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A new external ionization multisample MALDI source for Fourier transform mass spectrometry

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Abstract

A new external ionization source for matrix-assisted laser desorption/ionization (MALDI) Fourier transform mass spectrometry is described. This design allows a number of samples to be introduced simultaneously. This capability is important for studying metal ion-coordinated species produced by MALDI. The source is used to observe the effects of alkali metal ion affinities of neutral oligosaccharides on the intensity of the respective quasimolecular ions. (Int J Mass Spectrom 204 (2001) 23–29) © 2001 Elsevier Science B.V.

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1. Introduction

Matrix-assisted laser desorption/ionization (MALDI) [1] has proven to be a sensitive and reliable method for producing gas-phase oligosaccharide ions [2]. Neutral oligosaccharides typically coordinate alkali metal ions when ionized by MALDI to produce quasimolecular ions [2,3]. The respective alkali-metal-coordinated species are readily produced by the addition of the appropriate alkali-metal chloride to the oligosaccharide solution. This method has been extensively used in this laboratory and elsewhere to produce various ionic species and to study the effect of alkali metals on fragmentation during ionization [2,3]. It has also been used to obtain relative dissociation thresholds of alkali-metal ions coordinated to oligosaccharides [4,5]. The method is facilitated by MALDI, which is not as sensitive to salts as, say for example, electrospray ionization.

Fourier transform mass spectrometry (FTMS) has long been an important tool for studying ion/molecule chemistry and specifically metal ion chemistry. The external source represented a major innovation in the advancement of (FTMS) as it allowed the coupling of various ionization sources including MALDI [6–8] to FTMS. When MALDI was coupled as an external source for FTMS, it provided a versatile tool for producing and analyzing metal coordinated species [8].

The determination of intrinsic properties such as the binding energies of metal ions coordinated to oligosaccharides is difficult due to the complexity of the system. For this reason, relative values are often obtained. However, reproducibility is often a problem with MALDI as various factors including, for example, the rate of crystallization, can affect the intensi-

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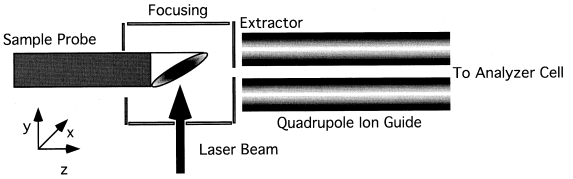


Scheme 1.

ties of the ions. For this reason it is preferable to maintain specific conditions such as those pertaining to ionization and detection. The development of a multisample probe for external MALDI source would be of value in this regard as it allows several samples to be produced at once and introduced into the MALDI source simultaneously. This greatly facilitates the analysis and helps maintain the experimental conditions through the course of several experiments.

Most external source FTMS instruments rely on ion guides to transfer ions produced in a low vacuum source to an ultrahigh vacuum analyzer chamber to gain the high-resolution and high-mass-accuracy capabilities of FTMS. However, the position of the ion guide poses several technical problems. Although multipole ion guides such as quadrupole and octapole have large angular acceptance, MALDI produces sufficiently divergent beams that the entrance of the guide must be directly in front of the MALDI probe (Scheme 1). For multipole ion guides, the simplest and most effective orientation is to position the laser and the ion guide 90° relative to each other. The probe surface is made accessible to the laser beam by orienting it 45° to the incidence beam and 45° to the extractor electrode. Because of the relative orientation of the laser and the ion guide, the number of samples that can be accommodated in this design is severely limited. Time-of-flight (TOF) instruments do not need highly collimated ion beams. For this reason, the development of the multisample MALDI source for TOF instruments was relatively simple. Commercial TOF-MS instruments routinely come equipped with multisample probes. Recently, FTMS manufacturers have began offering multisample probes for external MALDI sources but these arguably lack the flexibility or the performance of the TOF sample probes.

In this article, we present a versatile design for an external ionization source that will accommodate multisample probes and even microchip-based separation devices. This design readily allows a large number of sample wells to be incorporated into a single probe. It will also be useful for analyzing separation devices that spread the sample over a relatively large surface area such as thin layer chromatography or gel electrophoresis that are currently not amenable to direct analysis. The multisample probe will not only benefit the study of metal coordinated species, but will generally allow the analyses of multiple samples to meet other needs such as the analysis of products from combinatorial libraries where dozens or hundreds of samples need to be analyzed in a relatively short period of time.

