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Development of cryostage for matrix-assisted laser desorption/ionization Fourier transform ion cyclotron resonance mass spectrometry

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Abstract

Matrix-assisted laser desorption/ionization (MALDI) is generally carried out at ambient temperature. Lowering the temperature of a MALDI target is vitally important to IR-MALDI and the image analysis of biological tissues, and is expected to improve the interface between MALDI and Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) by reducing a large kinetic energy distribution of MALDI-generated ions. A new MALDI source equipped with a cryostage capability has been developed for attaching to a commercial FT-ICR instrument. The lowest target temperature of −20 °C was achieved by the cryostage based on a Peltier device. MALDI-generated ions can be focused and be confined by an originally designed quadrupole ion guide which is pressurized with a collisional buffer gas introduced via a pulsed valve. The effect of lowered target temperature on gaining mass spectrometric peak magnitudes was examined. It was found that the observed average molecular weight of MALDI-generated polyethylene glycol 600 (PEG 600) ions was increased upon decreasing the target temperature. Cryo-MALDI has become a feasible technique and may boost the performance and versatility of MALDI FT-ICR MS. (Int J Mass Spectrom 221 (2002) 83–92)

Keywords: MALDI; FT-ICR MS; Cryostage; Quadrupole ion guide; Collisional focusing

1. Introduction

Matrix-assisted laser desorption/ionization (MALDI) has become one of the most widely used techniques for the analysis of large biological molecules [1]. Incorporation of MALDI into Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) was done in an early stage of the history of MALDI [2,3]. Since then, MALDI FT-ICR MS has been playing a unique and important role in the biological mass spectrometry, owing mostly to its ultra-high mass resolving power and mass accuracy [4–7]. Basically, the combination of MALDI with FT-ICR MS is desirable; both the techniques are based on a pulse sequence, thus the combination is accomplished without a loss of the duty cycle. This relationship is analogous to that between MALDI and time-of-flight mass spectrometry (TOF MS).

In general, MALDI is conducted at ambient temperature. Lowering the temperature of a target plate

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for MALDI is recently gaining interest for several reasons. MALDI with a non-UV wavelength, particularly IR, is one of the strongest motifs for developing low-temperature MALDI [8–10]. IR-MALDI is a prospective technique for the analysis of double-stranded DNA [11]. Typical matrices for IR-MALDI are water [8] and glycerol [12]. Both matrices can be used as a solvent of sample solutions in the same time; however, it is difficult to introduce a liquid matrix into a MALDI source, which is a vacuum system. A MALDI target plate at very low temperature allows a liquid matrix to be crystallized and to be introduced into a vacuum system. Such an apparatus may facilitate the IR-MALDI applications.

It is also feasible to load a frozen sample on a MALDI target plate at very low temperature. This feature is particularly suitable to probe polyacrylamide gels of gel electrophoresis [10] or biological tissue sections [13], which can be preserved in good condition before/during being subjected to the measurements. Moreover, MALDI MS has been shown to be effective as an image analysis technology, which depicts a spatial distribution of one or more selected mass values in a sample [14,15]. Applying the technology to tissues, specific biological molecules such as peptides and proteins in the tissues can be localized and be traced. The image analysis with MALDI MS for blots of tissues and tissues themselves has been energetically pursued by Caprioli's group, using a time-of-flight instrument [16]. Although the coupling of MALDI with TOF MS is an adequate choice for the image analysis on the ground of high sensitivity, the mass resolving power and the mass accuracy are not always enough to identify molecules with the measured mass values alone. Viewing the image analysis with FT-ICR MS, laser microprobe mass spectrometry (LM MS) has been pursued by several groups on the basis of FT-ICR instruments [17–21]. LM MS utilizes a UV laser beam focused to 1–5 μm to desorb small ions from a microvolume at the surface of a solid sample and subsequently acquires their mass-to-charge ratios (m/z). Van Vaeck et al. applied FT-ICR LM MS to the analysis of a botanical specimen and obtained the high-resolution mass spectra containing peaks of small organic species [22]. They characterized and localized pigment molecules in the sample by employing the superior identification capabilities of FT-ICR MS.

