohydrates, small oligonucleotides, synthetic polymers, and lipids. ESI produces gaseous ionized molecule directly

from droplets in the presence of an electric field and allows polar and thermally labile compounds to be introduced into a mass spectrometer without thermal degradation. But the important characteristic of ESI is that it is able to produce multiply charged ions from large molecules. The formation of ions is a result of the electrochemical process and of the accumulation of charge in the droplets. The electrospray current is limited by the electrochemical process that occurs at the probe tip and is sensitive to concentration rather than to the total amount of sample. As shown in the experiment,[16] the sensitivity will increase somewhat when the sample flow entering the source is reduced. This remains true up to flows as low as some tens of nl/min. When flow rates higher than about 500 μl/min are used, the sensitivity is reduced. Lower flow rates also allow less analyte and buffer in the source to be injected, reducing contamination. Furthermore, for the same amount of sample, an injecting column with a lower diameter, and using smaller flow rates, will give an increased sensitivity because the concentration of the sample in the elution solvent is increased.[17–21]

For experiments, the spray tips of ESI were made by a 50 μm inner diameter, 360 μm outer diametet fused-silica capillary. A potential up to 4 kV was applied to the sample solution through a copper alligator clip attached to the metal union before the spray tip. The length of the silica emotter from the union to the tip is 25 mm. For ESI, the position of the spray tip was controlled by a three-dimensional linear stage.

2. AP-MALDI

AP-MALDI has become a widespread analytical tool for peptides, proteins, and most other biomolecules (oligonucleotides, carbohydrates, natural products, and lipids). The AP-MALDI produces singly charged ions. The efficient and directed energy transfer during a matrix-assisted laser-induced desorption event provides high ion yields of the intact analyte and allows for the measurement of compounds with subpicomole sensitivity. The advantages of AP-MALDI include the following: sample handling under normal atmospheric pressure conditions, softer analyte ionization compared with conventional vacuum MALDI, and increased resolution of individual components of complex analyte mixtures.[22–24]

The AP-MALDI ion source described here is a reflectron structure.[25] A pulsed nitrogen laser (337-Si air-cooled nitrogen laser system, P/N337203-01, Spectra-Physics), at a wavelength of 337 nm, was employed. The laser pulse duration was about 4 ns. The laser beam was focused on the end of an optical quartz glass fiber cable. The other end of the 1 m long optical cable was connected to the optical flange of the AP/MALDI source. The optical channel used for directing and focusing the laser pulse from the fiber end onto the target included a 25.4 mm focal length quartz lens and a fused-silica high accuracy turning mirror. The beam was focused on the stainless steel target area closest to the extended capillary inlet tip. The beam incidence angle was approximately 45°. The target plate was placed under high voltage varying in the 1.5–2.5 kV range. The position of the sample plate was optimized by using a hand-controlled of *x-y* direction adjusting knob.

III. RESULTS AND DISCUSSION

A. The experimental results with ESI

1. Mass range

Theoretically speaking, the mass range of TOF MS analyzer does not have the upper limit and the lower limit. But because of the limit of instrument component performance, the mass range has the limit. Usually, the lower limit of TOF MS is mainly decided by the system of ion transmission and modulation. While using lower pressure rf-only quadrupole can cause lower transmission efficiency of small molecular. The upper m/z limit of TOF MS is mainly influenced by the detector. The upper m/z limit of MCP itself is about 100 000, and it is related to the ion accelerating voltage. Moreover, the memory ability of data acquisition also can affect the mass spectrum width in a single record. So the theoretical lower mass limit of our instrument is 45 Da, and the theoretical

