The investigation of the toroidal electric sector multi-turn time-of-flight mass spectrometer 'MULTUM II'

Daisuke Okumura

Department of physics, Graduate school of science Osaka University

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Chapter 1 Introduction

Introduction to time-of-flight mass spectrometry

Time-of-flight mass spectrometry (TOF-MS) was first postulated in 1946^[1] by W. E. Stephens. The principle of TOF-MS is quite simple; ions of different m/z are dispersed in time during their flight along a field-free drift path. Provided all ions start their journey at the same time, the lighter ions will arrive earlier at the detector than the heavier ions. This demands that they emerge from a pulsed ion source which can be realized either by pulsing ion packets out of a continuous beam or more conveniently by employing a true pulsed ionization method. Since the introduction of matrix-assisted desorption/ionization (MALDI)^[2] which make it possible to generate large molecules, such as peptides and proteins, the TOF mass spectrometer has evolved rapidly as a routine analytical tool for the analysis of them. The main advantages of general TOF mass spectrometers are: 1) in principle, the m/z range is unlimited; 2) from each ionization event, e.g., a single laser shot in MALDI, a complete mass spectrum is obtained within several tens of microseconds; 3) the transmission of a TOF analyzer is very high, giving rise to high sensitivity; 4) the construction of a TOF instrument is comparatively inexpensive; 5) recent instruments allow for accurate mass measurements and tandem MS experiments.

Time of flight

Independent of the ionization method, the electric charge q of an ion of mass m is equal to an integer number z of electron charge e, that is q = ez. The energy uptake E by moving through a voltage U is given by

$$E = qU = ezU \tag{1.1}$$

Thereby, the former potential energy of a charged particle in an electric field is converted into kinetic energy

$$E = ezU = \frac{1}{2}mv^2.$$
 (1.2)

The ion will have velocity

$$v = \left(\frac{2zeU}{m}\right)^{1/2} \tag{1.3}$$

that depends on its mass. The time (T) required to traverse the drift length L

$$T = \left(\frac{m}{2ezU}\right)^{1/2} L \tag{1.4}$$

also depends on the mass of the ion, so that the time spectrum can be converted directly to a mass spectrum

$$\frac{m}{z} = 2eU\left(\frac{T}{L}\right)^2.$$
(1.5)

To obtain timing information, the time of ion extraction (or formation) must be known. Thus TOF mass spectrometers generally use pulsed extraction (or ionization). The time interval between ion extraction (or ionization) and detection is measured as t. The entire time/amplitude record is digitized by an analog-to-digital converter.

Mass resolving power of TOF mass spectrometer

The mass resolution of the TOF mass spectrometer is given in Eq. 1.6

$$R = \frac{T}{2\Delta t} \tag{1.6}$$

where Δt is peak width at half maximum. The mass resolution is proportional to its total flight time and inversely proportional to peak width. Flight time T is a function of flight length and acceleration voltage. Peak width is a function of the time distribution of ion bunch and the time resolution of a detection system. This time distribution results from the spatial and velocity distribution of initial ion bunch. In order to minimize the effects of spatial and velocity distributions, two-stage acceleration, time-lag focusing methods^[3] and orthogonal acceleration ion sources^[4] were developed. The ion mirror^[5] was devised to compensate the flight time distribution due to the initial kinetic energy distribution. Finally, electric sectors were proposed by Poschenrieder to achieve isochronous focusing and space focusing^{[6][7]}. Obviously, however, the simple and clear way to increase the resolution of TOF analyzers is to elongate the flight path length, because the mass resolution is proportional to the flight time under the assumption of a constant width of ion packet.

In 1971, Poschenrieder proposed the use of sector fields to achieve isochronous focusing, in order to improve resolution. Space focusing should be also achieved to increase sensitivity. Several sector type TOF mass spectrometers have been developed. A TOF mass spectrometer consists of four 269° toroidal electric sector fields of 50 mm radius was developed at Osaka University^[8] in 1985. Its shape of the ion orbit resembles a four-leaf clover and it has 1.7 m total flight path length. Triple isochronous and triple space focusing are satisfied and a mass resolution of 2,000 was achieved at m/z 1147.1^[9]. A multi-passage TOF mass spectrometer 'OVAL' was constructed at JAIST^[10] in 1999. The spectrometer consists of six electrostatic analyzers and the shape of the ion orbit resembles a racetrack oval. The size of the instrument is $2.5 \text{ m} \times 3.5 \text{ m}$ and the length of the circular orbit is 7.4m. Triple isochronous and triple space focusing are satisfied and a mass resolution of 7,200 was achieved at m/z 133 after 5 passages^[11]. In our laboratory, multi-turn TOF mass spectrometers have been developed. The first multi-turn TOF mass spectrometer^[12] 'MULTUM Linear plus' was designed and constructed in 1999 as a laboratory model of the 'COSAC' project of the 'ROSETTA'^[13] mission cometary study. It has four electric sectors and 28 quadrupole lenses. These elements were fixed on a 40 cm \times 40 cm plate and the flight path length of one cycle is 1.3 m. The shape of the ion orbit resembles the symbol for infinity. Six space and triple isochronous focusing are satisfied and a mass resolution of 350,000 was achieved at m/z 28 after 501.5 cycles^[14]. The 'MULTUM Linear plus' analyzer is not simple, because 28 quadrupole lenses are used. In order to reduce the number of ion optical parts, an improved multi-turn TOF mass spectrometer 'MULTUM II' consisting of only four toroidal electric sector fields and Q-lens doublet for ion injection was designed and constructed^[15] in 2001. The design parameters, simulated characteristics and several experimental results are presented in this thesis.

Outline of this thesis

The purpose of this thesis is the investigation of the multi-turn TOF mass spectrometer 'MULTUM II' consisting of four toroidal electric sector fields and application of it to organic compounds ionized by MALDI. Ion optics of 'MULTUM II' and the technical data for construction and measurement system are described in Chapter 2. In Chapter 3, as an investigation of performance of 'MULTUM II,' the increase in the mass resolution with the number of cycles was evaluated experimentally using gaseous samples with an EI ion source. Variation of the ion transmission and tolerance of voltage applied to MATSUDA plates^[16] were also investigated. Additionally as an application of a multi-turn TOF mass spectrometer, the combination of the ion mirror and 'MULTUM II' was examined. Based on these investigations, improvements of the ion source, power supplies and detectors were described in Chapter 4. Higher mass resolution and separation of a doublet peak of $m/\Delta m = 54, 162$ were confirmed. It is the first time that such a separation has been achieved by TOF mass spectrometry. In Chapter 5, an application of 'MULTUM II' to organic compounds was described. TOF mass spectra of biomolecules were obtained with good mass resolution using MALDI. The results demonstrate that the multi-turn TOF mass spectrometer allows not only high resolution but also very high separation of the ions of molecular species from organic compounds. Additionally, a mass determination method for MALDI was investigated.

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Chapter 2

Instrumentation

2.1 Introduction

A compact multi-turn TOF mass spectrometer 'MULTUM Linear plus'^[1] was constructed in our laboratory. It was developed as a laboratory model of the 'COSAC' project of the 'ROSETTA' mission^[2]. It consists of four cylindrical electric sectors and eight quadrupole lenses (Q-lens). In order to inject and eject ions, linear-type TOF mass spectrometers were combined. Four Q-lens triplets were used to achieve space focusing in linear part. There were two injection and ejection parts, the number of Q-lenses was 28. It was too complex to carry on a space craft. In order to reduce weight and the number of parts of the instrument, 'MULTUM II'^[3] consisting of only four toroidal electric sector fields have been designed and constructed by using the principle described in Appendix A and B. The size is similar to 'MULTUM Linear plus,' path length of one cycle was 1.308 m. In this chapter the technical data of the whole assembly for applications of laboratory experiments are reported.

2.2 Ion optics of a multi-turn time-of-flight mass spectrometer 'MULTUM II'

A simple outline of the ion optical system of 'MULTUM II' is given in this section. Detailed discussions about ion optical system of a multi-turn TOF mass spectrometer were given in Appendix B. 'Perfect focusing'^[4] condition should be achieved to increase the mass resolution and the ion transmission for multi-turn systems. 'Perfect focusing' means that absolute values of the position and angle at the final position should be the same as at the initial position in both the horizontal and vertical directions. By introducing symmetry in the arrangement of the ion optical components, multiple focusing is easily achieved under some circumstances^[5]. If the 'perfect focusing' is not satisfied, the ion beam will diverge. Consequently the mass resolution and the ion transmission will decrease according to the number of cycles. For example, the ion optical system of 'MULTUM Linear plus' satisfies this condition every cycle. But the ion optical system of 'MULTUM Linear plus' was complex. In order to simplify the ion optical system, 'perfect focusing' ion optical systems consisting of toroidal electric fields (described in section 2.3) instead of Q-lenses and cylindrical electric fields was investigated. As a result, a system comprised only four toroidal electric sector fields was developed. The c-value^[6] of the four toroidal electric sector fields is 0.033. Here the c-value is defined as;

$$c = \rho_0 / R_0 \tag{2.1}$$

where ρ_0 is the mean radius in the horizontal direction and R_0 is the radius of curvature of equipotential surface in the vertical direction.

The first and second order transfer matrix elements; R(i|j) of multi-turn part of 'MUL-TUM II' calculated by 'TRIO-DRAW'^[7] are shown in Table 2.1, where the unit of element is 1 m. The first order 'perfect focusing' condition is almost satisfied. However, there are small non-zero off-diagonal terms of the first order transfer matrix. Since initial position vector is very small, for example $x_i = 0.0005$, $\alpha_i = 0.005$ estimated from experimental condition, these non-zero terms can be neglected. Moreover two non-zero terms, $(l|\alpha) = -0.003$ and $(x|\delta) = -0.002$, are vanished after even cycles. The second order aberration may affect the peak shapes. This effect was evaluated by the computer simulation. The peak shapes after different number of cycles (1, 20, 60 and 100 cycles) calculated by 'TRIO-DRAW' are shown in Fig. 2.2. It is clearly shown that the peak width hardly change even after 100 cycles. Ion trajectories of 'MULTUM II' simulated by 'TRIO-DRAW' are shown in Fig. 2.2; (a) is a top view and (b) is x-, (c) is y-direction views and (d) is path length deviation mode.

i∖j	x	α	γ	δ	xx	$x \alpha$	$x\gamma$	$x\delta$	$\alpha \alpha$
\overline{x}	-1.000	0.000	0	-0.002	92.863	-0.010	0	0.144	-0.250
α	0.008	-1.000	0	0.000	-21.785	-185.516	0	92.104	0.006
l	0.000	-0.003	0.654	0.000	-36.642	-0.298	0.000	0.100	-0.037
i∖j	$lpha\gamma$	$\alpha\delta$	$\gamma\gamma$	$\gamma\delta$	$\delta\delta$	yy	yeta	etaeta	
x	0	-0.094	0	0	0.051	0.121	0.000	-0.011	-
α	0	-0.153	0	0	0.073	0.016	7.027	-0.004	
l	-0.002	0.164	-0.163	0.000	0.368	-0.001	0.011	0.000	
i∖j	y	eta							
\overline{y}	-1.000	0.001							
β	-0.004	-1.000							

Table 2.1: The first- and second-order transfer matrix elements; R(i|j) calculated by 'TRIO-DRAW'.