Lowering the temperature of a MALDI target is vitally important to IR-MALDI and the image analysis of biological tissues; however, introduction of a cryostat into MALDI FT-ICR MS has not been emphasized ever to the best of our knowledge. Several works on laser desorption FT-ICR MS with a cooled sample stage were carried out in mid-1980s by using the Nicolet FTMS instrument provided for circulation of cooled gas through the solids insertion probe. In this article, we describe a new MALDI source equipped with a cryostage, to be coupled with a commercial FT-ICR instrument. Apart from the feasibility of MALDI applications which require the capability of cryostage, this apparatus may improve the combination of MALDI with FT-ICR MS. We already mentioned the compatibility of MALDI and FT-ICR MS on the basis of the technical point of view. Practically, there are, however, some problems in the MALDI FT-ICR MS combination, particularly for high mass ions. Unlike MALDI TOF MS, which accelerates ions toward a certain direction with a very high voltage, FT-ICR MS does not allow a high accelerating voltage to be applied on the external MALDI source because ions must be statically captured in an ICR-trapped ion cell. This constraint makes MALDI FT-ICR MS susceptible to a large kinetic energy distribution of MALDI-generated ions. There are a few studies on the performance of MALDI FT-ICR MS in a high mass region [23]. Our cryostage apparatus may improve the performance of MALDI FT-ICR MS, if the bulk temperature of target might cool down the kinetic energy distribution of MALDI-generated ions. This is actually the primary aim of the present development.

For the same purpose, the new MALDI source attaches an originally designed ion guide, which enables ions to be focused toward the central axis by using collisional relaxation [24,25]. The same concept of a collisional ion guide for MALDI-generated ions has been proved by O'Connor and Costello [26] and has been adopted in the latest design of the Bruker MALDI FT-ICR mass spectrometer [27–29].

2. Experimental

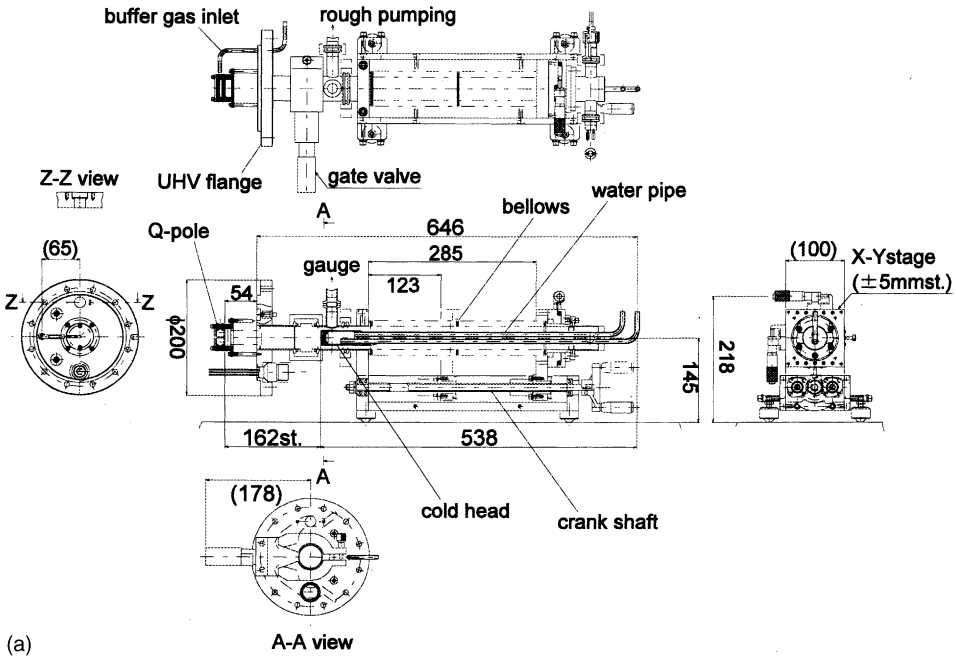
2.1. Mass spectrometer

All measurements were carried out using a BioAPEX70e FT-ICR mass spectrometer (Bruker Daltonics) equipped with a 7-T superconducting magnet. The standard ion source of the instrument is ESI (Analytica of Branford), but other types of ionization including MALDI are also implemented by the manufacturer. A nitrogen laser and optics were used as being supplied. The conventional MALDI source assembly was replaced by a custom designed one, which will be mentioned in the following subsection. The pulse sequence controlling the MALDI experiment was written in a user-programmable language and was run on a Bruker APEX II console. We slightly modified the default pulse sequence to enable the MALDI-generated ions to be accumulated externally in a quadrupole ion guide for several shots of the pulsed laser. The modified pulse sequence is similar to that of ESI experiments, except for adding a pulse output which gives the timing of introduction of a collisional cooling gas (dry nitrogen gas) into the quadrupole ion guide region via a pulsed valve (Series 9, General Valve Corp.).