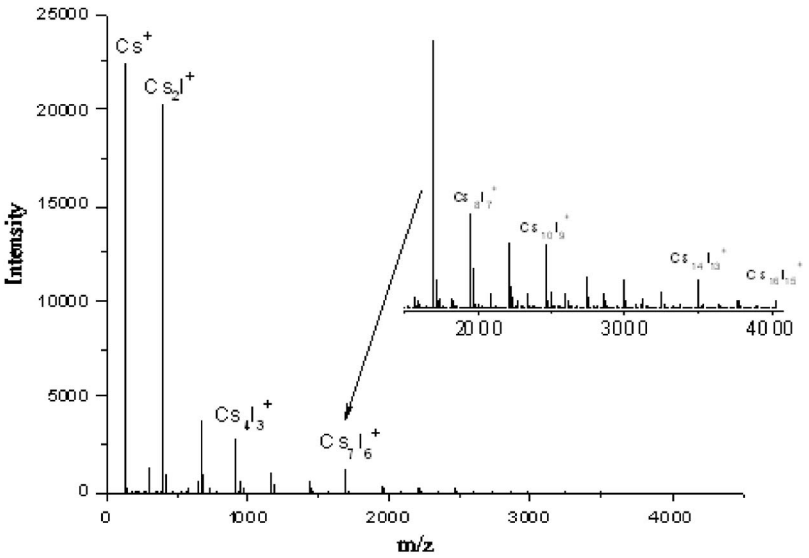

FIG. 3. ESI mass spectrum of CsI in positive mode. Experimental conditions were sample CsI, solvent composition methanol, sample concentrations of 7.7×10^{-4} mol/l, flow rates of 1 μl/min, filter of 60 s, ESI needle voltage=4 kV, the length of the silica emotter from the union to the tip 25 mm, the temperature of the heated capillary of 125 °C, and external calibration.

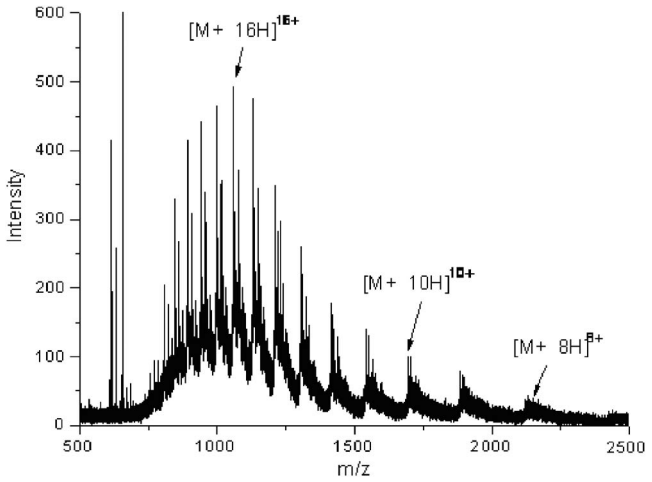

FIG. 4. ESI mass spectrum of saturated myoglobin in positive mode. Experimental conditions were sample saturated myoglobin in methanol (including 0.2% formic acid) flow rates of 1 μl/min, ESI needle voltage =4 kV, the length of the silica emotter from the union to the tip of 25 mm, the temperature of the heated capillary of 125 °C, and external calibration.

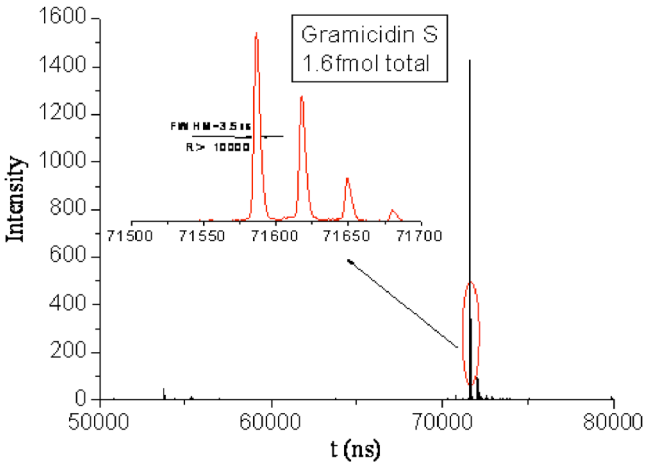

FIG. 5. (Color online) ESI mass spectrum of 10^{-7} mol/l gramicidin S. Experimental conditions were sample gramicidin S, solvent composition methanol, sample concentrations of 1×10^{-7} mol/l, flow rates of 1 μl/min, filter of 1 s, ESI needle voltage=4 kV, the length of the silica emotter from the union to the tip of 25 mm, and the temperature of the heated capillary of 125 °C.

upper m/z limit is 8000 in a single scan of entire spectrum. Next the actual mass range of our instrument would be examined through experiment.