Fig. 2.1: Flight time image shape after 1, 20, 60 and 100 cycles: initial pulse width 25 ns, $x_{max} = 0.0005$ m, $\alpha_{max} = 0.005$ rad, $y_{max} = 0.0005$ m, $\beta_{max} = 0.005$ rad, $\delta = 0.005$, velocity of reference particle 1.02×10^{-5} m/s, (for example m/z = 28, acceleration voltage 1500 V).





 $x \max = 0.0015 \text{ m}$, $\alpha \max = 0.0250$, $\gamma \max = 0.0000$, $\delta \max = 0.0200$





Fig. 2.2: Ion trajectories of 'MULTUM II' simulated by 'TRIO-DRAW'. (a) Top view, (b) x-direction, (c)y-direction, (d) path length deviation mode.

2.3 Toroidal electric field

Figure 2.3 (a) shows a cross section of toroidal condenser. The toroidal electric field is characterized by the field index $c = \rho_0/R_0$, where ρ_0 is the mean radius in the horizontal direction and R_0 is the radius of curvature of equipotential surface in the vertical direction. Toroidal condenser is more advantageous than cylindrical condenser. Toroidal condenser has the property of focusing not only in the radial but also in the axial direction. Therefore, the combination of cylindrical condenser and Q-lenses can be replaced to toroidal electric fields and 'MULTUM II' consists of only four toroidal electric fields.



Fig. 2.3: Cross sections of (a) toroidal electrodes and (b) cylindrical electrodes and MAT-SUDA plates.

Although toroidal electric field is produced by toroidal condenser, it can be produced by the combination of a cylindrical condenser and MATSUDA plates^[8]. Figure 2.3 (b) shows a cross section of cylindrical condenser terminated with MATSUDA plates, where l is the height of cylinder condenser, 2k is the gap distance between cylinders. In case of small *c*-value, if a l/2k is small and terminated by MATSUDA plates, the electric field in the cylinder electrodes is effectively modified. Its shape looks something like the field produced by toroidal electrodes. It has been shown that *c*-value of such an electric field can be controlled by changing the electrostatic potential applied to the MATSUDA plates. Cylindrical condenser has a simple shape, and can be easily manufactured. Moreover, the focal length of the electric field can be easily changed by varying the applied voltage to the MATSUDA plates, because the value of the field index, *c*, is adjusted electrically. Because of these merits, the combination of simple cylinders and MATSUDA plates was employed as sector electrodes of 'MULTUM II.'

2.4 Whole assembly

A schematic drawing and photograph of 'MULTUM II' with an ion mirror are shown in Fig. 2.4 and 2.5, respectively. The instrument has three housings; analyzer housing, ion source housing and detector housing.

2.4.1 Analyzer housing

A technical drawing of sector electrodes is shown in Fig. 2.6 and a photograph of analyzer part of 'MULTUM II' is shown in Fig. 2.7, respectively. The analyzer consists of a symmetrical array of four units. The each unit is comprised of two field-free drift spaces and a toroidal electric sector field. Two drift length of the basic unit are 80.9 mm and 108.9 mm. The four toroidal electric sector fields were produced using cylindrical electric sectors and MATSUDA plates. The mean radius of the toroidal electric sector field is 50 mm and the deflection angle is 157.1°, the gap between the electrodes is 10.0 mm and the height of the electrodes is 43 mm. The gap between the MATSUDA plates and cylinder electrodes is 2 mm. The outer sector electrodes have holes for ion injection and ejection. At the fringing



Fig. 2.4: A schematic drawing of 'MULTUM II.'



Fig. 2.5: A photograph of 'MULTUM II.'

of the sector electrode, Herzog shields were placed, so that the ideal field boundaries can coincide with the front faces of the cylindrical electrodes. The distance between the shield and the electrode was 1.3 mm and the aperture of the shields was 7.2 mm \times 30 mm. The total pass length of one cycle was 1.308 m. All elements were hung on the top plate of the analyzer housing.

2.4.2 Ion source housing

In 'MULTUM Linear plus,' the ion source and the analyzer are installed in the same vacuum chamber. Only an EI ion source can be attached. As a result, the background pressure in a whole chamber rises when a gaseous sample is introduced through a needle valve. However, high vacuum is required to obtain good ion transmission, because the collision probability of ions with residual gas increases at low vacuum. Therefore, 'MULTUM II' ion source housing was separated from the analyzer housing and a differential vacuum pumping system was introduced. Various ion sources (EI, fast atom bombardment (FAB), MALDI, electrospray ionization (ESI) and inductively coupled plasma (ICP)) can be attached to the ion source housing. The Q-lens doublet was introduced between the ion source housing and



Fig. 2.6: A technical drawing of sector electrodes.



Fig. 2.7: A photograph of multi-turn part of 'MULTUM II.' The elements were hung on the top plate of the vacuum chamber.



Fig. 2.8: A photograph of the ion mirror with the MCPs.

the analyzer housing to improve ion transmission in the linear part of the instrument. In this study, EI and MALDI ion sources were used. The detailed features were given in Chapter 3, 4 and 5.

2.4.3 Detector housing

In the detector housing, there were two detectors and an ion mirror. A photograph of the ion mirror with the MCPs is shown in Fig. 2.8. When the ion mirror was off, the ions ejected from the multi-turn part flew towards a two-stage micro channel plate (MCP) of 14.5 mm diameter (F4655-13, Hamamatsu photonics K.K., Shizuoka, Japan) attached at the position of Detector 1 in Fig. 2.8. In the case of using the ion mirror, a two-stage MCP of 27 mm diameter with center hole (F4292-09, Hamamatsu photonics K.K., Shizuoka, Japan) attached at Detector 2 in Fig. 2.8 was used. The ion mirror was used to achieve energy focusing in the linear part of the insturument. The ion mirror consisted of 11-ring electrodes and 85 % transmission meshes were attached to the first, fourth and last electrodes. The output signals from the detector are accumulated by a digital oscilloscope LC564DL; sample rate: 2 GS/s, bandwidth: 1 GHz, memory: 1 Mpts (LeCroy Japan, Osaka, Japan).



Fig. 2.9: A block diagram of ion flight control program.

2.4.4 Ion flight control program

A block diagram of the timing control system is shown in Fig. 2.9. A digital pattern generator (Model 555 Pulse Generator, Berkeley Nucleonics, CA, USA) supplies the timing signals for the ion source, Sectors I and IV, and the digital oscilloscope. This generator provides timing signals of eight channels with 1 ns resolution. Before ion extraction from the ion source, appropriate voltages are applied to Sector I, Sector II and Sector III but that of Sector IV is zero. A pulsed voltage is applied to the push-out chamber at time (T_1) to extract the ions from the ion source. The pulsed ions enter the analyzer part via the Q-lens doublet. After completion of the injection cycle (T_2) , the voltage is applied to Sector IV. On the completion of the predetermined number of cycles around the instrument (T_3) , the voltage in Sector I is turned off and the ions are allowed to pass into the detector. The voltages in Sector II and Sector III are always applied. The time of flight is measured from time T_1 by the digital oscilloscope.

Generally, light ions catch up with and pass heavy ions in the multi-turn portion. If the ions are overtaken, it is difficult to determine m/z from the flight time because one cannot decide the number of cycles of each peaks from only one TOF spectrum. In order to avoid such complicating phenomena, only ions that can be observed in the same number of cycles are injected into the multi-turn part. An ion gate was introduced for this purpose.

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Chapter 3

Investigation of the performance of 'MULTUM II' with an electron ionization ion source

3.1 Introduction

The mass resolution of the TOF mass spectrometer is given in Eq. 3.1

$$R = \frac{T}{2\Delta t} \tag{3.1}$$

where T is the flight time and Δt is the peak width at half maximum. The mass resolution is directly proportional to its total flight time under the assumption of a constant width of ion packet. Under the 'perfect focusing'^[1] condition, Δt is kept constant in the first-order approximation. If higher order aberrations are large, the mass resolution may decrease after many cycles. In our previous study, the performance of 'MULTUM Linear plus' was evaluated using CO-N₂ doublet with an EI ion source. The mass resolution increased according to the number of cycles and , in fact, a mass resolution of 350,000 (FWHM) was achieved after 501.5 cycles^[2]. In this chapter, as an investigation of the performance of 'MULTUM II,' the increase in the mass resolution with the number of cycles was evaluated experimentally using gaseous samples with an EI ion source. Variation of the ion transmission with number of cycles was also evaluated. The effect of the potential of the MATSUDA plates to the ion transmission efficiency was investigated. Additionally as an application of a multi-turn TOF mass spectrometer, the combination of the ion mirror and 'MULTUM II' was examined.

3.2 Instrumentation

3.2.1 Electron ionization ion source

A two-stage acceleration ion source^{[3][4]} of electron ionization (EI) type was used for preliminary experiments. A schematic drawing and photograph of this ion source are shown in Fig. 3.1 and Fig. 3.2 respectively. This type of the ion source can compensate for the flight time deviation caused by the distribution of the initial position of ions. This focusing effect was called 'space focusing' in Ref [3], but in this thesis this is called 'spatial focusing.' The spatial focusing condition is given as

$$L = 2S_0 k_0^{3/2} \left(1 - \frac{D}{(k_0 + k_0^{1/2})S_0} \right),$$
(3.2)

$$k_0 = \frac{S_0 E_s + D E_d}{S_0 E_s}$$
(3.3)

where L, S_0 , D, E_s and E_d are the focal length, the length of the first acceleration field, the length of the second acceleration field, the electric field strength of the first acceleration field and the electric field strength of the second acceleration field, respectively. The focal length is optionally fixed by the ratio of the first acceleration voltage to the second acceleration voltage. This makes the two-stage ion source much easer to operate, because only a fraction of the total acceleration voltage is pulsed. In this study, $S_0 = 3.5$ mm, S = 6.0 mm, D =8.0 mm and L = 760 mm, respectively. Thus the appropriate voltage ratio applied to the Push-out chamber (V_p) and the Grid 1 (V_{float}) can be calculated as

$$V_{float} \approx 14 V_p \tag{3.4}$$

Because of turn-around-time, in practice, this voltage ratio may be chosen so that the mass resolution is increased. Small E_s resulted in long turn-around-time, so that if spatial focusing is satisfied, good mass resolution was not obtained. Experimentally, the condition of $V_{float} \approx$ $8V_p$ gave the improved mass resolution.