2.2. Cryostage MALDI source

The custom designed cryostage MALDI source assembly is mounted on the same vacuum flange as the Bruker original one, as being directly attached to the vacuum chamber of BioAPEX70e. Fig. 1 shows the factory drawing of the cryostage MALDI source. Numbers on the diagram denote the dimensions (in millimeter) of labeled components. The heart of the new source is a cold head consisting of a Peltier device and a heat sink which is in contact with cold water. The MALDI target plate is made of copper and is placed on the cold head with solder. This plate works as an endothermic side of the Peltier device and can be electrically biased up to tens of volts to push generated ions off. Temperature of the plate is monitored by a thermocouple. The Peltier device (Ferrotec Inc.) has dimensions of 7.98 mm × 7.98 mm × 2.08 mm and its maximum temperature difference achieves 70 °C. The interface between the cold head and the cooling water housing is sealed by a Viton O-ring, allowing a fall in temperature of the cooling water well below zero. The cooling water is circulated through supply and drain pipes, which are made of stainless steel and form a solid probe to support the cold head. A vacuum chamber consisting of bellows, whose one end is mounted on an *x–y* stage with micrometer heads in the precision of 0.5 μm, surround the cold head and the solid probe. The chamber has two ports for a Pirani gauge head and a rough-pumping line, and is isolated from the source chamber by a gate valve (VAT, 01232-BA06) to establish a load-lock system. The *x–y* stage rides on a screw gear whose stroke is 162 mm. By rotating a crank joined to the screw gear the cold head can be smoothly inserted into the source chamber.

The electrode block of the MALDI source assembly was modified from the conventional design, aiming at enhanced efficiency of the ion extraction. In the original Bruker MALDI source assembly, the electrode block consists of housing, pusher, and a pair of extraction electrodes to form a slit-like gap from which MALDI-generated ions are extracted. This design allows only ions of a small divergence to selectively pass through the slit. Consequently, a large part of MALDI-generated ions which have an initial distribution of the takeoff angle cannot be extracted. The modified electrode block has a short quadrupole ion guide driven in the rf-only-mode. The quadrupole rods are 14 mm in diameter and 12 mm in length. The distance between the centers of opposite rods is 22.8 mm. With the assistance of collisional cooling, by introducing a gas via a pulsed valve, the quadrupole ion guide can collimate MALDI-generated ions of large takeoff angles. After inserting the cold head, the target plate is positioned at a distance of 2 mm from the ion guide. The other end of the ion guide has an extraction plate with a small orifice. The sign of the electric potential applied on the extraction plate can be switched, allowing MALDI-generated ions to be trapped for an extended period. During an external trapping period, ions collide with buffer neutrals and are focused into the center of the ion guide. This