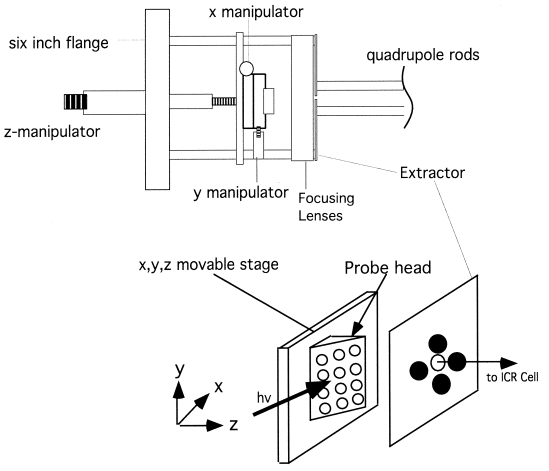
2. Experimental

The oligosaccharides and the MALDI matrix, 2,5-dihydroxybenzoic acid, were obtained from Sigma-Aldrich (St. Louis, MO). All compounds were used without additional purifications. Experiments were performed using a homebuilt external source FTMS described in detail in earlier publications [9,10]. The instrument consists of two vacuum chambers (a dual chamber) with one dedicated to MALDI and another dedicated to electrospray ionization. The two chambers share a 5.2 T Oxford superconducting magnet but each has a separate IonSpec (Irvine, CA) data system. A Laser Science Inc. (Franklin, MA) nitrogen laser operating at 337 nm is used for the desorption experiments. The MALDI source was previously a single sample source. Modifications to the instrument to accept multisample MALDI probe are discussed in the following section. MALDI samples of the oligosaccharides were prepared using standard procedures also described in an earlier publication [3].

3. Results

3.1. Construction of new multisample ionization source for MALDI/FTMS

The MALDI source region of the instrument was modified from a single sample to a multiple sample



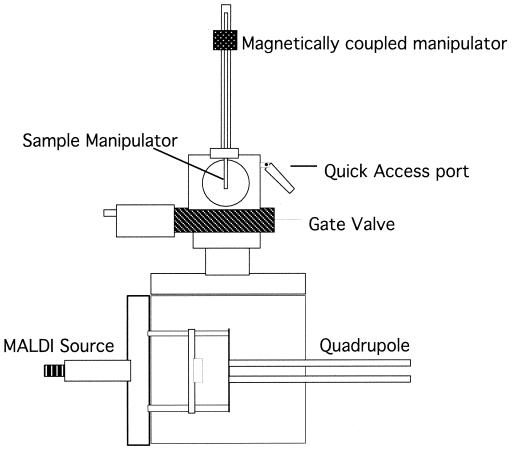
Scheme 2.

configuration. The previous MALDI source consisted of an array of stainless steel lenses fashioned into a cube with the sample probe positioned in the middle of the cube and in front of the extractor plate (Scheme 1). The entire assembly was mounted on a six-inch flange. The flange was bolted to a six-inch vacuum cube. The probe was made from a stainless steel shaft and is removed through a load-lock system.

The new MALDI source is similarly mounted on a six-inch flange and includes movable *x*, *y*, and *z* stages (Scheme 2). The flange is attached to the front end of the six-inch cube that serves as the main ionization source chamber. The *x*- and *y*-movable stages are made of aluminum and are translationally adjusted by two manipulators, one for each stage. The *z* stage is controlled by a linear manipulator with a micrometer dial on the ambient side of the flange (MDC Corp., Hayward, CA). The *x*- and *y*-stage manipulators (Newport Corp., Irvine, CA) have a linear range of 12.5 mm and are normally used for the mounting of laser optics. They were not designed for UHV applications. However, we observed no detrimental effects either to the manipulators or to the vacuum when they are placed directly inside the vacuum chamber and incorporated into the translational stages. Control is performed externally using electrical feedthroughs. Except for their large sizes and only moderate resolution (0.1 mm), the manipulators appear to work well in this application. Because the laser beam is positioned to impact the probe directly in front of the extractor aperture, the position of the probe must be adjusted in all three dimensions. The next stage in the development of this source is to use computer-controlled manipulators that will allow each well to be addressed automatically.