Gaseous samples were introduced into the ionization region through a needle valve and ionized by an electron beam.



Fig. 3.1: A schematic drawing of the two-stage ion source of EI type.



Fig. 3.2: A photograph of the two-stage ion source of EI type.



Fig. 3.3: The coaxial ion mirror and the two MCPs installed in the detector housing.

3.2.2 Detectors and Ion mirror

The detailed feature of the detector housing is described in Chapter 2. A schematic drawing of the detector housing is shown in Fig. 3.3. The ion mirror^[5] and the two MCPs were installed in the detector housing. When the ion mirror was off, the MCP at the position of Detector 1 in Fig. 3.3 was used. When the ion mirror was on, the MCP with center hole at position of Detector 2 in Fig. 3.3 was used. Generally, the ion mirror is used to improve the mass resolution by compensating of ion energy spread and expanding flight path length in the TOF mass spectrometer. In the multi-turn TOF mass spectrometer, the flight path length is increased by increasing flight cycles. The ion mirror was employed for the purpose of the energy focusing in the linear part of 'MULTUM II'. The ion mirror reverses the flight path of the ions in a retarding electrostatic fields so that those ions with higher kinetic energies penetrate further into the retarding field and follow a slightly longer path length as their trajectories are reversed. Higher-energy ions emerging from the ion mirror lag behind lower-energy ions, but catch up at a position in the flight tube which defines the ion mirror's focal point and is normally the optimal location for the detector.

It is noted that the combination of the two-stage ion source and the ion mirror gives advantages to improve the mass resolution. It is possible to decrease turn-around-time by strong acceleration electric field of the first stage. Then thin ion bunches can be formed near the ion source. If ions are detected at this position, although Δt in Eq. 3.1 is decreased, T is also shortened, so that the mass resolution is not dramatically improved. The energy focusing property of the ion mirror can realize the transmission of thin ion bunches to a different position without expansion due to the spread in the initial ion energies. Therefore turn-around-time decreasing and energy focusing are achieved simultaneously and provide the improved mass resolution.

3.2.3 Power supplies for sectors

MP1P/24 and MP1N/24 voltage supplies (Spellman, UK) were used for the voltage source of the sectors and Q-lens doublet. Output voltages from MP1P/24 and MP1N/24 were inputted to the negative and positive regulation circuits shown in Fig. 3.4 (a) and (b), respectively. The reference voltage from the reference circuit shown in Fig. 3.4 (c) is also inputed to the regulation circuits. For electrostatic sectors; namely, Sector II, III and Qlens doubles, the output voltages from the regulation circuits were applied to the sector electrodes. For Sector I and IV, the high voltage MOSFET circuits shown in Fig. 3.5 were used. These circuits were created on a printed circuit boards.





(c)

Fig. 3.4: A (a) negative, (b) positive regulation circuits and (c) a reference circuit.



Fig. 3.5: A high voltage switching circuit

3.3 Experimental and Results

3.3.1 Measurements of Xe

A preliminary experiment^[6] was carried out by using xenon as a sample. The experimental conditions were shown in Table 3.1. TOF spectra of Xe⁺ acquired using different flight

electron energy	69	eV
emission currents	90	μA
pulse voltage	180	V
total acceleration voltage	1.5	kV
back ground pressure (ion source housing)	2.1×10^{-5}	Pa
vacuum pressure (sample in ion source housing)	2.6×10^{-5}	Pa
back ground pressure (analyzer housing)	5.0×10^{-5}	Pa
voltage supplied to the MCP	-1.7	kV
sampling rate	2	GS/s
repetition rate	100	Hz
number of spectrum accumulated	10000	

Table 3.1: Experimental conditions

path length (1, 4, 7 and 10 cycles) are shown in Fig. 3.6. It is clear that the mass resolution increases with increasing the number of cycles. The relation between the number of cycles and the mass resolution 136 Xe⁺ is shown in Fig. 3.7. The peak width were approximately the same in the range 29.5 to 31.0 ns. Accordingly the mass resolution increased in proportion to the number of cycles. The mass resolution 5,100 at m/z 136 was achieved after 10 cycles. Hence, we can conclude that 'MULTUM II' satisfies the time focusing. The variation of the ion intensity, which is the sum of all Xe isotopes, with the number of cycles is shown in Fig. 3.8. The ordinate was normalized so that intensity of linear mode (0 cycle) was 1 unit. A large number of ions cannot circulate the multi-turn part owing to the gap of sector electrodes and slits in the multi-turn part. Therefore, there was great reduction of intensity after one cycle. In contrast to this, from 1 to 10 cycles no significant decrease in the ion intensity was observed. Hence we can conclude that 'MULTUM II' has stable multi-turn orbits.







Fig. 3.6: TOF spectra of 134 Xe⁺. (a) 1 cycle (the total flight path length was 2.108 m), (b) 4 cycles (6.032 m), (c) 7 cycles (9.956 m) and (d) 10 cycles (13.880 m).



Fig. 3.7: The relation between the number of cycles and the mass resolution of Xe⁺.



Fig. 3.8: The variation of the ion intensity which was the sum of all Xe⁺ isotopes with the number of cycles. The ordinate was normalized so that the intensity of the linear mode was 1 unit.

3.3.2 Measurements of CO and N₂

In previous experiments, according to the definition, a mass resolution, i.e., $T/2\Delta t$ 5,100 was confirmed using xenon as a sample. Separation of xenon isotopes need the practical mass resolution, i.e., $m/\Delta m$ 260. It was not proved whether or not the high mass resolution of 5,100 was really achieved. In order to confirm this, CO-N₂ doublet ($m/\Delta m$ 2,500) was used as a sample^[7]. The experimental conditions were shown in Table 3.2.

electron energy	74	eV
emission currents	80	μA
pulse voltage	180	V
total acceleration voltage	1.5	kV
back ground pressure (ion source housing)	1.5×10^{-5}	Pa
vacuum pressure (sample in ion source housing)	1.6×10^{-5}	Pa
back ground pressure (analyzer housing)	2.1×10^{-5}	Pa
voltage supplied to the MCP	-1.7	kV
sampling rate	4	GS/s
repetition rate	100	Hz
number of spectrum accumulated	25000	
	1	

 Table 3.2: Experimental conditions

TOF spectra of CO-N₂ doublet acquired using different flight path lengths (10, 20, 60, 100 and 150 cycles) are shown in Fig. 3.9. The peak width at half maximum was almost 26~31 ns. The relation between the number of cycles and the mass resolution N₂⁺ is shown in Fig. 3.10. It is clearly shown in Fig. 3.10 that the mass resolution increased proportion to the number of cycles. The mass resolution of 33,000 was achieved after 150 cycles. A mass resolution $T/2\Delta t$ 5,100 was achieved after 20 cycles. CO-N₂ doublet was really separated. The practical mass resolution 5,000 was also achieved and it accorded with theoretical one. Therefore the practical mass resolution 33,000 could be expected.

The variation of the ion intensity (the sum of CO-N_2 doublet) with the number of cycles is shown in Fig. 3.11. The ordinate was normalized so that intensity of 10 cycles was 1 unit. The ion transmission through the system was more than 99% after every one-cycle. Taking into consideration vacuum conditions, it could be expected that ions were mainly lost by collisions with neutral residual gas in the vacuum chamber. This ion transmission shows that the 'perfect focusing' condition was achieved.





Fig. 3.9: TOF spectra of CO⁺ and N_2^+ ions. (a) 10 cycles (the total flight path length was 13.880 m), (b) 20 cycles (29.960 m), (c) 60 cycles (79.280 m), (d) 100 cycles (131.60 m) and (e) 150 cycles (197.00 m).



Fig. 3.10: The relation between the number of cycles and the mass resolution for N_2^+ ions.



Fig. 3.11: The variation of intensity which was the sum of CO and N_2 with the number of cycles. The ordinate was normalized so that intensity of 10 cycles was 1 unit.
3.3.3 Tolerance of the voltage applied to the MATSUDA plates

The *c*-value of the toroidal field can be varied by changing potential applied to MAT-SUDA plates. Since an electric field produced by the combination of the cylinders and MATSUDA plates cannot be solved analytically, it was calculated numerically by the computer program 'ELECTRA'^[8]. Then *c*-value was derived as following manner. Generally, a radius of curvature of equipotential surface in the vertical direction of a function; f(r, z) = 0is given as follows:

$$R(r,z) = \left| \frac{(f_r^2 + f_z^2)^{3/2}}{2f_{rz}f_r f_z - f_{rr} f_z^2 - f_{zz} f_r^2} \right|,$$
(3.5)

$$f_i = \frac{\partial f}{\partial i}, f_{ij} = \frac{\partial^2 f}{\partial i j}, i, j = r, z$$

A network of points are taken with equal distances $\Delta = \Delta r = \Delta z = 0.001$ as shown in Fig. 3.12. Electric fields of *r*-direction: ER_i and *z*-direction: EZ_i , i = 0, 1, 2, 3, 4 are calculated in the 'ELECTRA.' Applying Eq. 3.6 to Eq. 3.5, the radius of curvature of equipotential surface in the vertical direction; R(r, z) is derived.



Fig. 3.12: Coordinate system and electrode structure. Representative mesh points are also shown.

$$f_{r} = ER_{0}$$

$$f_{z} = EZ_{0}$$

$$f_{rr} = (ER_{1} - ER_{3})/(2\Delta)$$

$$f_{rz} = (EZ_{1} - EZ_{3})/(2\Delta)$$

$$f_{zz} = (EZ_{2} - EZ_{4})/(2\Delta)$$
(3.6)

Table 3.3 shows distribution of values of $\rho(r)/R(r,z)$. Figure 3.13 shows an example of potential map. For the designed value c = 0.033, 82% of the potential of the cylindrical sector electrodes (V_s) is demanded. For the case of 4.5 kV ion acceleration, the voltage of the MATSUDA plates (V_m) corresponds to 740 V.

To estimate the effect of the MATSUDA plate, the N_2^+ signal intensity of three different flight cycles (1, 10 and 100 cycles) were measured. Experimental result is shown in Fig. 3.14. The acceleration voltage was 4.5 kV. The voltage applied to the outer sector electrodes was just +900 V, and the voltage applied to the inner electrodes was just -900 V. The signal intensity was maximum around 1400 V after 1 cycle. The results show that the tolerance became narrow as the number of cycles increases. After 100 cycles the ion transmission efficiency was maximum around 700 V. This voltage is in good agreement with the designed one. It is suggested that voltages of the instrument should be tuned after many cycles.

		r (mm)				
	49.0	49.5	50.0	50.5	51.0	
4	0.0358	0.0381	0.0400	0.0404	0.0403	
3	0.0333	0.0351	0.0370	0.0374	0.0372	
2	0.0314	0.0332	0.0345	0.0354	0.0347	
1	0.0299	0.0322	0.0335	0.0338	0.0337	
z (mm) 0	0.0299	0.0317	0.0330	0.0338	0.0332	
-1	0.0299	0.0322	0.0335	0.0338	0.0337	
-2	0.0314	0.0332	0.0345	0.0354	0.0347	
-3	0.0333	0.0351	0.0370	0.0374	0.0372	
-4	0.0358	0.0381	0.0400	0.0404	0.0403	

Table 3.3: The distribution of values of $\rho(r)/R(r, z)$ in the toroidal electric field produced by cylinder electrodes (±900 V) and MATSUDA plates (+740 V).