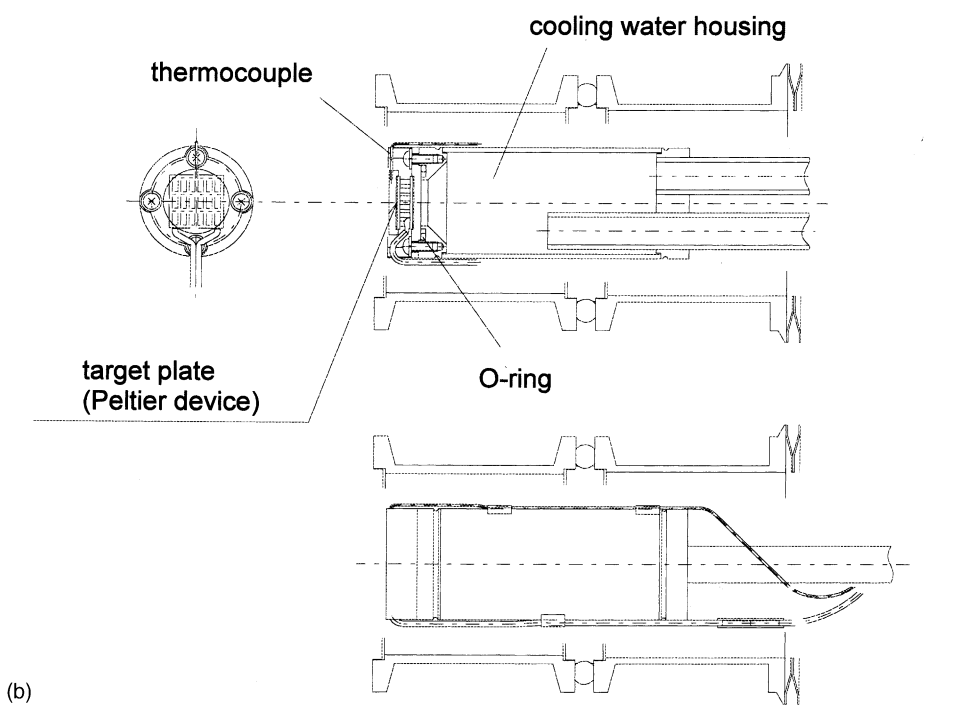


Fig. 1. (a) Factory drawing of the new MALDI source equipped with a cryostage. (b) Enlarged part of the drawing for the cold head.

process may improve not only the extraction efficiency but also the distribution of ionic velocities, which is important to the stable transport of ions from the source to the analyzer region. Moreover, MALDI-generated ions can be accumulated in the ion guide for several shots of the pulsed laser.

The cryostage MALDI source was built at a manufacturer (ARIOS Inc.). The rf-waveform for the quadrupole ion guide was generated by a function generator (Iwatsu Electric Co., Ltd., SG-4111) and was amplified by a bipolar power supply (NF Corp., HSA4101). A pair of waveforms of reversed phases was obtained from a pulse transformer (O.E.P. Inc.).

2.3. Sample preparation

Methylene blue and 2,5-dihydroxy benzoic acid (DHB) were purchased from Wako Chemicals. Polyethylene glycol 600 (PEG 600) was purchased from Nacalai Tesque. Methanol (Water Analysis Grade) was obtained from Kanto Chemicals and was used as supplied. Methylene blue was dissolved in methanol in the concentration of 10 mg/mL. Matrix solution was prepared by dissolving 15 mg of DHB in 1 mL of methanol/water (1:1). PEG 600 was dissolved in methanol in the concentration of 0.08 mg/mL, then diluted with the matrix solution in the volume ratio of 1:10. For the methylene blue solution 1 μL was pipetted and was loaded on the target. For the solution of PEG 600/matrix mixture 2 μL was dispensed then was lightly tapped with a pipette tip to facilitate forming the polycrystine on the target. In each case, the target was completely dried up in the ambient temperature.

3. Results and discussion

3.1. New MALDI source in the ambient temperature

Operational conditions of the ion guide were explored and were optimized experimentally by using a target in the ambient temperature. There are additional parameters involved with replacing the conventional electrode block by the ion guide; an amplitude and a frequency of the rf-waveform to drive the quadrupole, and on-time and backing pressure of the pulsed valve to introduce a collisional cooling gas.

Fig. 2 shows a variation of the magnitude of m/z 284 from methylene blue by changing the dc-potentials applied on the extraction and target plates of the new MALDI source assembly. These electric potentials correspond to the parameters of the conventional MALDI electrode block. A collisional cooling accumulation was not employed in this experiment. The measurements were repeated five times under the same conditions; the average values and standard deviations are indicated as markers and bars, respectively. There was a small gain in the peak magnitude by lowering the pusher potential, but a rather flat response to the extraction potential has been observed. Fig. 3 shows a variation of the same signal as above by changing the rf-amplitude applied to the quadrupole. In contrast to the influence of dc-parameters, the rf-amplitude was found to be significant for the peak magnitude. A 10-fold gain was achieved in the examined range of amplitude (20–140 V_{p-p}). A further improvement in the performance of the ion guide is expected with