To accommodate the adjustable stages and the new probe head, the ion focusing elements were greatly simplified. Although the extractor was essentially left intact, the steering electrodes (or focusing lenses in Scheme 2) were made considerably smaller so as not to interfere with the manipulators and the laser beam. MACOR spacers were used to keep the stainless steel electrodes in place. The dimensions of the steering electrodes, as shown in the diagram in Scheme 2, are 6.5 mm×23 mm. There was some concern whether these elements would be sufficient to guide the ions out of the source, however as we will show below the size of the steering lenses do not appear to affect the quality of the spectra.

A sample introduction device that allows the sample probes to be exchanged rapidly (in a matter of minutes with pumpdown time) was also added to the ionization source region. A commercially available load-lock system was attached to the top of the six-inch vacuum cube. The sample introduction device consists of a small chamber with a quick access port, a magnetically coupled manipulator and a gate valve to isolate the device from the main ionization source chamber (Scheme 3). To place a sample in the source, the gate valve is closed and the device vented to allow the quick-access port to be opened. The sample holder is placed on the end of the magnetically coupled manipulator by a grappling device that was built in-house. The window of the port is closed and the sample introduction device chamber is pumped down by a small mechanical pump (with a pumping speed of 9 $ft^3\,min^{-1}$). When the pressure of the load-lock chamber reaches base pressure (approximately 10^{-3} Torr), the gate valve is opened and the sample is lowered into the translational stage of the ionization source. The sample probe head is released and the magnetically coupled manipulator is raised. The gate valve is shut again to isolate the load-lock chamber from the ionization source. The pressure in

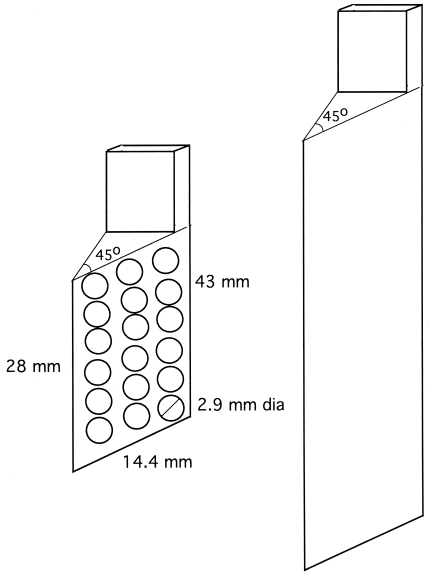


Scheme 3.

the source during the analysis is between 10^{-6} and 10^{-8} Torr.

3.2. Sample probe head

The MALDI probe head is made of aluminum and shaped as a wedge to expose the surface at 45° incidence to the nitrogen laser (Scheme 4). Using this design, every part of the 14×28 mm surface is accessible to the beam. For maximum sensitivity, it is desirable for the laser spot to be always in front of the extractor hole. In the present configuration, 18 wells with 3 mm diameter are machined on the surface. The number of wells is increased by decreasing the diameter of the wells. We have increased the number of wells up to 100, however there is a limitation based on the effective amount of material that can be conveniently placed on the surface. To accommodate still more sample wells, the size of the probe head can be increased by elongating it as shown in Scheme 4. The elongated probe head is not expected to alter the performance of the instrument relative to the shorter version. The length of the probe is currently limited only by the manipulator travel in the vertical direction. In the present vacuum configuration, probe heads as long as four inches can potentially be analyzed. Longer probe heads would need only a vacuum chamber extension above the six-inch cube to move the load-lock chamber further up. Finally, for convenience a video camera with a zoom lens is mounted near the source to monitor the position of the laser spot relative to the sample well.



Scheme 4.

3.3. MALDI/FTMS of oligosaccharides on multisample probe

To examine the performance of the probe, permethylated β-cyclodextrin [heptakis-(2-3-6)-tri-O-methyl-β-cyclodextrin], a cyclic heptasaccharide, was analyzed with MALDI/FTMS. This compound is commonly used in this laboratory for diagnostic purposes. It usually generates strong signals with MALDI. We examined whether the design, specifically with regard to the small focusing elements, was sufficient to provide strong signals with MALDI/FTMS. We also wanted to determine the sensitivity at various positions on the probe head.

Fig. 1 shows the MALDI/FTMS spectrum of β-cyclodextrin doped with NaCl to produce primarily the sodium-coordinated parent. The quasimolecular ion is the base peak (*m/z* 1453) and there is little fragmentation in the spectrum. The ion at *m/z* 727 (designated by an asterisk) corresponds to an overtone of the quasimolecular ion—a consequence of the strong ion signal and the cubic analyzer cell design.