Fig. 3.13: An example of potential map. Boundary condition is $V_m = 0.82V_s$



Fig. 3.14: The relation between the number of cycles and the peak area for N_2^+ . The ordinate was normalized so that a peak area at 1400 V was 1 unit.

3.3.4 Measurements of CO and N_2 by using the ion mirror

It is possible to shorten turn-around-time by strong acceleration electric field. Then thin ion bunches can be formed at the beginning of the drift space (near the ion source or far from the detector). If ions are detected at this position, although Δt in Eq.3.1 is decreased, T is also shortened, so that the mass resolution is not dramatically improved. The basic unsolved problem is the transmission of these thin bunches to the end of the drift space without expansion due to the spread in the initial ion energies. The ion mirror solves this problem by its energy focusing property. Therefore turn-around-time decreasing and energy focusing are achieved simultaneously, so that the mass resolution is improved. Our ion mirror is consisting of 11 ring electrodes and the potentials of each electrodes can be changed independently. As a result, the ion mirror can be used as a single- or a dual-stage ion mirror. The functions as a single- and a dual-stage ion mirror were tested by using the linear part of 'MULTUM II' with the two-stage ion source. And the combination of the two-stage acceleration ion source, the dual-stage ion mirror and 'MULTUM II' was also investigated.

In case of the single-stage ion mirror, ions are focused at a point in space satisfying the condition $L = 4D^{[9]}$, where L is the total field-free drift length from a focal point to a detector and D is the distance the ions travel into the ion mirror. On the other hand, the dual-stage ion mirror has a flexibility about its focal length. The focusing properties of a single- and a dual-stage ion mirror were confirmed experimentally. The way of experiment was as follows. In order to observe the mass resolution improved by minimizing the turn-around-time, it is preferred that the flight time is the same. Therefore the appropriate total acceleration voltage was constant value of 1750 V; $V_p/2 + V_{float} \approx 1750$, where V_p is the pulsed voltage applied to the Push-out chamber and V_{float} is the voltage applied to the Grid 1 of the ion source. Voltages in the ion mirror were adjusted to make peak sharp every change of V_p and V_{float} .

Figure 3.15 shows a relation between V_p and peak width of N₂⁺. Variation of calculated turn-around-time was plotted in closed diamond. Experimental results of single- and dualstage ion mirror were plotted in closed circle and closed square, respectively. Short focal length means strong first acceleration electric field. As strong acceleration electric field provides short turn-around-time, calculated turn-around-time becomes short according to decrease of the focal length. In case of the single-stage ion mirror, according to decrease of the focal length, the peak width at focal length > 100 mm became small. However peak width at focal length < 100 mm became wide again. This reason is as follows. Ions are focused at a point in space satisfying the condition L = 4D. In this experimental condition, the distance from the ion source to the MCP was 760 mm. The length of the ion mirror was 100 mm. L and D are designated as

$$L = 760 - [focal length], \quad D < 100 \tag{3.7}$$

Since L was >660 mm (> 4D) at focal length < 100 mm, thin ion bunches focused before the MCP was transmitted to the MCP with expansion due to the spread in the initial ion energies. Initial energy spread was defocused at focal length < 360 owing to the condition L = 4D. The peak width increased with an decrease in focal length. A dramatically increase of the peak width was observed at focal length 80 mm. In case of the dual-stage



Fig. 3.15: The relation between pulse voltage (or the focal length from the ion source) and peak width at half maximum for N_2^+ ions by using the ion mirrors as a singleand a dual-stage one.

ion mirror, energy focusing was satisfied regardless with L. The experimental results were in good agreement with the decrease of turn-around-time. It was concluded that dual-stage ion mirror had a possibility to achieve the high mass resolution with 'MULTUM II.'

Next, the combination of the two-stage acceleration ion source, the dual-stage ion mirror and 'MULTUM II' was investigated. In order to find better operating conditions of this combination, variation of the ion intensity and the ion transmission ratio were measured. Figure 3.16 shows first acceleration pulse voltage dependence of the ion intensity and the ion transmission ratio. Variation of the ion intensity of linear part was depend on ion draw-out efficiency and lens effect of the ion source. A good ion intensity was obtained at pulse voltage 250 V. The ratio of ion intensity after 1 and 0 cycle was defined as the ion transmission of the instrument. The ion transmission ratio increased to 30% around pulse voltage of 250 V. In addition, the ion intensity was also maximum around pulse voltage of 250 V. Therefore this condition was used for operating the instrument. Figure 3.17 shows TOF spectra obtained with and without the ion mirror. A mass resolution of 7,000 was achieved with the ion mirror after 10 cycles, while 30-cycles was necessary without the ion mirror to achieve similar mass resolution. A higher mass resolution of 18,000 was achieved after 30 cycles with the ion mirror. The relation between the number of cycles and peak width, and the mass resolution of N_2^+ from 0 to 100 cycles is shown in Fig. 3.18 and 3.19, respectively. The peak width acquired with the ion mirror was almost the same, $11\sim14$ ns, and that without the ion mirror was $24\sim25$ ns. The energy focusing was satisfied by the ion mirror and turn-around-time was shorted by higher acceleration electric field, so that the peak width was narrow compared to that acquired without the ion mirror. The mass resolution increased with increasing the number of cycles and a mass resolution of 47,000 was achieved after 100 cycles.



Fig. 3.16: The variation in (a) peak area and (b) ion transmission ratio as a function of pulse voltage: V_p . Total acceleration voltage: V_{total} was 1500 V. The ordinate was normalized so that peak area at $V_p=250$ was 1 unit.



Fig. 3.17: TOF spectra of CO⁺-N₂⁺ obtained with the ion mirror after (a) 10 cycles, (b) 30 cycles and without the ion mirror after (c) 10 cycles, (d) 30 cycles.



Fig. 3.18: The variation in the peak width as a function of the number of cycles.



Fig. 3.19: The variation in the mass resolution as a function of the number of cycles.

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Chapter 4

Improvement of 'MULTUM II' and high-resolution TOF spectra

4.1 Introduction

In preliminary EI experiments without the ion mirror described in Chapter 3, a mass resolving power of 33,000 after 150 cycles was achieved. However, a mass resolving power was not proportionally increased after 150 cycles. One of the possible reasons was instability of the electric sector fields. In this chapter, some improvements: the ion source, the detector, the power supplies for sectors were achieved. As a result, it was confirmed that increasing the ion flight cycles increased the mass resolution. Moreover, a doublet peak of CH_2Cl_2 and $CDCl_2$ ($m/\Delta m = 54, 162, \Delta m$: mass difference of doublet, m: mass of lighter ion of doublet) was separated. When one peak of this doublet was used as a calibrant, the mass of the other peak was determined within a few ppm by mass accuracy.

4.2 Instrumentation

4.2.1 Improved electron ionization ion source

The mass resolution of TOF mass spectrometer is proportional to its total flight time and inversely proportional to the peak width. The peak width mainly consists of the distribution of the initial position of ions. Described in Chapter 3, the two-stage ion source satisfies the spatial focusing. In practice, the spatial focusing condition must be compromised with the effect of the turn-around-time to achieve good mass resolution. In other words, the spatial focusing condition is not satisfied experimentally. In order to compensate this defocusing effect, the distribution of the initial position of ions was to be small by thinning the electron beam. An electron beam slit was attached to the push out chamber of the EI ion source. A technical drawing and a photo of these parts is shown in Fig. 4.1 and Fig. 4.2.



Fig. 4.1: A technical drawing of the electron beam slit and the push-out chamber of the EI ion source.



Fig. 4.2: A photograph of the electron beam slit and the push-out chamber of the EI ion source.



Fig. 4.3: TOF spectra of $\rm N_2^+$ obtained by using the 0.8 mm and ϕ 3.0 mm electron beam slit.

TOF spectrum of N_2^+ obtained by using the 0.8 mm and ϕ 3.0 mm electron beam slit shown in Fig. 4.3. It is clear that the peak width becomes small by using the electron beam slit.

4.2.2 Improved ion detection

A schematic drawing of 'MULTUM II' without the ion mirror is shown in Fig. 4.4. The coaxial ion mirror having the MCP with a center hole was installed in the detector housing in the study reported in Chapter 3. Using the ion mirror actually increased the mass resolving power. However, the ion transmission decreased because of the requiring the ions to pass through meshes four times. Moreover ions passing again through the center hole of the MCP could not be detected. Therefore, it was decided that the ion mirror should be removed. If the distance between the detector and the ion source is long, the focal length of the two-stage acceleration ion source becomes long. It means that the electric field strength of the first acceleration field becomes weak. Therefore turn-around-time becomes long, so that the peak width will then be wide. In order to remove the effect of turn-around-time to the greatest extent possible, the distance between the ion source and the detector of the present instrument was made shorter than that in earlier study. The path length of the linear part



Fig. 4.4: A schematic drawing of 'MULTUM II' without the ion mirror.

of 'MULTUM II' was shortened from 76 cm to 65 cm.

For general mass spectrometers, ions are converted to electrons and electrons are amplified by the detection system. The conversion efficiency is quite bad for low speed ions. In the TOF mass spectrometry, ions have same kinetic energy. Because the speed of heavy ions is slow, the conversion efficiency for them is decreased. To improve the conversion efficiency, ions must be post-accelerated^[1]. Therefore, the post acceleration detector consisting of a conversion dynode and the MCP (F4655-10, Hamamatsu, Japan) was employed. Figure 4.5 shows a schematic view of the system. A is a mesh connected to the earth. B is a mesh of the conversion dynode unit and C is a conversion dynode. Voltage of B is slightly larger than that of C to avoid electron emissions from C to A. D is the MCP.

4.2.3 Improved power supplies for sectors

MP3P/24 and MP3N/24 voltage supplies (Spellman, UK) were used for the electrostatic sectors; namely, Sector II, Sector III, and all Matsuda plates. For Sector I and Sector IV, MOSFET switching modules (HTS41-03GSM, Behlke, Germany) and MP3P/24 and



Fig. 4.5: A schematic view of the post-acceleration detector.