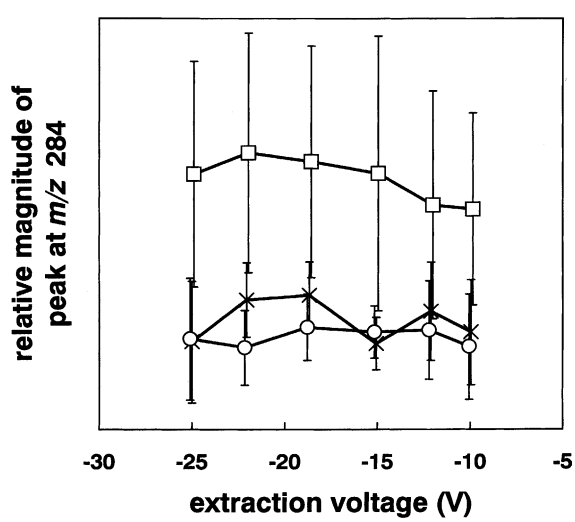


Fig. 2. Mass spectrometric peak magnitude of methylene blue $m/z = 284$ as a function of extraction voltage for pusher voltages of 6.03 (□), 10.27 (×), and 15.39 V (○). Markers and bars indicate the average values and the standard deviations of five individual measurements, respectively.

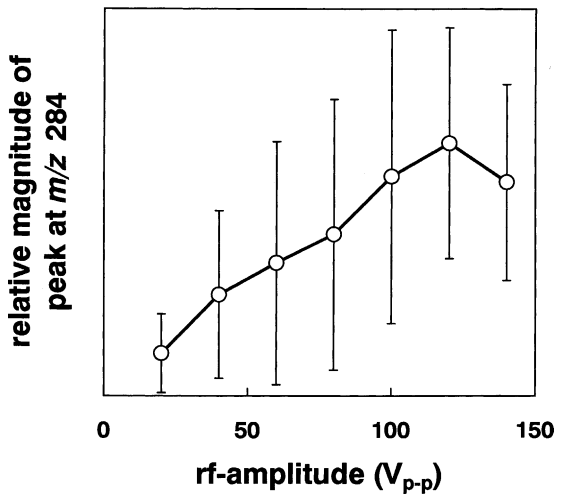


Fig. 3. Mass spectrometric peak magnitude of methylene blue $m/z = 284$ as a function of rf-amplitude applied to the quadrupole ion guide. Markers and bars indicate the average values and the standard deviations of five individual measurements, respectively.

a larger rf-amplitude, which is not available in the present apparatus. The peak magnitude shows some dependence upon also a frequency of the rf-waveform (Fig. 4). There is a threshold frequency around 330 kHz for the quadrupole device to function. The

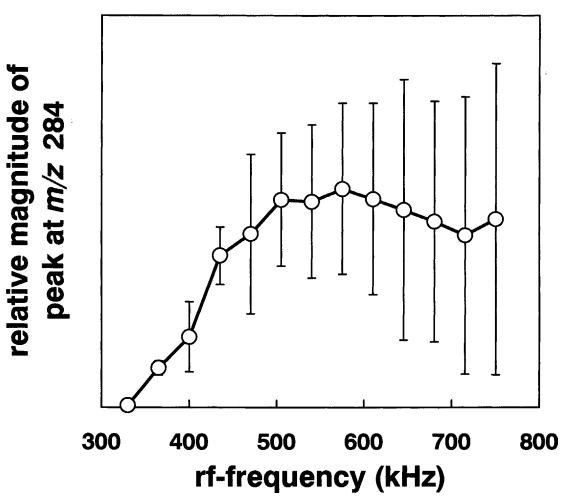


Fig. 4. Mass spectrometric peak magnitude of methylene blue $m/z = 284$ as a function of rf-frequency applied to the quadrupole ion guide. Markers and bars indicate the average values and the standard deviations of five individual measurements, respectively.

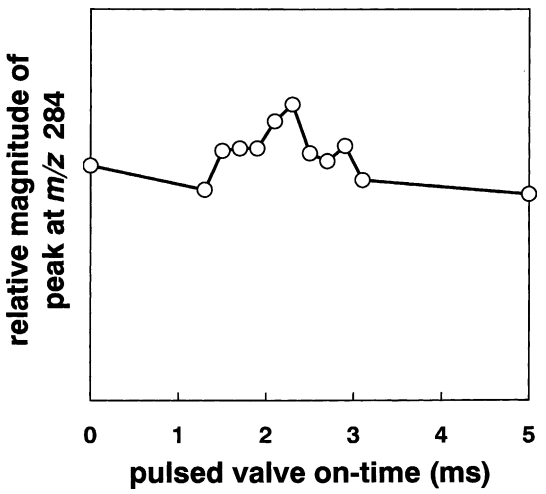


Fig. 5. Mass spectrometric peak magnitude of methylene blue $m/z = 284$ as a function of the pulsed valve on-time for introducing a buffer nitrogen gas.

frequency which had given the maximum peak magnitude was used throughout the following experiments.