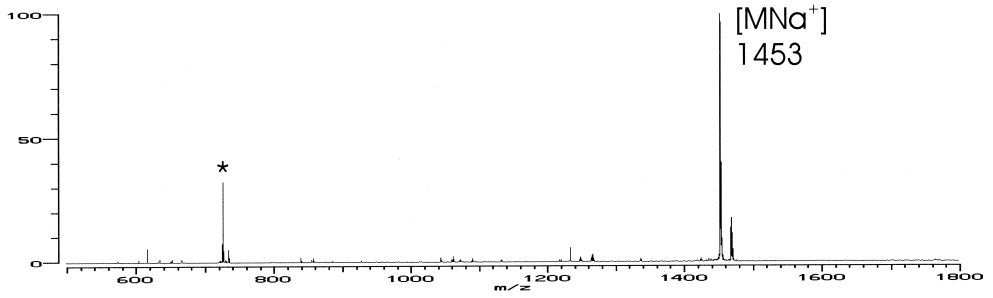


Fig. 1. MALDI-FTMS spectrum of β-cyclodextrin doped with NaCl employing the multisample probe. The quasimolecular ion is the base peak with *m/z* 1453.

Although we have not performed thorough comparisons between the single sample source and the multisample source, indications are that the resolution, the mass accuracy, and the sensitivity are relatively unchanged. The ion transport and ion trapping are apparently not affected by the new design. To obtain the spectrum in Fig. 1, the extractor voltage was set to −90 V and all the steering plates were set to 160 V to collimate the beam toward the center of the extractor plate. The manipulators appeared to function under vacuum as they do in atmosphere. Outgasing of the manipulators in the source was not readily observed and did not appear to affect the performance of the instrument.

We find only small variations in the intensity of the quasimolecular ion between the wells on the same column (Spectra not shown). For example, the variation in the signal intensity in first column (the one closer to the extractor plate) from the five wells is less than 10%. This is the natural variation in MALDI intensities even under identical conditions. There is similarly little variation between spectra from the first and the second column. However, there is a slight but general attenuation of signals from samples placed on the third column. The intensities of ions produced from samples in the third column are consistently 30%–40% lower than those from the first two columns. We suspect that the metal probe head affects the electric field produced by the extractor plate and the steering lenses as the sample well is brought closer to the extractor plate.

3.4. MALDI/FTMS of oligosaccharides with different alkali metal ion dopants

A sample probe containing β-cyclodextrin doped with the chloride salts of Li, Na, K, Rb, and Cs was placed in the ionization source and examined by MALDI. The aliquots from the same oligosaccharide sample solution were placed on five wells of the first column of the probe and to each well different chloride salts were added. Fig. 2 shows the MALDI/FTMS spectra of samples, scaled to the LiCl doped sample. The spectrum for Na was shown previously in Fig. 1. Each spectrum was obtained from the sum of ten scans. There is some variation in the abundances of the quasimolecular ions, but in each case the quasimolecular ion is the base peak. The ionization conditions for each sample was optimized for minimal fragmentation, hence there are no fragment ions in the spectra. Only the overtone at one half the quasimolecular ion and some electrical noise are observed. The signals corresponding to the oligosaccharide coordinated to Li (*m/z* 1437), Na, and K (*m/z* 1469) all have similar intensities. The relative deviation between each dopant is less than 5%. However, the signals for Rb (*m/z* 1515) and for Cs (*m/z* 1563) are attenuated by about 10%–15%.

Although seemingly simple, these types of comparative experiments would be difficult with single-sample probes. In an earlier publication, we demonstrated that Rb and Cs are not as strongly bound as the smaller alkali metal ions to neutral oligosaccharides [3]. We suspected that the lower affinity of the