MP3N/24 voltage supplies were initially used. However, they were influenced by the MOS-FET switching. In practical terms, we could not measure a stability of the applied voltage on an equal time-scale with the time of flight. It was measured by the digital multimeter (DMM2000, KEITHLEY) on conditions: the integration time of A/D converter is 16.67 ms, the usable digits is $6^{1/2}$. The result indicated a stability of 200 ppm by voltage. Then 410B voltage supplies (Fluke, USA) was used. By using the 410B type, stable voltage (20 ppm) was obtained regardless of whether the MOSFET switch was on or off.

4.3 Experimental and Results

The operating conditions were shown in Table 4.1. Gaseous samples are directly introduced into the ionization region through a needle valve. Liquid samples are introduced into the heated sample reservoir (JEOL Ltd., Akishima, Japan). The evaporated samples then enter the ionization region of the EI ion source.

For measuring N_2^+ , air is introduced into the ionization region through a needle valve. A solution of pyridine/benzene was prepared by mixing in the ratio of 1:10. One μl of this solution was introduced into the heated sample reservoir by a microsyringe. A 1:1 solution of chloroform-d/dichloromethane was introduced into the reservoir in the same manner.

electron energy	69	eV
emission currents	90	μA
pulse voltage	700	V
total acceleration voltage	4.5	kV
back ground pressure (analyzer housing)	3.0×10^{-5}	Pa
voltage supplied to the conversion dynode	-4.0	kV
voltage supplied to the MCP	-1.7	kV
sampling rate	2	GS/s
repetition rate	20	Hz
number of spectrum accumulated	300	

Table 4.1: Experimental conditions

4.3.1 High resolution TOF spectra

Figure 4.6 shows high-resolution TOF spectra of N_2^+ obtained after 600 and 1200 cycles. Even after 1.57 km flight length, high resolution TOF spectra was achieved with good signalnoise ratio. Figure 4.7 shows flight-time dependence of the mass resolution of N_2^+ .

The mass resolution increases linearly up to 3.0 ms (flight cycles: 400, flight length: 520 m). Moreover a mass resolution greater than 250,000 was achieved after 9.0 ms. After 3.0 ms, the mass resolution still increases but not proportionally because the peak width became wide. Three main reasons why peak width became wide are presumed. The first reason is the influence of higher order aberrations. The ion optics of one cycle of 'MULTUM II' satisfy the 'perfect focusing' condition. However, this condition is satisfied only in the first-order approximation and higher order aberrations remain. Such aberrations are small in one cycle. Even so, they are accumulated for every cycle. After multiple cycles, the accumulated aberrations create distortion of the peak, so that the peak width becomes wide and the mass-resolving power decreases. The second reason involves the stability of the sector electrode potentials while the TOF spectra are being obtained. The flight time of the multi-turn orbit is proportional to the potential of the sector electrodes. Therefore, if the voltage applied to the sector electrodes varies during accumulation of a TOF spectrum, the peak position will also vary. As a result, the accumulated TOF spectrum will consist of the accumulation of different flight times in spite of the same mass-to-charge ratio. This variation has a greater impact on a longer flight time. The third reason is the influence of ion scattering^[2]. Figure 4.8 shows the flight-length dependence of the peak area of N_2^+ . The solid circles show the peak area of N_2^+ and the line was obtained by fitting the following function to the data from 400 to 1600 m:

$$I = I_0 \exp(-l/\lambda) \tag{4.1}$$

where I is the ion signal intensity, l is the flight length and λ is the mean free path. The derived value of λ was 660 m. This is longer than the length of 300 m estimated by N₂ - N₂ collision under 3.2×10^{-5} Pa. This estimation was carried out for case in which the flying N₂ is assumed to move and all other molecules remain stationary. In practice, cross section of N₂⁺ - N₂ collision might be larger than that of N₂ - N₂ collision; that is, the mean free path would become short. However, the experimental results showed longer mean free path than estimated one. Low-angle scattered ions are expected to be able to fly in a multi-turn orbit. These ions might have deviations of flight time, so that the peak width would be broad.



Fig. 4.6: High-resolution TOF spectra of N_2^+ showing (a) achievement of a mass resolution of 150,000 after 600 cycles and (b) achievement of a mass resolution of 250,000 after 1200 cycles.



Fig. 4.7: Measured relation between the mass resolution and flight time of N_2^+ .



Fig. 4.8: Flight-length dependence of the peak area of N_2^+ . The ordinate was normalized such that the intensity after 54 m was 1. The solid circles show the experimental results and the line was obtained by fitting the exponential function to the data from 400 to 1600 m.

Next, we measured pyridine/benzene 1:10 mixture to confirm separation of $m/\Delta m$ 9,746. Figure 4.9 (a) shows the TOF spectra of a doublet of m/z 79.04220 of ${}^{12}C_{5}H_{5}{}^{14}N$ and m/z79.05031 of ${}^{13}C^{12}C_{5}H_{6}$ produced by EI. The mass resolution of 27,000 (FWHM) was obtained after 1.0 ms (flight cycles: 80, flight length: 170 m). We also recorded this doublet with a mass resolution of 91,000 (FWHM) after 4.0 ms, as shown in Fig. 4.9 (b). A 1:1 mixture of chloroform-d and dichloromethane was measured to confirm the separation of $m/\Delta m$ 54,162. Figure 4.10 shows the TOF spectrum of a doublet of m/z 83. 95181 of CDCl₂ and m/z 83.95336 of CH₂Cl₂. A mass resolution of 115,000 (FWHM) was obtained after 8.5 ms (flight cycles: 660, flight length: 860 m). This is the first time such a separation has been achieved in TOF mass spectrometry.





Fig. 4.9: TOF spectra of ${}^{12}C_5H_5{}^{14}N$ and ${}^{13}C^{12}C_5H_6$ mass doublet showing (a) separation of the doublet peaks after 1.0 ms and (b) achievement of a mass resolution of 91,000 after 4.0 ms.



Fig. 4.10: TOF spectrum of CH₂Cl₂ and CDCl₂ mass doublet obtained after 8.5 ms. The peaks were separated and a mass resolution of 115,000 was achieved.

4.3.2 Mass determination

Mass calibration in TOF mass spectrometry is the conversion of a recorded TOF spectrum to a mass spectrum. This conversion is usually achieved using the following equation^[3]:

$$T = a\sqrt{m/z} + b \tag{4.2}$$

where T is the time of flight, a is a constant that can be calculated from the geometry of the mass spectrometer and the accelerating voltage, and b is the total time shift introduced by the registration system. In practice, however, measuring the flight time of at least two peaks of known masses (calibrants) and determining these constants achieves better results. Eq. 4.2 is then used to determine the mass of the unknown peaks. In the case that z is 1, Eq. 4.2 for an unknown peak is expressed as

$$T = a\sqrt{m} + b \tag{4.3}$$

and that for the calibrant is expressed as

$$T_{ref} = a\sqrt{m_{ref}} + b \tag{4.4}$$

Using Eq. 4.3 and Eq. 4.4, the mass of the unknown peak is expressed as

$$m = m_{ref} \frac{(T-b)^2}{(T_{ref}-b)^2}$$

= $m_{ref} \left(\frac{T^2}{T_{ref}^2} + 2 \frac{T(T_{ref}-T)^2}{T_{ref}^3} b + \frac{(5T+T_{ref})(T-T_{ref})}{T_{ref}^4} b^2 + \cdots \right)$ (4.5)

The second term of Eq. 4.5 contains the time shift b. If a calibrant is prepared close to the known peaks, the term $T_{ref} - T$ is very small compared to T_{ref} and time shift b is generally also very small compared to the TOF. As a result, the second and later terms are small enough to be ignored. We can therefore determine the mass of the unknown peak using only one calibrant. In this study, the order of $T_{ref} - T$ was a few hundred nanoseconds, b was about 200 ns, and the flight time was on the order of milliseconds. The ion masses could thus be determined by the following equation:

$$m = m_{ref} \left(T/T_{ref} \right)^2 \tag{4.6}$$

 ${}^{13}C^{12}C_5H_6$ benzene (calculated exact mass is 79.05030) was treated as an unknown, while pyridine ${}^{12}C_5H_5{}^{14}N$ (calculated exact mass is 79.042199) was used as a calibrant. The measured accurate mass of ${}^{13}C^{12}C_5H_6$ was 79.05017 (-1.6 ppm by mass) in the TOF spectrum of Fig. 4.9. To compare a long flight time with a short flight time in this procedure, a linear TOF spectrum of benzene -in other words, a short flight time- was used. ${}^{12}C_5H_6$ benzene (calculated exact mass is 78.04695) was used as a known, while ${}^{13}C^{12}C_5H_6$ benzene was treated as an unknown. The TOF of these ions was around 6.8 μs and $T_{ref} - T$ was 40 ns. The measured accurate mass of ${}^{13}C^{12}C_5H_6$ was 79.030 (-250 ppm). To extend the flight time, the accuracy of mass determination in this procedure was improved. In the same manner, the mass of dichloromethane (CH₂Cl₂) (calculated exact mass is 83.9536) was measured to be 83.95305 (-3.7 ppm) using chloroform-d (CDCl₂) (calculated exact mass is 83. 95181), as shown in Fig. 4.10.

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Chapter 5

Application of 'MULTUM II' to organic compounds ionized by matrix-assisted laser desorption/ionization (MALDI)

5.1 Introduction

Matrix-assisted laser desorption/ionization (MALDI)^{[1][2][3]} is currently a powerful tool for analyzing large molecules such a synthetic polymers or biomolecules. All ions drew out from an ion source can be detected by a TOF mass spectrometer. In addition, it has, in principle, an unlimited mass range. Given these advantages, the combined use of TOF mass spectrometers and MALDI allows the molecular mass measurement of large molecules with good sensitivity. In Chapter 4, the performances of 'MULTUM II' were evaluated using an electron ionization source. Mass resolution increased according to the number of cycles, and, in fact, a mass resolution of 250,000 (FWHM) was achieved at m/z 28 after 1200 cycles. In this chapter, the feasibility of 'MULTUM II' for separating biomolecule ions was evaluated^[4] by MALDI.

5.2 Instrumentation

5.2.1 MALDI ion source

In order to effectively evaluate 'MULTUM II,' a MALDI ion source with a 337 nm nitrogen laser was opted to use. A TLC-FAB probe (JEOL Ltd., Akishima, Japan) was

used as a stage for the MALDI sample target, since it enabled us to move the sample target in a range of 0 and 50 mm under the laser beam. The space-velocity correlation focusing (SVCF)^[5] method which is based on time-lag focusing and space focusing was employed to compensate temporal, spatial and velocity distributions in the ion source. The ion source is illustrated in Fig. 5.1 and its photograph is in Fig. 5.2.