The collisional cooling effect in the ion guide was examined with pulsed-in-dry nitrogen gas. The backing pressure, which was a direct readout of a Pirani gauge at a nitrogen reservoir, was maintained at 1×10^3 Pa and the magnitude of m/z 284 was recorded for various on-time periods of the pulsed valve. The result is shown in Fig. 5, where an only small variation of the peak magnitude can be seen. Fig. 6 shows the spectra of MALDI-generated PEG 600 for various on-time periods. The variation of the mass spectrometric peak magnitudes appeared to be within a fluctuation between each measurement. We believe the collisional cooling effect should be obtained with the proposed ion guide in appropriate conditions. We were not able to read precisely the temporal pressure in the source chamber because the cooling gas was immediately pumped out and the base pressure ($<10^{-4}$ Pa) was recovered. The readable highest pressure was about 10^{-3} Pa, which may be order-of-magnitude lower than the actual pressure considering the location and the response time of the cold cathode gauge attached to the source chamber. Nevertheless, this estimated pressure region seems not to be high enough to realize

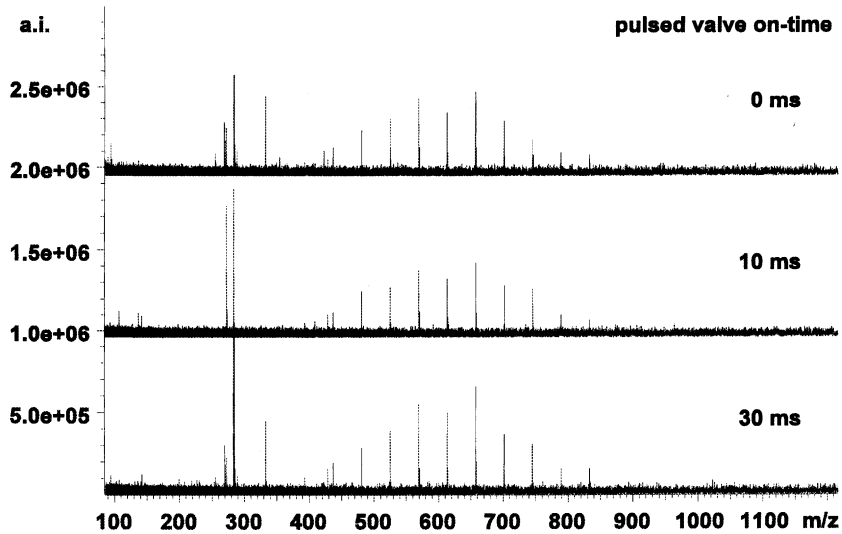


Fig. 6. MALDI-generated PEG 600 spectra for various on-time periods of the pulsed valve: (top) 0 ms; (middle) 10 ms; (bottom) 30 ms.

the collisional cooling effect. Our previous simulation study shows that a pressure around 1–10 Pa (mean free path $= 10^{-1}$ to 10^{-2} mm) is necessary for a sufficient number of ion/neutral collisions to occur in the ion guide for the collisional cooling effect [24]. For this reason, we have not established the external accumulation of MALDI-generated ions with the proposed ion guide. The present ion source cannot be pressurized to the desirable pressure range necessary for an effective collisional cooling and the external accumulation, due to a large conductance between the ion guide region and the cryo pump. To overcome the above mentioned problem an airtight tube will be necessary to cover the ion guide in the revised version of the new MALDI source whose development is being undertaken.

3.2. Effects of lowered MALDI-target temperature

To avoid frosting the cold head, samples were deposited on the target at ambient temperature and the target was cooled by the Peltier device following the introduction of the solid probe into the vacuum chamber. The lowest target temperature achieved by the present instrumentation was about −20 °C, despite the maximum temperature difference of 70 °C found in the specifications of the Peltier device. Lower temperature may be obtained by improving the heat sink efficiency. In the present paper, we will report the effects of the target temperature ranging from 30 to −15 °C.