In Fig. 3, the spectrum of CsI in positive mode is given. In positive mode, CsI forms a series of complex peak in $Cs_{(n+1)}I_n^+$ format. The four highest peaks of complex were chosen as internal standard to confirm m/z of other peaks. As shown in Fig. 3, the range of m/z of the singly charged ions of CsI complex is distributed between 100 and 4000.

Figure 4 shows that the parent ions of myoglobin (molecular weight=16952 Da, purchased from SIGMA), are distributed as multiply charged peaks. The charged numbers of parent ions are distributed in 8–22, and m/z is mainly distributed between 700 and 2200.

The preliminary debugging result demonstrates that the mass range of our instrument is wider, the samples of which mass between 100 and 17000 Da can be detected, and the m/z of measured samples is mainly distributed less than 2000.

2. Mass resolution

Usually, mass resolution refers the capacity of separating two neighboring mass spectra peaks in the condition of sample assigned. According to the principle of TOF analyzer, it is one kind of time resolution, the time of flight $T_{\mathrm{TOF}} \propto \sqrt{M/q}$. The computational method of resolution [full width at half maximum (FWHM)] is showed in formula (1),

$$R_{\mathrm{FWHM}} = \frac{M}{\Delta M} = \frac{T}{2\Delta T}. \quad (1)$$

Regarding as TOF MS, the value of mass resolution may be not the same if using different sample debugging. Here using gramicidin S to inspect the mass resolution of our instrument.

As shown in Fig. 5, the resolution of gramicidin S, $R_{\mathrm{FWHM}} > 10\,000$.

3. Detection limit

The detection limit refers to the smallest quantity of sample which can be detected in the condition of ensuring a certain signal to noise ratio. The primary factors of influencing absolute sensitivity measured are the ionized efficiency of ion source, the transmission efficiency of ions, the actual utilizable efficiency of ions pushed, the flux of grid, the gain of MCP, and the trigger threshold value of CFD. The different natures of samples decide that there are differences in ions efficiency, transmission characteristics, and the contributions of MCP in producing secondary electrons. Therefore for the different samples, the instrument may have different detection limits.

Figure 6 shows that a total of 1.6 amol Gramicidin S was actually used in obtaining the detection limit of the instrument. In this spectrum, the area of main peak (m/z

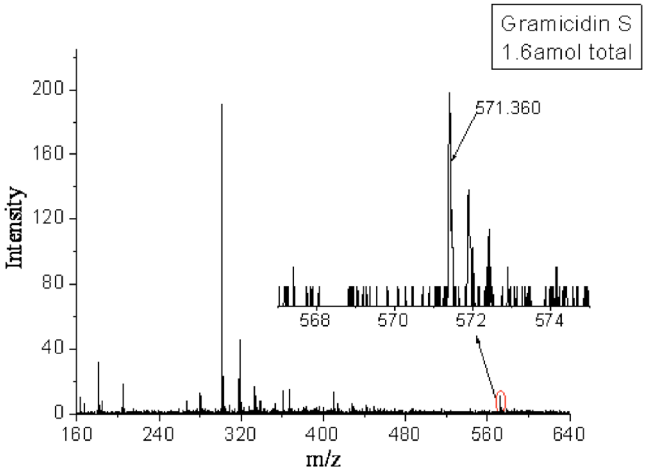

FIG. 6. (Color online) ESI mass spectrum of 10^{-10} mol/l gramicidin S. Experimental conditions were sample gramicidin S, solvent composition methanol, sample concentrations of 1×10^{-10} mol/l, flow rates of 1 μl/min, filter of 1 s; ESI needle voltage=4 kV, the length of the silica emotter from the union to the tip of 25 mm, the temperature of the heated capillary of 125 °C.

TABLE II. The mass accuracy of the O-TOF MS.

	Theoretical value (T)	Experimental value (E)	Accuracy (ppm) $(E\text{-}T)/T$
Cs^+	132.905 429	132.9057	2.0
Cs_2I^+	392.715 334	392.7159	−1.4
$Cs_4I_3^+$	912.335 144	912.3332	−2.1
$Cs_5I_4^+$	1172.145 049	1172.1494	3.7
$Cs_6I_5^+$	1431.954 954	1431.9614	4.5
$Cs_7I_6^+$	1691.764 859	1691.7800	8.9
$Cs_8I_7^+$	1951.574 764	1951.5548	−10
$Cs_9I_8^+$	2211.384 669	2211.4036	8.6
Ions of gramicidin S	571.360	571.362	3.5

=571.360) is 30, the signal-to-noise ratio ⩾10:1. We obtained that the lowest detection limit of our instrument is 1.6 amol.