The acceleration chamber and the sample plate were held at the same potential. The distance from the surface of the sample plate to Grid 1 was 5.5 mm, and that from Grid 1 to Grid 2, which was held at ground potential, was 9.5 mm. Two mirrors were attached for laser reflection and sample monitoring. An aluminum sample plate $(7 \text{ mm} \times 53 \text{ mm})$ was attached to the TLC-FAB probe in Fig. 5.3. A pulsed laser beam from a 337 nm nitrogen laser VSL-337ND-S (Laser Science, MA, USA) passed through a Lens 1 (f = 450 mm) and was attenuated by a variable ND filter wheel located close to the focal point of laser beam. In case that the filter is located in front of the focus lens, as the output laser size of 35 mm^2 is larger than the homogeneity area of the variable ND filter, the portions of laser beam are attenuated at different rate. Because of inhomogeneity of laser density and this different attenuation rate, the laser intensity finally irradiated at a sample spot is not stabilized each laser shot. Because the attenuation of focused laser beam whose area is 1 mm^2 can compensate inhomogeneity of laser density, the variable ND filter was located close to the focal point. Since the Lens 1 was held to the 3-axis translation stage, fine adjustment of the optical axis was possible. The optical axis was adjusted by moving the Lens 1. The pulsed laser beam was reflected by Mirror 1 and focused on a sample spot. The irradiation at the sample spot was observed by using Mirror 2 and a video camera. The sample plate, the acceleration chamber and Grid 1 were kept at the same voltage during laser irradiation. The pulse voltage was applied to them $2\sim3 \ \mu s$ after laser irradiation. During the application of pulsed voltage to the sample plate and the acceleration chamber, ions were extracted from the ion source.







Fig. 5.2: A photograph of the two stage MALDI ion source.



Fig. 5.3: A photograph of the TLC-FAB prove with sample plate.

5.3 Experimental and Results

5.3.1 Sample preparation

Protoporphyrin IX (PpIX) and angiotensin I (AgtI) were purchased from ICN Biomedicals Inc. (Aurora, OH, USA) and Bachem AG (Bubendorf, Switzerland), respectively. Other several compounds were proffered by Dr. Yoshinao Wada of Osaka medical center and research institute for maternal and child health.

A liquid matrix^[6] was mainly used for MALDI in this study. As reported by Kumar Kolli et al., this viscous liquid with a self-healing surface provides very long-lasting and reasonably constant ion currents for over 1 hour. This property favors the fine tuning of instrumental parameters. The matrix solution was prepared by dissolving 35 mg of 3-aminoquinoline (Sigma-Aldrich, St. Louis, MO, USA) in 150 μ L of a saturated solution of α -cyano-4hydroxycinnamic acid (CHCA) (Sigma-Aldrich) in methanol. The matrix solution was then mixed with 0.2 parts each of a saturated PpIX solution in acetone or a 10 pmol/ μ L AgtI solution (dissolved in 50% v/v acetonitrile/H₂O and with an addition of 0.1% trifuloroacetic acid (TFA)).

A solid matrix of CHCA was also used. For the case of solid sample, 1 μ L of CHCA solution (dissolved in 50% v/v acetonitrile/H₂O and with an addition of 0.1% TFA) was mixed with 1 μ L of sample solution. 0.5 μ L of the mixture was placed on the sample plate and the resulting mixture was slowly air-dried to form sample-matrix 'co-crystal'.

5.3.2 Measurements of biomolecules by using a liquid matrix

The operating conditions were shown in Table 5.1.

Table 5.1: Experimental conditions of MALDI/SVCF-multi-turn-TOF

pulse voltage	$800 \sim 900$	V
total acceleration voltage	$6.8 \sim 6.9$	kV
delay time	$2 \sim 3$	$\mu { m s}$
back ground pressure (ion source housing)	2.1×10^{-5}	Pa
back ground pressure(multi-turn housing)	2.5×10^{-5}	Pa
voltage supplied to the conversion dynode	-4.0	kV
voltage supplied to the MCP	-1.7	kV
sampling rate	2	GS/s
repetition rate	2	Hz
number of spectrum accumulated	100	

TOF spectrum of PpIX is shown in Fig. 5.4. The most abundant signal represented the MH⁺ with m/z 563.3. The 34 cycles spanning a total 45.1 m flight provided a resolution of 32,000 (FWHM).



Fig. 5.4: TOF spectrum of protoporphyrin IX after 34 cycles. The total flight was 45.1 m.

Next, AgtI was measured. In this case, the resolution of the MH⁺ ions $(m/z \ 1296.7)$ after various cycles of flight was examined. As shown in Fig. 5.5 the peak width was nearly

constant in a range of $8.5 \sim 12.0$ ns between cycles 4 to 40, with flight times of 180 μ s to 1600 μ s, respectively. The resolution increased as the ions flied the circuit, and finally reached > 60,000 after 30 cycles, or a 40.0 m total flight. It was also noted worthy that the signal intensity, or the abundance of detected ions, was not significantly reduced during cycles 11 through 40. This was consistent with our previous studies carried out under similar conditions including vacuum level, where the signal intensity of CO or N₂ ions was only minimally diminished after the initial several cycles of flight.

In the present study, the mass resolution to flight path length ratio was 1860 for AgtI after 4 cycles. This value was low compared with that of 10000 obtained by the conventional TOF instruments with a $1\sim2$ m reflector-type flight path. This would be due to the





Fig. 5.5: TOF spectra of angiotensin I. (a) 4 cycles (5.9 m the total flight path length),
(b) 11 cycles (15.0 m), (c) 24 cycles (32.0 m), (d) 30 cycles (40.0 m), and (e) 40 cycles (52.3 m). The scales unit of the vertical axes are comparable in these graphs.

following factors. First, the liquid matrix was used to facilitate the operation of this prototype instrument while monitoring long-lasting signals. As described by Cornett et al^[7]., the liquid matrix causes significant peak broadening. Depending on the thermal conductivity of the liquid matrix, the time required for the solution to cool below the critical evaporation rate would be much longer, perhaps even longer than the laser pulse. This would increase the time over which organic ions are formed, resulting in a wider peak for the TOF mass spectrum.

Next, the accelerating voltage was 6.8 kV in the present study. The broad energy distribution^[8] of the ions produced by MALDI is a dominant factor that widens the peak width. This effect can be reduced by supplying a high enough acceleration voltage for the contribution of the initial kinetic energy to be rendered a small portion of the total energy after acceleration. However, the higher accelerating potential results in a shorter flight time in a fixed path length, and thus the improvement of resolution is limited with the conventional TOF instruments. In contrast, as demonstrated in the present study, the multi-turn TOF mass spectrometer has an unlimited path and is able to improve resolution by extending the flight length without compromising the compact size of the instruments. Also, the present study indicates that this type of mass spectrometer is capable of separating the ions of molecular species from organic compounds to an extremely high degree. It is possible to devise a high performance tandem TOF mass spectrometer by introducing the technology of multi-turn TOF mass spectrometers. A new tandem TOF mass spectrometer for structural analysis of peptides or proteins has been designed and constructed in 2004^[9]. A multi-turn TOF mass spectrometer has been adopted for the first mass spectrometer (MS1), which has same dimensions as the 'MULTUM II', to achieve the high mass resolution necessary for the monoisotopic precursor ion selection. A TOF mass spectrometer with a quadratic-field ion mirror was adopted for the second mass spectrometer (MS2), which has been developed at the University of Warwick, to achieve time focusing independent of ion energy. A combination of a multi-turn TOF mass spectrometer and a quadratic-field ion mirror constitute a powerful instrument for structural analysis of peptides or proteins.

Figure 5.6 shows TOF spectra of rhodamin 6G (Rhod6G), angiotensin II (AgtII), β cyclodextrin (β CD) and a peptide VEADIAGHGQEVLIR. The high mass resolutions were also achieved for these compounds.




5.3.3 Measurements of biomolecules by using a solid matrix

Mentioned above, the liquid matrix increases the time of ion formation, resulting in a wider peak for the TOF mass spectrum. This effect cannot be removed by a TOF mass spectrometer. In order to improve the mass resolution, it is necessary to use a solid matrix which decreases the time of ion formation. Figure 5.7 shows TOF spectra of AgtI obtained with solid matrix of CHCA.

Because of the short time of ion formation, the peak widths were narrow compared to the TOF spectra shown in Fig. 5.5. The peak width (FWHM) was 5.0 ns in the linear TOF spectrum (Fig. 5.7(a)) and separation of ¹³C isotope ions of AgtI was observed. Because the solid matrix cannot provide constant ion currents over many laser shots, it was difficult to





Fig. 5.7: TOF spectra of angiotensin I obtained by using solid matrix. (a) after linear (b) 3 cycles, (c) 6 cycles, (d) 13 cycles and (e) 15 cycles. The scales unit of the vertical axes are comparable in these graphs without (a).

finely tune the instrumental parameters.

5.3.4 Mass determination for MALDI

Theory

Mass calibration in TOF mass spectrometry is the conversion of a recorded TOF spectrum to a mass spectrum. The flight time obtained by a multi-turn TOF mass spectrometer is expressed as

$$T = NT_{cycle} + T_{linear} + b \tag{5.1}$$

where T_{cycle} is a flight time per cycle, T_{linear} is flight time in the linear portion, and N is the number of cycles. N can be calculated by the flight time of linear part and a few cycles. In case of general MALDI/DE^[10]-TOF, T_{linear} contains the information of sample position, sample surface inhomogeneity^{[11][12]} and small acceleration energy difference^{[12][13]} owing to DE. The sample plate planarity imperfections and sample surface inhomogeneity vary the distance between the plate and the first extraction grid and can result in variations of ion flight times. Acceleration energy difference also results in variations of ion flight times. On the other hand, flight path length of multi-turn orbit cannot vary and ion optics of multiturn portion of 'MULTUM II' satisfies energy focusing condition, so that T_{cycle} is proportion to root m/z in the first order approximation. In other words, T_{cycle} does not include the information of sample position and small energy difference. If the same sample spot was used, the time shift of b and T_{linear} don't be changed for same ions. By measuring TOF mass spectra using at least two different flight cycles, T_{cycle} is obtained and expressed as follows:

$$T_{cycle} = a_{cycle} \sqrt{m/z} \tag{5.2}$$

where a_{cycle} is a mass independent constant per cycle. In this equation the number of calibration constants is only one. Therefore we can determine the mass of unknown peak by using only one calibrant. Then mass of analyzed substances is described by

$$m = \frac{(T_{cycle})^2}{(T_{cycle})_{ref}^2} m_{ref}$$
(5.3)

where citation ref belongs to the calibrant. This method has a merit can be applied to wide mass range. Because a calibrant and sample are prepared on separated sample spots, optimal experimental conditions, i.e., delay time and matrix, are decided independently. Therefore high sensitivity and high resolution can be obtained. The combination of MALDI and TOF mass spectrometer is capable of producing mass spectra of proteins of about 100,000 u molecular weight. It is advantage that wide range mass spectrum is calibrated accurately by only one calibrant.