Fig. 7 shows the relationship between the magnitude of $m/z = 284$ and the target temperature. It is clear that

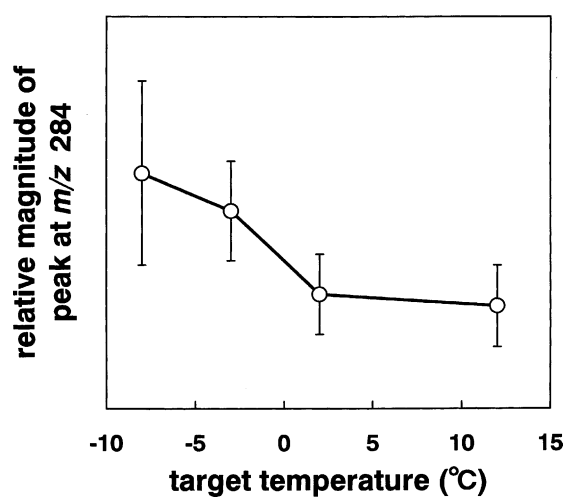


Fig. 7. Mass spectrometric peak magnitude of methylene blue $m/z = 284$ as a function of the target temperature. Markers and bars indicate the average values and the standard deviations of five individual measurements, respectively.

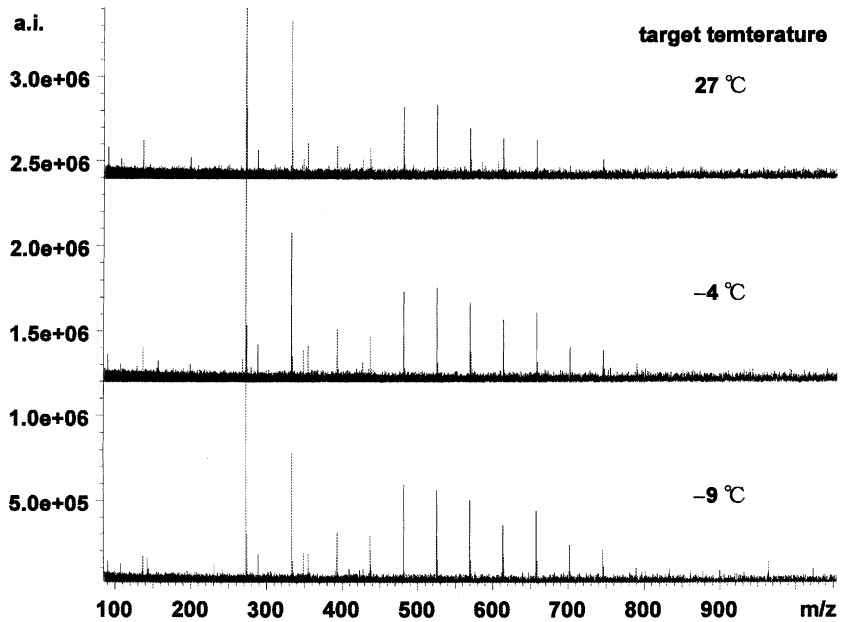


Fig. 8. MALDI-generated PEG 600 spectra for various target temperatures: (top) 27 °C; (middle) −4 °C; (bottom) −9 °C.

a decrease in the target temperature enhances the peak magnitude. This effect was presumably attributed to sustainable spots on the low-temperature target. The signal of methylene blue ions desorbed from a spot on the usual target was diminished after a few shots of laser. In contrast, the signal obtained from a spot on the low-temperature target kept a substantial magnitude for the examined number of shots. The lowered temperature may reduce sputtering and/or bulk ablation of an irradiated spot.