4. Mass accuracy

The mass accuracy refers the conforming degree between the experimental value and the theoretical value. It is related to the accuracy of power source, the change of ambient temperature, the transformation precision of CFD and preamplifier, the time measuring precision of TDC, and the calibration method of spectrum peaks.

Taking CsI and gramicidin S as samples, the table of the mass accuracy of the O-TOF MS is given in Table II. As shown in Table II, the mass accuracies of each peaks of CsI and gramicidin S all surpass 10 ppm.

B. The experimental results with AP-MALDI

Except experiment for the mass range taking PPG as the sample, gramicidin S (the molecular weight is 1140.704) was used as the sample in other experiments. Dissolving 2×10^{-2} mol/l gramicidin S in methanol, we diluted the sample into the concentrations of 2×10^{-6} and 2×10^{-5} mol/l. The matrix solution was saturated α-cyano-4-hydroxycinnamic in 1:1 of acetonitrile:H_2O. The sample and matrix were not processed previously.[26] The sample solution and the matrix solution were mixed evenly according to the physical volume of 1:1. Then the mixed solution was dropped to the target plate and dried in the air after 20–30 min.

In the experiment, we aimed the laser beam at a point on the sample target, until one sample point was ionized completely by laser, we can get the sample consumption. The sample consumption (N) is obtained from Eq. (2),

$$N(\text{mol}) = \frac{C}{2} V \frac{A_2}{A_1}, \tag{2}$$

C is the concentration of the sample solution (because in the experiment we mixed the sample solution and matrix solution according to the physical volume of 1:1 and we dropped the mixed solution to the target, so in the equation we need to divide 2 to obtain sample consumption), V is the volume of the mixed solution dropped on the target plate, A_1 is the area of the mixed solution dropped on the target plate, and A_2 is the area of the solution consumed at one laser point (i.e., the area of the laser point).