Flight time reproducibility

Figure 5.8 contains data on the flight time reproducibility of ions generated by MALDI. Three spectra, which each consisted of 100 averaged laser shots, were taken from each sample spot, each flight cycle and each sample. However, the position of laser irradiation was different in the same sample spot. The data points in Fig. 5.8 represent the deviation as Eq.5.4, expressed in parts par million, of each flight time from the average flight time for $[AgtI+H]^+$, $[\beta CD+Na]^+$ and $[Rhod6G-Cl]^+$.

$$Deviation(ppm) = [(flight time - avg.flight time)/(avg.flight time)] \times 10^6$$
(5.4)

These flight time variation resulted from errors of decision on flight time and shift of flight time. The errors of flight time decision resulted from peak shape or signal to noise ratio and limitation of the digitizer (2Gs/s) of the flight time recording system. Shift of flight time resulted from drift in the power supplies and inhomogeneity of sample surface. For example, voltage applied to outer electrode of Sector II was changed from 1400 to 1401 V, shift of flight time of 33 ns was observed after 39 cycles. Including these effects, the reproducibility of TOF was within 3 ppm. Figure 5.9 shows the reproducibility of T_{cycle} of each sample. The reproducibility within 4 ppm was appropriate to the flight time reproducibility.

The sample position layout of the MALDI sample plate is shown in Fig. 5.10 and flight time reproducibilities of AgtI obtained from sample spot $1 \sim 4$ are shown in Table 5.2. Four different sample spots were made close to each other. The errors were large for spot 1 and 4, which were made most in the distance. At the nearest sample spots, the errors were comparatively small. Sample surface and sample plate planarity are probable source of this error.

Variations in the sample thickness and the plate planarity vary the distance between the plate and the first extraction grid and result in variations of kinetic energy. Described



Fig. 5.8: A plot of the variation in flight time (expressed as ppm) of AgtI, β CD and Rhod6G taken from a each sample spot.



Fig. 5.9: A plot of the variation in difference of T_{cycle} derived from the date plotted in Figure 5.8



Fig. 5.10: Sample position layout of the MALDI sample plate. Orange closed circle indicates the position of sample

Spot	T_{24}	T_{30}	T_{cycle}	Error (ppm)
1	985.5052	1226.5507	40.17427	17
2	985.5063	1226.5481	40.17363	2
3	985.5007	1226.5423	40.17362	1
4	985.5097	1226.5463	40.17277	-20
Average			40.17357	

Table 5.2: Flight time reproducibilities of AgtI obtained from different sample spots.

above, although the energy focusing is theoretically satisfied in the first order approximation, Table 5.2 shows that flight time dependence of kinetic energy was experimentally observed. A variation of 100 μ m in the ion source produces an acceleration voltage of 15 V. Detailed flight time dependence of kinetic energy was investigated. A plot of the variation in flight time (expressed as ppm) of AgtI is plotted against a variation of float voltage in Fig. 5.11. Acceleration voltage variation of 15 V resulted in 5 ppm flight time difference.

Influence of pulsed electric sector

Table 5.3 shows the preliminary results of mass determination. The mass of $[AgtI + H]^+$ was derived by using $[\beta CD + Na]^+$ as a calibrant. The flight times of 4 and 11 cycles of each sample were used. The average errors of accurate mass of $[AgtI + H]^+$ was -44 ppm. According to the flight time reproducibility of the instrument, this was systematic error of the instrument. One possible reason was a stability of the pulsed voltage of Sector I and Sector IV, which are on and off during ion injection and ejection.

Then the stability of the pulsed voltage was investigated by measuring flight time shift of ions. The ion flight control program for MALDI is shown in Fig. 5.12 and Table 5.4 shows the flight time dependence of the duration between the rise time of pulse voltage of Sector I and trigger of flight time measurement; T_I in Fig. 5.12. It is clearly shown that this flight time shift depends on T_I . This result indicates that pulsed voltage required more than 1.0 ms to be stable. The same phenomena might be occurred to Sector IV. Table 5.5 shows the flight time per cycle at higher and lower cycles. The flight time difference was 36 ns and it was obviously flight time shift depending on the instability of the applied voltage of Sector IV.

The instability of applied voltages of Sector I and Sector IV provide mass dependence with mass accuracy. In order to minimize these effects, 5.0 ms was chosen as T_I . On the other hand, we can not extend T_{IV} because ions returns to Sector IV within T_{cycle} , which is around 40 μ s for example AgtI. Therefore pair of larger number of cycles whose TOF were more than 900 μ s was used. Table 5.6 shows results of mass determination by using the flight times of 24 and 30 cycles. The each experiment was carried on the different day. The errors attached to mass were of the values of -4, -17 and 10 ppm. 13 ppm of the experimental uncertainties for this mass determination method is derived from the reproducibility of TOF (< 4 ppm)



Fig. 5.11: A plot of the variation of flight time (expressed as ppm) of AgtI in a variation of float voltage.

and sample position difference (< 5 ppm). In addition there is a time lapse between the acquisition of the analyte and calibrant data, additional errors could arise from drift of the voltage supplies. A kinetic energy difference owing to delayed extraction also results in mass accuracy errors. It is preliminary concluded that using TOF difference between large number of cycles vanished the systematic error resulted from instability of voltages in pulsed sectors.

	Mass accuracy (ppm)	-54 -29 -48	-44	
Н + I п	m/z	1296.6147 1296.6474 1296.6227	1296.6283	1296.6853
Angiotensiı	s) 1 cycles	$\begin{array}{c} 40.24928 \\ 40.24954 \\ 40.24925 \end{array}$	40.24935	
	ght time (μ 11 cycles	463.7593 463.7606 463.7596	463.7598	
	Fli 4 cycles	182.0143 182.0139 182.0149	182.0143	
	m/z	1 1 1	I	1157.3595
xtrin + Na	us) 1 cycles	38.02654 38.02630 38.02639	38.02641	
qe				
β-Cyclo	ght time (µ 11 cycles	$\begin{array}{c} 438.1769\\ 438.1754\\ 438.1756\\ 438.1756\end{array}$	438.1760	
<i>β</i> -Cyclo	Flight time (<i>j</i> 4 cycles 11 cycles	171.9911438.1769171.9913438.1754171.9909438.1756	171.9911 438.1760	

Table 5.3: Mass accuracy of MALDI/SVCF-multi-turn-TOF in the case of 4 and 11 cycles.



Fig. 5.12: The ion flight control program for MALDI-multi-turn-TOF

Table 5.4: Flight time of β CD dependence of the duration between the rise time of pulse voltage of Sector I and trigger of TOF measurement; T_I in Figure 5.12.

Flight time (μs)	$T_I \ (\mu s)$
590.324	220
590.273	320
590.236	420
590.221	620
590.213	1020
590.213	2020
590.213	3020
590.208	4020
590.213	5020

Table 5.5: Difference of flight time of AgtI; $T_{i+1} - T_i$, i=2, 30.

T_2	T_3	T_3 - T_2
101.6628	141.9519	40.2891
T_{30}	T_{31}	T_{31} - T_{30}
1229.0163	1269.2690	40.2527

		β-Cyclodex	ttrin + Na				Angiotensin	I I + H	
	F	light time $(\mu$	s)	m/z	FI	ight time $(\mu$	s)	m/z	Mass
Pair of spectra	24 cycles	30 cycles	1 cycles		24 cycles	30 cycles	1 cycles		accuracy (ppm)
1	932.1352	1160.1577	38.00375	I	986.6528	1228.0077	40.22582	1296.6577	-21
2	932.1373	1160.1556	38.00305	I	986.6500	1228.0039	40.22565	1296.6942	2
3	932.1348	1160.1530	38.00303	ı	986.6500	1228.0032	40.22553	1296.6879	2
Average	932.1358	1160.1554	38.00327	ı	986.6509	1228.0049	40.22567	1296.6799	-4
1	932.0364	1159.9951	37.99311	ı	986.5032	1227.7910	40.21463	1296.6616	-18
2	932.0334	1159.9926	37.99321	I	986.5025	1227.7894	40.21449	1296.6464	-30
3	932.0339	1159.9910	37.99285	ı	986.5047	1227.7927	40.21467	1296.6820	-3
Average	932.0346	1159.9929	37.99306	I	986.5035	1227.7910	40.21460	1296.6633	-17
1	931.5679	1235.3656	37.97471	ı	945.8213	1227.1924	40.19587	1296.7082	18
2	931.5599	1235.3582	37.97478	I	945.8212	1227.1900	40.19554	1296.6821	-2
3	931.5625	1235.3604	37.97474	ı	945.8142	1227.1853	40.19587	1296.7062	16
Average	931.5635	1235.3614	37.97474	ı	945.8189	1227.1892	40.19576	1296.6988	10
Exact mass				1157.3595				1296.6853	

Table 5.6: Mass accuracy of MALDI/SVCF-multi-turn-TOF in the case of 24 and 30 cycles.

Discussion

The errors attached to mass were results from the reproducibility of TOF and the difference of the sample spots. Kinetic energy difference was caused by combined effect of sample position and delayed ion extraction. The first order energy focusing is satisfied in the multi-turn orbit of 'MULTUM II.' However, the experimental results with the different sample position suggested that kinetic energy deviations caused the flight time deviations. Therefore the effect of energy deviations was measured. Figure 5.13 shows a relation between flight time of AgtI (4 and 14 cycles) and float voltage (same data in Fig. 5.11). These flight time variations consisted of variations of TOF of linear part and multi-turn part. Figure 5.14 shows the relation between the difference of flight time after 4 and 14 cycles and float voltage. The closed square was experimental result. The flight time shift was about 8/100 as compared to Fig. 5.13. It shows the effect of energy focusing of the multi-turn part. A minimum was observed at 6060 V. It is considered to be due to the second order energy deviation. The flight time deviation caused by the second order energy deviation of one cycle can be estimated by following equation:

$$(T_{cycle})_{\delta} = \frac{T_{cycle}}{l_{cycle}} \times (l|\delta\delta) \times \delta^2$$
(5.5)

where T_{cycle} is a flight time of one cycle, l_{cycle} is the path-length of one cycle, $(l|\delta\delta)$ is the second order energy coefficient of one cycle and δ is an energy deviation. In Fig. 5.14, the



Fig. 5.13: A plot of flight time of AgtI after (a) 4 and (b) 14 cycles versus float voltage.



Fig. 5.14: A relation between the difference of flight time after 4 and 14 cycles and float voltage

solid line was fitted by following;

$$y = \left(40.118815 + \frac{40.18815}{1.308} \times (0.368) \times \left(\frac{x - 60}{6400}\right)^2\right) \times 10$$
(5.6)

where T_{cycle} is decided to 40.118815 which is the flight time at the float voltage of 6060 V, l_{cycle} is 1.308 m, $(l|\delta\delta)$ is 0.368 and the total acceleration voltage is decided to 6400 V. The experimental results were comparable to the solid line in the range 40 to 80. The ion optics is originally realized only in the minute range. If the first order energy focusing is satisfied, the effect of the second order is surely observed. Since the minimum point (namely, second order energy focusing) depends on kinetic energy of ions, not on m/z, same kinetic energy could be found for all m/z. This method can compensate accurate mass errors resulted from kinetic energy deviations caused by delayed ion extraction and sample position. A decrease of flight time with float voltage < 6040 V perhaps resulted from the higher order aberration. More detailed experiments are necessary to draw the conclusion.