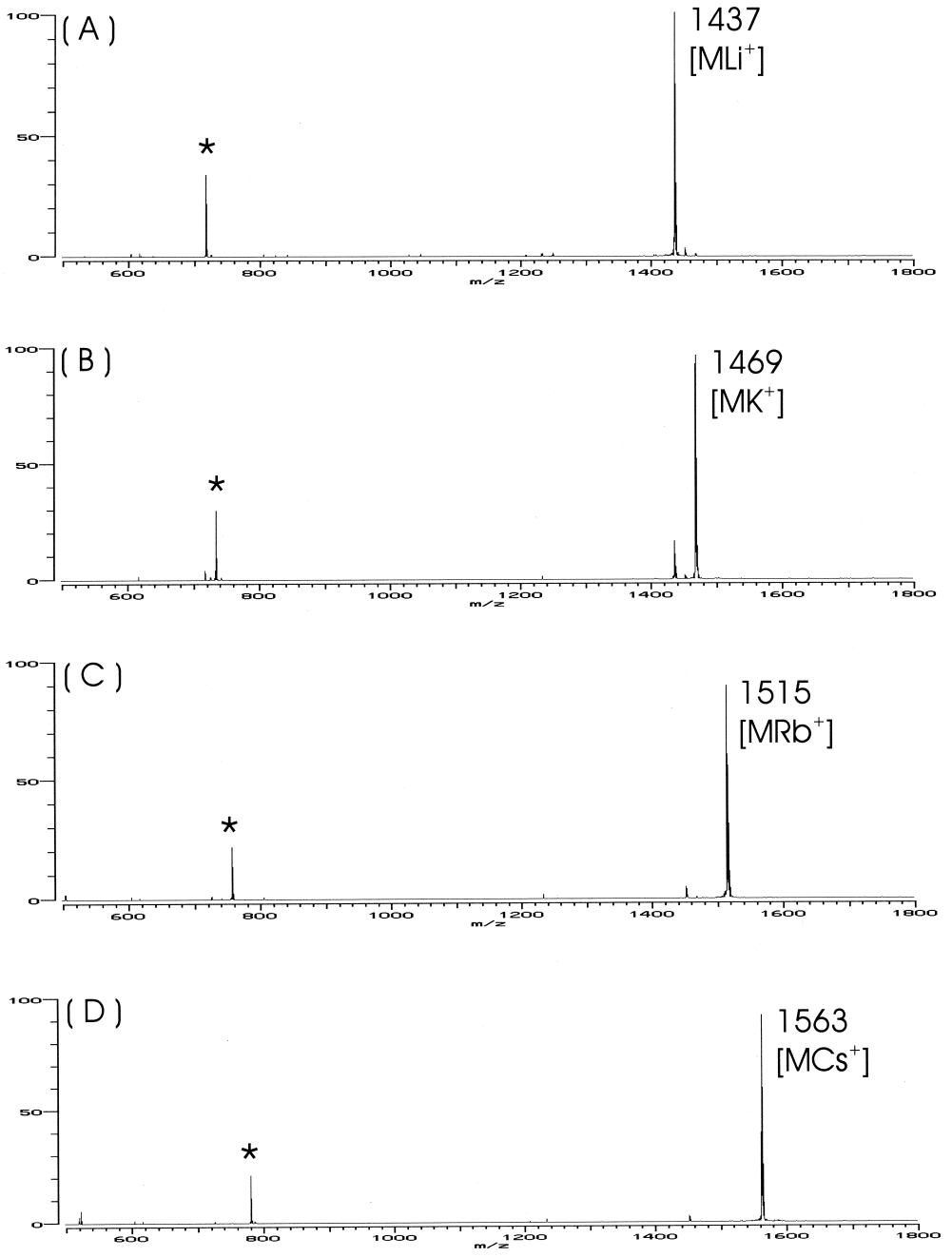


Fig. 2. MALDI-FTMS spectra of β-cyclodextrin doped with various alkali metal chlorides. The spectra were all obtained with samples placed on the first column. The spectra show that Li and K (and Na) produce similarly intense quasimolecular ions while Rb and Cs show somewhat decreased intensities. The results are consistent with the notion that alkali metal ions that are not strongly bound to the oligosaccharide do not similarly exhibit intense quasimolecular ions.

oligosaccharides for large alkali metal ions such as Rb and Cs would affect the sensitivity and the detection limit. However, because of the multitude of conditions for preparing MALDI samples and the nature of

the single-sample probe, we could not perform direct comparisons between different dopants to illustrate that some worked better than others. The new probe allows specifically these types of experiments. The new results are consistent with the notion that because Li, Na, and even K are more strongly bound, they also produce more intense signals.

4. Conclusion

A simple and easily implemented multisample MALDI external source is described for a Fourier transform mass spectrometer. The ionization source is versatile and does not diminish the performance of the instrument relative to the single-sample device. On the contrary, the multisample probe adds additional capabilities that are well suited for ion/molecule reactions. Moreover, the probes will find wide applications in the analysis of biomolecules such as oligosaccharides. The probe heads are currently being adapted to accept parts of separation devices such as gels, thin layer chromatography, and microchips for capillary electrophoresis.

Acknowledgements

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