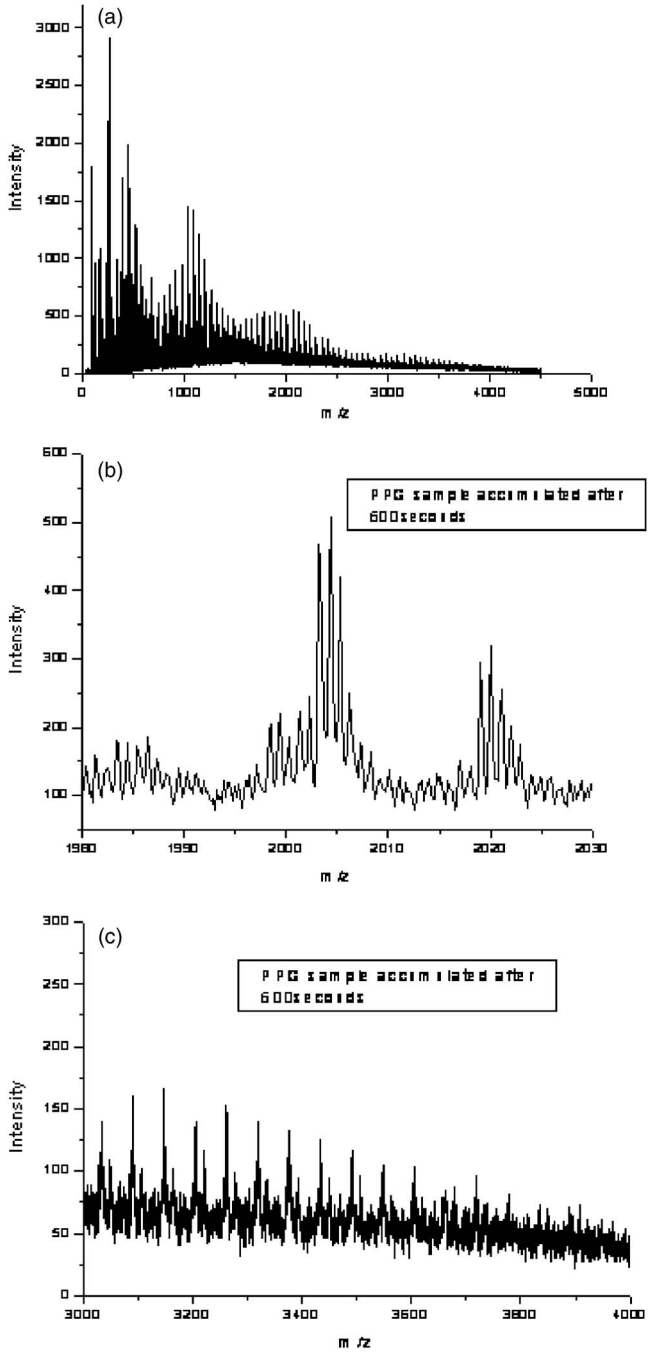

FIG. 7. AP-MALDI mass spectrum of PPG in positive mode. Experimental conditions were sample PPG, laser wavelength of 337 nm, laser frequency of 650 Hz, laser energy of single pulse of 300 μJ, AP-MALDI target plate voltage=2 kV, the distance between target plate and heated capillary inlet of 2 mm, the temperature of the heated capillary of 130 °C, and external calibration.

1. Mass range

In the experiment for detecting the mass range, the sample of PPG (purchased from ABI Corporation) was used as the standard sample. It is a mixed sample of polypropylene glycol oligomers and the mass of it is distributed from 200 to 4000.

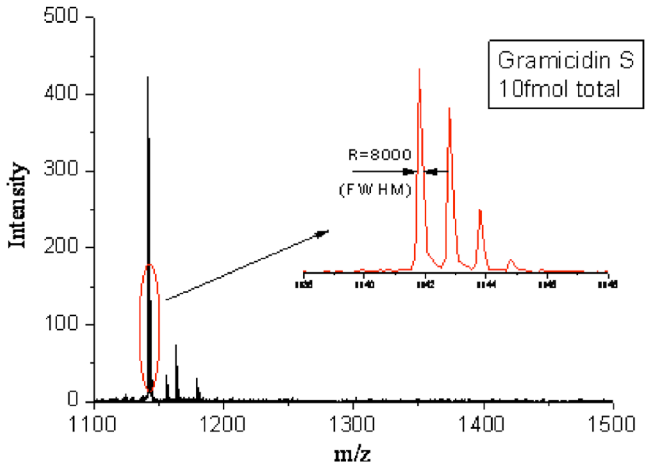

FIG. 8. (Color online) AP-MALDI mass spectrum of 10 fmol gramicidin S. Experimental conditions were sample gramicidin S, laser wavelength of 337 nm, laser frequency of 650 Hz, laser energy of single pulse of 300 μJ, AP-MALDI target plate voltage=2 kV, the distance between target plate and heated capillary inlet of 2 mm, and the temperature of the heated capillary of 130°C.

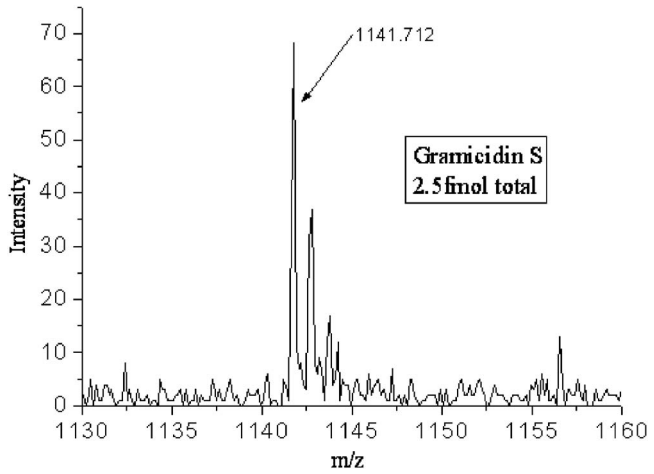

FIG. 9. AP-MALDI mass spectrum of 2.5 fmol gramicidin S. Experimental conditions were sample gramicidin S, laser wavelength of 337 nm, laser frequency of 650 Hz, laser energy of single pulse of 300 μJ, AP-MALDI target plate voltage=2 kV, the distance between target plate and heated capillary inlet of 2 mm, and the temperature of the heated capillary of 130°C.