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Chapter 6 Summary

A new toroidal electric sector multi-turn time-of-flight mass spectrometer 'MULTUM II' was investigated. In order to improve the mass resolution and the ion transmission efficiency, the ion optical system of the instrument satisfies 'perfect focusing' condition in the first order approximation. The instrument having flight path length of 1.3 m per cycle was constructed. The variations of mass resolution and ion transmission efficiency with an increase in flight cycles were experimentally examined. The ion mirror was introduced to the instrument. Then improvements of the ion source, the power supplies and the detection system were achieved. An application to biomolecules were also examined. A new technique for mass determination was developed. The present investigation leads to the following conclusions:

- 1. The preliminary performance of the instrument was evaluated using Xe and CO/N_2 doublet with an EI ion source. The linear relation between the mass resolution and the number of flight cycles was experimentally confirmed. A mass resolution 33,000 was achieved at m/z 28 after 150 cycles. The ion transmission efficiency through the system was more than 99% after every one-cycle. The relation between the ion transmission efficiency and the *c*-value was measured. The *c*-value which provided good ion transmission efficiency was in good agreement with the designed one. Hence, the conclusions was reached that 'MULTUM II' had stable multi-turn orbits or 'perfect focusing' condition was satisfied.
- 2. In order to compensate energy dispersion in the linear part of 'MULTUM II,' the ion mirror was combined with the instrument. By minimizing turn-around-time and satisfying energy focusing, the mass resolution was improved and that of 47,000 was

achieved at m/z 28 after 100 cycles.

- 3. Some improvements of the instrument were achieved in consideration of above results and an application for biomolecules. The ion mirror was removed for higher ion transmission efficiency. In order to achieve higher mass resolution, the power supplies for electric sectors were replaced with more stable ones and the initial ion packet was to be thin. A mass resolution of 250,000 was achieved at m/z 28 and a doublet of $m/\Delta m$ 54,162 was separated for the first time in TOF mass spectrometry.
- 4. The feasibility of 'MULTUM II' for separating biomolecule ions was evaluated by MALDI. High mass resolutions > 60,000 were obtained at m/z 1296 with liquid matrix. The narrow peak widths were obtained with solid matrix and good mass resolution of 37,000 was also obtained. Because the solid matrix cannot provide constant ion currents over many laser shots, it was difficult to finely tune the instrumental parameters.
- 5. A mass determination method which employes the energy focusing of 'MULTUM II' efficiently was discussed. The systematic error owing to stability of pulsed sector fields was removed and then improved mass accuracy. The mass errors was obtained in comparable order ~ 13 ppm to the experimental uncertainties for this method. A higher order energy focusing method was also discussed to improve mass accuracy measurement dramatically. However it is preferred to do more detailed experiments.

In the present study, the instrument has been developed to obtain high mass resolution stably. The multi-turn TOF mass spectrometer has an unlimited path and is able to improve resolution by extending the flight length without compromising the compact size of the instruments. Also, the present study indicates that this type of mass spectrometer is capable of separating the ions of molecular species from organic compounds to an extremely high degree. The introduction of multi-turn TOF mass spectrometer into the first stage (MS1) of MS/MS instruments makes it possible to select the monoisotopic precursor ion. A combination of a multi-turn TOF mass spectrometer e.g. a quadratic-field ion mirror constitute a powerful instrument for structural analysis of peptides or proteins.

Appendix A Transfer matrix method

The transfer matrix method is used to calculate ion trajectories in electromagnetic fields. The coordinate system (x, y, z) is defined with its origin on the optic axis, with z direction along the optical axis as shown in Fig. A.1. In principle, ion trajectories in eletromagnetic



Fig. A.1: Definitions of the coordinate system and the elements of optical position

fields can be calculated by solving the equation of motion. The geometric trajectory of an arbitrary particle can be expressed by an ion optical position vector $(x, \alpha, y, \beta, \gamma, \delta, l)$, where x, y and α, β denote the lateral and angular deviations, and l is the path length deviation of the ion under consideration from a reference ion at the object. The mass and energy deviations, γ and δ , are defined as

$$m/z = (m_0/z_0)(1+\gamma), \quad U/z = (U_0/z_0)(1+\delta)$$
 (A.1)

where U is energy, citation $_0$ belongs to a reference ion. The position vector at an arbitrary profile plane is related to the initial position vector $(x_0, \alpha_0, y_0, \beta_0, \gamma, \delta, l_0)$ in a first order approximation by a transfer matrix A in the following manner:

$$\begin{bmatrix} x\\ \alpha\\ y\\ \beta\\ \gamma\\ \delta\\ l \end{bmatrix} = \begin{bmatrix} A(x|x) & A(x|\alpha) & 0 & 0 & A(x|\gamma) & A(x|\delta) & 0\\ A(\alpha|x) & A(\alpha|\alpha) & 0 & 0 & A(\alpha|\gamma) & A(\alpha|\delta) & 0\\ 0 & 0 & A(y|y) & A(y|\beta) & 0 & 0 & 0\\ 0 & 0 & A(\beta|y) & A\beta|\beta) & 0 & 0 & 0\\ 0 & 0 & 0 & 0 & 1 & 0 & 0\\ 0 & 0 & 0 & 0 & 0 & 1 & 0\\ A(l|x) & A(l|\alpha) & 0 & 0 & A(l|\gamma) & A(l|\delta) & 1 \end{bmatrix} \begin{bmatrix} x_0\\ \alpha_0\\ y_0\\ \beta_0\\ \gamma_0\\ \delta_0\\ l_0 \end{bmatrix}$$
(A.2)

A transfer matrix of each optical component can be calculated numerically when its physical parameters are given. If the system consists of several ion optical components, such as electric sectors, quadrupole lenses and drift spaces, the total transfer matrix R can be simply obtained by multiplying the transfer matrix of the individual elements as

$$R = A_n \times A_{n-1} \times \dots \times A_2 \times A_1 \tag{A.3}$$

Focusing conditions of an entire system will be discussed using the above overall transfer matrix elements R(i|j). In case of using only electric sectors and electric quadrupole lenses, the conditions $R(x|\gamma)=0$ and $R(\alpha|\gamma)=0$ are always fulfilled. Additionally, for example, x, lis expressed in a second order approximation as

$$x = R(x|x)x_0 + R(x|\alpha)\alpha_0 + R(x|\delta)\delta + R(x|xx)x_0^2 + R(x|x\alpha)x_0\alpha_0 + R(x|x\delta)x_0\delta$$
$$+ R(x|\alpha\alpha)\alpha_0^2 + R(x|\alpha\delta)\alpha_0\delta + R(x|\delta\delta)\delta^2$$
$$+ R(x|yy)y_0^2 + R(x|y\beta)y_0\beta_0 + R(x|\beta\beta)\beta_0^2$$

$$l = R(l|x)x_{0} + R(l|\alpha)\alpha_{0} + R(l|\gamma)\gamma + R(l|\delta)\delta + R(l|xx)x_{0}^{2} + R(l|x\alpha)x_{0}\alpha_{0} + R(l|x\gamma)x_{0}\gamma$$
$$+ R(l|x\delta)x_{0}\delta + R(x|\alpha\alpha)\alpha_{0}^{2} + R(l|\alpha\gamma)\gamma_{0}\delta + R(l|\alpha\delta)\alpha_{0}\delta + R(l|\gamma\gamma)\gamma^{2} + R(l|\gamma\delta)\gamma\delta$$
$$+ R(l|\delta\delta)\delta^{2} + R(l|yy)y_{0}^{2} + R(l|y\beta)y_{0}\beta_{0} + R(l|\beta\beta)\beta_{0}^{2}$$
(A.4)

Appendix B 'MULTUM Linear plus'

For MULTUM TOF mass spectrometer, 'perfect focusing'^[1] condition is very important to increase the mass resolution and the ion transmission. 'Perfect focusing' means that absolute values of the position and angle at the final position should be the same as at the initial position in both the horizontal and vertical directions. Moreover, the flight time aberration should be zero. The 'perfect focusing' condition is represented in a first order approximation as follows:

$$\begin{bmatrix} x_f \\ \alpha_f \\ y_f \\ \beta_f \\ \gamma_f \\ \delta_f \\ l_f \end{bmatrix} = \begin{bmatrix} \pm 1 & \underline{0} & 0 & 0 & 0 & \underline{0} & 0 \\ \underline{0} & \pm 1 & 0 & 0 & 0 & \underline{0} & 0 \\ 0 & 0 & \pm 1 & \underline{0} & 0 & 0 & 0 \\ 0 & 0 & 0 & \pm 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 \\ \underline{0} & \underline{0} & 0 & 0 & (l|\gamma) & \underline{0} & 1 \end{bmatrix} \begin{bmatrix} x_i \\ \alpha_i \\ y_i \\ \beta_i \\ \gamma_i \\ \delta_i \\ l_i \end{bmatrix}$$
(B.1)

The subscripts *i* and *f* indicate conditions existing before and after a cycle, respectively. It should be noted that the character $\underline{0}$ (zero with underline) means the matrix element which should be forced to be zero and that 0 (zero without underline) always means zero. Accordingly the nine $\underline{0}$ elements should be zero i.e., the 'nine-fold focusing' condition is required. Ion optical system of 'MULTUM Linear plus' satisfies this condition every cycle. Ion trajectories of 'MULTUM' simulated by 'TRIO-DRAW'^[2] are shown in Fig. B.1; (a) is a top view and (b) is *x*-, (c) is *y*-direction views and (d) is pathlength deviation mode.





Fig. B.1: Ion trajectories of 'MULTUM Linearplus' simulated by 'TRIO-DRAW'. (a) Top view, (b) x-direction, (c)y-direction, (d) path length deviation mode.

'MULTUM Linear plus' whose flight path length per cycle of 1.284 m was constructed. Figure B.2 is a photograph of the instrument in the vacuum chamber. The performance of 'MULTUM Linear plus' was evaluated using CO/N_2 doublet. The mass resolution increased with the number of cycles and that of 350,000 was achieved after 501.5 cycles (shown in Fig. B.3). It consists of many elements, i.e., 4 electric sectors and 28 Q-lenses. In order to reduce weight and the number of parts of the instrument, 'MULTUM II' consisting of only four toroidal electric sector fields and quadrupole doublet lens have been designed and constructed.



Fig. B.2: A photograph of 'MULTUM Linear plus' in the vacuum chamber.



Fig. B.3: TOF spectrum of CO⁺-N₂⁺ doublet for 501.5 cycles.

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