Fig. 8 shows spectra of PEG 600 recorded by changing the target temperature. The total abundance of PEG 600 ions was plotted for a range of the target temperature (Fig. 9). It was found that the mass spectrometric peaks of the MALDI-generated ions increased with decreased target temperature, as similar to the case of methylene blue ions which were generated by the laser desorption ionization (LDI). More interestingly, the apparent distribution of molecular weights of PEG 600 was dependent upon the target temperature. The number average molecular weight (M_W) was obtained from each spectrum in Fig. 8 and was plotted as a function of the target temperature (Fig. 10). Upon decreasing the temperature, M_W shifts to a higher value, which is more close to that of PEG 600. A possible explanation for these effects is a reduction in laser-induced dissociation of the polymers, and/or relaxation of a large kinetic energy distribution of the MALDI-generated

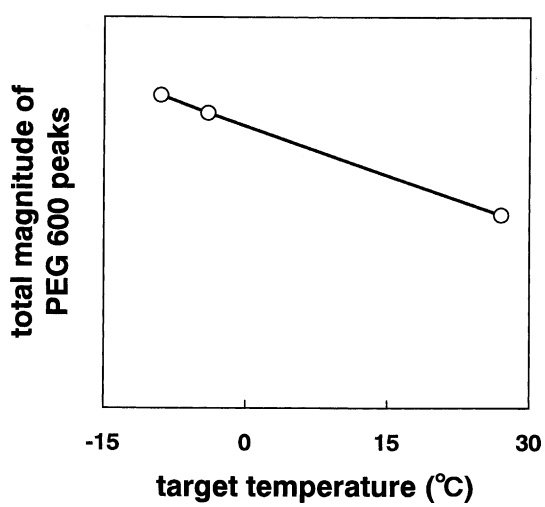


Fig. 9. Total mass spectrometric peak magnitude of PEG 600 as a function of the target temperature.

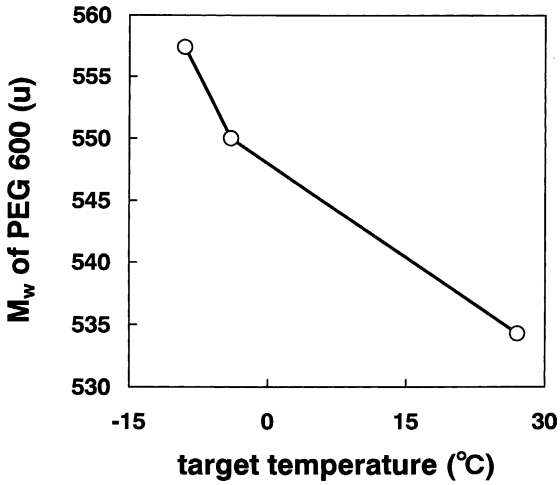


Fig. 10. Observational number average molecular weight (M_W) of PEG 600 as a function of the target temperature.

species. Usually, it is difficult to detect heavy ions with MALDI FT-ICR MS [23]. The motion of ions trapped in an ICR cell consists of three modes: cyclotron, magnetron, and trapping oscillation. The magnetron mode is a rotational motion of the guiding center around the center of an ICR cell. The magnetron radius is directly proportional to both the mass-to-charge ratio and the velocity of ions. In the principle of FT-ICR MS, ions of too large magnetron radii are not detectable. It is suggested that a smaller ionic velocity at lower target temperature may help to detect heavier ions. In addition, a Coulombic effect may be one of the sources for the observed M_W shifts. Lowering the temperature increases the total number of ions, then the ion number may effect on the molecular weight distribution.

4. Conclusions

We have developed a new MALDI source equipped with a collisional focusing ion guide and cryostage capability, coupled with a commercial FT-ICR mass spectrometer. We have found that at lower temperature of the target plate an increase of the magnitudes of both MALDI-generated (PEG 600) and LDI-generated (methylene blue) ions takes place. The observed average molecular weight of MALDI-generated PEG 600 ions increases with a decrease of the target temperature. There are several possible mechanisms to rationalize these effects. A detailed elucidation of the low temperature effects will be discussed in a future work with further lowering the target temperature. We have explored the optimum conditions for the collisional focusing ion guide. Mass spectrometric peak intensities were dependent upon a frequency and an amplitude of the rf-waveform applied on the quadrupole of the ion guide. The present pulse gas system did not fulfill the idea of collisional focusing of ions in the ion guide. In order to achieve both effective collisional focusing of ions and extremely low temperature of the target plate, the revised designs of an airtight housing for the ion guide and a heat sink for the Peltier device are being undertaken. Completing the development of this apparatus may put forward MALDI FT-ICR MS to a versatile technique applicable to IR-MALDI and direct probing of biological tissues.

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