Experimental total spectrum like Fig. 7(a) shows the samples which mass between 100 and 3800 Da can be detected. Figure 7(b) is an enlarged graph of Fig. 7(a) at m/z between 1980 and 2030, and Fig. 7(c) is a zoomed in graph of Fig. 7(a) at m/z between 3000 and 4000. Because of higher resolution, Fig. 7(b) explains the isotope peaks of ions which m/z is around 2000 can be separated well. Figure 7(c) indicates that the ions which m/z is up to 3800 can also be obtained a better detection, the intensity of ions peak is almost over 100, and the main ions signal can be separated well with noise signal.

2. Mass resolution

In this experiment, we mixed the 2×10^{-5} mol/l gramicidin S solution and the matrix solution according to the physical volume of 1:1, dropped 0.25 μl mixed solution on the target plate, then a circle of 1.5 mm radius was formed. The radius of the laser point is 0.1 mm, so the sample consumption is 10 fmol, the formula is as follows:

$$\frac{2\times10^{-5}}{2}\ \text{mol/l}\times0.25\times10^{-6}\,\text{l}\times\frac{\pi\times0.1^2}{\pi\times1.5^2}\approx10\ \text{fmol}.$$

Figure 8 shows, with consuming sample of 10 fmol, the signal-to-noise ratio $\geqslant$200:1. So taking AP-MALDI as the ion source of our instrument, the resolution of gramicidin S surpasses 8000 (FWHM).

3. Detection limit

The 2×10^{-6} mol/l gramicidin S solution and the matrix solution were mixed evenly according to the physical volume of 1:1, we dropped 0.4 μl solution on the target plate to form a circle of 1.25 mm radius. The radius of the laser point is 0.1 mm, so the sample consumption is 2.5 fmol, the formula is shown as the following:

$$\frac{2\times10^{-6}}{2}\ \text{mol/l}\times0.4\times10^{-6}\,\text{l}\times\frac{\pi\times0.1^2}{\pi\times1.25^2}\approx2.5\ \text{fmol}.$$

As shown in Fig. 9, a total of 2.5 fmol sample was actually used to obtain this spectrum, and the signal-to-noise ratio $\geqslant$10:1 in the spectrum. The 2.5 fmol is the lowest detection limit of our instrument coupling with AP-MALDI.

4. Mass accuracy

Figure 10 reflects that the spectrum of gramicidin S was obtained in the condition of having no standard sample calibrating. As shown in Fig. 10, the m/z of the main peaks of spectrum, respectively, are 1141.802 and 1141.825. The difference is 0.023/1141=20 ppm. These data can illustrate that the temperature stability and the electricity stability of the instrument are both good. Once our instrument was calibrated, it may maintain the accuracy of 20 ppm in the continuous multiday experiments.

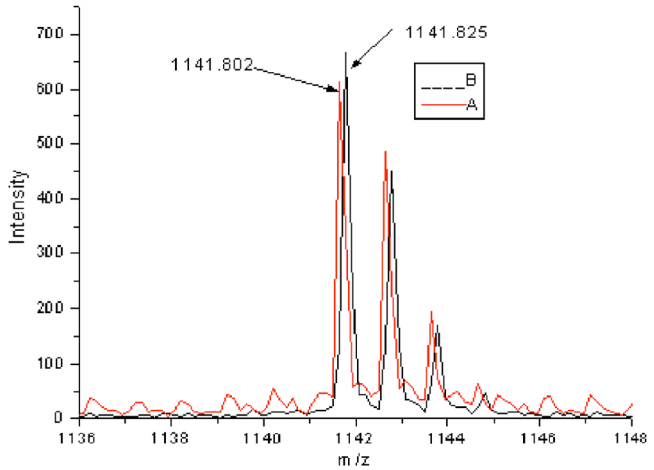

FIG. 10. (Color online) AP-MALDI mass spectrum of 30 fmol gramicidin S. The spectrum B was obtained two days after the spectrum A. Experimental conditions were sample gramicidin S, laser wavelength of 337 nm, laser frequency of 650 Hz, laser energy of single pulse of 300 μJ, AP-MALDI target plate voltage=2 kV, the distance between target plate and heated capillary inlet of 2 mm, and the temperature of the heated capillary of 130°C.

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